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ANOTHER MILESTONE

The XXIst World Conference of the International Society for Fluoride Research will be held later this year in Budapest, Hungary. The date will be August 25-29 (later than the date announced in our last issue - see announcement on following page). The Society’s Vice President, Dr Miklós Bély, is organizing an interesting program. All Society members, and other readers of our journal, are urged to make it to Budapest for this historic event. Those who attended our XIXth Conference in 1989 will recall the unique beauty of the ancient cities of Buda and Pest straddling the Danube river in central Europe.

The first world conference on fluoride research, leading to the founding of our Society, was held in Bern, Switzerland, in 1962. The next world conference, the first in the name of our Society, was held in Frankfurt, Germany, in 1967. A year earlier the American Society for Fluoride Research, the forerunner of our Society, had held a meeting in Detroit that included participants from Europe and Asia as well as North America. In 1978 Dr George Waldbott, founding editor of this journal, wrote of our Society:

“Through its conferences and its journal Fluoride, it has been contributing materially to the advancement of research on fluoride, especially on matters biological and medical. At no time has the Society been involved in the politics of fluoridation. Its members hold widely differing views on the subject and on the role of fluoride in air and water pollution. Biochemists, physicians, dentists, veterinary scientists, botanists, physicists, chemists, and engineers from many parts of the globe have participated in its meetings.”¹ Science is an international exercise, engaging the human intellect in a continuing search for truthful interpretations of our world and universe. Such an enterprise is not easily silenced. Since Waldbott wrote the above words, the Society and its journal have continued to flourish and meet at world conferences, in spite of some powerful influences against it, also described in Waldbott’s book.¹ The last (XXth) conference, held in Beijing, capital of China, was jointly co-sponsored by the Chinese Ministry of Health and the World Health Organization.

Of course, the full history of fluoride research predates that of our Society. After the isolation of elemental fluorine in France in 1886 by Professor Henri Moissan,² the greatest advance was the classic work of Danish medical scientist Kaj Roholm, published in 1937.³ Roholm’s masterly exposition of fluoride pollution inspired those scientists who later founded our Society. His work is memorialized on the cover of each issue of our journal. In 1978 Waldbott also drew attention to the decay of scientific inquiry which “has spread like a cancer in many areas.” He cited the science writer P M Boffey⁴ who had documented an astonishing variety of scientific problems issuing from an illicit merger of government/industrial interests. Six major areas were identified: radioactive waste disposal, the supersonic transport, defoliation, the safety of food additives, persistent pesticides, and airborne lead - to which, wrote Waldbott, “we must add the fluoride problem.”
While primarily devoted to the publication of quality research, our journal is also available for the expression of the varying views of Society members and other readers. Waldbott concluded his book with the observation that no more satisfying nor humane goal can be attained than the truth which alleviates human suffering. Our Society and its journal contribute to the pursuit of that goal. May our XXIst Conference be another milestone on the way.

References

2. Same (pages 16-20).

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**XXIst WORLD CONFERENCE**

of the INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH

in association with the

HUNGARIAN SOCIETY FOR FLUORIDE RESEARCH

BUDAPEST, HUNGARY. AUGUST 25 - 29, 1996

**Venue:** Aquincum Thermal Hotel, on the Danube

**Scientific Program:** will include discussions on effects of fluoride on humans, animals, plants, and the environment

**Language:** All proceedings will be in English

**Registration fee:** Delegate US$300, accompanying guest US$150 (includes lunches and coffee breaks)

**Accommodation:** (at Aquincum Hotel) for 4 nights: single room US$149 per night
double room US$97 each person per night

Book direct with hotel: H-1036 Budapest, Árpád fejedelem útja 94
(Phones: 36 1 250 3360, 36 1 250 4177. Fax: 36 1 250 4672)

**Farewell Banquet:** US$100

**Social:** Conference days will be free after 2 pm, with special programs planned

**Post-Conference Tours**

A. 3 days to northern Hungary
B. 4 days to Vienna and Prague
C. 7 days to both above

Direct enquiries to: Dr Miklós Bély, Department of Morphology, National Institute of Rheumatology, PO Box 54, H-1525 Budapest 114, Hungary
EFFECTS OF FLUORIDE ON GROWTH AND SOLUBLE SUGARS IN GERMINATING MUNG BEAN (Vigna radiata) SEEDS

Ming-Ho Yu
Bellingham, Washington, USA

SUMMARY: The influence of NaF on the growth and soluble sugar contents of mung bean (Vigna radiata) seedlings was studied. One-day-old seedlings were treated with 0, 0.1, 1.0, or 5.0 mM NaF at 25°C for 72 h, and the wet weight, root elongation, and soluble sugar levels of the seedlings were determined. Wet weight of the cotyledons was increased while the weight and length of radicles were significantly depressed in seedlings exposed to NaF at and above 1.0 mM. Total soluble sugars and, particularly, reducing sugars, decreased with increase in F concentration. The results suggest that an impaired sugar metabolism may be an important factor contributing to the observed inhibition of germination in mung bean seedlings exposed to NaF.

Key Words: Fluoride; Growth; Mung bean seedlings; Radicle elongation; Soluble sugars.

Introduction

The importance of seed germination in plant growth is widely recognized. Seed germination has increasingly been used as a model for studying the toxic effects of environmental pollutants on plants. Sodium fluoride at concentrations as low as 0.1 mM was shown to inhibit root elongation and phytase during corn germination. When exposed to 10 mM NaF, a reduction in RNA contents of 3-mm root tips of corn seedlings occurred. In addition, the ratios of acid soluble nucleotide species were altered in the seedlings. These changes were attributed to F-induced inhibition of ATPase and 5' nucleotidase.

We showed previously that NaF inhibited the germination of mung bean (Vigna radiata) seeds and that such inhibition appeared to be correlated with lowered amylase activity caused by fluoride. Because addition of Ca ions partly restored amylase activity lowered by F, it was concluded that the inhibitory effect of F on amylase may be through its removal of Ca needed for amylase activity. This paper is concerned with a further study on F-induced growth inhibition of the germination process of mung bean seeds. A marked decrease in root elongation and a concomitant reduction in soluble sugar levels were observed in seedlings exposed to NaF.

Materials and Methods

Seed germination

Mung bean (Vigna radiata) seeds, locally obtained, were soaked in water for 24 h. The seedlings were transferred to petri dish lined with filter paper and treated with 10 ml of 0 (control), 0.1, 1.0, or 5.0 mM NaF. They were then incubated at 25°C for 72 h, with the addition of each of the treatment solutions every 24 h.

Determination of seedling growth

Growth of the seedlings was determined by their fresh weight and root elongation. For fresh weight determination, 20-40 seeds were routinely used. The cotyledons and radicles were separated and weighed. For studying root elongation, 15-20 seedlings were randomly selected and the length of each radicle was measured.

Center for Environmental Sciences, Western Washington University, Bellingham, WA 98225-9181 USA.
Extraction and determination of soluble sugars

At the end of the experimental period, 40-50 seedlings were harvested and the cotyledons and radicles separated. Soluble sugars of the tissues were extracted in hot water. The aqueous extract was filtered through 4-layers of cheesecloth, and the resultant filtrate was analyzed for total and reducing sugars by the method of Pomeranz and Meloan, using alkaline copper reagent followed by titration with 0.005 M sodium thiosulfate solution. Differences between total and reducing sugars were considered as non-reducing sugars. A calibration curve was constructed using standard glucose solutions.

Data analysis

Where applicable, statistical analysis of the experimental data were carried out using a students' t-test software at Western Washington University, Bellingham, Washington.

Results

Exposure to NaF at 1.0 and 5.0 mM caused significant changes in the fresh weight of mung bean seedlings. While the weight of radicles decreased with increase in F concentrations, that of cotyledons increased (Table 1). A marked decrease in root elongation was manifested in the F-exposed seedlings. Compared with the control, radicles from seedlings exposed to 1.0 and 5.0 mM NaF were 33% and 73% shorter, respectively (Table 2).

Total sugars in cotyledons from F-exposed seedlings remained unchanged until the F concentration reached 5.0 mM, when the level increased about 56% over the control. A slight increase in reducing sugars was also observed in these tissues, but a more pronounced increase (71%) occurred in the non-reducing sugar content. The ratios of non-reducing to reducing sugars were thus markedly increased (Table 3a).

The levels of soluble sugars in radicles from F-treated seedlings were markedly different from those of the control. Both reducing and non-reducing sugars in the F-exposed tissues exhibited a steady reduction coinciding with increase in F concentrations (Table 3b). The ratios of non-reducing to reducing sugars were thus altered. For radicles exposed to 1.0 and 5.0 mM NaF, the ratios increased by 16% and 61%, respectively (Table 3b).

Discussion

Results obtained from this study support earlier findings that NaF inhibited seed germination. Morphologically, the inhibitory effect was manifested in reduced growth and impaired root elongation. Interestingly, the F-induced inhibition of mung bean germination was manifested in a different way depending on the tissues. While the radicles from the F-exposed seedlings were much shorter than those of the control, their cotyledons were heavier (Tables 1 and 2). The observed increase in the biomass of the cotyledons exposed to NaF may be the result of an impaired breakdown of reserve materials, including fats, proteins, and carbohydrates. In support of this consideration is our earlier observations that both lipase and amylase from NaF-treated seedlings were markedly depressed compared to those of the control.

The pronounced reduction in the growth of radicles from F-treated seedlings (Tables 1 and 2) support published results. In a germinating seedling, the radicle is known to be an area where rapid synthesis of cellular components and cell division take place. Reduction in radicle growth, therefore, implies that these vital
processes were severely impaired. Obviously, such impairment is detrimental to the germination itself, as it limits subsequent plant growth and development.

The steady decrease observed in the soluble sugar content in the radicles from F treated seedlings is also striking. Importantly, such decrease was exhibited in radicles exposed to NaF at a concentration as low as 0.1 mM (Table 3b). This observation points out to the pronounced effect NaF has on sugar metabolism in the

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<th>TABLE 1. Fluoride effect on fresh weight of mung bean seedlings</th>
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<td><strong>NaF (mM)</strong></td>
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Values are the mean ± S.D. (N=20).  * p < 0.05  *** p < 0.001.

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<th>TABLE 2. Fluoride effect on root elongation</th>
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<td><strong>NaF (mM)</strong></td>
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Values are the mean ± S.D. (N=15).  *** p < 0.001.

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<th>TABLE 3a. Fluoride effect on soluble sugars in mung bean cotyledons</th>
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<td><strong>NaF mM</strong></td>
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ᵃ mg/seed; values are average of two determinations

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<th>TABLE 3b. Fluoride effect on soluble sugars in mung bean radicles</th>
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<td><strong>NaF mM</strong></td>
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ᵃ mg/seed; values are average of two determinations
germinating seedlings. Although both reducing and non-reducing sugar contents of the F-exposed radicles showed decreases, the extent of such decrease exhibited by the two types of sugars was found to be different from each other. In radicles exposed to NaF at 1.0 and 5.0 mM, the reduction of reducing sugar levels is greater than that of non-reducing sugars (Table 3b). Such difference resulted in a marked increase in the ratios of non-reducing to reducing sugars (NR/R) by 16% and 61% for the 1.0 mM and 5.0 mM groups, respectively (Table 3b).

The mechanism involved in the observed changes in sugar composition is not known. Because sucrose is widely known as a transport sugar in plants, an increase in sucrose content in the cotyledons may imply an enhanced synthesis of the sugar at the site of synthesis, or an impaired transport of the sugar to the growing tissue such as the radicle. Furthermore, since germination is an energy requiring process, a lowered availability of reducing sugars would be detrimental to the process, as they are immediate sources of energy and carbon for the growing axis.

Acknowledgement

This work was supported in part by the Bureau for Faculty Research, Western Washington University, Bellingham, Washington, U.S.A.

References

RELATIONSHIP BETWEEN TOTAL FLUORIDE INTAKE AND DENTAL FLUOROSIS IN AREAS POLLUTED BY AIRBORNE FLUORIDE

Y X Chen, a M Q Lin, a Z L He, a Y D Xiao, a C Chen a
D Min, a Y Q Liu a and M H Yu b
Nanchang, China and Bellingham, Washington, USA

SUMMARY: The relationship between fluoride (F) exposure and dental fluorosis in three (two experimental and one reference) villages in Jiangxi Province, China, has been studied. Total daily F-intakes by adults in the two F-exposed areas, Changping Village and Wenpan Village, were 3.16 and 3.01 mg, whereas the incidence of dental fluorosis was 51.8% and 57.4%, respectively. Airborne F levels of the two experimental villages were similar to each other, but the SO2 level in the air was higher in Wenpan Village than in Changping Village. On the other hand, food borne F levels in Changping Village was higher than those in Wenpan Village. The levels of kitchen airborne F and SO2 in the experimental areas were much higher than those in the reference area. Results from animal experiments showed that more dental lesions occurred in rats exposed to both F and SO2 than in those exposed to F alone. These results suggest that SO2 may be important in enhancing F-induced dental fluorosis.

Key words: Airborne fluoride; Dental fluorosis; Total fluoride intake, SO2.

Introduction

In fluorosis resulting from consumption of high fluoride (F) levels in drinking water, F enters the human body through the digestive tract. But, in fluorosis induced by airborne F, F enters the human body through both the respiratory and digestive tracts. The latter clearly involves contamination of both water and food by airborne F. The total fluoride intake of a patient with air-pollution-type fluorosis is, thus, the sum of fluoride intake through the respiratory and the digestive tracts. We studied the relationship between the occurrence of dental fluorosis and total fluoride intake in two disease areas and a reference area in southern China. Laboratory experiments were also conducted to study the influence of SO2 on F-induced dental fluorosis.

Materials and Methods

Study sites

Three villages, Changping Village, Wenpan Village, and Nankan Village, in the Province of Jiangxi, China, were chosen for this study. Residents in both Changping Village and Wenpan Village burn coal for heating and cooking purposes, and many are known to be afflicted by F pollution.1,2 Nankan Village, an area with no known F pollution except that from firewood combustion, was chosen as the reference area.

Fluoride analysis

To determine the F levels in drinking water, samples were taken from both source water and boiled water. To study airborne F levels, air samples were taken from five representative households and outdoor samples at least four times daily in the presence and absence of burning coal or firewood, for five days consecutively.

a Jiangxi Institute of Labor Hygiene and Occupational Medicine, Nanchang, Jiangxi, 330006, People’s Republic of China. b Center for Environmental Sciences, Huxley College of Environmental Studies, Western Washington University, Bellingham WA 98225-9181, USA. Correspondence to Professor Y X Chen or Professor M H Yu.
To determine the F intakes through inhalation, air samples were taken from five representative households. Another 50 households were chosen for studying the gaseous F intake through both indoor and outdoor activities. A daily respiratory volume of 12 m³ for an adult was used as the basis for calculation.

To assess the dietary F-intake, drinking water and tea samples were taken from the above-mentioned 50 households for F analysis using the method described previously.¹ Grain, vegetables and other food items consumed by the residents were also collected from 50 families residing in the study areas. Some of the samples were dried and ground into powder before analysis, while others were used without the drying process. The amount of F intake from food was determined using the “inquiry-calculation” method described by UNEP/FAO/WHO.³

Incidence of fluorosis

The incidence of fluorosis in Changping Village and Wenpan Village, based on a method similar to Dean’s classification of human dental fluorosis, has been reported to be 51.8% and 57.4%, respectively.¹²

Animal studies

To investigate the influence of SO₂ on F toxicity in humans, experiments were carried out using laboratory animals. A total of 108 SD rats, with a mean body weight of 105 g and consisting of both males and females in equal number, were used. The animals were randomly divided into three groups (A, B, and C), each consisting of 18 male and 18 female rats. Rats in Group A were placed in an exposure room where high F and high sulfur-containing coal was burned; Group B animals were placed in another room where high F and low sulfur containing coal was burned, whereas group C animals (control) were placed in a room where no coal was burned. All animals were fed fodder and water containing low F levels. To imitate the mode of coal-burning in a farmer's house, an exposure room with an area of 12 m² was constructed. Experimental animals were placed in the room where coal was burned in a chimney-less stove located in the center. The animals were exposed in the room 8 h during daytime daily for a period of five months.

Monitoring and observation

The levels of F, SO₂, and CO in the exposure rooms were monitored daily. The extent of dental fluorosis of the animals was examined and designated as normal, questionable, mild, moderate, and severe, based on a method similar to Dean’s classification of human dental fluorosis.

Results

Fluoride levels in drinking water, air, and foods

The levels of fluoride in all drinking water samples from the study areas were found to be relatively low. However, the levels were about 40% higher in the disease areas than in the reference village (Table 1). Boiled water raised the F content by about 50%. The levels of airborne F in Wenpan and Changping villages were much higher than those in Nankan Village. In particular, F levels of the kitchen air of the two disease areas were more than 3 times higher than those of the reference area. More strikingly, the kitchen SO₂ levels of the two disease areas were more than 20 times as high as those of the reference area (Table 2).

The F contents of rice and some of the vegetable samples differed from each other in the study areas. Marked differences were particularly evident in dry pepper and sweet potatoes (Table 3).
### TABLE 1. Fluoride content (mg/L) in drinking water

<table>
<thead>
<tr>
<th>Source of water</th>
<th>Study site</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wenpan Village</td>
<td>Changping Village</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Tap water (unboiled)</td>
<td>n</td>
<td>$\bar{x} \pm SD$</td>
<td>10</td>
<td>0.17 ± 0.07</td>
</tr>
<tr>
<td>Tap water (boiled)</td>
<td>10</td>
<td>0.25 ± 0.13</td>
<td>10</td>
<td>0.23 ± 0.12</td>
</tr>
</tbody>
</table>

### TABLE 2. Fluoride and SO$_2$ levels (mg/m$^3$) in air samples

<table>
<thead>
<tr>
<th>Source of pollution</th>
<th>Study site</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wenpan Village</td>
<td>Changping Village</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>n</td>
<td>$\bar{x} \pm SD$</td>
<td>100</td>
<td>0.0210 ± 0.0132</td>
</tr>
<tr>
<td>Bedroom</td>
<td>30</td>
<td>0.0034 ± 0.0023</td>
<td>28</td>
<td>0.0036 ± 0.0017</td>
</tr>
<tr>
<td>Outdoor</td>
<td>20</td>
<td>0.0025 ± 0.0021</td>
<td>10</td>
<td>0.0026 ± 0.0013</td>
</tr>
<tr>
<td>SO$_2$</td>
<td>Kitchen 100</td>
<td>0.691 ± 0.631</td>
<td>100</td>
<td>0.630 ± 0.421</td>
</tr>
</tbody>
</table>

### TABLE 3. Fluoride contents in rice and vegetables (mg/Kg)

<table>
<thead>
<tr>
<th>Vegetation</th>
<th>Study site</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wenpan Village</td>
<td>Changping Village</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Rice</td>
<td>n</td>
<td>$\bar{x} \pm SD$</td>
<td>47</td>
<td>1.85 ± 0.45</td>
</tr>
<tr>
<td>Sweet potato</td>
<td>16</td>
<td>1.20 ± 0.21</td>
<td>11</td>
<td>1.34 ± 0.23</td>
</tr>
<tr>
<td>Radish</td>
<td>38</td>
<td>0.40 ± 0.22</td>
<td>16</td>
<td>0.43 ± 0.20</td>
</tr>
<tr>
<td>Fresh pepper</td>
<td>29</td>
<td>1.33 ± 0.29</td>
<td>35</td>
<td>0.30 ± 0.13</td>
</tr>
<tr>
<td>Dry pepper</td>
<td>$^a$</td>
<td>30</td>
<td>50.13 ± 26.13</td>
<td>25</td>
</tr>
<tr>
<td>Chinese cabbage</td>
<td>34</td>
<td>0.59 ± 0.48</td>
<td>16</td>
<td>0.79 ± 0.61</td>
</tr>
<tr>
<td>Tea $^a$</td>
<td>47</td>
<td>191.20 ± 74.13</td>
<td>15</td>
<td>287.89 ± 97.85</td>
</tr>
</tbody>
</table>

$^a$ On dry basis
Daily F intake
Based on the analysis results of the air, drinking water, and vegetation samples and of studies of the time the residents spent on indoor and outdoor activities, daily average F intakes per person in the three villages were calculated and the results are presented in Table 4. Daily F intakes by residents of the two disease areas were found to be slightly greater than 3 mg per person, compared to about 1.7 mg for residents of the reference area.

<table>
<thead>
<tr>
<th>F source</th>
<th>Wenpan Village</th>
<th>Changping Village</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \bar{x} \pm SD )</td>
<td>%</td>
<td>( \bar{x} \pm SD )</td>
</tr>
<tr>
<td>Air</td>
<td>0.105 ± 0.008</td>
<td>3.5</td>
<td>0.105 ± 0.008</td>
</tr>
<tr>
<td>Water</td>
<td>0.438 ± 0.084</td>
<td>14.5</td>
<td>0.416 ± 0.061</td>
</tr>
<tr>
<td>Food</td>
<td>2.471 ± 0.187</td>
<td>82.0</td>
<td>2.643 ± 0.187</td>
</tr>
<tr>
<td>Total intake</td>
<td>3.014 ± 0.212</td>
<td></td>
<td>3.167 ± 0.301</td>
</tr>
</tbody>
</table>

Concentrations of F, SO\(_2\), and CO in exposure rooms

<table>
<thead>
<tr>
<th>Group</th>
<th>Fluoride</th>
<th>SO(_2)</th>
<th>CO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \bar{x} \pm SD )</td>
<td>( \bar{x} \pm SD )</td>
<td>( \bar{x} \pm SD )</td>
</tr>
<tr>
<td>A</td>
<td>0.631 ± 0.172</td>
<td>40.970 ± 1.741</td>
<td>98 ± 14</td>
</tr>
<tr>
<td>B</td>
<td>0.583 ± 0.184</td>
<td>5.086 ± 0.513</td>
<td>101 ± 12</td>
</tr>
<tr>
<td>C</td>
<td>0.008 ± 0.002</td>
<td>0.191 ± 0.044</td>
<td>&lt; 10</td>
</tr>
</tbody>
</table>

Incidence of dental fluorosis in experimental rats

<table>
<thead>
<tr>
<th>Degree of dental fluorosis</th>
<th>Percent incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
</tr>
<tr>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td>Questionable</td>
<td>5.6 (2)(^a)</td>
</tr>
<tr>
<td>Mild</td>
<td>8.3 (3)</td>
</tr>
<tr>
<td>Moderate</td>
<td>47.2 (17)</td>
</tr>
<tr>
<td>Severe</td>
<td>38.9 (14)</td>
</tr>
</tbody>
</table>

\(^a\) Values in parenthesis represent the number of animals with dental fluorosis
Animal experiments

a) Concentrations of air pollutants in exposure rooms: The levels of F and CO in each of the exposure rooms were similar to each other (Table 5). However, the level of SO₂ in Group A room was much higher than that in Group B room. Levels of these pollutants in the reference room were low but appeared to be normal, being comparable with those found in non-polluted atmosphere.

b) Growth, development, and weight of experimental animals: No significant differences in growth, development, or body weight were found between the experimental and control animals (p > 0.05).

c) Dental fluorosis: Table 6 shows dental changes exhibited by the experimental animals and the control. The teeth of the control rats remained normal, with creamy-white and translucent enamel, throughout the experimental period, and no dental fluorosis was observed. However, dental changes were observed in both Groups A and B rats during the 10th week of exposure. Increases in the incidence and severity of dental fluorosis, manifested by mottling and loss of translucency of the teeth, were observed. Their teeth were fragile and more susceptible to breakage. However, the incidence of dental fluorosis strikingly differed between the two experimental groups. The incidence of mild, moderate and severe dental fluorosis in Groups A and B was found to be significantly different from each other (p < 0.05). Lesions in Group A involving opaque stripes, pitting and fragility of the enamel were more severe than those in Group B.

Discussion

The two villages afflicted by F pollution chosen in this study were of high population-density areas and 30 km apart, whereas the reference area was about 50 km from either of the experimental areas. The socio-economic conditions of residents in the three villages are essentially similar to each other. As mentioned previously, firewood is used as a main source of fuel in the reference area, but during the past 10 years some residents have started to burn coal. In the experimental areas residents burned coal in stoves with no chimneys, the source of coal and the mud mixed with it were different, and their fluoride contents are different, also.

Results of the survey showed that F intakes by residents in the disease areas were much higher than those of the reference area. In the disease areas, the incidence of dental fluorosis in Wenpan Village was slightly higher than that in Changping Village, but the total F intakes by residents of Changping Village were slightly higher than those of Wenpan village.

As shown in Table 2, the kitchens in these two areas were highly polluted with airborne F and SO₂. Although F levels in these areas were more than twice as high as those in the reference area, a more striking difference was found in the kitchen SO₂ levels between the two comparison areas. These observations suggest a significant contribution of SO₂ to the incidence of dental fluorosis. The observations described above in human studies were supported by animal studies. As shown in Tables 5 and 6, rats exposed to air contaminated with high F and SO₂ levels had a higher incidence and more severe dental fluorosis than those exposed to air contaminated with high levels of F alone. These results suggest that exposure to high levels of SO₂ may promote F-dependent dental fluorosis.
Acknowledgement
The authors thank Professor Liang Chaoke for his assistance in animal experiments.

References
EFFECT OF SODIUM FLUORIDE ON THE TRANSMURAL POTENTIAL DIFFERENCE OF THE RAT STOMACH

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SUMMARY: Transmural potential difference (PD) was measured in vivo under continuous perfusion of the rat stomach. Luminal NaF (5 mM) perfusion elicited a net temporal decrease of PD, whereas lower NaF concentrations (1 and 2 mM) had no effect. The fall of PD induced by 5 mM NaF was abolished at pH 8.4 and was enhanced (161%) at pH 3.2. Compared to NaF alone, the decrease of PD induced by the simultaneous perfusion of NaF and salicylic acid (5 mM each) was more pronounced (209%) with a previous perfusion of salicylic acid alone than the direct perfusion of NaF and salicylic acid (136%). These results suggest that the observed variations of PD reflect the early functional alterations of the mucosa preceding the structural damaging effects of hydrofluoric and salicylic acids.

Key words: NaF; pH; Potential difference; Rat; Salicylic acid; Stomach.

Introduction

The major pathway by which fluoride enters the circulation is by absorption from the gastrointestinal tract.¹⁻³ Fluoride can be absorbed in appreciable amounts from the stomach due to the more lipid hydrofluoric acid (HF) formation.⁴⁻⁵ Acute and chronic studies in animals and human have shown that NaF causes gastrointestinal damage.⁶⁻¹³ The toxic effects of F⁻ and H⁺ on enzyme systems and structural damage were attributed to the rapid penetration of HF into cells.⁷⁻¹⁴,¹⁵ Fluoride acting as a proton ionophore can overcome the gastric mucosal barrier, a term accounting for the relative impermeability of gastric epithelium to passive movements of ions.¹⁶ The integrity of this diffusion barrier characterized by a high transmucosal potential difference (PD) and low conductance can be monitored with PD measurements.¹⁷

The aim of the present study is to examine the effect of NaF on PD and its behaviour as a barrier-breaking agent.

Material and Methods

Male Wistar rats weighing between 200 and 250 g were purchased from Institut Pasteur (Alger, Algeria). The animals were maintained on standard laboratory chow and tap water ad libitum. They were starved for 24 hours in cages with mesh bottoms to minimise coprophagy but allowed free access to drinking water. The rats were anesthetized with an intraperitoneal injection of urethane (1.5 g.Kg⁻¹).

Operative technique

After a tracheostomy was performed, a tracheal cannula was inserted to ensure free airways. Stomach perfusion was performed by inserting a polyethylene tubing (OD 1.78 mm, ID 1.02 mm) through the oesophagus into the gastric lumen. The abdomen was opened by a midline incision, the pyloro-duodenal junction exposed and a blind glass cannula (OD 5.0 mm, ID 3.5 mm) with lateral holes introduced through a cut in the duodenum into the stomach. The cannula was secured firmly by tying ligature around the pylorus, care being taken to avoid ligating blood vessels. The whole stomach was then brought forward, the wound covered with a moistened cotton wool pad. The animal was maintained along the experiment under a heating lamp.

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Continuous recording of transmucosal PD

The transmucosal PD was recorded between the gastric lumen and the peritoneal cavity with a potentiometric recorder (LKB Bromma, Sweden) via matched calomel half-cells (Taucussel, Lyon, France). The half-cells were connected by means of agar bridges (5% agar in 3 M KCl) with their distal ends in the gastric lumen and the peritoneal cavity, respectively. The luminal bridge was in fact introduced into the glass cannula through a small hole in the side of a soft rubber tubing attached to the cannula which served also for drainage of the perfused solution. Asymmetry between the two ends of the measuring system (≤ 0.7 mV) was determined by placing distal ends of the agar bridges in 0.9% NaCl. Potential difference was expressed as luminal face negative with respect to the serosal side. After completion of the operative procedures, the stomach was perfused with prewarmed isotonic solution (37°C) at a rate of 0.8 mL min⁻¹ by means of a peristaltic pump (LKB Bromma, Sweden). All the tested solutions were made isotonic by addition of NaCl.

Experimental protocols

To assess the stability of PD measurements, the stomach was perfused with 0.9% NaCl and PD recorded for 90 min after completion of abdominal surgery.

In order to study the effect of varying concentrations of NaF on PD, perfusion of the stomach was started with 0.9% NaCl until stabilisation of PD (basal PD). This solution was then replaced with isotonic saline containing 1, 2 or 5 mM NaF.

The influence of luminal pH on NaF-induced variations of PD was investigated at pH 3.2 and 8.4. After stabilisation of PD under isotonic NaCl perfusion, the stomach was perfused for 90 min with a test solution containing 5 mM NaF and either 3 mM HCl or 20 mM NaHCO₃.

A fourth experiment was undertaken in order to investigate the influence of salicylic acid (SA) on NaF-induced variations of PD. After stabilisation of PD under isotonic NaCl perfusion, the stomach was perfused for 30 min with isotonic solution containing 5 mM NaF and 5 mM SA just after 30 min perfusion of 0.9% NaCl or of 5 mM salicylic acid. To test the recovery of PD, the test solution was replaced with isotonic NaCl for a further 30 min period.

Statistical calculations: All data are expressed as means ± SE with the number of experiments between brackets. The significance of differences was evaluated by Student's t test for paired or unpaired data and probability values lower than 0.05 were considered statistically significant.

Results

The perfused stomach developed a PD, serosal side positive. Within 5 min after starting perfusion of isotonic NaCl, the average PD was 31.4 ± 2.2 mV, and subsequently stabilised at 35.0 ± 0.7 mV (48 experiments). Perfusion of the stomach with isotonic NaCl for 90 min resulted in a stable PD. In the subsequent studies, the experiments were undertaken with at least a 30 min period for stabilization. Since PD was not affected by the time course of perfusion, it was not necessary to correct the variations of PD induced by the test solutions.

Figure 1 shows that perfusion of the stomach with 1 or 2 mM NaF was without effect on PD (P > 0.05). But 5 mM NaF led to a temporal decrease of PD (Figure 1). Basal PD fell significantly from 36.5 ± 1.5 to 26.5 ± 1.6 (8) mV at time 25 minutes
(P < 0.05). Thereafter, PD increased slowly to attain a value not significantly different from the basal value.

The perfusion of the stomach with 5 mM NaF at pH 8.4 (20 mM NaHCO₃) had no significant effect on PD (Figure 2). When the luminal pH was lowered to 3.2 by addition of 3 mM HCl, 5 mM NaF caused a net decrease (P < 0.05) of PD from 37.7 ± 1.7 to 16.4 ± 1.4 (8) mV (Figure 2). Thereafter, PD began to increase. After 90 min, PD had not fully recovered and was significantly decreased compared with basal value.

Perfusion of 5 mM NaF simultaneously with 5 mM SA reduced significantly the PD from 32.7 ± 1.8 to 12.4 ± 0.9 (8) mV (Figure 3). The ease of PD was partially reversible since the replacement of the test solution with isotonic NaCl rapidly increased PD but without reaching the basal value (P < 0.05). After 30 min, PD was 18.3% lower than the basal value. Perfusion of 5 mM SA alone was able to induce a decrease of PD from 38.8 ± 1.8 to 21.1 ± 2.5 (8) mV (Figure 4). When 5 mM NaF was added to the medium, a further decrease of PF to 13.5 ± 2.4 (8) mV was observed. The replacement of the previous solution with NaCl was able to increase PD but without reaching the basal value (P < 0.05). After 30 min perfusion of NaCl, PD was 19.6% lower than the basal value.

Figure 5 shows comparisons between treatments of the maximal decrease of PD (ΔPD). Addition of 3 mM HCl or 5 mM SA to the NaF solution (5 mM) enhanced ΔDPmax by 161% and 136% respectively. Under perfusion of the stomach with SA and then with SA plus NaF, ΔDPmax was successively 116% and 209% greater than that induced by NaF alone.

**Figure 1.** Effects of luminal perfusion of various concentrations of NaF on gastric transmural PD. Upper panel: 1 mM (○), 2 mM (●); lower panel: 5 mM. Vertical lines represent one SE (n = 8). * Significant difference from basal value (B).
Figure 2. Effects of luminal perfusion of 5 mM NaF at pH 3.2 (○) and 8.4 (*) on gastric transmural PD. Vertical lines represent one SE (n = 8). * Significant difference from basal value (B).

Figure 3. Effect of luminal perfusion of 5 mM NaF and 5 mM salicylic acid on gastric transmural PD. Vertical lines represent one SE (n=8). * Significant difference from basal value (B).
Figure 4. Effect of luminal perfusion of 5 mM NaF and 5 mM salicylic acid on gastric transmural PD following a 30 min perfusion of salicylic acid alone. Vertical lines represent one SE (n=6). * Significant difference from basal value (B).

Figure 5. Maximal variations of PD induced by perfusion of: 1) NaF; 2) NaF + 3 mM HCl; 3) salicylic acid; 4) salicylic acid + NaF just after isotonic NaCl; 5) salicylic acid + NaF just after salicylic acid. NaF and salicylic acid concentrations were set to 5 mM. Vertical lines represent one SE. * Significantly different from 1), unless otherwise indicated.
Discussion

The gastric preparation used in this study showed that NaF was able to change the electrical properties of the mucosa. The extent and reversibility of the fall of PD induced by NaF were dependant upon concentration, luminal pH, and time of exposure of the stomach to SA.

A temporal decrease of PD was observed with 5 mM NaF but not with lower concentrations. The decrease of PD was 2.6 times at pH 3.2 and completely abolished at pH 8.4. Fluoride is a weak acid with a pKa of 3.5. At pH 3.2, 67% of fluoride exists as unionized hydrofluoric acid (HF), the main form of passive transport by epithelial cells.4,5,18-20 Once inside the cell (pH 7.4), the HF is buffered by intracellular proteins and rapidly dissociates releasing the fluoride anion.21 The pH-dependant decrease of PD seems resulting from the increasing amounts of F⁻ and H⁺ entering the cell interior. Similarly, SA reduced the PD, except that it is twice more potent than NaF at the same concentration. Salicylic acid belongs to the category of the barrier-breaking agents that act synergistically to enhance the noxious effects of H⁺.22,23 It was shown that acetylsalicylic acid (10-20 mM) induced a marked fall of PD and electrical resistance of frog gastric mucosa preparation.24,25 Furthermore, oral administration of aspirin to humans resulted in a drastic fall of the gastric transepithelial PD.26 The actual enhanced ΔPDmax (30%) observed following pre-exposure of the stomach to SA may result from the decrease of the electrical resistance, rendering the mucosa more permeable to fluoride.

Acute and chronic studies in animals and human have shown that NaF causes extensive gastroduodenal mucosal damage: petechiae, erosion, erythema, denudation of the mucosa, and degeneration of the epithelial cells.6-13 The toxic effects of F⁻ and H⁺ on enzyme systems and structural damage were attributed to rapid penetration HF across lipid cell membranes.7,14,15

The present study suggests that the decrease of PD induced by relatively low concentrations of NaF may reflect the early functional modifications of the gastric mucosa before the appearance of structural injury. These changes may be altered by manipulation of the luminal environment and the epithelial resistance.

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THE EFFECT OF NUTRITION ON THE DEVELOPMENT OF ENDEMIC OSTEOMALACIA IN PATIENTS WITH SKELETAL FLUOROSIS

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SUMMARY: The aim of the study was to study the relationship between nutrition and endemic osteomalacia, resulting in bone deformation with hump back, spinal curvature and "O" legs, in persons living in high drinking water fluoride areas with skeletal fluorosis. A dietary survey was made of 30–50 families from each of three villages in high fluoride areas of China, and in 273 persons in whom skeletal fluorosis was diagnosed clinically, clinical and radiological assessments were made for the presence of endemic osteomalacia with bone deformation together with measurement of the serum calcium and total protein. A higher incidence of osteomalacia was found in persons with a lower intake of energy, protein, calcium and Vitamin C, particularly multiparous women. Poor nutrition was seen to be an important cause of endemic osteomalacia in high fluoride areas and that prevention would be aided by a better diet.

Key words: Calcium; China; Diet; Endemic osteomalacia; Epidemiology; Fluoride; Nutrition; Protein; Skeletal fluorosis; Vitamin C.

Introduction

Although a relationship between nutrition and endemic osteomalacia is recognized, few epidemiological studies have been made.1,2 In an animal study, monkeys given a diet with low calcium and high fluoride for 60 months developed osteomalacia, while control groups with low calcium or high fluoride developed osteoporosis and osteosclerosis respectively.3 In the USA, it has been suggested that no clinically significant, adverse, physiological or functional effects, with the exception of dental fluorosis, are to be anticipated in persons whose water supply contains up to 8 parts per million (ppm) of fluoride, while, in India, skeletal fluorosis has been described with 3–4 ppm of fluoride in the drinking water.4 In China, skeletal fluorosis is widespread with variations in incidence being related to differences in the diet and economic conditions. In the present study the relationship between nutrition and endemic osteomalacia was examined in three villages with high drinking water fluoride levels but different diets.

Materials and Methods

Thirty to fifty families from each of three high fluoride areas were randomly selected for study. In two villages - Beichengzi, drinking water fluoride 5.9 ppm, in the eastern mountain part of Lainyuan county, and Dongjingji, drinking water fluoride 4.5 ppm, in the western basin of Yangyuan county, - the people were very poor and worked mainly in agriculture without secondary occupations. Their main crops were maize and millet with little access to fruit or meat. Dongjiao, drinking water fluoride 5.2 ppm, in the eastern suburb of Tianjin city was used as a control.

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b Institute of Endocrinology, Tianjin Medical College, China.
c Institute of Endemic Disease Prevention in Zhangjiako Region, China.

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village. There the residents had a higher standard of living with wider employment opportunities and better nutrition. The food intake for each family under study was weighed for three days and the sex, age and ability to work for each family member recorded. The average intakes of energy and nutrients for each person were calculated and compared to the Recommended Daily Allowance (RDA) for China. An assessment for the presence of skeletal fluorosis was made according to the Standard for the Prevention and Treatment of Endemic Fluorosis of China and when found, pelvic, forearm and leg X-ray examinations were made and the serum calcium and total protein measured. The Chi-square Test and Fisher’s Exact Test were used for testing for statistical significance.

Results

In the poorer villages of Beichengzi and Dongjingji, there was less Vitamin C in the diet and the intakes of energy, protein and calcium were significantly less than the RDA, while in Dongjiao they were significantly higher as shown in Tables 1 and 2.

Vitamin A intake, expressed as Retinol equivalents, was less than the RDA in all three villages but especially so in Beichengzi and Dongjingji.

The incidence of endemic bone deformation, diagnosed clinically, was higher in Beichengzi and Dongjingji than in Dongjiao, where no cases of hump back or spinal curvature occurred, as shown in Table 3.

Twenty two (29%) of the cases of bone deformation were male and 54 (71%) female giving a male:female ratio of 1:2.5.

The serum calcium and total protein levels were significantly lower in Beichengzi and Dongjingji compared to Dongjiao as shown in Table 4.

X-rays showed that osteosclerosis was significantly less prevalent in persons with skeletal fluorosis in Beichengzi and Dongjingji than in Dongjiao as shown in Table 5. Radiological evidence of osteomalacia was not found in Dongjiao but was present in the poorer villages of Beichengzi and Dongjingji, this difference being significant with a one sided test. Twenty four of the 28 persons with radiological evidence of osteomalacia were women (85.7%).

Discussion

Using energy, protein and calcium as indicators, the villages of Beichengzi and Dongjingji had significantly lower levels of nutritional intake than Dongjiao and a higher incidence of skeletal fluorosis. Protein contains amino acids and is required for growth, replacement of body tissues and the synthesis of hormones, enzymes, haemoglobin, antibodies and phagocytes. As well as being a basic component of bone, it promotes the absorption of calcium. The intake of animal protein in the form of meat and eggs was less in Beichengzi and Dongjingji at 2.7 g and 30 g a day, compared to Dongjiao with 79.7 g a day. Because animal proteins usually have a higher concentration of essential amino acids that most vegetable proteins, the people living in Beichengzi and Dongjingji had poorer nutritional levels and together with a deficiency in energy and protein was a deficiency in calcium. Calcium is needed throughout life with 99% of the 1300 g in the human body being in bone. At least 1000 mg a day is recommended to build bones and teeth. The intakes of calcium in Beichengzi and Dongjingji at 327 mg (54.5% of RDA) and 241 mg (40.1% of RDA) were significantly lower than in Dongjiao at 762 mg (127.0% of RDA).
Vitamin C is required for the formation of collagen, the protein fibres that bind cells together and form the body's connective tissue. Vitamin C is necessary for the step in collagen synthesis that gives collagen its rigid and stable structure and thus gives strength to the bones, cartilage, teeth, gums, muscles, vascular tissues and skin. The intakes of Vitamin C were significantly lower in Beichengzi and Dongjingji at 43 mg (71.1% of RDA) and 63 mg (105% of RDA) than in Dongjiao at 164 mg (273% of RDA).

Persons in Beichengzi and Dongjingji with skeletal fluorosis and poor nutrition had a higher incidence of osteomalacia than residents of Dongjiao, as well as lower calcium and total protein levels in the serum and less osteosclerosis.

### TABLE 1. Daily food intake per person for residents of the three villages

<table>
<thead>
<tr>
<th>Food</th>
<th>Beichengzi</th>
<th></th>
<th>Dongjingji</th>
<th></th>
<th>Dongjiao</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intake</td>
<td>% of total intake</td>
<td>Intake</td>
<td>% of total intake</td>
<td>Intake</td>
<td>% of total intake</td>
</tr>
<tr>
<td>cereals</td>
<td>495.0</td>
<td>68.2</td>
<td>509.4</td>
<td>51.3</td>
<td>582.3</td>
<td>45.7</td>
</tr>
<tr>
<td>starchy tubers</td>
<td>23.6</td>
<td>3.3</td>
<td>76.7</td>
<td>7.7</td>
<td>118.0</td>
<td>9.3</td>
</tr>
<tr>
<td>legumes</td>
<td>4.4</td>
<td>0.6</td>
<td>1.5</td>
<td>0.2</td>
<td>2.6</td>
<td>0.2</td>
</tr>
<tr>
<td>legume products</td>
<td>1.2</td>
<td>0.2</td>
<td>21.2</td>
<td>2.1</td>
<td>11.2</td>
<td>0.9</td>
</tr>
<tr>
<td>meat and eggs</td>
<td>2.7</td>
<td>0.3</td>
<td>30.3</td>
<td>3.0</td>
<td>79.7</td>
<td>6.3</td>
</tr>
<tr>
<td>vegetables</td>
<td>177.2</td>
<td>24.4</td>
<td>335.3</td>
<td>33.7</td>
<td>439.8</td>
<td>34.5</td>
</tr>
<tr>
<td>oils</td>
<td>1.4</td>
<td>0.2</td>
<td>0.5</td>
<td>0.1</td>
<td>16.6</td>
<td>1.3</td>
</tr>
<tr>
<td>other</td>
<td>20.6</td>
<td>2.8</td>
<td>18.8</td>
<td>1.9</td>
<td>23.9</td>
<td>1.9</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>726.1</td>
<td>100.0</td>
<td>993.7</td>
<td>100.0</td>
<td>1274.1</td>
<td>100.0</td>
</tr>
</tbody>
</table>

### TABLE 2. Daily nutrient intake per person for residents of the three villages

<table>
<thead>
<tr>
<th>RDA</th>
<th>Beichengzi</th>
<th></th>
<th>Dongjingji</th>
<th></th>
<th>Dongjiao</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>2600 kcal</td>
<td>1839*</td>
<td>70.7</td>
<td>2005*</td>
<td>77.1</td>
<td>2741**</td>
</tr>
<tr>
<td>Protein</td>
<td>75 g</td>
<td>51*</td>
<td>68.0</td>
<td>65*</td>
<td>86.7</td>
<td>85**</td>
</tr>
<tr>
<td>Fat</td>
<td>23</td>
<td></td>
<td>21</td>
<td></td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>270</td>
<td>389</td>
<td>483</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>600 mg</td>
<td>327*</td>
<td>54.5</td>
<td>241*</td>
<td>40.1</td>
<td>762**</td>
</tr>
<tr>
<td>Iron</td>
<td>12 mg</td>
<td>22</td>
<td>183.3</td>
<td>12</td>
<td>100.0</td>
<td>25</td>
</tr>
<tr>
<td>Retinol equivalents</td>
<td>1000</td>
<td>219</td>
<td>21.9</td>
<td>214</td>
<td>24.4</td>
<td>315</td>
</tr>
<tr>
<td>Vitamin B1</td>
<td>1.3 mg</td>
<td>2.1</td>
<td>161.5</td>
<td>1.9</td>
<td>146.2</td>
<td>2.5</td>
</tr>
<tr>
<td>Vitamin B2</td>
<td>1.3 mg</td>
<td>0.7</td>
<td>53.8</td>
<td>0.5</td>
<td>38.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>60 mg</td>
<td>43.0***</td>
<td>71.7</td>
<td>63.0***</td>
<td>105.0</td>
<td>164.0***</td>
</tr>
</tbody>
</table>

* Significantly lower than the RDA (National Standard of China), Chi-square test, p<0.05
** Significantly higher than the RDA, Chi-square test, p<0.05
*** Significantly lower in Beichengzi and Dongjingji compared to Dongjiao, χ² test, p<0.05
### TABLE 3. Endemic bone deformation in the villages

<table>
<thead>
<tr>
<th>Fluoride in drinking water</th>
<th>Beichengzi 5.9 ppm</th>
<th>Dongjingji 4.5 ppm</th>
<th>Dongjiao 5.2 ppm</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>Cases of hump back</td>
<td>13§</td>
<td>24.1</td>
<td>20§</td>
<td>10.5</td>
</tr>
<tr>
<td>Cases of spinal curvature</td>
<td>2</td>
<td>3.7</td>
<td>7</td>
<td>3.7</td>
</tr>
<tr>
<td>Cases of &quot;O&quot;-legs</td>
<td>4</td>
<td>7.4</td>
<td>28</td>
<td>14.6</td>
</tr>
<tr>
<td>Total with bone deformation</td>
<td>19*</td>
<td>35.2</td>
<td>55**</td>
<td>28.8</td>
</tr>
<tr>
<td>Total without bone deformation</td>
<td>35</td>
<td>64.8</td>
<td>136</td>
<td>71.2</td>
</tr>
<tr>
<td>Total with skeletal fluorosis</td>
<td>54</td>
<td>100</td>
<td>191</td>
<td>100</td>
</tr>
</tbody>
</table>

§ comparing Beichengzi and Dongjingji together with Dongjiao, Fisher’s Exact Test, 2 sided p = 0.0069
* comparing Beichengzi with Dongjiao, Fisher’s Exact Test, 2 sided p = 0.0069
** comparing Dongjingji with Dongjiao, Fisher’s Exact Test, 2 sided p = 0.0117
* and ** comparing Beichengzi and Dongjingji together with Dongjiao, Fisher’s Exact Test, 2 sided p = 0.0074

### TABLE 4. Serum calcium and total protein levels in persons with skeletal fluorosis

<table>
<thead>
<tr>
<th></th>
<th>Beichengzi</th>
<th>Dongjingji</th>
<th>Dongjiao</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum calcium &lt; 6 mg/dL</td>
<td>16.7%*</td>
<td>11.4%*</td>
<td>2.0%</td>
</tr>
<tr>
<td>Serum total protein &lt; 3.1 g/dL</td>
<td>15.3%*</td>
<td>68.4%*</td>
<td>0.7%</td>
</tr>
</tbody>
</table>

* comparing Beichengzi and Dongjingji with Dongjiao, Chi-square test, p < 0.005

### TABLE 5. The presence of osteosclerosis and osteomalacia, determined radiologically, in persons with skeletal fluorosis in the villages

<table>
<thead>
<tr>
<th></th>
<th>Beichengzi n = 54</th>
<th>Dongjingji n = 191</th>
<th>Dongjiao n = 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteosclerosis present</td>
<td>23§</td>
<td>104**</td>
<td>22</td>
</tr>
<tr>
<td>%</td>
<td>42.6%</td>
<td>54.5%</td>
<td>78.6%*</td>
</tr>
<tr>
<td>Osteomalacia present</td>
<td>4§</td>
<td>24§</td>
<td>0</td>
</tr>
<tr>
<td>%</td>
<td>7.4%</td>
<td>12.6%</td>
<td>0%</td>
</tr>
</tbody>
</table>

§ comparing Beichengzi and Dongjingji together with Dongjiao, Fisher’s Exact Test, 2 sided p = 0.0915, 1 sided p = 0.0407
* comparing Beichengzi with Dongjiao, Fisher’s Exact Test, 2 sided p = 0.0023
** comparing Dongjingji with Dongjiao, Fisher’s Exact Test, 2 sided p = 0.0230
* and ** comparing Beichengzi and Dongjingji together with Dongjiao, Fisher’s Exact Test, 2 sided p = 0.0085
Of those with radiological evidence of osteomalacia, 85.7% were women, who were particularly at risk when multiparous. The extra energy and protein requirements for pregnancy were not met by the diet available for women in Beichengzi and Dongjingji. In contrast in Dongjiao, with better nutrition, no cases of hump back or spinal curvature were found and the skeletal fluorosis had symptoms and signs of lesser severity.

The results are consistent with the primary factors, in the aetiology of osteomalacia in a high fluoride environment, being an insufficient intake of energy, protein, calcium and Vitamin C. It is seen to be essential that, during pregnancy and lactation, mothers have a good supply of dietary calcium to cover their needs and those of the growing baby. With a high fluoride intake, calcium fluoride is formed in the intestine and excreted in the faeces thus increasing the likelihood of a low blood calcium if there is an insufficient dietary intake. In turn, this may lead to parathyroid stimulation with a secondary hyperparathyroidism, bone matrix absorption, osteoporosis, and osteomalacia. With the pressure from the body weight, pregnancy and labour, deformation may occur with curvature of the lumbar spine, hump back, scoliosis and “O” legs. An adequate intake of Vitamin D, a healthy diet with a wide variety of foods and regular exercise can assist with calcium absorption.7

Thus poor nutrition is seen to be an important cause of endemic osteomalacia in a high fluoride environment, and increasing dietary energy, calcium, protein and Vitamin C may help in prevention, especially in pregnant and nursing women, and children.

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7 The Health Aspects of Food and Nutrition: a manual for developing countries in the Western Pacific Region. 3rd ed. Western Pacific Regional Office, World Health Organization, Manila 1979 pp 27-60.
SKELETAL CHANGE IN OSTEOFLUOROSIS PATIENTS AFTER DEFLUORIDATION OF DRINKING WATER

X C Meng, P Z Chen, Y P Qin and H X Li
Jinan, Shandong, China

SUMMARY: Serial X-ray studies of cases of osteofluorosis over 12 years following defluoridation of drinking water show that signs of reversion may be seen as early as 4 and 5 years, and are found in all cases after 12 years, with a significant percentage reverting to an apparently normal state. Changes are found predominantly in cancellous bone.

Key words: Defluoridation; Osteofluorosis; Skeletal change.

Introduction

Shandong Province is the chief area in China in which fluorosis associated with drinking water is endemic. For the past 12 years, defluoridation of water supplies had taken place for a population of 5.6 million people.

Fluoride concentrations in drinking water were decreased in Xi Liukou village, from 6.4-20.0 mg/L to 0.5-0.9 mg/L; and in Hou Zhu village from 2.4-7.6 mg/L to 0.71-0.75 mg/L. The prevalences of dental fluorosis and osteofluorosis had been, respectively, 100% and 57.3% in Xi Liukou; and 87.7% and 34.7% in Hou Zhu.

Method

Serial observations of skeletal changes over the 12-year period, by means of X-rays, were carried out annually for the first 3 years, and then on the 4th, 5th and 12th year after exposure to the low-fluoride drinking water.

The study sample was 200 cases (110 male, 90 female) in Xi Liukou and 51 cases (34 male 17 female) in Hou Zhu. Ages ranged from 42-72 years.

X-ray studies consisted of: posterior-anterior view of pelvis; right forearm, including elbow joint; and right leg, including knee joint.

Results

During the first 3 years, (30 cases per year) no significant skeletal changes were found. In the 4th year (50 cases) 9 osteofluorosis patients showed some mitigation. In the 5th year (60 cases) 56 patients with osteofluorosis showed reversion of various degrees (Table 1). In the 12th year, 51 sclerotic osteofluorosis cases were re-examined and showed a reversion rate of 100% (Table 2).

Apparent reversion was seen in cancellous bones in the 5th lumbar vertebra, sacrum, ilium, sacro-iliac joints, trochanters of the femur, and medial and lateral condyles of the femur and tibia.

In this group of patients, pelvic ligaments and the periosteum of the right forearm and leg showed ossification. There were no apparent changes in this finding on follow-up. Changes of joint degenerative diseases increased with age; but this was not thought to have any specific association with osteofluorosis.

Shandong Provincial Institute of Endemic Diseases, Jinan, Shandong, 250014 China.
Presented to the XXth Conference of the International Society for Fluoride Research, Beijing, China, September 5-9, 1994.
1. Subject G X. Male, 64 years before defluoridation.
   Note: Pelvis, III sclerotic osteofluorosis.

2. Same subject as in 1 twelve years post-defluoridation.
   Note: almost normal. Reduced bone density, bone texture reappears, trabeculae thinning and improved distribution.
3. Subject C T. Male age 63, before defluoridation. II degree osteofluorosis.

4. Same subject as in 3 12 years after defluoridation, reversed to normal. Note: bone density returning to normal, trabeculae thinning and better distributed.
### TABLE 1. Skeletal changes in 56 patients in 5th year after defluoridation of drinking water

<table>
<thead>
<tr>
<th>Degree</th>
<th>Cases</th>
<th>Normal</th>
<th>Early</th>
<th>Reversion</th>
<th>Unchanged</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Early</td>
<td>8</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>28</td>
<td>9</td>
<td>16</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>II</td>
<td>15</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>5</td>
<td></td>
<td>4</td>
<td>1*</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>13</td>
<td>18</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

* slight reversion, but no reduction in degree

### TABLE 2. Skeletal changes in 51 osteofluorosis patients in the 12th year after defluoridation of drinking water

<table>
<thead>
<tr>
<th>Degree</th>
<th>Cases</th>
<th>Normal</th>
<th>Early</th>
<th>Reversion</th>
<th>Unchanged</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>I</td>
<td>13</td>
<td>13</td>
<td></td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>20</td>
<td>12</td>
<td></td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>III</td>
<td>18</td>
<td>2</td>
<td></td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>27</td>
<td></td>
<td>22</td>
<td></td>
</tr>
</tbody>
</table>

#### Discussion

It has been considered, generally, that it takes more than 5 years to see any improvement in changes of osteofluorosis seen in plain X-ray films. This study shows that some mitigation may be expected in 4 years. Other Chinese studies have shown even longer periods, up to 15 years, for reversion. This study shows a reversion rate of 86.6% after 5 years with a 21.6% reversion to normal. It shows, also, that after 12 years, the reversion rate was 100% with 52.9% of cases reverting to normal including 2 cases with III degree sclerotic fluorosis.

Bone damage caused by fluorosis is not irreversible. Exchange of minerals (calcium, phosphorus, magnesium, etc and fluoride) in bone can take place at any time. When body intake exceeds excretion, these accumulate.

The higher metabolic rate of cancellous bone, compared to compact bone as well as ligaments and periosteum, probably accounts for this being the major site of reversion.

#### References


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CT EXAMINATION OF PATIENTS WITH OSTEOFLUOROSIS

P Z Chen and X C Meng
Jinan, Shandong, China

SUMMARY: This paper reports CT (Computerized Tomography) scan findings of the examination of the vertebral canals of 12 patients with severe osteofluorosis diagnosed by X-ray and with typical signs of spinal nerve damage. The examination showed various degrees of ossification of the rear longitudinal ligament (RLL) (aka posterior longitudinal ligament) in all 12 cases and of the yellow ligament (YL) (aka ligamentum flavum) in 3 cases. Stricture of the intervertebral foramen was found in one case. These results suggest that neurological findings indicative of spinal cord compression in patients with osteofluorosis are chiefly created by vertebral canal stricture caused by ossification of the RLL and YL rather than stricture of the canal by bone overgrowth. The CT scan clearly reveals the shape of the vertebral canal and the RLL and YL. Reconstruction of sagittal pictures may be used to localize the site of compression of the dural sac and nerve root, thereby obtaining results that can not be found by any other technique.

Key words: CT scan; Osteofluorosis; Vertebral canal.

Introduction

The clinical manifestations of osteofluorosis patients with nerve damage has been reported previously. Previous reports of the findings of Computerised Tomography (CT) scanning of the vertebral canal (VC) in these cases have not been found. To investigate the relationship between CT findings of examination of the vertebral canal and the clinical symptoms and signs found in patients with osteofluorosis, patients were selected with typical manifestations of nerve damage and subjected to CT scan in an attempt to identify the location of damaged nerves and the clinical significance.

Materials and Methods

The subjects examined in this study are residents of Bingzhuang Village, Jiaxiang county, Shandong province in China. Twelve patients, 6 male and 6 female aged 51-70 years of age, all had osteofluorosis, diagnosed by X-ray, and manifestations of nerve damage. The concentration of fluoride in drinking water to which they had been exposed for more than 30 years was 4.5-9.95 mg/L.

The vertebrae of these patients were examined by CT scan using a Siemens Somatron DR3 machine. The patients were examined in the supine position with the scanning plane parallel with the axis of the vertebral canal. The major scanning site was C2-C7, with some patients scanned from C2-T10. Scanning slice was 5-8 mm in thickness at intervals of 5-10 mm. The sagittal picture was reconstructed for further observation according to need. The window width was 1400-2046 and its center 300-500. Intravenous contrast medium was not used.

Shandong Provincial Institute of Endemic Diseases, Jinan, Shandong, 250014, China.
Presented to the XXth Conference of the International Society for Fluoride Research, Beijing, China, September 5-9, 1994.
Results and Analysis

The CT pictures of the normal osseous canal of cervical vertebrae show round obtuse isosceles triangularity at transversal scan, its base side is the rear (posterior) edge of the vertebral body and its base angle is the side crypt. The normal sagittal diameter (SD) of the vertebral canal is not less than 11.5 mm.

The 12 patients studied suffered from different degrees of ossification of the RLL (posterior longitudinal ligament). CT pictures revealed that osteoid high density reflection protruded into the vertebral canal at the center or side rear (posterior) edge of the vertebra. The transversal section showed different shapes: small round lump; transversal strip; semi-round; and triangles. On bone window observation a narrow line-like low density gap could be found between the high density reflection and the rear edge of the vertebra in some patients. The degree of ossification of the RLL varied so that the shapes on various CT scan slices differed. The appearance of the ossified reflection on the scan confirmed the constitution and extent of the ossification of the RLL. The major ossified portion was mainly of the continuous type at the rear centre of the vertebral canal and extended from C₃ to T₈. In 10 patients, ossification of the RLL was confined to the cervical vertebrae (C₃ - C₇); in two patients, this extended to the thoracic vertebrae (C₄ - T₄).

The reduction of the SD of the vertebral canal by the ossification of the RLL can be seen clearly on the CT image. Each was less than 11.5 mm. The most obvious osseous narrow position was located at C₆. The SD of 3 patients was only 2 mm. Three patients showed an ossification of YL which presented as a semi-round shape protruding to the front inside the canal; thereby, causing a reduction in the SD. One patient had a stricture of the intervertebral foramen caused by the ossification of the RLL which protruded to the left. Three patients exhibited osteohyperplasia and ossification of the appendages. Three patients had dural compression and one patient showed herniation of the intervertebral disk. The vertical distance between the ossified rear edge of RLL and the front edge of the vertebral plate was 2-9 mm, mean 5.8 mm.

The ossification of the RLL and YL cause the cervical and thoracic vertebral canal, normally concave, to assume a rhomboidal shape, or a horse-shoe shape in severe cases. The obvious stricture of the side crypt at the level of the upper articular process was not found because of the central location of the ossification of RLL. There was no obvious thickening of the YL and no obvious hypertrophy of the small articular process.

The maximum ossified thickness of RLL was 9 mm on CT scan in this group. The stricture rate of the vertebral canal was 69% (ratio of the ossified thickness to SC of the vertebral canal). The total stricture rate of the vertebral canal in each patient ranged from 29% to 69%.

CT transversal scanning can reveal clearly the shape of the vertebral canal, the RLL and YL and can permit observation of the changes in side crypt, intervertebral foramen and lower and upper articular processes. The reconstruction of sagittal pictures show a longitudinal section image of the vertebral canal. It is possible, sometimes, to find the compression of the dural sac and nerve root. These results could not be determined by any other method. Therefore, CT scanning is superior to other methods in determining the pathological location and details required in the selection of a treatment program.
Discussion

Most patients in this group exhibited sensory deficits in "glove" and "stocking" distribution and atrophy of small muscles of the hands. This was considered to be an indication of damage to nerves supplying the areas affected. These clinical findings are similar to those found in infectious multiple neuritis; but the pathological changes in the cases under investigation were assumed to be caused by damage to the nerve root by osteofluorosis.

Singh\(^1\) found that osteofluorosis caused muscle atrophy, sensory deficits in arms and legs and pain distributed along nerve pathways. Singh interpreted this to mean that a pathological process was taking place at the nerve root as it leaves the spinal cord.

Zhao\(^2\) examined six patients with osteofluorosis by means of spinography and found that the contrast medium showed a marked "honeycomb" appearance and flowed slowly. The spinogram and pathological examination demonstrated that hypertrophy of the YL was present and causing stricture of the vertebral canal. Zhao found that the concentration of fluoride in ligaments was much above the normal limit. CT scanning of this group demonstrated that spinal damage caused by osteofluorosis was chiefly related to the ossification of RLL and YL in cervical and thoracic vertebrae. This caused a stricture of the vertebral canal which resulted in the compression of the spinal cord to various degrees to produce spinal nerve symptoms and signs. These results are identical to the findings of our present study.
This may be generalised as indicating that spinal stricture was mainly in the vertebral canal, the side crypt and the intervertebral foramen.\textsuperscript{3} Ossification of the RL was found to be 100% and YL 25%. It is can readily be seen that compression of the spinal cord in this way can produce all of the presenting neurological signs and symptoms.

The neurological symptoms and signs produced by stricture of cervical vertebrae are related closely to the location and the degree of stricture. If the stricture occurs above the brachial plexus, where the SD of the vertebral canal is large, clinical symptoms may be minor or absent. If the stricture is in the lower cervical vertebrae, even slight compression of the cord produces symptoms and signs.\textsuperscript{4} Major stricture at the C\textsubscript{6} level produces severe symptoms.

Conclusion

This study confirms that signs and symptoms of nerve damage found in this group of osteofluorosis patients are caused by ossification of ligaments in cervical and thoracic vertebrae, especially in the RLL and YL. This ossification of ligaments results in stricture of the vertebral canal and the compression of the spinal cord and nerve root.

References

LUNG X-RAY CHANGES IN SKELETAL FLUOROSIS CAUSED BY COAL COMBUSTION

Bing-Kun Liu
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SUMMARY: Lung X-ray findings are reported in 45 cases with skeletal fluorosis in an area contaminated by coal combustion. The findings include chronic bronchitis, with diffuse interstitial fibrosis and pulmonary emphysema. The degree of pulmonary pathological findings and skeletal fluorosis is correlated with patient age. Among the 45 cases were 5 with cardio-pulmonary disease and 5 with tuberculosis.

Key words: Coal combustion; Fluorosis; Pulmonary changes; Skeletal fluorosis.

Introduction

Since the discovery, in the early 1980s, that contamination from coal combustion caused skeletal fluorosis, the phenomenon has been reported in the mid-south and southern provinces of China. It is now known that China has a large area of endemic fluorine toxicosis not associated with high levels of fluoride in drinking water.1-5

In these areas fluoride in drinking water conforms to the country’s standard level. Fluoride levels are high in coal used for domestic heating and for cooking food. Cooking stoves without chimneys pollute the atmosphere and contaminate food. The chief cause of fluorosis is the contaminated food. Air pollution probably causes lung injury, but at the present time in China, this type of injury has not been reported.

Method

Radiological investigation

Forty five persons from an endemic fluorosis area of a coal combustion city, EnShi Autonomous Prefecture, had X-rays taken of the pelvis, right forearm and leg (including elbow and knee joints), to disclose various degrees of skeletal fluorosis. Chest X-rays were also taken to reveal lung changes.

Environmental investigation

Air fluoride concentrations around indoor stoves were found to be 0.03-0.32 mg/m³, averaging 0.12 mg/m³. Sulfur dioxide concentrations were 0.1-8.93 mg/m³, averaging 2.5 mg/m³. These exceeded the national standard for the “highest concentration of poisonous substances in a residential area atmosphere” of 0.02 mg/m³ for fluoride and 0.5 mg/m³ for sulfur dioxide. (Determinations were according to The Determination of Pernicious Substances in Air, Institute of Hygiene, Chinese Academy of Medical Sciences.)

Control

Chest X-rays were carried out on a random sample of 45 persons of similar sex and age living in a non-coal combustion and non-endemic fluorosis area of EnShi.

Results

Skeletal findings

All of the persons from the endemic fluorosis area (22 males and 23 females whose ages ranged from 22 to 64) had some manifestation of skeletal fluorosis. Thirty were classified as “mild”; 13 as “medium”; and 2 as “severe”.

Lung findings

For the most part, these were diffuse interstitial fibrosis with different degrees of lung striations. Pulmonary emphysema changes were also encountered.

The chest X-ray findings were classified as: "mild" (irregular shadows did not exceed 4 zones of 6 zones of the 2 lungs) 28 cases; "medium" (involved all 6 zones) 12 cases; and "severe" (involved all 6 zones, shadows were of high density and associated with cor pulmonale) 5 cases.

The severity of degree of both skeletal fluorosis and lung changes was directly related to age; that is, to duration of exposure (Table 1).

<table>
<thead>
<tr>
<th>Age</th>
<th>Degree of lung change</th>
<th>Degree of skeletal fluorosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mild</td>
<td>medium</td>
</tr>
<tr>
<td>20-</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>30-</td>
<td>12</td>
<td>1</td>
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<td>40-</td>
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<td>50-</td>
<td>4</td>
<td>8</td>
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<tr>
<td>60-</td>
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</tr>
</tbody>
</table>

Five of the cases in the study group were diagnosed as having cardio-pulmonary disease, 5 with tuberculosis. Eleven had cost-phrenic angle obliteration and pleural thickening. Lymph node calcification was seen in 38 cases.

The control group chest X-rays were interpreted as "normal" in 40 cases. Five cases showed "mild" findings (Table 2).

<table>
<thead>
<tr>
<th>Age</th>
<th>20-</th>
<th>30-</th>
<th>40-</th>
<th>50-</th>
<th>60-</th>
<th>Total</th>
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<tr>
<td></td>
<td>mild</td>
<td>3</td>
<td>12</td>
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<td>4</td>
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<tr>
<td></td>
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<td>2</td>
<td>8</td>
<td>1</td>
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<tr>
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<td>severe</td>
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<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>3</td>
<td>13</td>
<td>11</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>Control group</td>
<td>normal</td>
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<td>13</td>
<td>10</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td></td>
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<td>1</td>
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<td>2</td>
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</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>total</td>
<td>3</td>
<td>14</td>
<td>11</td>
<td>15</td>
<td>2</td>
</tr>
</tbody>
</table>
Discussion

Coal of poor quality in mountain areas of China has been increasingly used as domestic fuel by local residents. It is used not only for cooking and heating but also for drying food after the autumn harvest. Coal combustion is, therefore, a principal factor harmful to human health. In addition to skeletal fluorosis caused by contamination of food, harm to the respiratory tract occurs due to atmospheric pollution.

To date, in the poverty stricken mountainous areas, skeletal change is recognized but respiratory disease has been neglected.

Due to the poor function of the lungs and heart, especially as a result of obstructive emphysema, many residents of the endemic area prematurely lose their ability to work. The prevention of respiratory disease in this population may be of more importance than the prevention of skeletal fluorosis.

References

TREATMENT OF POSTMENPAUSAL OSTEOPOROSIS
WITH SLOW-RELEASE SODIUM FLUORIDE:
Critique of Final Report from CYC Pak et al
John R Lee MD
Sebastopol, California, USA

This final report* of a randomized controlled trial of slow release fluoride given for osteoporosis, is a continuation of the study by Dr. Pak et al first described in 1989 and follows an interim report published in 1994. In this final report the authors describe the results of 54 postmenopausal osteoporotic patients receiving 25 mg of slow-release sodium fluoride twice daily (in cycles of 12 months of medication followed by 2 months not receiving it) and 400 mg of calcium citrate twice daily continuously, comparing their results with 56 similar patients receiving only the calcium citrate and a placebo capsule (without sodium fluoride) twice daily. The authors concluded that slow-release sodium fluoride and calcium citrate administered for 4 years inhibits new vertebral fractures (but not recurrent fractures), augments spinal and femoral neck bone mass, and is safe to use.

In my earlier comment (Fluoride 27 (4) 227-228 1994) I described this study as an exercise in controlled osteofluorosis (osteosclerosis), a well-known phenomenon of excess fluoride intake which augments bone mass and prevents compression deformities but at the risk of eventual spinal stiffness and the formation of imperfectly formed bone with loss of tensile (torsion) strength. Earlier studies of fluoride supplementation at similar dosages resulted in increased bone density but an unacceptable incidence of gastrointestinal problems and an eventual increase in hip fractures incidence.1-3 The slow-release fluoride avoided gastrointestinal problems but leaves open the possibility of eventual increase in hip fracture incidence. Dr Pak et al believe that the lower serum concentrations of fluoride obtained by their slow-release form of sodium fluoride achieve a “therapeutic window” which will also avoid the fluoride-induced damage to bone quality seen in these earlier studies. Since hip fractures produce more serious morbidity than vertebral compression fractures, this matter is of greater importance than merely demonstrating increased bone mass and a decrease in the more minor vertebral compression changes.

In the Pak et al study, the mean duration of treatment was 3.57 years. In the earlier fluoride studies, the increase in hip fracture incidence occurred after 3 years of treatment. Thus the present study may simply be a year or so short of seeing a similar rise in hip fracture incidence. It is likely that, given the lower serum fluoride

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levels, an increase of hip fractures is somewhat delayed by the slow-release form of sodium fluoride given. The authors agree that their treatment: (1) did not alter the rate of fractures occurring on already fractured vertebra (recurrent fractures); (2) “only marginally” affected spinal fracture rate in patients with severe bone loss; and (3) did not appear to inhibit non-spinal fractures. Thus, the jury is still out on the question of hip fractures, especially in women with fairly advanced osteoporosis.

It is clear from a considerable mass of scientific research that osteoporosis is not a disease of fluoride deficiency. Osteoporosis is a multi-factorial metabolic bone disorder involving improper nutrition, lack of exercise, and lack of appropriate hormones. In this regard, Pak et al found no discernible benefit from estrogen supplementation. In the US and other industrially advanced countries, estrogen supplementation is the cornerstone of postmenopausal osteoporosis treatment. This is a major error, as the Pak et al study and others demonstrated and is the primary reason why osteoporotic fractures occur at such a high rate in these countries. As I have described in previous reports, treatment that includes attention to nutrition, exercise, and progesterone supplementation results in bone mass improvement and fracture prevention at least as impressive as Pak’s slow-release fluoride treatment and without potential fluoride-induced deleterious side effects.

As is the case in medical problems, the most effective treatments result when the cause of the condition is corrected. Fluoride deficiency is not the cause of osteoporosis. In my opinion, fluoride treatment as Pak et al recommend should still be regarded as controlled osteofluorosis and the 3.5 year trial is insufficient to evaluate the potential increased risk of hip fracture. Further research along the lines I have pioneered is far more likely to achieve proper prevention and treatment of postmenopausal osteoporosis.

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Published by the International Society for Fluoride Research
Editorial Office: 81A Landscape Road, Mount Eden, Auckland 4, New Zealand
TREATMENT OF POSTMENOPAUSAL OSTEOPOROSIS
WITH SLOW-RELEASE SODIUM FLUORIDE.
FINAL REPORT OF A RANDOMIZED CONTROLLED TRIAL
C Y C Pak, K Sakhaee, B Adamshuert, V Piziaik, R D Peterson and J R Poindexter
Dallas, Texas, USA

Abstract from Annals of Internal Medicine 123 (6) 401-408 1995

Objective: To test whether slow-release sodium fluoride inhibits spinal fractures and is safe to use.

Design: Placebo-controlled randomized trial.

Interventions: Slow-release sodium fluoride, 25 mg twice daily, in four 14-month cycles (12 months receiving sodium fluoride followed by 2 months not receiving it) compared with placebo. Calcium citrate, 400 mg calcium twice daily, continuously in both groups.

Patients: 48 of 54 patients who received sodium fluoride and 51 of 56 patients who received placebo completed at least 1 year of the study. All patients had postmenopausal osteoporosis.

Results: Compared with the placebo group, the fluoride group had a lower individual vertebral fracture rate (0.064 ± 0.182 per patient-year compared with 0.205 ± 0.297 per patient-year; P = 0.002), a higher unadjusted fracture-free rate (85.4% compared with 56.9%; P = 0.001), and a greater survival estimate (relative risk, 0.3 [95% CI, 0.12 to 0.76]) for new fractures. The recurrent spinal fracture rate did not differ between the two groups. The fluoride group had a substantial increase in L2-L4 bone mass of 4% to 5% per year for 4 years, a mean increase in femoral neck bone density of 2.38% ± 3.33% per year, and no change in radial shaft bone density. The frequency with which minor side effects and appendicular fractures occurred was similar in the two groups; no patients developed microfractures or gastric ulcers.

Conclusion: Slow-release sodium fluoride and calcium citrate administered for 4 years inhibits new vertebral fractures (but not recurrent fractures), augments spinal and femoral neck bone mass, and is safe to use.

Key words: Calcium compounds; Delayed-action preparations; Fractures; Osteoporosis, postmenopausal; Sodium fluoride.

Reprints: C Y C Pak, University of Texas, Center for Mineral Metabolism and Clinical Research, 5323 Harry Hines Blvd, Dallas, TX 75235 USA.

FLUOROSIS: EXPERIENCE BASED ON TWO CASES
I Tollefsen, G Duus and F Johannessen
Stavanger, Norway

Abstract from Tidsskrift for Den Norske Lægeforening (Journal of the Norwegian Medical Association) 115 (21) 2648-2651 1995

Since 1961 sodium fluoride has been an alternative in the treatment of osteoporosis, although there is still some difference of opinion between endocrinologists regarding the effect on pain and occurrence of fracture of the vertebral column.

Two cases are reported, both treated for postmenopausal osteoporosis with calcium, vitamin D and sodium fluoride for longer periods over many years, and with good effect on pain and tendency to lumbar vertebral body fracture. In both patients the diagnosis of skeletal fluorosis was delayed for several years, mainly because information about this treatment never reached the radiologist. When the
diagnosis was eventually established after the radiologist himself had made inquiries to the referring physician, the patients had in the meanwhile undergone several unnecessary supplementary examinations because of suspected cancer metastasis.

Key words: Fluoride therapy; Osteoporosis; Skeletal fluorosis; Sodium fluoride.
Reprints: I Tollefsen, Department of Radiology, Central Hospital Rogaland, 4011 Stavanger, Norway.

FLUORIDE REDUCES BONE STRENGTH IN OLDER RATS
C H Turner, K Hasegawa, W Zhang, M Wilson, Y Li and A J Dunipace
Indianapolis, Indiana, USA

Abstract from Journal of Dental Research 74 (8) 1475-1481 1995

In response to recent concerns about the effect of water fluoridation on hip fracture rates, we studied the influence of fluoride intake on bone strength. Four groups of rats were fed a low-fluoride diet ad libitum and received 0, 5, 15, or 50 ppm of fluoride in their drinking water. Animals were euthanized after 3, 6, 12, or 18 months of treatment. Mechanical strength of the right femur was measured by three-point bending. Fluoride content for the left femur was measured, and static histomorphometric measurements were made on a lumbar vertebra. Femoral failure load was not significantly decreased in rats treated for 3 and 6 months, but was decreased as much as 23% in rats treated 12 and 18 months at 50 ppm fluoride. Extrapolation from regression equations predicted that older rats lose 36% of femoral bone strength when bone fluoride content is increased from 0 to 10,000 ppm, while younger rats will lose only 15%. Thus, the decreased strength appeared to be due to the combined effects of fluoride intake and age on bone tissue and was not associated with a decrease in bone density or mineralization defects. There were only small effects of fluoride on bone histomorphometry. Fluoride intake at high levels had no negative effects on bone mineralization. Fluoride intake was associated with slight increases in trabecular bone volume and trabecular thickness, but these effects could not be demonstrated consistently. The mechanism by which large amounts of fluoride affect bone strength more severely in older animals is unknown.

Key words: Bone; Fluoride; Rats.
Reprints: C H Turner, Indiana University, School of Medicine, Department of Orthopaedic Surgery, 541 Clin Dr, Indianapolis, IN 46202 USA.

A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR FLUORIDE UPTAKE BY BONE
H V Rao, R P Beliles, G M Whitford and C H Turner
Hartford, Connecticut, USA

Abstract from Regulatory Toxicology and Pharmacology 22 (1) 30-42 1995

A sex-specific, physiologically based pharmacokinetic (pbpk) model has been developed to describe the absorption, distribution, and elimination of fluorides in rats and humans. Growth curves generated by plotting mean body weights (kg) against age (weeks or years) are included in the simulation model to allow the integration of chronic fluoride exposure from birth to old age. The model incorporates age and body weight dependence of the physiological processes that control the
uptake of fluoride by bone and the elimination of fluoride by the kidneys. Six compartments make up the model. These are lung, liver, kidney, bone, and slowly and rapidly perfused compartments. The model also includes two bone subcompartments: a small, flow-limited, rapidly exchangeable surface bone compartment and a bulk virtually nonexchangeable inner bone compartment. The inner bone compartment contains nearly all of the whole body content of fluoride, which, in the longer time frame, may be mobilized through the process of bone modeling and remodeling. The model has been validated by comparing the model predictions with experimental data gathered in rats and humans after drinking water and dietary ingestion of fluoride. This physiological model description of absorption, distribution, and elimination of fluoride from the body permits the analysis of the combined effect of ingesting and inhaling fluorides on the target organ, bone. Estimates of fluoride concentrations in bone are calculated and related to chronic fluoride toxicity. The model is thus useful for predicting some of the long-term metabolic features and tissue concentrations of fluoride that may be of value in understanding positive or negative effects of fluoride on human health. In addition, the pbpk model provides a basis for across-species extrapolation of the effective fluoride dose at the target tissue, bone, in the assessment of risk from different exposure conditions.

Key words: Bone; Fluoride uptake; Physiologically based pharmacokinetic model.
Reprints: H V Rao, Department of Public Health and Addiction Services, 150 Washington St, Hartford, CT 06106 USA.

OSTEOPOROSIS: DRUG AND NONDRUG THERAPIES
FOR THE PATIENT AT RISK [REVIEW]

C L Gamble
Fort Smith, Arizona, USA

Abstract from Geriatrics 50 (8) 39-43 1995

Preventing bone loss and avoiding fractures are the most effective therapies for osteoporosis. Nondrug measures include weight-bearing exercise, adequate calcium intake, and the prevention of falls. Estrogen replacement therapy can protect bone from rapid demineralization typical of the early post-menopausal period. New research has provided more data on estrogen's safety and efficacy. Calcitonin is an option when estrogen is contraindicated. Although calcitonin requires frequent injections, it does provide some analgesic effect for patients with osteoporosis-related fracture. Fluoride and etidronic acid have shown promise but remain investigational due to questions about long-term effects on bone mass. Potent third-generation bisphosphonates are being studied and may be available soon.

Key words: Drug therapies; Non-drug measures; Osteoporosis.
Reprints: C L Gamble, Cooper Clinic Osteoporosis Center, Fort Smith, AR USA.
PRETREATMENT WITH LOW DOESES OF NORETHINDRONE POTENTIATES THE OSTEOGENIC EFFECTS OF FLUORIDE ON HUMAN OSTEO SARCOMA CELLS

J Takada, D J Baylink and K H W Lau
Loma Linda, California, USA


We recently reported that picomolar doses of norethindrone (NET), a synthetic analog of 19-nortestosterone, significantly stimulated human TE85 osteosarcoma cell proliferation, differentiation, and activity in vitro. In the present study, we investigated the possibility that NET interacts with another osteogenic agent, i.e., fluoride, to stimulate human TE85 osteosarcoma cell proliferation, differentiation, and activities. Bone cell proliferation as measured by the stimulation in [H-3] thymidine incorporation. Differentiation was monitored by the increase in alkaline phosphatase-specific activity. Osteoblastic activity was assessed by the stimulations in collagen synthesis and in osteocalcin secretion (in the presence of 1 nM 1,25-dihydroxyvitamin D-3). When the human TE85 cells were incubated with mitogenic doses of NET and fluoride concurrently, the stimulatory effects of the two agents on these parameters exhibited no significant interaction. The enhancing effect of NET on the osteogenic effect of fluoride was not due to a shift of the fluoride dose response curve. Pretreatment with NET for 24 h followed by a treatment with a mitogenic dose (i.e., 100 μM) of fluoride for an additional 24 h significantly and synergistically potentiated the effects of fluoride on the [H-3] thymidine incorporation, alkaline phosphatase-specific activity, collagen synthesis, and osteocalcin secretion, compared with those with the subsequent vehicle (0.05% ethanol) treatments. In contrast, pretreatment, with fluoride for 24 h before the addition of NET for 24 h did not produce significant synergistic stimulations in the test parameters. Pretreatment of TE85 cells with the same doses of dihydrotestosterone or progesterone prior to treatment with fluoride under the same conditions did not induce synergistic potentiation of fluoride in [H-3] thymidine incorporation, suggesting that the synergistic interaction with fluoride is probably not a common property of anabolic sex steroids. In summary, we found that: (1) the osteogenic effects of fluoride and NET were additive when cells were treated with both agents concurrently; (2) a 24-h pretreatment with picomolar doses of NET potentiated the osteogenic actions of fluoride in human TE85 osteosarcoma cells; and (3) pretreatment with NET produced a subsequent fluoride response that was synergistic. In conclusion, these findings led us to speculate that the osteogenic actions of NET and fluoride act through different mechanisms, and that NET at low doses has a permissive effect on the osteogenic effects of fluoride, and as such NET may be used in concert with fluoride to increase osteoblast proliferation, differentiation, and activity.

Key words: Bone; Fluoride; Norethindrone; Osteogenic; Osteosarcoma.
Reprints: K H W Lau, Jerry L Pettis Memorial Veterinary ADM Medical Center, Mineral Metabolism Unit 151, 11201 Benton St, Loma Linda, CA 92357 USA.
PRESENT AND FUTURE OF OSTEOPOROSIS THERAPY

E Seeman, C Tsalamandris, S Bass and G Pearce
Heidelberg, Victoria, Australia

Abstract from Bone 17 (2) Suppl. S23-S29 1995

In the 50-year “modern” history of osteoporosis, there have been about 17 anti-fracture studies with sufficient attention to design to allow inference regarding efficacy. Antivebral fracture efficacy has been reported with etidronate, estrogen patch, calcitonin, and 1,25-dihydroxyvitamin D. Two studies using fluoride were positive, and two were negative. Hip fractures have been neglected. One study showed efficacy of hip protectors, one showed efficacy of vitamin D and calcium in nursing home dwellers. The source of most hip fractures is the community. One community based antihip fracture efficacy study using annual injections of vitamin D was positive. There have been no antivebral or antihip fracture studies in men, or in corticosteroid-related osteoporosis in men or women. Lack of independently repeated demonstration of efficacy, small fracture numbers, and data pooling in some of these (the best) studies leave great uncertainty. Estrogen and bisphosphonates appear to be the best options at this time. New data suggest that calcium supplementation is likely to reduce the rate of bone loss and perhaps reduce fracture rates. The challenge is to maintain and restore the constituents of bone mineral density (BMD), that is: to promote periosteal and endosteal bone formation; reduce endosteal bone resorption and cortical porosity; and increase trabecular thickness, number, and connectivity. There are many opportunities, for instance, intermittent parathyroid hormone (PTH) increases bone strength and, with estrogen, may increase connectivity. The anabolic effects of PTH may be partly mediated by IGF-1. IGF-1 increases periosteal, endosteal, and trabecular bone formation, cortical and trabecular width, and trabecular and endocortical connectivity. With bisphosphonate, IGF-1 may increase bone area and strength as the bisphosphonate decreases medullary area while IGF-I increases subperiosteal area. Anabolic effects of fluoride warrant further study provided that the study design addresses the issue of bone strength, the narrow toxic therapeutic window, and cortical bone loss. Aluminum, a constituent of zeolite, has anabolic effects which may be partly mediated by TGF-P. Prostaglandin E(2) increases periosteal and endosteal bone formation but may increase cortical porosity. More data are needed regarding these growth factors, silicon compounds, strontium salts, and flavonoids. The effects of medroxyprogesterone and 19-norprogestins on BMD have not been compared. Raloxifene, a new estrogen agonist free of endometrial hyperplastic effects, is being studied. Most treated individuals with osteoporosis (i.e., low BMD with or without a fracture) will not suffer a fracture so treatment must be safe. Success — absence of fracture — will be measured by the epidemiologist because it is difficult to distinguish efficacy from chance in an individual as the peak incidence of fractures in the community is usually only about 1-4/100 per year.

Key words: Osteoporosis; Study design; Treatment.
Reprints: E Seeman, Austin Hospital, Department of Endocrinology, Heidelberg, Vic 3084, Australia.
NO INCREASES IN CHROMOSOME ABERRATIONS IN HUMAN DIPLOID FIBROBLASTS FOLLOWING EXPOSURE TO LOW CONCENTRATIONS OF SODIUM FLUORIDE FOR LONG TIMES

T Tsutsui, Y Tanaka, Y Matsudo, A Uehama, T Someya, F Hamaguchi, H Yamamoto and M Takahashi
Tokyo, Japan

Abstract from Mutation Research - Environmental Mutagenesis and Related Subjects 335 (1) 15-20 1995

To study whether exposure to fluoride at low concentrations for long times induces chromosome aberrations in human cells, human diploid fibroblasts in the quiescent phase were treated with sodium fluoride (NaF) at 1-10 µg/mL (equivalent to fluoride ion at 0.45-4.5 ppm) for 1-3 weeks. Quiescent cells were obtained by a 10-day culture in medium containing 1% serum following overnight incubation of cells in the logarithmic phase. Significant levels of cytotoxicity, as determined by a decrease in the number of cells, were not induced by treatment of the cells with NaF at 5 or 10 µg/mL for 1-3 weeks. No increase in the frequency of chromosome aberrations was elicited in cultures treated for 1-3 weeks with NaF over the range of doses examined. In contrast, a dose-dependent increase in the frequency of chromosome aberrations was observed in cultures treated with N methyl-N'-nitro-N-nitrosoguanidine, used as a positive control. The results indicate that fluoride might be not clastogenic to human fibroblasts when exposed at low levels, equivalent to those in the communal water supplies.

Key words: Chromosome aberrations; Clastogenicity; Human diploid fibroblasts; Sodium fluoride.
Reprints: T Tsutsui, University of Tokyo School of Dentistry, Department of Pharmacology, Chiyoda Ku, 1-9-20 Fujimi, Tokyo 102, Japan.

LONG TERM EXPOSURE TO FLUORIDE IN DRINKING WATER AND SISTER CHROMATID EXCHANGE FREQUENCY IN HUMAN BLOOD LYMPHOCYTES

Y Li, C K Liang, B P Katz, E J Brizendine and G K Stookey
Indianapolis, Indiana, USA

Abstract from Journal of Dental Research 74 (8) 1468-1474 1995

The genetic toxicity of fluoride has been investigated extensively by various test systems. However, results obtained have been inconsistent. Fluoride has been reported to be non-genotoxic, genotoxic, and synergistic or antagonistic with certain mutagens. To date, there are no published human studies on the genotoxicity of fluoride. The purpose of this investigation was to determine genotoxic risks of long-term exposure to various concentrations of fluoride in drinking water in humans with normal or inadequate nutrition. Six groups of subjects with either normal or inadequate nutritional intakes were selected from areas of approximately 0.2, 1.0, or 4.8 ppm (10.5, 52.6, or 252.6 µmol/L) fluoride in water. The subjects had been continuous residents in the area for at least 5 years. Samples of drinking water, plasma, and urine were analyzed for fluoride content. Blood lymphocytes were
examined to determine the frequency of sister chromatid exchange (SCE). Blood chemistry and electrolytes were also analyzed. The results showed that average daily fluoride intake as well as urine and plasma fluoride levels increased with increase in the fluoride content of the drinking water. The blood chemistry and electrolyte values were within the normal range for all populations, but several parameters were significantly different. While the numerical differences were small, the subjects with low fluoride in the water (0.11 and 0.23 ppm or 5.8 and 12.1 μ mol/L) had significantly higher SCE frequencies than those with higher fluoride exposures. Reasons for the reduced SCE frequency in subjects with higher fluoride exposure are unclear; however, the data demonstrated that long-term exposure to fluoride in the drinking water, even at an elevated level, does not have genotoxic effects in humans.

Key words: Genotoxicity; Human blood lymphocytes; Sister chromatid exchange; Water fluoride.
Reprints: Y Li, Indiana University, School of Dentistry, 1121 W Michigan St, Indianapolis, IN 46202 USA.

[The above research, which was supported by a grant from the US government's National Institute of Dental Research, was presented at the XXth Conference of the International Society for Fluoride Research, Beijing, in September 1994. Since then other studies - one of them also presented at the XXth ISFR Conference - reporting human genetic damage associated with endemic fluorosis, have been published (Fluoride 27 215-219 1994, and Fluoride 28 125-127 1995). The different research designs partly account for the differing conclusions. The studies published in Fluoride compared SCE rates of normal, non-fluorotic, patients with those of patients with symptoms of fluorosis. The above NIDR study compared SCE rates of residents, rather than normal and fluorotic individuals, from areas with differing water fluoride levels. - JC]

SERUM LIPOPROTEIN LEVELS IN GENETICALLY HYPERCHOLESTEROLAEMIC RICO RATS:
EFFECTS OF A HIGH-SUCROSE-CHOLESTEROL DIET WITHOUT OR WITH ALTERED MAGNESIUM AND FLUORIDE CONTENT

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Genetically hypercholesterolaemic Rico rats (male, 6 weeks old) were randomly distributed into 6 experimental groups. The zero-time basal group A was sacrificed at the start of the experiment while the other groups were fed for 6 weeks and then sacrificed. Group B was fed a stock diet. Control group C was fed a high-sucrose (45%) diet with 0.5% added cholesterol. In the diet of group D, only the magnesium (Mg) content was reduced from the level of group C (883 ppm) to 200 ppm. The diet of group E was the same as that of group D with the addition of 12 ppm of fluoride (F) and the diet of group G was the same as that of group E, but with its Mg content elevated from 200 ppm to 300 ppm. Analysis of aortic blood samples, taken before sacrifice, indicated significant increases in total serum cholesterol (p<0.01), very low density lipoprotein (VLDL) (p < 0.001) and low density lipoprotein (LDL), (p < 0.001) cholesterol, and a trend to lower high density lipoprotein (HDL) cholesterol in group C, as compared to group B. Significantly lower total (p<0.05), VLDL (p<0.01) and LDL (p<0.01) triglycerides were observed in group C when
compared to group B. The LDL phospholipids were significantly higher in group C (p < 0.001) than in group B. When cholesterol levels in groups D, E and G were compared with group C, the VLDL cholesterol in group E and the LDL cholesterol in group G were slightly but significantly (p < 0.05) reduced, while total cholesterol and the other subfractions were unaltered. The LDL triglycerides of groups E and G were significantly smaller still than the already small fraction in group C. The VLDL triglyceride in group E was significantly lower than that of group C (35% reduction, p < 0.001), D and G (p < 0.05). Phospholipids were slightly but significantly reduced in the VLDL fraction of group E and in the LDL fraction of group G (p < 0.05 and 0.01, respectively), as compared to those of group C.

Key words: Cholesterol; Dietary fluoride; HDL; LDL; Magnesium; Phospholipids; 
Rico rat; Serum; Triglycerides; VLDL.

Reprints: H Luoma, University of Kuopio, Department Preventive Dentistry and 
Cariology, POB 1627, SF-70211 Kuopio, Finland.

RENAL FUNCTION IN PATIENTS WITH HIGH SERUM 
FLUORIDE CONCENTRATIONS AFTER PROLONGED 
SEVOFLURANE ANESTHESIA

H Higuchi, H Sumikura, S Sumita, S Arimura, 
F Takamatsu, M Kanno and T Satoh 
Saitama, Japan 

Abstract from Anesthesiology 83 (3) 449-458 1995

Background: In studies of methoxyflurane-induced nephrotoxicity, renal-concentrating impairment has been observed only when serum inorganic fluoride concentrations exceed 50 μM. Prolonged sevoflurane anesthesia can result in serum inorganic fluoride concentrations in excess of 50 μM. The authors compared renal function after prolonged sevoflurane anesthesia with that after isoflurane anesthesia. In addition, they measured urinary excretion of N-acetyl-beta-glucosaminidase (NAG), a sensitive index of renal tubular damage, during the S-day period after anesthesia.

Methods: Thirty-four healthy patients who underwent either sevoflurane (23 patients) or isoflurane (11 patients) anesthesia at a total gas flow of 61/min for orthopedic surgery scheduled to last at least 5 h were studied. At 16.5 h after cessation of anesthesia, patients were administered 10 units of vasopressin, and urine was collected frequently thereafter for evaluation of urinary osmolality. In addition, urinary excretion of NAG was measured before and on days 1-3 after anesthesia. Based on whether peak fluoride concentrations exceeded 50 μM, 23 patients anesthetized with sevoflurane were assigned to a sevoflurane (high) (> 50 μM) group or a sevoflurane (low) (< 50 μM) group.

Results: The eight patients in the sevoflurane (high) group had a mean peak fluoride concentration of 57.5 ± 4.3 μM. A significant, albeit weak, inverse correlation was found between peak fluoride concentration and maximal urinary osmolality after the injection of vasopressin (r = -0.42, P < 0.05). Mean maximum urinary osmolality tended to be lower in the sevoflurane (high) group (681 ± 60 m Osm/kg) than in the other two groups after administration of vasopressin, although the difference among the three groups did not quite reach statistical significance (P = 0.068). One patient had a transient concentrating defect (maximum urinary osmolality = 390 m Osm/kg) on day 1 after anesthesia. Urinary excretion of NAG in both the
sevoflurane (high) and sevoflurane (low) groups was greater on days 2 and 3 after anesthesia than before anesthesia. The increase in urinary NAG excretion was dose related with sevoflurane, but there was no difference in results of routine laboratory renal tests on days 2 and 3 after anesthesia among the three groups.

**Conclusions:** The authors concluded that sevoflurane anesthesia results in increased serum fluoride concentration, a tendency toward decreased maximal ability to concentrate urine, and increased excretion of NAG. However, the increase in urinary NAG excretion was not indicative of clinically significant renal damage in these patients with no preexisting renal disease.

Key words: Anesthetics; Fluoride ions; Isoflurane; Kidney function; Nephrotoxicity; Sevoflurane; Urinary concentrating mechanism; Vasopressin.

Reprints: H Higuchi, National Defence Medical College, Department of Anesthesiology, 3-2 Namiki, Tokorozawa, Saitama 359, Japan.

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**RENA L FUNCTION AND SERUM FLUORIDE CONCEN TRATIONS IN PATIENTS WITH STABLE RENAL INSUFFICIENCY AFTER ANESTHESIA WITH SEVOFLURANE OR ENFLURANE**

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Abstract from *Anesthesia and Analgesia* 81 (3) 569-575 1995

Sevoflurane is metabolized to hexa-fluoro-isopropanol and inorganic fluoride by the human liver. Its use as an anesthetic may lead to peak plasma fluoride concentrations exceeding those seen after enflurane. Although there is no nephrotoxicity after sevoflurane anesthesia in humans with normal kidneys, those with chronically impaired renal function might be at increased risk because of increased fluoride load due to prolonged elimination half-life. In this study, measures of renal function after sevoflurane anesthesia were compared to those after enflurane in patients with chronically impaired renal function. Forty-one elective surgical patients with a stable preoperative serum creatinine concentration greater than or equal to 1.5 mg/dL were randomly allocated to receive sevoflurane (n = 21) or enflurane (n = 20) at a fresh gas inflow rate of 4 L/min for maintenance of anesthesia. Serum fluoride concentrations were measured by ion-selective electrode. Renal function (creatinine, urea, sodium, osmolality) was assessed in serum and urine preoperatively and for up to 7 days postoperatively. Peak serum inorganic fluoride concentrations were significantly higher after sevoflurane than after enflurane anesthesia (25.0 ± 2.2 vs 13.3 ± 1.1 μM; mean ± SEM). Laboratory measures of renal function remained stable throughout the postoperative period in both groups. No patient suffered a permanent deterioration of preexisting renal insufficiency and none required dialysis. Thus, neither sevoflurane nor enflurane deteriorated postoperative renal function in these patients with preexisting renal insufficiency. There is no evidence that fluoride released by metabolism of sevoflurane metabolism worsened renal function in these patients with stable, permanent serum creatinine concentrations more than 1.5 mg/dL. Our data also suggest that the peak fluoride concentrations measured in peripheral blood may not be a good predictor of nephrotoxic potential after sevoflurane anesthesia in these patients.

Key words: Anesthesia; Fluoride; Kidney function; Sevoflurane.

Reprints: P F Conzen, University of Munich, Klinikum Grosshadern, Institute of Anesthesiology, Marchioninistr 15, D-81377 Munich, Germany.
Much of the dental literature on fluoride effects continues to assume a dental "optimal" (1 ppm) water fluoride level. Much contains little original research of interest to other researchers. Heading the following list is the subject of our last editorial: an inaccurate personal attack on a prominent opponent of water fluoridation. The second on the list reports a non-blind examination of 344 children residing in communities with negligible (NF: 0.2 ppm), optimal (OPF: 1.0 ppm), and four-times optimal (4X OPF: 4.0 ppm) naturally occurring fluoride in their water systems. Predictably it concludes: "The ingestion of water containing 1 ppm or less fluoride during the time of tooth development may result in dental fluorosis, albeit in its milder forms. However, in these times of numerous products containing fluoride being available, children ingesting water containing 1 ppm fluoride continue to derive caries protection compared to children ingesting water with negligible amounts of fluoride. Thus, the potential for developing a relatively minor unesthetic condition must be weighed against the potential for reducing dental disease." After this list we reprint some of the more interesting abstracts, commenting on one of them. - JC


DENTAL FLUOROSIS AND CARIES PREVALENCE IN CHILDREN RESIDING IN COMMUNITIES WITH DIFFERENT LEVELS OF FLUORIDE IN THE WATER.


DENTAL CARIES EXPERIENCE AND DEFECTS OF DENTAL ENAMEL AMONG 12-YEAR-OLD CHILDREN IN NORTH LONDON, EDINBURGH, GLASGOW AND DUBLIN
M C Downer, A S Blinkhorn, R D Holt, C Wright and C Attwood London, England

Abstract from Community Dentistry and Oral Epidemiology 22 283-285 1994

A multi-center study of caries experience and defects of dental enamel was conducted among 12-year-old children in north London, Edinburgh, Glasgow and Dublin. None of the cities had water fluoridation except Dublin, which was included in the national program introduced in the Republic of Ireland in 1964. A random sample of children was drawn from state schools in each location and identical methods of clinical examination were used throughout under the same standardized conditions. All examiners were trained and calibrated with a reference examiner and achieved high levels of inter- and intra-examiner consistency. Mean DMFT values for the 4 cities were 1.27 (London), 1.39 (Edinburgh), 2.70 (Glasgow) and 1.48 (Dublin) (P < 0.001). Proportions of subjects free from caries in the same order were, 50, 47, 24 and 43% (P < 0.001), and child prevalence of diffuse opacities, 28,
29, 7 and 17% ($P < 0.001$), respectively. The relatively low caries levels recorded in London and Edinburgh (lower than Dublin) were considered to be related most probably to fluoride effects other than water fluoridation.

Key words: Defects of dental enamel; Dental caries; Epidemiology; Fluoride.
Reprints: M C Browne, Department of Dental Health Policy, Institute of Dental Surgery, Eastman Dental Hospital, 256 Gray's Inn Road, London WC1X 8LD, England.

Comment: Although two of the three unfluoridated cities surveyed had less tooth decay than the fluoridated city surveyed, the authors in their Discussion section argued that other forms of fluoride exposure were probably responsible for the reduced decay. Because their results also showed that "diffuse enamel opacities" (their term for dental fluorosis) were more prevalent in the unfluoridated cities with low tooth decay rates than in the high-decay unfluoridated city and the fluoridated city, they concluded that greater use (and swallowing) of fluoridated toothpaste could be a cause. Though they reported that the low-decay unfluoridated cities were more affluent, with possibly differing "dietary propensities", they did not discuss the possibility that better nutrition, rather than differing fluoride exposure, could have contributed to the lower decay rates. - JC

THE EFFECT OF A LOW FLUORIDE CONTAINING TOOTHPASTE ON THE DEVELOPMENT OF DENTAL CARIES AND MICROBIAL COMPOSITION USING A CARIES GENERATING MODEL DEVICE IN VIVO

L G Petersson, S Edwardsson, G Koch, J Kurol and A Lodding
Halmstad, Sweden

Abstract from Swedish Dental Journal 19 (3) 83-94 1995

The purpose of the study was to evaluate the effect of daily use of a low fluoride containing toothpaste (250 ppm F) on the uptake of fluoride and development of enamel lesions as well as the prevalence of lactobacilli and mutans streptococci in dental plaque compared to the use of placebo toothpaste. 16 children were selected with homologous premolar teeth. The teeth were cemented with orthodontic bands ad modum Ogaard for plaque accumulation and enamel lesion development. The plaque accumulated during 4 weeks was collected and analysed for lactobacilli and mutans streptococci. The teeth were further analysed by secondary ion mass spectrometry (SIMS), determining the concentration profiles of fluoride and other elements in the outermost enamel and in the lesion. The results show that although significant amounts of fluoride were taken up in the surface enamel from the fluoride toothpaste, the extent of the lesions was not influenced compared to teeth brushed with a non F-toothpaste. Neither were microbiological differences in the dental plaque found between the groups. An interesting observation was that early demineralization of enamel took place without detectable levels of mutans streptococci in the overlaying dental plaque. The conclusion is that fluoride taken up in enamel from F-toothpaste has no significant influence on enamel lesion development if a cariogenic dental plaque with high levels of acid producing microorganisms is continuously attached to the enamel surface.

Key words: Dental caries; Dental plaque; Fluoride toothpaste.
Reprints: Department of Preventive Dentistry, Medical and Dental Center, Halmstad, Sweden.
FORMATION OF PHOSPHATE-CONTAINING CALCIUM FLUORIDE AT THE EXPENSE OF ENAMEL, HYDROXYAPATITE AND FLUORAPATITE

J Christoffersen, M R Christoffersen, J Arends and E S Leonardsen
Copenhagen, Denmark

Abstract from Caries Research 29 (3) 223-230 1995

During the caries process complex reactions involving calcium, phosphate, hydrogen and fluoride ions as main species take place. In this study the precipitation and dissolution reactions occurring in suspensions of enamel, hydroxyapatite (HAP) and fluorapatite (FAP) on addition of fluoride were investigated under well-defined conditions. pH and pF were monitored; calcium and phosphate concentrations were measured at selected times; the solid phases were examined by infra-red, X-ray diffraction and transmission electron microscopy. Precipitation of phosphate-containing calcium fluoride crystals, CaF\(_2\)(P), can cause severe reduction in the calcium ion concentration and release of hydrogen ions from the precipitated phosphate. These reactions result in considerable dissolution of enamel, HAP and even of FAP. More of the added mineral dissolves with 50 mmol/L fluoride than with 10 mmol/L fluoride, mainly due to the greater reduction in calcium ion concentration. This work shows that phosphate-containing calcium fluoride is most likely an important compound to be considered in the caries process.

Key words: Calcium fluoride; Dental enamel; Fluorapatite; Hydroxyapatite.
Reprints: Department of Medical Biochemistry and Genetics, Panum Institute, Copenhagen N, Denmark.

THREE-YEAR RANDOMIZED TRIAL OF PROFESSIONALLY APPLIED TOPICAL FLUORIDE GEL COMPARING ANNUAL AND BIANNUAL APPLICATIONS WITH WITHOUT PRIOR PROPHYLAXIS

D W Johnston and D W Lewis
London, Ontario, Canada

Abstract from Caries Research 29 (5) 331-336 1995

The twice yearly application to children's teeth of acidulated phosphate fluoride (APF) gel in dental trays preceded by a professionally rendered 'dental prophylaxis' has become the standard and most commonly used dental chairside procedure for prevention of dental caries. This study was a randomized, 3-year, community-based clinical trial of professionally applied APF gel involving the use and non-use of a prior dental prophylaxis and annual and biannual APF applications for children in age groups 6-7 (n = 176) and 10-11 (n = 153) years initially, who are likely at high risk of future dental caries. The 3-year results of this study show no significant effect on dental caries reduction of either a prior prophylaxis or annual versus biannual APF gel applications. A significant reduction in the frequency of provision of these dental services, limited to high caries risk patients only, is recommended.

Key words: Acidulated phosphate fluoride; Dental caries prevention; Dental prophylaxis; Topical fluoride application.
Reprints: D W Johnston, University of Western Ontario, Health Science Center, Dental Science Bldg Room 1007B, London ON N6A 5C1, Canada.
EFFECT OF LOW LEVELS OF FLUORIDE ON CALCIUM UPTAKE BY DEMINERALIZED HUMAN ENAMEL

C D Gibbs, S E Atherton, E Huntington, R J M Lynch and R M Duckworth
Wirral, England

Abstract from Archives of Oral Biology 40 (9) 879-881 1995

The effect of fluoride (ca. 0.1 parts/10^6) on calcium uptake by enamel was examined under alternating remineralizing and demineralizing conditions. The remineralizing solutions contained either 0, 0.058, 0.104 or 0.138 parts/10^6 fluoride (ex NaF), while the demineralizing solutions contained no added fluoride. During the demineralizing periods, calcium loss was similar for all groups. However, during the remineralizing periods, all levels of added fluoride were found to promote calcium uptake. Calcium levels taken up by the artificial lesions were found to increase with increasing fluoride concentration in solution, and were independent of surface area of exposed enamel. In the absence of fluoride, even under conditions that are considered to be remineralizing, further demineralization took place.

Key words: Fluoride; Enamel; Demineralization; Remineralization.
Reprints: C D Gibbs, Unilever Dental Research, Quarry Rd E, Wirral L63 3JW, Merseyside, England.

TOOTHPASTE TECHNIQUE: STUDIES ON FLUORIDE DELIVERY AND CARIES PREVENTION.

K Sjogren
Goteborg, Sweden

Abstract from Swedish Dental Journal - Supplement 110 1-44 1995

The aim of the investigations was to evaluate the cariostatic effects of a modified toothpaste technique using fluoride (F) toothpaste. The modification consisted of an active mouthrinse with the toothpaste slurry and a sip of water for one minute after brushing. Toothpaste technique and salivary F concentration after toothbrushing were recorded in a carries active and a carries inactive group. The level of F in whole saliva, the concentration of F in plasma, the effect on demineralised enamel and dentine samples, and the accumulation of F in interdental plaque when using the modified toothpaste technique were studied. In a 3-year clinical trial, 4-year old children were trained in the toothpaste technique. The results showed that in the carries active group, the water rinsing was more thorough and more water was used compared to a carries inactive group. Rinsing with water and eating immediately after toothbrushing decreased the F level in whole saliva. Mouthrinising with either a NaF solution or a slurry of toothpaste foam and water increased the F concentration in saliva compared to when a single or double water rinse was performed. The degree of F absorption in plasma, the accumulation of F in approximal plaque and the interdental clearance after toothbrushing were strongly related to the mode of water rinsing. The degree of demineralisation of enamel and dentine at approximal sites was also related to the mode of water rinsing. The clinical study showed that the cariostatic effect of the modified toothpaste technique resulted in 26% less approximal caries in the test group. It is concluded that a toothpaste technique where a slurry rinse was carried out after brushing increased the efficacy of F toothpaste.

Key words: Dental caries; Dental plaque; Fluoride toothpaste.
Reprints: Department of Cariology, Faculty of Odontology, Goteborg University, Sweden.
CHARACTERIZATION OF FLUOROSE HUMAN ENAMEL
BY COLOR REFLECTANCE, ULTRASTRUCTURE,
AND ELEMENTAL COMPOSITION

N J Giambro, K Prostak and P K Den Besten
Boston, Massachusetts, USA

Abstract from Caries Research 29 (4) 251-257 1995

Mature fluorosed human enamel has been described as a subsurface enamel hypomineralization, with porosity increasing relative to the degree of fluorosis. The purpose of the current study was to quantitatively measure the color of the fluorosed enamel by light reflectance, and to further characterize the enamel by scanning electron microscopy. Teeth with varying degrees of fluorosis were obtained and divided in groups of mild, moderate and severe fluorosis using Dean's index for fluorosis. The color of the labial enamel surface was measured using a Minolta Chroma Meter CR241 (Minolta, Ramsey, NJ, USA). The teeth were further characterized for elemental composition using an energy-dispersive spectrometer, and imaged in both secondary and backscattered electron modes. The results of this study showed that the moderately and severely fluorosed enamel contained an uneven distribution of areas which were more electron-absorbent with a relatively increased carbon content. The changes in the physical characteristics of the teeth could be quantitated by measurements of light reflectance. The color of the teeth was significantly different between groups, with all groups significantly different than normal.

Key words: Color reflectance; Dental enamel composition; Dental fluorosis; Ultrastructure.
Reprints: N J Giambro, Forsyth Dental Center, 140 Fenway, Boston MA 02115 USA.

EFFECTS OF FLUORIDE-SUPPLEMENTED SUCROSE ON EXPERIMENTAL
DENTAL CARIES AND DENTAL PLAQUE pH

T W Cutress, C H Sissons, E I Pearce, L Wong,
K Anderssen and B Angmar-Mansson
Wellington, New Zealand

Abstract from Advances in Dental Research 9 (1) 14-20 1995

Sucrose, 5% and 10% (w/v), supplemented with between 0 and 5 ppm fluoride (F), was tested for its influence in vitro on plaque-induced experimental in vitro enamel caries and plaque pH. Plaque growth on bovine enamel was initiated from saliva inocula and sustained in a multiple plaque growth system for up to 31 days by means of a basal medium with periodic applications of sucrose or sucrose supplemented with F. Change in enamel mineralization was assessed, before and after plaque growth, by microhardness testing and microradiography; pH was monitored with microelectrodes. It was found that enamel demineralization was inversely related to the F concentration in the range 2 to 5 ppm, for both 5% and 10% sucrose. Plaque pH responses were unaffected by the F supplements.

Key words: Dental caries; Dental plaque; Sugar fluoridation.
Reprints: Dental Research Unit, Health Research Council, PO Box 27007, Wellington, New Zealand.
FLUORIDE AND SUGAR INTAKE AMONG ADULTS AND YOUTH IN MAURITIUS: PRELIMINARY RESULTS
S M Lahti, U Uusitalo, E Feskens, U Haw, J Tuomilehto and H Luoma
Kuopio, Finland

Abstract from Advances in Dental Research 9 (1) 21-25 1995

The potential use of different vehicles for delivering fluoride to prevent dental caries has been discussed recently in Mauritius. Water fluoridation was found not to be feasible, and extending the fluoride tablet program would not be easy. Thus, sugar fluoridation as one possibility was considered. For these purposes, the average fluoride and sugar intake was estimated in Mauritius. The results are based on two studies - a Survey on Diet, Health and Lifestyle of Youth in Mauritius (1990) and the Mauritius Diet and Health Survey. Information was collected by trained interviewers using food-frequency and 24-hour-recall questionnaires. The daily total sugar intake, manufactured and natural, was found to be 62 g per day in young people and 50 g per day in adults. In the younger groups, daily frequencies of raw sugar, sweets, and biscuit (cookie) consumption were 1.5, 0.2, and 0.2 times a day, respectively. For adults, the mean daily frequency of consuming sugar-containing foods was 2.6 (SD = 1.3). The daily sucrose intake was rather high, representing about 10% of the daily energy intake. The fluoride levels of foods were calculated by use of Finnish and other available fluoride tables. The mean fluoride intakes per day were 0.64, 0.72, and 0.62 mg per day for 8-17-year-, 18-24-year-, and 30-64-year-old groups, respectively. The median fluoride intake for the oldest group was 0.62 mg/day. The estimated fluoride intake from food did not correspond with the proposed level for the prevention of caries (Murray, 1986) except for the 18-24-year-olds, where it might have been just above the lower recommended limit. However, further data based on analysis of the fluoride contents of Mauritian food samples, especially of whole daily diet, are needed.

Key words: Fluoride intake; Mauritius; Sugar fluoridation; Sugar intake.
Reprints: S M Lahti, WHO Collaborating Centre, Faculty of Dentistry, University of Kuopio, Finland.

EFFECTS ON DEMINERALIZATION OF ENAMEL BY FLUORIDATED SUCROSE: A PILOT STUDY IN AN IN SITU CARIES MODEL
P Carlsson, B Angmar-Mansson, I M Redmo-Emanuelsson and K Anderssen
Malmo, Sweden

Abstract from Advances in Dental Research 9 (1) 9-13 1995

Blocks of human enamel, placed in removable partial dentures, were allowed to acquire natural plaque for seven days and were exposed extra- orally to a cariogenic challenge by repeated periods in a fluoride-sucrose solution. As a control, enamel blocks were exposed extra- orally to a sucrose solution. After two weeks of cariogenic challenge, the blocks were examined for mineral loss by quantitative micro-radiography on thin sections of the enamel. The results from six subjects showed that no significant effect on demineralization could be detected by the addition of fluoride corresponding to fluoride/sugar content of 1 mg/kg, 5 mg/kg, or 10 mg/kg (dry weight). One subject did not develop lesions at all, either with fluoride-sucrose or with sucrose exposure alone.

Key words: Dental caries; Sugar fluoridation.
Reprints: P Carlson, Department of Cariology, University of Lund, Malmo, Sweden.
ADDING FLUORIDE TO SUGAR - A NEW AVENUE TO REDUCE DENTAL CARIES, OR A "DEAD END"?

D Brathall and D E Barmes
Geneva, Switzerland

Abstract from Advances in Dental Research 9 (1) 3-5 1995

A study group was formed in 1989 by the Oral Health Program of WHO, Geneva, to consider the possibility of reducing dental caries by adding fluoride to sugar. Although a few promising clinical reports were available for review, the group found that information was too scarce for field trials to be recommended at this stage. Among the many items to be considered was what concentration of fluoride in sugar could reasonably be regarded as cariostatic. Thus, the committee decided to initiate studies to obtain further background information. Unlike fluoridated salt, the concept of fluoridated sugar does not involve trying to give the individual a certain daily amount of fluoride, since daily consumption varies considerably. Instead, the idea is to elaborate on recent fluoride research showing that low concentrations of fluoride may also be beneficial, particularly for remineralization, if present at the sites where caries occurs. This paper is an introduction to a set of papers describing the background for the project, attempting to define optimal concentrations for a clinical trial, and concluding that, although dental caries prevalence continues to decrease in industrialized countries, the potential for large increases remains in the huge populations in developing countries. All avenues must be searched for a system which optimizes preventive efficiency. However, the possible introduction of fluoridated sugar on the market is not related only to oral health. Safety aspects are of high priority, and several ethical, political, and economic factors must also be considered.

Key words: Dental caries; Sugar fluoridation.
Reprints: Department of Cariology, WHO Collaborating Centre for Education, Training and Research in Oral Health, University of Lund, Geneva, Switzerland.

REDDUCING THE CARIOGENIC EFFECT OF SUGAR BY ADDING FLUORIDE TO SUGAR - PROJECT BACKGROUND

D M O'Mullane
Dublin, Ireland

Abstract from Advances in Dental Research 9 (1) 6-8 1995

For various reasons, the use of the more widely used fluoride vehicles - such as water, salt, and toothpastes - is not feasible in some communities. There is some theoretical and laboratory evidence to support the view that adding fluoride to sugar and sugar products has potential to reduce their cariogenic effect. However, the clinical evidence is minimal. The minimum concentration of fluoride in sugar which will bring about a caries-preventive effect needs to be determined. Logistical issues requiring investigation include the most efficient method of adding fluoride to sugar and the distribution and consumption patterns of sugar in target populations. The practical application of this knowledge to large populations is the priority focus of this investigation.

Key words: Ireland; Sugar fluoridation.
Reprints: Oral Health Services Research Centre, Department of Preventive Dentistry, University Dental School, Dublin, Ireland.
FLUORIDE, CALCIUM AND PHOSPHORUS LEVELS IN BEE HONEY AND WATER
I J Dutoit, S R Grobler, T J V Kotze and N J Basson
Tygerberg, South Africa


We wished to learn whether any correlation exists between concentrations of calcium, phosphorus and fluoride in honey and the water used in its processing. Calcium concentrations were determined by flame \( \text{N}_2\text{O/C}_2\text{H}_2 \) atomic-absorption spectrophotometry, phosphorus by flameless atomic-absorption spectrometry and fluoride potentiometrically. Statistical analysis revealed no direct correlation between the different elements in water and honey. Furthermore, reduced variability of these three elements in honey with respect to their concentration in water was found. It is concluded that the elemental composition of water does not contribute substantially towards the levels of calcium, phosphorus or fluoride of honey.

Key words: Calcium; Fluoride; Honey; Phosphorus.
Reprints: S R Grobler, University of Stellenbosch, Faculty of Dentistry, Oral and Dental Research Institute, Private Bag X1, Tygerberg 7505, South Africa.

INFANTS' FLUORIDE INTAKE FROM DRINKING WATER ALONE, AND FROM WATER ADDED TO FORMULA, BEVERAGES, AND FOOD
S M Levy, F J Kohout, N Guhachowdhury, M C Kiritsy, J R Heilman and J S Wefel
Iowa City, Iowa, USA

Abstract from Journal of Dental Research 74 (7) 1399-1407 1995

In infants, the majority of total ingested fluoride is obtained from water, formula and beverages prepared with water, baby foods, and dietary fluoride supplements. Few studies have investigated the distribution of fluoride intake from these sources among young children at risk for dental fluorosis. The purpose of this study was to assess estimated water fluoride intake from different sources of water among a birth cohort studied longitudinally from birth until age 9 months. Parental reports were collected at 6 weeks, 3 months, 6 months, and 9 months of age for water, formula, beverage, and other dietary intake during the preceding week. Fluoride levels of home and child-care tap and bottled water sources were determined. This report estimates daily quantities of fluoride ingested only from water-both by itself and used to reconstitute formula, beverages, and food. Daily fluoride intake from water by itself ranged to 0.43 mg, with mean intakes < 0.05 mg. Water fluoride intake from reconstitution of concentrated infant formula ranged to 1.57 mg, with mean intakes by age from 0.18 to 0.31 mg. Fluoride intake from water added to juices and other beverages ranged to 0.67 mg, with means < 0.05 mg. Estimated total daily water fluoride intake ranged to 1.73 mg fluoride, with means from 0.29 to 0.38 mg.

Key words: Dental fluorosis; Fluoride ingestion; Fluoride intake; Water fluoride.
Reprints: S M Levy, University of Iowa, College of Dentistry, Department Preventive and Community Dentistry, DSB N330 Iowa City, IA 52242 USA.
FLUORIDE CONTENT OF LOS ANGELES COUNTY WATER.
D F Duperon, J R Jedrychowski and J Kong
Los Angeles, California, USA

Abstract from *Journal of the California Dental Association* 23 (2) 45-48 1995

A series of tap water samples were collected from residents in various areas of Los Angeles County. Three samples were collected each week over a period of three weeks, for a total of nine samples from each of the 45 residences. Samples were analyzed for fluoride content, and the data compared to information received from the agencies supplying the source water. Fluoride levels varied an average of 0.11 ppm during the three-week collection period; mean levels were consistently higher than values reported by the various agencies.

Key words: Fluoride content; Los Angeles; Water fluoride.
Reprints: Section of Pediatric Dentistry, School of Dentistry, University of California at Los Angeles, Los Angeles, CA 90024-1668 USA.

GRAMINE AND FREE AMINO ACIDS AS INDICATORS OF FLUORIDE-INDUCED STRESS IN BARLEY AND ITS CONSEQUENCES TO INSECT HERBIVORY
E L Hautala and J K Holopainen
Kuopio, Finland

Abstract from *Ecotoxicology and Environmental Safety* 31 (3) 238-245 1995

Barley leaves were sprayed with aqueous NaF, which caused accumulation of fluoride in the foliage, but no visible symptoms were detectable. No significant correlation was observed between foliar fluoride concentration and content of the indole alkaloid gramine after exposure to fluoride levels of 20 to 60 mg F liter\(^{-1}\). Fluoride exposure did not explicitly affect the performance of Carausius morosus or Rhopalosiphum padi on barley. After exposure to fluoride levels of 100 and 200 mg F liter\(^{-1}\), as NaF, fluoride treatment had a significant effect on gramine concentration of the first leaf of barley, being highest at a fluoride treatment of 200 mg liter\(^{-1}\), and there was a slight, but significant positive correlation between the log-transformed foliar fluoride concentration and log-transformed gramine concentration of the first leaf. Fluoride treatment increased levels of some individual free amino acids in barley foliage. Exposure of young barley to NaF in aqueous form caused accumulation of fluoride in barley foliage and resulted in increased levels of gramine in the first leaf and levels of some free amino acids in foliage. It is possible that the fluoride-induced concurrent increase in gramine concentration in barley leaves could override the eventual increase in nutritive value to herbivorous insects after fluoride exposure. More detailed biochemical studies of the induction of gramine production are needed to understand the fluoride effects in secondary metabolism of barley.

Key words: Amino acids; Barley; Fluoride; Gramine.
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INHIBITION OF MAIZE ROOT $H^+$-ATPase BY FLUORIDE AND FLUOROALUMINATE COMPLEXES

A R Facanha and L Demeis
Rio de Janeiro, Brazil

Abstract from Plant Physiology 108 (1) 241-246 1995

Vesicles derived from maize roots retain a membrane-bound $H^+$-ATPase that is able to pump $H^+$ at the expense of ATP hydrolysis. The $H^+$ pumping and the ATPase activity of these vesicles are inhibited by lithium fluoride and by the complex formed between fluoride and aluminum. The inhibition promoted by lithium fluoride increases as the $\text{MgCl}_2$ concentration in the medium is increased from 2 to 20 mM. The inhibitory activity of both lithium fluoride and aluminum fluoride increases as the temperature of the medium is increased from 20 to 35 degrees C. Inorganic phosphate (10-40 mM) inhibits the $H^+$-ATPase at pH 6.5 but not at pH 7.0, and at both pH values, it antagonizes the inhibition promoted by lithium fluoride and fluoroaluminate complexes.

Key words: Fluoride; Fluoroaluminate complexes; $H^+$-ATPase; Maize.
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