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The International Society for Fluoride Research will hold its Sixth Conference in historic Williamsburg, Virginia, November 7 to 9, 1974. This town is easily accessible by air to foreign and U. S. participants. It offers an unusual opportunity to observe, at first hand, the way of life experienced during the early years of our nation. The following are the main subjects to be discussed: Clinical features of fluoride intoxication and its relationship to dietary mineral intake; Fluoride pollution abatement and control; Enzymatic and cellular effects of fluoride in both plant and mammalian tissues; Genetic and teratogenic effects of fluoride. For reservations contact The Motor House, Reservations Manager, Colonial Williamsburg Visitor Accomodations Services, P. O. Drawer B, Williamsburg, Virginia 23185.

The program committee is now soliciting abstracts up to 300 words of papers dealing with any phase of fluoride research. They should be submitted in triplicate to the Society's office, P. O. Box 692, Warren, Michigan 48090. The deadline is June 15, 1974.

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MANUSCRIPTS for publication should be submitted in English, double-spaced with generous margins. References should be arranged according to the order in which they are cited in the text, and written as follows: Author, title, journal, volume, pages and year. Each paper must contain a summary of not more than 12 lines.

Contributors will receive copies of the issue of FLUORIDE containing their paper, free of charge.

FLUORIDE is listed in Current Contents Agricultural Food and Veterinary Sciences
EDITORIAL

SODIUM FLUORIDE IN OSTEOPOROSIS

Increased density of the skeleton following intake of fluoride is believed to be the result of the incorporation of fluoride into the hydroxyapatite crystal of the bone. Fluorapatite is presumably more stable and more resistant than hydroxyapatite to parathyroid hormone which constitutes the normal stimulus to resorption of calcium (1). A direct action of fluoride on the function of bone cells, resulting in a decreased osteoclastic activity, was postulated by Roholm (2).

Some investigators have shown an increase in bone mass, a positive calcium balance and clinical improvement (3, 4, 5) whereas others have failed to discern any positive effect in their patients (6, 7) as the result of sodium fluoride therapy.

On May 4 and 5, 1973 a symposium on the treatment of osteoporosis with sodium fluoride took place in Baden near Vienna, Austria.

This conference concluded that, in the majority of cases, fluoride treatment promotes formation of new bone and increased bone density and that it relieves bone pain. On several points, however, the experience of the conferees differs from observations of U.S.A. investigators, particularly with reference to the technique of the treatment and to the question of side effects.

Such conflicting results are undoubtedly due to the lack of common indices, especially to the failure to establish distinct biological endpoints which can be quantified and measured accurately.

The symposium failed to bring out that, under certain conditions, treatment with sodium fluoride results in a negative calcium balance which has been described repeatedly in studies in the U.S.A. (8, 9). That skeletal fluorosis may be accompanied by osteomalacia has already been pointed out by Roholm (2) and more recently by Jowsey (10). In alcoholics, for instance, severe osteoclastic activity due to fluoride results in dissolution of bone substance associated with osteomalacia and spontaneous fractures. Such lesions occur following intake of fluoride in daily doses greater than 8 to 10 mg as illustrated by Soriano in an earlier issue of FLUORIDE (11). Ramberg and Olsson (12) demonstrated that in cattle increased skeletal mineralization following fluoride intake is only the initial phase of a pathological process which is followed by a suppression of calcium absorption from the gastrointestinal tract. This decreased utilization of alimentary calcium is accompanied by increased resorption of calcium from bone which, in turn, accounts for the calcium loss. The reasons for the negative calcium balance are not understood.

Another area of disagreement is the dose of the drugs to be ad-
Some investigators have advocated treatment with daily doses in the range of 75 to 150 mg whereas others recommend much smaller amounts namely 20 to 60 mg daily. One author pointed out that most European pharmacopeias designate 20 mg per day as the maximum daily dose of fluoride.

The question of immediate side effects, such as nausea, vomiting and intestinal disturbances was brought up at the conference. These symptoms were recorded in 1949 by Black, Kleiner, and Bolker (13) who were among the first to advocate sodium fluoride treatment for children with leukemia and cancer; furthermore by Rich (14) who was first to propose this treatment in osteoporosis. Rich also recorded subacromial bursitis in 2 of 9 patients so treated (6). To prevent immediate side effects, Black, Kleiner, and Bolker employed aluminum salts simultaneously with fluoride; this antidote retards absorption of fluoride from the upper intestinal tract. At the Baden symposium, Jesserer suggested the use of enteric-coated tablets to eliminate untoward effects from fluoride treatment. Because of the occurrence of such reactions it is likely that a certain segment of patients, who cannot tolerate fluoride, abandon treatment after the first trial and are thus eliminated from the study, a fact which would affect the statistical results.

Of greater concern than the immediate effect of long-term administration of sodium fluoride in large doses is the deposition of calcium in areas of the body where it can produce irreparable harm. Experience with skeletal fluorosis has shown repeatedly (11, 15, 16, 17, 18) that the bones and teeth are not the only organs susceptible to increased calcification. Fluoride accumulates in ligaments, joints and tendons where it causes arthritic changes, particularly in arteries where it leads to serious calcifications of the Menkeburg type. Observations by Duffy et al (19) which, to date, have not been followed up raise the possibility of future malignancies. These authors noted the appearance of a certain kind of megalocyte in the bone marrow which has not been described heretofore. The two patients in whom these cells were found had received treatment with 16 to 159 mg of sodium fluoride daily for 1 to 36 months.

The conferees discussed the retardation of absorption of sodium fluoride in individuals with low acidity of the stomach. However, no mention was made in the report of the meeting concerning the effect of therapy with sodium fluoride on patients with gastric ulcer. Excess hydrochloric acid, usually encountered in such cases, is likely to react with sodium fluoride and to form hydrofluoric acid, an extremely corrosive agent. Indeed, gastric hemorrhages have been reported in infants (20) receiving minute doses (1 mg/day) of fluoride.

It is difficult to reconcile the experience of the European investigators with those of Jowsey (21) who states that microradiographic studies on bone tissue have shown an increase in bone mass when fluoride (50 mg
NaF/day) is combined with calcium (1000 mg/day) and vitamin D (50,000 units twice per week). At the Baden conference the addition of calcium to treatment with sodium fluoride was not recommended by the conferees.

Another point requiring clarification is the question of bone strength in treated cases. Most authors in the U.S.A. question the quality of fluoride-induced bone substance.

Whereas treatment with sodium fluoride adds new bone substance to the osteoporotic skeleton and thus induces temporary relief to patients with osteoporosis one cannot help but recall the warning by Albright (22), one of the pioneers in bone research, that administration of fluoride in bone disease is tantamount to "playing with fire".

Bibliography


FLUORIDE


** * * * **

Volum 7  Number 2
April, 1974
ELUOFUDE EFFECTS ON CHLOROPHYLL BIOSYNTHESIS
IN Nicotiana tabacum

by

W. J. Wallis, 1 G. W. Miller, 2 M. Psenak, 3 and J. Shieh 4
Bellingham, Washington

SUMMARY: The effect of fluoride and chloride at different concentrations (0, 10^{-4}M, 10^{-3}M, 10^{-2}M) was studied on the incorporation of 14C-6-aminolevulinic acid (ALA) into coproporphyrin and protoporphyrin fractions, and ether-extractable pigments (chlorophyll a and pheophytin a) in tobacco leaf discs.

Both chloride and fluoride at concentrations of 10^{-4} to 10^{-2} M inhibited the incorporation of ALA into chlorophyll a and pheophytin a. At 10^{-2} M the effect of fluoride on ALA incorporation was greater than that of chloride. The inhibition on formation of pheophytin a by fluoride was similar to that found on chlorophyll a, indicating an effect on synthesis of chlorophyll rather than degradation. The effect of fluoride on individual enzymes in the chlorophyll biosynthetic pathway is discussed in relation to the action of fluoride on cellular ultrastructure.

INTRODUCTION

It is well known that some plant species have a very low tolerance to atmospheric fluorides (1-4). The symptoms of injury may be manifested by an initial chlorosis of the leaves (5) which is characterized by a yellow to brown bleaching of the tips and upper margins of plant leaves, with subsequent tissue collapse, cupping, and other distortions (5, 6). Despite the absence of precise mechanisms by which fluorides induce injury in plants, much is known concerning the alteration of plant cell ultrastructure and physiological processes by fluoride (7, 8). Effects on photosynthesis, respiration, RNA structure, and on cellular metabolites involved in the reactions of certain enzymes have been well documented (9-12).

Little is known regarding the influence of fluorides on the biosynthesis or degradation of chlorophyll. Since succinate and glycine were pro-

1 Present address: 6027 - 41st Avenue, S.W., Seattle, Washington 98136.
3 Present address: Comenius University, Department of Biochemistry and Microbiology, Faculty of Pharmacy, Bratislava, Czechoslovakia.
4 Present address: Department of Biochemistry and Biophysics, University of Hawaii, Honolulu, Hawaii 96822.
posed as precursors for synthesis of δ-aminolevulinic acid and for the subsequent biosynthesis of porphyrin (13), several investigators have shown the effects of fluoride and chloride in vitro on the activity of various enzymes which participate in biosynthesis pathway of porphyrin (14-16). Newman (17) has shown that fluoride in vivo causes a reduction in the content of chlorophyll in green tissue and also in etiolated leaf tissue following a light treatment. He theorized that fluorides were either affecting early stages in the biosynthesis pathway of chlorophyll or inducing degradation of chlorophyll.

In these experiments the incorporation of δ-4-14C-aminolevulinic acid (ALA) into chlorophyll and several of its precursors were studied in tissues exposed to chloride and fluoride to determine any effect on the biosynthesis of chlorophyll.

MATERIALS AND METHODS

Chemicals

δ-aminolevulinic acid was purchased from Sigma Chemical Co. (St. Louis, Missouri), δ-4-14C-aminolevulinic acid (20-30 mCi/mmole) from New England Nuclear Corporation (Boston, Massachusetts), and PCS (liquid scintillation cocktail) from Amersham/Searle Corporation (Arlington Heights, Illinois). All other chemicals were A. R. grade.

Plant Material

Nicotiana tabacum L. (variety Havana 38) was selected for this study because of its short growth period, ease of maintenance, and large leaf areas with high concentrations of chlorophyll. Seeds were germinated in moist vermiculite. The seedlings were irrigated with distilled water regularly, and with Hoagland's nutrient solution I(18) once a week. When they attained a height of about 5 cm, the seedlings were transferred to aerated polyethylene pots containing Hoagland's solution in full strength. Plants were grown at controlled temperature (28±2°) and light intensity (240 lux) with a day length of 16 hours. When the plants attained a height of about 40 cm (half mature) the second and third leaves from the apex were taken.

Chlorophyll Labeling

A cork borer (1 cm diameter) was used to prepare discs from the inter-veinal areas of the leaves. Two hundred eighty leaf discs were soaked for 10 minutes in 1/10 strength Hoagland's nutrient solution without Ca(NO₃)₂ (to prevent complexing with fluoride), and then randomly distributed adaxial side up in Petri dishes containing filter paper and 1 ml of the same solution. To each dish was added 2.2 x 10⁵ counts/min ¹⁴C-ALA (20-30 mCi/mmole) and 1 ml (1.2 mM) of carrier ALA in 4 ml of the aforementioned nutrient solution. Treatments contained KF or KCl at 0, 10⁻², 10⁻³ or 10⁻⁴M.

A zero time control was also prepared by soaking leaf discs in abso-
solute methanol for 20 minutes, rinsing in water, and placing them in the treatment mixture for incubation. All treatments were incubated for 8 hours at a light intensity of 150 lux at 32°(±1°) and the pH value was maintained at 6.2.

**Chlorophyll Extraction**

Pigments were extracted by grinding the leaf tissue in absolute methanol. The methanol extract was filtered and the residue washed again with absolute methanol. Pigments were transferred to diethyl ether by shaking the methanolic extracts in a separatory funnel with ether. A 10% NaCl solution was used to separate the two layers and finally the ether layer was washed 3 times with deionized water.

**Chromatography**

Chlorophyll a and pheophytin were separated by concentrating the ether-extracted pigments and spotting aliquots on thin-layer chromatographic plates (0.25 mm thickness), using silica gel-G as an absorbent, and an isooctane-acetone-ether (3:1:1, v/v) mixture as developing solvent (19). Good resolution of chlorophyll a and pheophytin was obtained on the chromatograms.

**Porphyrin Labeling**

Half mature leaf discs were incubated in the dark at room temperature for 12 hours. Petri dishes were used containing leaf discs and a treatment mixture containing KCl or KF at concentrations of 0, 10^{-2} or 10^{-3} M. As in the chlorophyll studies, each incubation medium contained 2.2 × 10^5 counts/min ALA (S-4-14C) and 1 μ mole of carrier ALA in 5.0 ml of 1/10 strength Hoagland's solution without Ca(NO3)_2.

**Porphyrin Extraction**

Porphyrins were extracted by the procedures outlined by Dresel and Falk (20). Metabolic activity was stopped by homogenizing each sample in a mixture of ethyl acetate-acetic acid (3:1, v/v). The homogenate was filtered through glass wool and the residue rewashed with ethyl acetate-acetic acid (3:1, v/v). The filtrate was washed twice with saturated aqueous sodium acetate; the washings were back extracted with ethyl acetate and the combined ethyl acetate layers were rewashed once with 3% sodium acetate. The combined aqueous extracts were saved for the determination of uroporphyrin. Coproporphyrin and protoporphyrin were extracted from ethyl acetate by means of 15% HCl.

The acid extracts were combined and adjusted to pH 3.1 with saturated aqueous sodium acetate. The porphyrins were extracted from the aqueous solution into anhydrous ether. After concentration, the ether solution
was twice extracted, first with 0.2% HCl for the removal of coproporphyrin and then with 10% HCl for removal of protoporphyrin.

The pH of the sodium acetate washings was adjusted to a value of 3.1, followed by extraction 3 times with ethyl acetate. The combined ethyl acetate layers were then extracted twice with 2% HCl (uroporphyrin removal).

The fractions of protoporphyrin IX, uroporphyrin and coproporphyrin were dried, dissolved in 2M KOH, and chromatographed on t.i.c. plates with silica gel (0.25 mm thick) or Whatman No. 1 paper by means of a solution of 2.6 lutidine in water (5:3, v/v) as a developing agent (21). Coproporphyrin and protoporphyrin IX fractions showed the presence chromatographically of only these porphyrins with no 14C-ALA contamination. The uroporphyrin fraction was found to have some 14C-ALA present as reported previously by Rebeiz et al. (22).

Scintillation Counting

Radioactivity of the extracted compounds was determined by means of a tri-carb liquid scintillation spectrometer. Aliquots of the extracted pigments were mixed with prepared scintillation solutions (23), and porphyrins were mixed with PCS liquid scintillation cocktail. All samples were counted for 10 minutes. Both the channels ratio method and internal standards were used to determine quenching and efficiency. Efficiency of counting of isolated pigments was 50% or more, of total pigments 55% or more, and of porphyrins over 65%.

EXPERIMENTAL RESULTS

The rates of incorporation of 14C-ALA into chlorophyll in both chloride and fluoride-treated leaf tissues were investigated to determine whether treatment with fluoride resulted in a block in the biosynthesis of chlorophyll using a known precursor. Fluoride had a significantly greater inhibitory effect than chloride on the incorporation of δ-aminolevulinic acid into total pigments, chlorophyll a and pheophytin a (Table I). Chloride at concentrations from 10⁻⁴ - 10⁻² M inhibited the incorporation of ALA into chlorophyll a 33 to 56%. Similar effects of chloride were found in the incorporation of ALA into pheophytin a and ether-extracted pigments.

Fluoride at a concentration of 10⁻² M inhibited the incorporation of ALA into chlorophyll a, pheophytin a and ether-extracted pigments 81%, 90%, and 68% respectively. The inhibition was markedly greater than that found at similar concentrations of chloride. Fluoride also inhibited more than chloride at lower concentrations but the differences were not as pronounced as at 10⁻² M.

When the incorporation of ALA into uroporphyrin, coproporphyrin and protoporphyrin was studied, increased concentrations of fluoride resulted in decreased incorporation of ALA into porphyrins (Table II). Although
TABLE I

Incorporation of $\delta$-4-14C Aminolevulinic Acid into Pigments in Fluoride and Chloride Treated Tobacco Leaf Discs

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (M)</th>
<th>14C incorporated into chlorophyll (disintegrations/min)</th>
<th>Inhibition</th>
<th>14C incorporated into chlorophyll (disintegrations/min)</th>
<th>Inhibition</th>
<th>14C incorporated into chlorophyll (disintegrations/min)</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>45147</td>
<td>--</td>
<td>14423</td>
<td>--</td>
<td>8702</td>
<td>--</td>
</tr>
<tr>
<td>Chloride</td>
<td>$10^{-4}$</td>
<td>35197</td>
<td>22</td>
<td>9660</td>
<td>33</td>
<td>8483</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>$10^{-3}$</td>
<td>27632</td>
<td>39</td>
<td>7560</td>
<td>48</td>
<td>5675</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>$10^{-2}$</td>
<td>24828</td>
<td>45</td>
<td>6282</td>
<td>56</td>
<td>5325</td>
<td>39</td>
</tr>
<tr>
<td>Fluoride</td>
<td>$10^{-4}$</td>
<td>32832</td>
<td>27</td>
<td>7528</td>
<td>48</td>
<td>6647</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>$10^{-3}$</td>
<td>28933</td>
<td>47</td>
<td>7090</td>
<td>51</td>
<td>4685</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>$10^{-2}$</td>
<td>14533</td>
<td>68</td>
<td>2715</td>
<td>81</td>
<td>902</td>
<td>90</td>
</tr>
</tbody>
</table>

-Discs were incubated at 32°C under 150 lux for 8 h with $\delta$-4-14C aminolevulinic acid, $2.2 \times 10^5$ counts/min. The results are the average of three experiments, expressed as disintegrations/min per forty leaf discs. For details see text.

TABLE II

Incorporation of $\delta$-4-14C Aminolevulinic Acid into Porphyrins in Fluoride and Chloride Treated Tobacco Leaf Discs

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (M)</th>
<th>14C incorporated into coproporphyrin (disintegrations/min)</th>
<th>14C incorporated into protoporphyrin (disintegrations/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloride treated</td>
<td>$10^{-3}$</td>
<td>690</td>
<td>1759</td>
</tr>
<tr>
<td></td>
<td>$10^{-2}$</td>
<td>682</td>
<td>1354</td>
</tr>
<tr>
<td>Fluoride treated</td>
<td>$10^{-3}$</td>
<td>534</td>
<td>1459</td>
</tr>
<tr>
<td></td>
<td>$10^{-2}$</td>
<td>340</td>
<td>687</td>
</tr>
</tbody>
</table>

-Discs were incubated at room temperature in the dark for 12 h with $\delta$-4-14C aminolevulinic acid, $2.2 \times 10^5$ counts/min. The results are the average of three experiments, expressed as disintegrations/min per forty leaf discs.
a marked reduction of incorporation of ALA into the apparently predominant uroporphyrin fraction was noted, this fraction was found to be contaminated by $^{14}$C-ALA. No attempt was made to purify it further (and the results are not reported). In samples treated with $10^{-2}$M fluoride, incorporation of ALA into coproporphyrin and protoporphyrin was approximately 50% of that of comparable chloride treated tissues. Incorporation of ALA into coproporphyrin was lower with incorporation into protoporphyrin roughly twice as high. As noted in Materials and Methods these fractions were not contaminated with $^{14}$C-ALA.

DISCUSSION

Newman (17) has shown that the content of chlorophyll is reduced by 43% in Phaseolus vulgaris (bush bean) when exposed to fluoride. He concluded that fluorides affect the early stages in the synthesis of chlorophyll or induce the degradation of the structure of chloroplast. In our studies with Nicotiana tabacum it was demonstrated that fluoride at concentrations as low as $10^{-4}$ M inhibited the incorporation of a known precursor, $\delta$-4-$^{14}$C aminolevulinic acid, into chlorophyll by 48%.

The incorporation of $^{14}$C-ALA into pheophytin a was studied to determine any effect of fluoride on the degradation of chlorophyll. With increasing concentrations of fluoride or chloride there was a decrease in the incorporation of $^{14}$C-ALA into pheophytin a similar to the effect found with incorporation of $^{14}$C-ALA into chlorophyll. This finding indicates that fluoride did not significantly affect the removal of magnesium from the tetrapyrrole ring under the conditions of these experiments. Conditions of the assay such as long exposure to high light intensity (24) may have been the primary determinant for the transformation of chlorophyll to pheophytin. However, it has been noted (17) that when the turn-over rate of pheophytin is very great, then a large accumulation of pheophytin would not be found under any condition, providing the conversion of pheophytin to other degradation products still occurred at a normal rate. Since no estimate of the turn-over rate of pheophytin is available, an accurate evaluation of this theory is not possible.

A number of investigations have demonstrated the effect of chloride on the proposed biosynthesis pathway of tetrapyrrole. In vitro chloride inhibits the metabolism of porphobilinogen (14), and decarboxylation of uroporphyrinogen to coproporphyrinogen (14, 15, 25, 26). These studies support our findings that chloride exerts an inhibitory effect on the incorporation of $^{14}$C-ALA into chlorophyll.

In vitro, fluoride has been shown to inhibit the decarboxylation of uroporphyrinogen to coproporphyrinogen (14, 15), with no effect upon the conversions of ALA to porphobilinogen (27) or porphobilinogen to uroporphyrinogen (14). Dresel and Falk (16) found a 90% inhibition in the formation of protoporphyrinogen when $^{14}$C-labeled glycine was incubated with $0.12 \text{ M NaF}$.

Volume 7 Number 4
April, 1974
Our investigations indicate that tissues of tobacco leaf treated by fluoride at a concentration of $10^{-2}$M incorporated 50% less $^{14}$C-ALA into coproporphyrinogen fractions than similarly exposed chloride-treated tissue. These results suggest that, in vivo, fluoride can exert an inhibitory effect on the incorporation of $\delta$-aminolevulinic acid into the coproporphyrinogen pool of the tetrapyrrole biosynthesis pathway.

When the oxidative activity of intact, coupled mitochondria from soybean hypocotyls was measured after 2 minutes' exposure to fluoride ($6.6 \times 10^{-3} - 6.6 \times 10^{-2}$M; with succinate, malate and NADH as substrates), a significant decline of respiratory rate and ADP/P ratio was observed (28). After exposure of mitochondria to fluoride for ten or more minutes a flocculation of mitochondria occurred. Exposure of hypocotyl sections to a $10^{-2}$ M solution of KF for 3 and 10 hours decreased the respiratory rate to only 57% and 34% respectively, compared to that of the control (28). The explanation of these results might be related to the destruction of the structural integrity of mitochondrial membranes as proposed by Ramagopal et al. (29). Taking into account the effect of fluoride on the ultrastructural integrity of mesophyll cells (7) or on the protein structures (30), the observed effect of fluoride on incorporation of $^{14}$C-ALA into porphyrins, including chlorophyll and pheophytin, may be attributed to changes in organelle structures and the integrity of membranes.

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BIBLIOGRAPHY


Volume 7  Number 2
April, 1974


* * *
SUMMARY: Simultaneous determination of fluoride and calcium by proton microanalysis revealed a simultaneous accumulation in the injured tip, and a large increase in the calcium level throughout the polluted needles. The ratio of the chemical forms of calcium also changes with the accumulation of fluoride; calcium oxalates particularly show an increase. The results suggest that calcium precipitates with fluoride as CaF₂.

The effect of calcium on the fluoride tolerance of plants is now well known. It has been established that damage to leaves by fluoride is most severe when calcium levels in leaves are low (1, 2). Our studies indicate that translocation and accumulation of fluoride are modified effectively by the calcium content of the leaves (3). Fluoride injury to the growing tips of leaves is similar to the symptoms of severe calcium deficiency.

It has also been recognized that lime sprays are most effective in preventing fluoride damage (4, 5). The protection afforded by the lime was attributed to the formation of insoluble CaF₂ at the surface of the leaf. An increase in the calcium content inside the leaf tissue might also be involved.

In view of these findings, we conducted the following experiments in order to study the fluoride-calcium interaction in the leaf tissues of fir needles growing in a polluted area. The fluoride and the calcium distribution were established in relation to the effect of fluoride on the chemical forms of calcium.

**Method**

We determined the content of fluoride by nuclear reaction and that of calcium by the characteristic X-ray changes (6). We employed the same experimental approach as that used in our previous studies which yielded an accurate localization of fluoride (7), namely the proton microanalysis.

The characteristic X-rays of calcium from 3.688 keV (ka) and 4.02...
keV (k$^\beta$) are detected by a Si(Li) junction, 30 cm distant from the target. With a 1369 keV proton beam, the distribution of fluoride and calcium are analyzed over a depth of 40$\mu$m in biological tissues. The needles can be measured every 5/10 millimeter and the sensitivity for calcium is better than 50 $\mu$g per gram of dry matter. Figure 1 shows the apparatus used for the experiments.

\begin{figure}
\centering
\includegraphics[width=1\textwidth]{fig1}
\caption{Scheme of Experimental Apparatus}
\end{figure}

For the chemical separation, the fir needles are dried at 80°C and then ground. Estimates of the chemical forms of calcium are obtained from sample (taking 1 g dry matter/100 cm³ solvent) by successive extraction in hot water (water soluble compounds), 0.5 N NaNO₃ (exchangeable Ca), 10% CH₃COOH (phosphates, carbonates) and 2 N HCl (calcium oxalates) according to the method of Abutiloff as described by Schilling (8). The different fractions are collected after stirring for one hour and centrifugation for 10 minutes. The analysis is carried out by a flame photometer.

**Results**

**Simultaneous Determination of Fluoride and Calcium**

In healthy needles, the calcium content increases from the tip to the base. On the other hand, the injured tip shows two distinct areas of

**FLUORIDE**
accumulation of calcium. The simultaneous determination of calcium and fluoride indicates that their highest levels occur exactly at the same site in the needle (Fig 2). The highest accumulation takes place in the injured area of the needles. Less pronounced is the accumulation between the injured and the healthy tissues.

Fig. 2

**Distribution of Calcium and Fluoride in Fir Needles**

<table>
<thead>
<tr>
<th>In Polluted Needles</th>
<th>In Healthy Needles</th>
</tr>
</thead>
<tbody>
<tr>
<td>(grown in 1971)</td>
<td>(grown in 1971)</td>
</tr>
</tbody>
</table>

![Graphs showing distribution of calcium and fluoride in polluted and healthy needles.](image)

Distance to needle tip

At the tip of the needle where, normally, the calcium level is low, we found nearly equal weights of calcium and fluoride. Since CaF$_2$ has the same weight ratio, our results imply that fluoride is precipitated in the plant as calcium fluoride (9, 10). We also observed that, in the green tissues of injured needles, the calcium level is twice as high as in healthy needles.

**Effect of Fluoride on Chemical Forms of Calcium**

Chemical forms of calcium were studied in relation to the needle's age (Fig. 3). In a polluted needle, the fluoride content increases with the
Fluoride in Fir Needles

Fig. 3

Effect of Fluorine on the Chemical Forms of Ca

Water soluble calcium: Normally, this fraction decreases between the first and the second year and stabilizes throughout the following years. In a polluted needle, the water soluble calcium fraction increases with age and with the leaf's content of fluoride, but the ratio is nearly the same as in a low polluted needle.

Exchangeable calcium: This fraction generally increases between the first and the second years and becomes stable during the following years. Like water-soluble calcium, it increases with the fluoride content of the leaf without modifying the ratio.

Calcium carbonates and phosphates: This fraction also increases between the first and the second years and stabilizes during the following years. In polluted needles, calcium carbonates and phosphates increase slowly with the fluoride content of the leaf.

Calcium oxalates: This fraction, the most important one, in-
creases with the age of the needle, particularly after the first year, and also with the fluoride content of the leaf. Fluoride has a marked influence on formation of calcium oxalate. We noted a large increase in the relative distribution of calcium oxalates in polluted needles.

**Total calcium:** In low-polluted plants, the older needles contain more than the younger ones. In polluted needles all chemical forms of calcium increase and, therefore, the total calcium is twice as abundant as in non-polluted needles.

**Discussion**

Abutalybov et al. (11) pointed to the fact that fluoride influences the accumulation of calcium. Invariably we found that needles with a higher content of fluoride exhibit a correspondingly higher content of calcium. Only in the tip of the needle the calcium/fluoride ratio suggests a simultaneous accumulation of the two elements as CaF₂, in agreement with several authors.

Among the different factors leading to necrosis such as enzymatic inactivation, chloroplastic alteration etc., Ramagopal et al. (10) state that accumulation of fluoride in plants can result in injuries of tissues by precipitating calcium which is in a free ionized state (water soluble and exchangeable calcium). Its concentration is essential for the maintenance of cellular stability. Our results on the whole needle showed no decrease of these fractions of calcium, but rather an increase following a rise in total calcium. Yet such results fail to account for what happens in the injured tip.

Fluoride increases the total calcium level but, among the other fractions of calcium present as insoluble salts, carbonates and phosphates seem to be slightly modified whereas oxalates, which are often considered waste products, increase notably. It is interesting to note that the changes induced by fluoride in distribution of calcium are similar (12) to those induced by aging namely, an increase in the total calcium and calcium oxalates.

**Conclusion**

Accurate determinations of fluoride and calcium reveal a parallel deposition of the two elements in polluted needles and suggest precipitation of CaF₂. The largest amounts of calcium and fluoride in equal proportion are localized in the injured area. This localization of the two elements in the tips suggests a direct relation between the possible precipitation of CaF₂ and apical necrosis.

The relationship between the fluoride content and the modification of the different chemical forms of calcium also suggests that formation of CaF₂ represents either a mode of inactivation of the halogen or a disturbing
fluoride in fir needles

factor in the cellular equilibrium of calcium.

bibliography


FLUORIDE-CONTAINING MINERAL SUPPLEMENTS IN AGRICULTURE

by

W. Oelschläger
Stuttgart-Hohenheim, West Germany

SUMMARY: In July, 1971, the commissioner of the European Communities recommended maximum allowable limits for fluoride contained in mineral feed supplements. This decision is of major importance to numerous industries, particularly to those confronted with the problem of fluoride emissions from their factories.

The authors demonstrated that, under "normal" circumstances in a non-polluted area, the maximum allowable limits are in the range of, or exceed, the values which are considered tolerable by domestic animals. In the environs of industries which emit fluoride, however, the maximum allowable limits are considerably exceeded even by the industrial facilities which are equipped with modern anti-pollution devices.

The establishment of maximum allowable limits for fluoride levels in mineral feed supplements is of major importance to numerous industries.

In March, 1971, the Agra-Europe published the recommendations of the Council of the European Communities concerned with the establishment of maximum allowable limits for undesirable constituents in animal feed (1). In accordance with these suggestions an ordinance was adopted on January 1st, 1971 and implemented on July 1, 1971.

Whereas the maximum allowable levels for such elements as arsenic, lead, molybdenum, etc., are considerably below their toxic thresholds, this is not the case with the element fluoride.

From the Institut für Tierernahrung der Universität Hohenheim, Stuttgart-Hohenheim, Germany.
Mineral Supplements

On the basis of the above-mentioned ordinance, the following amounts of fluoride are permissible in animal feed:

TABLE 1

<table>
<thead>
<tr>
<th>Individual feed supplements:</th>
<th>Fluoride (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Except for:</td>
<td></td>
</tr>
<tr>
<td>Feed derived from animals</td>
<td>500</td>
</tr>
<tr>
<td>&quot;Natural&quot; raw phosphate</td>
<td>3000</td>
</tr>
<tr>
<td>Clay-Calcium phosphate</td>
<td>3000</td>
</tr>
<tr>
<td>Other phosphates</td>
<td>2000</td>
</tr>
<tr>
<td>Mineral supplements for cattle, sheep, goats</td>
<td>2000</td>
</tr>
<tr>
<td>Mineral supplements for pigs</td>
<td>4500</td>
</tr>
<tr>
<td>Mineral supplements for poultry</td>
<td>6000</td>
</tr>
<tr>
<td>Supplementary feed mixtures:</td>
<td>150</td>
</tr>
<tr>
<td>Except for:</td>
<td></td>
</tr>
<tr>
<td>Mixed feed supplements for cattle, sheep, goats</td>
<td>100</td>
</tr>
<tr>
<td>Mixed feed supplements for calves, lambs and kids</td>
<td>50</td>
</tr>
<tr>
<td>Mixed feed supplements for poultry</td>
<td>350</td>
</tr>
<tr>
<td>Mixed feed supplements for chicks</td>
<td>250</td>
</tr>
</tbody>
</table>

related to 100% raw ash in the dry substance.

If such maximum allowable limits are maintained outside of fluoride emission areas, according to Wohlbier and Oelschläger (2) and to Bronsch and Lüders (3) the following amounts of fluoride in feed rations can be calculated in cows producing 25 liters of milk a day:

TABLE 2

Addition of 150 gm mineral supplements (Wohlbier, Oelschläger)

<table>
<thead>
<tr>
<th>Ration</th>
<th>Fluoride (ppm) in feed dry substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26 ppm</td>
</tr>
<tr>
<td>2</td>
<td>43 ppm</td>
</tr>
<tr>
<td>3a</td>
<td>47 ppm</td>
</tr>
</tbody>
</table>

Addition of 120 gm mineral supplements (Bronsch, Lüders)

<table>
<thead>
<tr>
<th>Ration</th>
<th>Fluoride (ppm) in feed dry substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31 ppm</td>
</tr>
<tr>
<td>2</td>
<td>50 ppm</td>
</tr>
<tr>
<td>3a</td>
<td>39 ppm</td>
</tr>
<tr>
<td>3b</td>
<td>43 ppm</td>
</tr>
<tr>
<td>4</td>
<td>58 ppm</td>
</tr>
</tbody>
</table>
These rather high levels of fluoride in feed rations are principally based upon the amounts permitted by the Europäische Werk Gemeinschaft (European Working Community) (E. W. G.) in mixed feed supplements.

In contrast with these values, the following tolerance thresholds are prevalent today:

Thirty ppm is the tolerable limit of consumption of fluoride in feed for milking cows over a prolonged period which will not induce damage of economic significance. The borderline level lies between 30 and 40 ppm. Damage and loss of productivity occurs at 40 ppm fluoride or more. All above values refer to dry substance. These are the tolerance limits based on both short and long-term experiments as well as on clinical observations of more than 70,000 animals in endemic fluoride areas according to Schmidt, et al. (4) and as shown at the Fourth International Session of the World Community for Buiastrak.

These findings agree with those of a number of other authors on the basis of which the National Academy of Sciences, Subcommittee on Fluorosis Problems, Washington, D. C. 1960, publication 824 established the following maximum limits (Table 3).

<table>
<thead>
<tr>
<th>Kinds of Animals</th>
<th>NaF or Other Soluble Fluorides</th>
<th>Phosphate, Calcium or raw Phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milking Cows</td>
<td>30-50 ppm</td>
<td>60-100 ppm</td>
</tr>
<tr>
<td>Feed Cattle</td>
<td>40-50</td>
<td>65-100</td>
</tr>
<tr>
<td>Sheep</td>
<td>70-100</td>
<td>100-200</td>
</tr>
</tbody>
</table>

The tolerance limit for uptake of phosphate, calcium, and raw phosphate, which is about twice as high as that for sodium fluoride, is related to the solubility of sodium fluoride which is between 50 to 60% greater than that of calcium fluoride and raw phosphates. The lower the threshold limit, the greater is the milk production in cows. In addition, other factors such as the general health and the uptake of minerals present in the forage play a role.

Bronsch (5) determined a somewhat lower tolerance value for raw phosphates, namely 0.5 mg F/kg body weight for beef and 2.0 mg F/kg body weight for sheep. He states: "for a milking cow weighing 500 kg, the established fluoride concentrations is equal to a minimum of 50 ppm in dry feed.

This value can be considered correct for the low limit of tolerance of the above-noted food rations in animals with high productivity. In these feed rations approximately 2/3 to 3/4 of the fluoride is derived from raw...
phosphates and the remaining amounts from fluoride contained in feed. The availability of the latter lies between that of fluoride from NaF and of fluoride from raw phosphates. For this reason, according to Bronsch (5), the commission of the E.W.G. dealing with this subject established 50 ppm fluoride as the upper limit for the fluoride content of dry substance.

As can be seen in the above-outlined examples of feeding, the maximum allowable limit of 50 ppm is reached and even exceeded in the various rations noted above.

However, a much greater excess of this value of 50 ppm occurs in every fluoride-emitting area, i.e., in the environment of such industries as hydrogen fluoride and superphosphate factories, aluminum smelters, glass and brick manufacturing, enamel and electro-metallurgical works, smelting and near other industrial centers. This is due to the fact that complete elimination of fluoride emission is unobtainable because of physical and economic reasons. Therefore, feed supplements grown in the environs of fluoride-emitting factories which have efficient wash and filtering equipment, contain excess fluoride in amounts as high as 30 ppm in dry substance. In addition, the availability of fluoride in such forage, which is taken up mainly via the stomata of the leaves, is greater than that of fluoride derived from raw phosphates.

Nowadays, the majority of the fluoride emission areas have achieved a substantial reduction in emission through installation of expensive air cleaning equipment. Therefore, the resultant damage to domestic animals is less severe because the forage grown in such areas is less toxic. On the other hand, regardless of the ordinances or laws which are already in effect in the German Federal Republic as well as in the E.W.G., fluoride damage is becoming much more acute. This is true particularly in Germany where a wide variety of industries is crowded into a relatively small area and where much of the non-ferrous metals are being produced including aluminum. There appears to be a lack of full appreciation of the extraordinary amounts of fluoride which reach the feed rations through mineral supplement mixtures, both by the fluoride-emitting industry and by the industry which manufactures feed supplements. For example, the management of a certain HF factory stated that the corporation had given to two farmers in the emission area—for a certain period of time without cost—mineral supplements for the purpose of increasing the productivity of their herds. An investigation on the basis of this information revealed that the mineral supplement contained the surprisingly high level of 1750 ppm fluoride.

Employment of different kinds of phosphates produces a mineral mixture with relatively low fluoride content which minimizes the danger of damage to animals. The feeding of fluoride-containing mineral mixtures caused such large amounts of fluoride to accumulate in the bones of cattle that even a slight rise in fluoride levels in hay and silage constitutes a threat of fluorosis.

FLUORIDE
Bibliography


* * *

EVALUATION OF SOME HEALTH PARAMETERS IN CHILDREN IN THE VICINITY OF AN ALUMINUM FACTORY

by

G. Balazova and V. Lipkova
Bratislava, Czechoslovakia

SUMMARY: The health status of children, aged 6-14 years, was investigated with special regard to the hematological indices and the content of fluorine compounds in urine and hair. The children had been living in a settlement in the environs of the aluminum factory in operation for 20 years. In the first years of the operation of the factory, the examinations of children aged 6-14 years showed evidence of potential hazard, induced by the influence of the factory on the environment. Therefore, certain sanitary measures were designed and implemented: A portion of the population

From the Research Institute for Hygiene, Bratislava, Czechoslovakia.
was translocated. Precautions were taken regarding consumption of crops grown in the polluted area, to ensure a safe diet for children. Regular visits to clinically suitable recreational areas were organized every year for children.

After ten years, the medical examinations were repeated. The values which were obtained were related with the concentration of fluorine compounds in the air and compared with the results obtained from a group of children of the same age during the first years of the operation of the factory.

A clear and total improvement of the studied indices resulted. Their values approach the values of the control group of children from other non-exposed areas. It can be concluded that the sanitary measures were effective.

The construction of new factories and their incorporation into dense population centers of Slovakia entailed, besides their positive social-economic advantages, also certain negative influences on the human environment.

The aluminum factory had started its operation in 1953. The problem concerned its placement in a densely populated valley, where gaseous and solid emissions were dispersed only in one direction, depending mainly on their quantities and on the complexity of a variety of meteorological conditions (1). This complicated situation has led to a decision on the part of the government to establish certain measures for:

a. Working out of a new zoning plan, based on the possible emissions and their effect on the human environment in case of a future increase in the production capacity of the aluminum smelter;

b. Translocation of populations from the most exposed site, where the maximum allowable fluoride concentration in the air has been continuously exceeded;

c. Restriction of new buildings and expansion of communities where the allowable fluoride concentration in the air occasionally exceeded the allowable limits;

d. Proposed rules concerning the consumption of agricultural products grown in the polluted areas by transporting vegetables and fruits which contained excess fluoride from the contaminated environment into uncontaminated areas and by importation of produce grown in distant regions;

e. Intensification of the preventive health measures of all popu-
lation groups residing in exposed regions, with special regard to children and teenagers.

f. Adoption of measures to insure a nutritional regime for children and regular visits to mountainous recreation centers;

g. A protective diet for the staff established in the dining facilities of the smelter as well as an extensive program of instructions about the principles of the proper nutrition for the remaining population.

We were interested in observing the state of health of children under the conditions of the new settlement, to which the population was transferred from the exposed areas in the environs of the factory. In this settlement we repeated in 1970, after an interval of 10 years, all investigations in the 6 to 10 year old children. In 646 children fundamental indices of the physical development were evaluated, in 399 children the blood picture and in 368 children the level of fluorine eliminated in the urine and in hair. The results were compared with the values found in exposed communities in the vicinity of the aluminum factory in 1960, before the adoption of the corrective measures.

The average fallout values in the settlement under study are moderately elevated and fluctuate from 200 to 300 tons/km² per year. The average fallout of fluorine compounds has reached 133-224 kg/km² per year. The content of fluorine compounds in the air was about twice the maximum permissible concentration (2).

We have compared the results of the physical development of the 6 to 10 year-old children from an exposed region with the average for all Slovakia standards of the year 1961 (graphs 1 and 2). In this locality the physical development of the child population showed a remarkable improvement. We attribute this to the improved socio-economic status and to changes in the standard of living due to the industrialization of this region.

**Red Blood Counts and Hemoglobin**

The average values in the erythrocyte count at present do not differ from the values of the year 1960. Regarding the hemoglobin content, levels of which were lower in 1960, a statistically significant improvement was observed at present. Improvement was manifested likewise in the color index value (graph 3).

In order to compare the current blood pictures of exposed children with those residing in uncontaminated areas, an equal group of children of the same age was examined in a submountain region of Slovakia, where no sources of air pollution exist (graph 4). The total erythrocyte count and the amount of hemoglobin were slightly higher in the children at a settle-
Graph 1
Comparison of Body Height and Weight in 1960-1970

Graph 2
Comparison of Body Height and Weight in 1960-1970

Graph 3
Average Hematologic Indices in Children

--- Whole-Slovak Average in 1961
--- Exposed Area in 1960
--- Exposed Area in 1970

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ment in the vicinity of the aluminum factory, but the differences were not significant statistically. The hematocrit values were also higher in the children from a settlement near the aluminum factory. A statistically significant difference in the average erythrocyte volume was noted, so that the children from the settlement under study near the aluminum factory had distinctly smaller erythrocytes. We see here the same results as we met in the regions covered with dust or polluted with SO₂ (3).

**Fluoride in Urine and Hair**

With respect to urinary fluoride eliminated by the children near the aluminum factory, the average value in 1970 was 0.36 mg/l (minimal 0.01 - maximal 1.05 mg/l). These results can be considered as occurring currently. In 1960, in the exposed communities, the average value was 0.91 mg/l with maximum values 4.75 mg/l, which were significantly higher.

The fluoride content in the hair of children had the average value of 0.4 mg/100 g, whereas we found in the year 1960 in an exposed community 1.6 mg/100 g. The average values of fluoride in hair ranged from 0.10 to 0.82 mg, in the exposed community in 1960 from 0.7 to 4.7 mg/100 g.(4).

The current results in the group of children under study in a settlement near the aluminum factory constitute conclusive proof that during the 10 year interval an essential improvement has taken place in comparison with the results in children from exposed localities during 1960.

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April, 1974
The proposed health measures which were achieved in form of a translocation of people and of regime adjustment produced a definite effect which was manifested by the favorable results of physical development, by the hemoglobin values and, by the level of fluorine compounds in the urine and hair of children.

Bibliography


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EFFECTS OF FLUORIDE ON THE KIDNEY
(A REVIEW)

by

J. Jankauskas
Pensacola Beach, Florida

SUMMARY: A review of the available literature on the effects of fluoride on kidneys indicates that fluoride is removed from the kidneys by glomerular filtration. Approximately 1/3 of ingested fluoride appears in the urine within 24 hours.

In experimental animals massive doses of fluoride induce tubular necrosis, especially in the convoluted portions of the tubules, and inflammation of glomeruli. These changes are associated with the clinical findings of impaired kidney function such as polyuria, polydipsia, increased non-protein nitrogen, etc.
In humans, in acute fluoride intoxication, the kidneys are affected adversely. Although data on the long-term effect of repeated small doses of fluoride are sparse, in areas of endemic fluorosis functional disturbances have been reported. Whereas epidemiological statistics in the U.S.A. have not revealed any effect of fluoridated water (1 ppm) on the kidneys, in persons with kidney disease increased retention of fluoride in the blood has been recorded. Prolonged anesthesia with methoxyflurane can lead to renal failure and death.

Fluoride ingestion and inhalation have been linked to structural and functional renal abnormalities, in both humans and animals, as far back as 1890 when Hewelke observed albuminuria and hematuria in dogs fed sodium fluoride. McClure and Mitchell, in 1931, noted that polyuria and polydipsia were major manifestations of acute and chronic fluorosis. In 1930 Goldemberg (1) reported a polyuric state in humans following administration of sodium fluoride for control of thyrotoxicosis and Roholm (2) suggested that polyuria was a prominent component of acute fluoride intoxication in humans.

Since then, numerous studies have been published on the effects of fluoride on the kidney. This paper will review the means of excretion of fluoride, its effect on the kidneys of animals and humans under a variety of circumstances and the iatrogenic renal dysfunction caused by the halogenated anesthetic agent, methoxyflurane.

Excretion of Fluoride

After ingestion of fluoride, absorption from the gastrointestinal tract is very rapid and probably starts in the stomach (3, 4, 5, 6). This seems to be an entirely passive process which does not require an active transport system (4). Using radiofluoride, Carlson (7) reported maximum plasma concentrations within 60 minutes. Once absorbed, plasma fluoride is readily distributed to the extracellular body water at rates of 30 to 40% per minute (5). From here, fluoride is either deposited in bones or teeth and, in variable quantities, in soft tissues or is excreted via the kidney (8). The other known routes of excretion, such as the gastrointestinal tract and sweat glands, seem to play only a minor role under most conditions. Approximately half of the fluoride ingested by adults is excreted in the urine within 24 hours and about half is deposited in the skeleton (4, 9) but there are tremendous variations. Children, who are actively laying down new bone, excrete less fluoride than adults (4, 5). Longwell (10) found that children 5 to 6 years old excreted 0.16 mg fluoride per 24 hours whereas children 10 to 12 years of age excreted 0.35 mg per 24 hours. Excretion of fluoride is also likewise lower during pregnancy and returns to pre-pregnancy levels 2 to 3 months postpartum (11).

Numerous clearance studies in humans have shown that fluoride clearance is less than that of inulin or creatinine but many times more ra-
Fluoride and Kidneys

Fluoride is removed from the circulation by glomerular filtration and variable amounts are reabsorbed by tubular reabsorption (3-6, 9, 12, 13). Hodge (5) in 1961 estimated a tubular fluoride reabsorption of 92% but Carlson (7), using radiofluoride techniques, reported values of 51% and 63%. Thus, fluoride is not reabsorbed to the same extent as sodium, chloride, or phosphate even though the same transport system or systems are thought to be utilized by all of these ions (7, 9). The tubules are not known to secrete fluoride (3, 9, 12).

In the United States, in persons drinking water that contains little or no fluoride urinary fluoride concentrations are in the range of 0.2 to 0.5 ppm whereas in people ingesting water that contains 1 ppm fluoride urinary concentrations are of the order of 0.5 to 1.5 ppm (4).

Structural and Functional Effects of Fluoride on Animal Kidneys

As mentioned previously, the effect of fluoride administration on both the structure and function of the kidneys of experimental animals has been extensively investigated. Marked discrepancies in pathologic findings can be attributed to use of different fluoride compounds and modes of fluoride analysis, various modes of administration, and species of animals. However, the conclusion can be drawn that sublethal and lethal doses, as well as large long-term doses, do produce structural alterations in the kidney. Ogilvie (14) using sublethal doses of fluoride in rats for periods up to three months produced marked tubular necrosis, especially in the convoluted portion of the tubule. He also noted inflammatory changes in the glomeruli and interstitial edema. With lethal doses of sodium fluoride (30-50 mg/kg) Kawahara (15) produced in rabbits and rats tubular degeneration, glomerular changes, and heterogenous types of tubular lumen casts. Similar changes (16) occurred in sheep and dogs, following lethal doses of fluoracetate. Poulsen and Ericsson (17) who administered large doses for up to 16 weeks, reported glomerular inflammation, casts, interstitial edema, and fibrosis. Roehm (2), Bond and Murray (18), employing various species of animals, found that fluoride dosage levels of 14 mg/kg/day or higher produce chronic renal lesions closely resembling those of interstitial nephritis. Bond and Murray (18) calculated that a total dosage of 250 mg sodium fluoride administered to the rat for periods of 18 to 60 weeks was needed to produce this pathologic change. The aforementioned studies and others (17, 19-24) have shown that large amounts of fluoride compounds adversely affect the various portions of the kidney.

Pindborg (25) in 1957 indicated that a diet containing 0.05% (500 ppm) Fluoride...
sodium fluoride precipitated renal lesions in rats after 21 to 28 days and Ramseyer (26) reported hypertrophy and hyperplasia in the tubules of rats receiving 1, 5, and 10 ppm fluoride in their drinking water for 500 days. The latter study was repeated by Bosworth and McCay (27) who attributed the morphologic effect on the kidney by such low dosages to old age but their interpretation of their findings has been challenged.

Reported derangements of the kidney function induced by the administration of fluoride to laboratory animals have been more homogenous than the pathologic studies. The most common alteration in renal function due to long-term fluoride ingestion has been an increase in urine volume; in most cases polyuria has been accompanied by polydipsia (1, 2, 21, 24, 25, 28, 29). Bond and Murray (18) found that rats given 2 to 7.5 mg of fluoride per day for 18 to 48 weeks showed polyuria, polydipsia, an increase in urinary nitrogen, and a lowering of the renal glucose threshold values; the urines are of low specific gravity. Kawahara (19, 20) employing rabbits, found that sodium fluoride in doses of 10 to 50 mg/kg/day for 5 months produced increased serum levels of non-protein nitrogen and of creatinine as well as decreased glomerular filtration rates and a reduction in urea clearance. Taylor also observed polyuria and polydipsia (21) from administering long-term doses, and Egyed (28) from lethal doses of fluoride compounds to their respective animals.

Franscino et al. (29) studied the effects of intravenous sodium fluoride on the renal function of dogs. Their serum concentrations of fluoride were similar to those observed in humans administered methoxyflurane anesthesia. They report that fluoride causes a defect in the generation of both maximally concentrated urine and tubular free-water reabsorption. The severity of these defects was related primarily to fluoride blood levels rather than to the duration of exposure. Fluoride apparently produces renal dysfunction by its inhibitory properties on various enzyme systems of the kidney as reported by numerous authors (3, 4, 16, 28, 29, 31-33) as well as by its vaso-dilatory action (29).

Structural and Functional Effects of Fluoride on Human Kidneys

The effects of fluoride on human renal structure and function have not been investigated as thoroughly as in animals. In cases of acute sodium fluoride poisoning resulting in death, renal findings have ranged from no abnormalities (34, 35) to distension of tubules (35) and cloudy swelling (36) to acute tubular necrosis (2, 34).

In areas of endemic fluorosis, impaired renal function of unknown etiology has been found in persons drinking water containing 5 to 16.2 ppm fluoride (37-39). These abnormalities included impaired urea clearance, decreased glomerular filtration rate, and increased blood urea nitrogen. Singh et al. (39) also found a generalized aminoaciduria in people with fluorosis. The amino acid content of the serum was normal. They concluded that
fluorosis produces a failure of tubular reabsorption of amino acids. Unfortunately, in the above reports, one does not know whether the renal dysfunction occurred independent of the chronic ingestion of fluoride or was an integral part of fluoride intoxication.

McClure (40) in 1946 found no differences in the urine of five healthy young men using natural fluoride water (2 to 5.2 ppm) from those of matched controls drinking low-fluoride water. Leone (41, 42) studied over 200 people in 1944 and again in 1954. About half of them had resided for 10 years in Bartlett, Texas where water contained 8 ppm fluoride naturally, the other half in Cameron where the water supply contained 0.4 ppm fluoride. He reported no differences in kidney function tests between the two groups.* Schlesinger (43) et al., who studied 100 boys in fluoridated Newburgh, N.Y., (fluoride in water 1.2 ppm) and 100 boys from Kingston, N.Y., where the water was not fluoridated found no significant differences in the quantity of excretion of albumin and formed elements between the two groups.** Geever (44, 45) collected a series of 904 necropsy reports of people who had been residing in Colorado Springs for 20 years or more. This community has a natural water fluoride level of 2.5 ppm. Comparative statistical analyses of the histologic findings showed no differences which could be related to prolonged residence in that location.* Call et al. (46) examined tissues from 88 persons known to have been exposed to increased concentrations of fluoride in the atmosphere but found no pathologic changes that could be attributed to fluoride exposure.*

Experimental and epidemiologic studies on the relationship of increased intake of fluoride and the occurrence of renal calculi have revealed conflicting results (39, 42, 47, 48, 49). In rats receiving 500 ppm fluoride for two months, fluoride was not an etiologic factor in urolithogenesis (50) but, in areas of endemic fluorosis, according to epidemiologic studies an increased incidence of renal calculi has occurred.

Diseased kidneys: The incidence of fluorosis and fluoride damage to various organs, including kidneys, in people with renal disease is unknown but systemic fluorosis in patients with diminished renal function seems a reasonable possibility. Hodge (51) has indicated that urinary excretion of fluoride tends to be reduced in patients with advanced kidney disease. Taves (52) and Call (46) have reported higher than normal concentrations of fluoride in serum and in bones of people with advanced renal disease. The question of increased retention of fluoride in patients with kidney disorders has not been

*Editor's Note: These surveys failed to consider fluoride intake through sources other than water, especially food. In areas where fluoride occurs in water naturally or where the air is polluted by fluoride, such intake is substantial.

**Editor's Note: Children with a history of "clinical illness no matter how mild, during the previous two weeks," were eliminated from the study.
resolved but perhaps in the future increased concentrations of serum fluoride will be added to the already long list of metabolic abnormalities observed in chronic renal failure (32).

**Hemodialysis**

The danger of the use of fluoridated water in long-term hemodialysis has been suggested and investigated by Taves et al. (8). Patients on hemodialysis with fluoridated water in the dialysate were found to have extremely high serum and vertebral fluoride values. The levels were in the range of, or higher than, those in patients with skeletal fluorosis. Although no definite conclusions can be drawn at this time, several investigators have advised the use of non-fluoridated dialysate baths for long-term hemodialysis (8, 52, 53).

**Methoxyflurane Anesthesia**

The last area to be considered is that of the effects of methoxyflurane anesthesia on the kidneys. Shortly after its initiation as a general anesthetic in 1959 (54) methoxyflurane (2, 2 dichloro-1, 1-difluoroethylmethyl ether) was suspected of causing renal dysfunction although studies in animals and humans failed to demonstrate a definite change in either renal morphology or function (55). In quick succession thereafter, many retrospective reports were published linking methoxyflurane to renal impairment (54, 56-60).

Mazze (60) and Fry (61) demonstrated that the biotransformation of methoxyflurane in man has two major metabolic products, fluoride and oxalic acid. Both serum fluoride and serum oxalic acid levels were increased in patients exposed to this anesthetic. Also an increased rate of oxalic acid excretion via the urine (30, 31, 60-64) was observed. Elevation of serum fluoride occurs in all patients anesthetized with methoxyflurane but the degree of elevation seems to depend upon the duration of anesthesia (64).

Pezzi (58) and Crandall (56), in 1966, described a syndrome of polyuric renal failure in 16% of 274 patients anesthetized with methoxyflurane. Mazze et al. (60) and many others (31, 63, 64) also found a high output renal insufficiency state in variable numbers of their patients anesthetized with this halogenated ether. It is now clear that there is a continuum of manifestations due to methoxyflurane on the kidney ranging from no demonstrable effect to subclinical renal dysfunction, clinical renal impairment with recovery, chronic renal failure, and death. The difference between laboratory evidence of impairment of kidney function and frank clinical dysfunction appears to be dose related (64): The larger the total dose of the anesthetic, the more likely is the possibility of developing signs and symptoms of renal failure. If the total duration of methoxyflurane anesthesia is less than 1 1/2 hours, the harmful effects on the kidney seem to be nonexistent. Taves et al. (65) suggested that concentrations of fluoride below 100 µM (1.9 ppm) would not produce renal changes. Why some patients go on to develop irreversible renal failure following methoxyflurane anesthesia is not known.
The clinical syndrome of transient renal dysfunction following methoxyflurane anesthesia is quite well defined. It is a high output renal insufficiency characterized by hypostenuria and polyuria which is resistant to vasopressin (antidiuretic hormone) (29–31, 62). The diuresis is not characterized by natriuresis or by an increase in total solute excretion (29). This defect in the renal concentrating mechanism closely resembles a form of nephrogenic diabetes insipidus. The offending agent, almost beyond doubt, is the fluoride ion (30, 31, 61–63). The syndrome usually commences a day or two postoperatively (61, 66) and closely corresponds with the maximal serum level of fluoride which occurs approximately 17 hours following anesthesia (61, 66). It differs from subclinical renal dysfunction by virtue of higher serum fluoride and oxalic acid concentrations and by a greater excretion rate of oxalic acid.

Paddock (57) and others (30, 31, 63, 64) have found increased deposition of calcium oxalate crystal in the renal tubules following anesthesia with methoxyflurane. It is possible that this substance is the causative agent of the renal dysfunction. Deposition of calcium oxalate is known to occur in the kidneys in a variety of diseases. Maculoso and Berg (67) found crystals of calcium oxalate in approximately half of 54 patients dying of renal failure. Tobey and Clubb (64) reported the crystals in 90% of methoxyflurane-treated patients in whom no evidence of renal impairment was found. The same authors obtained oxalate crystals in 43% of patients anesthetized with halothane. Calcium oxalate deposition occurs throughout the body in a rare condition called familial oxalosis (68) in which the renal failure is characterized by oliguria and anuria, not by polyuria (30). Moreover, polyuria has not been a feature of acute oxalate intoxication following ingestion of ethylene glycol (62). Therefore, oxalic acid is not likely to be the initiating agent of the polyuric renal syndrome following methoxyflurane (30, 31, 62, 63).

The inhibitory effect of methoxyflurane or, more correctly, of the fluoride ion on renal concentrating mechanisms could result from a biochemical lesion in either the vasopressin-sensitive regions of the collecting duct or in the ascending limb of the loop of Henle (32). Fluoride is known to inhibit many enzyme systems (3, 4, 28, 32, 33) and appears to have an, as yet, undefined inhibitory effect on the action of antidiuretic hormone (30). Intravenous sodium fluoride infusions in dogs have produced defects in the concentrating mechanism identical to that of methoxyflurane (29). Known inhibitors of anaerobic metabolism in the kidney, such as iodoacetamide, have produced analogous defects of water concentration (69).

Increasing numbers of cases of chronic renal failure and death following methoxyflurane anesthesia are being reported (55, 62, 63) and the original speculations of methoxyflurane's transient and benign effect on renal function have not been substantiated. Biopsies of kidney tissue and descriptions of necropsies in these situations are quite uniform. Hollenberg et al., (63) described glomerular hypercellularity, interstitial fibrosis, tubular degeneration, and deposition of oxalate crystal in tubules and interstitium. Halpern et al. (62) reviewed several post-methoxyflurane kidneys and found...
interstitial fibrosis, tubular degeneration, and deposition of calcium oxalate in tubules and interstitium. These histologic reports are not too dissimilar from those observed in experimental animals following large doses of sodium fluoride.

It seems not unreasonable with the information we now have to consider methoxyflurane a potential nephrotoxin, particularly if the duration of exposure is excessive.

Bibliography


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Fluoride and Kidneys


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REPORT OF A MEETING

SODIUM FLUORIDE THERAPY FOR OSTEOPOROSIS

Prof. H. Jesserer (Vienna) opened the conference by pointing out that the term osteoporosis applies to a large group of diseases. It is the most common bone disease, especially if one adds to it the disability and the invalidism induced by steroids administered in early life. Conventional methods of treatment such as anabolic agents, sex-hormones and large doses of calcium not only induce many side-effects but usually afford only temporary subjective relief of pain. To date, there is no indication that they produce new bone formation. The purpose of this symposium is to summarize the experience of different research groups and "allay the fear of toxic side-effects of sodium fluoride" which has been revealed in the available literature.

Presented at the 8th Working Meeting of "Forschungsgemeinschaft für Erkrankungen des Bewegungsapparates Wien" on May 4th and 5th, 1973 in Baden near Vienna.
Prof. E. Uehlinger (Zürich) presented a review of the pathology of osteoporosis and the possibility of its reparation. He emphasized that sodium fluoride is effective solely in cases of osteoporosis in which trabeculae of bone are still found. In completely "burned out" osteoporosis no form of therapy is effective.

Dr. J. Franke (Halle) presented clinical, roentgenological, pathological and histological studies on chronic industrial fluorosis. He pointed to the early stage of fluorosis (the "Schwachzeichen and stage O-I according to Fritz). Biochemically and roentgenologically as well as in two autopsies, he failed to find indications of secondary hyperparathyroidism, changes in the blood-picture or damage to parenchymatous organs,

Dr. W. G. J. Putschar (Boston) presented the findings of severe endemic fluorosis in India.

Dr. K. Chlud (Vienna) called attention to the legal implications arising from therapy with sodium fluoride. In many countries the maximum daily dose of sodium fluoride ranges between 2 and 20 mg. He demanded a re-assessment of this problem in the pharmacopeias, because this maximum is considerably exceeded in the therapy for osteoporosis.

Dozent H. G. Ilas (Basel) presented data on fluoride metabolism as related to the metabolism of calcium. After administering sodium fluoride, he found distinctly positive calcium balances in good correlation with the histo-morphometrical findings, an increase of serum alkaline phosphatase and of excretion of hydroxyproline in urine. Fluoride stimulated bone formation. Addition of calcium to the diet during fluoride therapy, reduces resorption of calcium due to formation of insoluble calcium fluoride.

Prof. F. Kuhlencordt (Hamburg) reviewed the clinical features of osteoporosis and its histo-morphometrical classification. He noted positive calcium balances and distinct apposition of bone substance. He rejected the addition of calcium and vitamin D to the treatment.

In a second report, Dr. J. Franke (Halle) related the results of his four-year experience with sodium fluoride therapy of osteoporosis. He presented roentgenological, densitometrical and histo-morphometrical studies. In contrast to large doses in the range of 75 to 150 mg NaF daily, which are often administered according to the literature, lower doses ranging from 30 to 60 mg NaF daily suffice to induce new bone formation. Twenty-six or two thirds of the thirty-seven patients experienced significant relief or freedom from pain. Minimal pathological changes were found in the newly formed bone produced by low doses of fluoride. Absorption of sodium fluoride into the blood stream decreases distinctly when the gastric juice is anacid or hypacid. Addition of calcium is ineffective. On the basis of physical examinations, he concluded that human fluorotic bone is not of infer-
ior value statically; even a distinct increase in bone strength was registered in cases of moderate fluorosis.

Dr. Haas (Basel) reported clinical and roentgenological findings in 30 patients with osteoporosis following sodium fluoride therapy. In 95 percent an increase of bone density was observed in the roentgenogram.

Dr. Krokowski (Kassel) achieved much success with sodium fluoride therapy in patients with steroid-induced osteoporosis. The increase of hydroxyapatite content was established by means of the analytical method devised by Krokowski. He suggested simultaneous prophylactic treatment with sodium fluoride in conjunction with or even prior to initiation of steroid therapy in patients in whom the bone mineral content is low. He also recommended intervals between periods of therapy.

Dr. F. Reutter (St. Gallen) discussed his eight year experience with sodium fluoride therapy in osteoporosis. He observed much newly developed osteoid in the microscopic picture following large doses of sodium fluoride, as high as 100 to 150 mg daily.

Dr. A. J. Olah (Basel) demonstrated distinctly new bone formation following fluoride therapy. He used microscopic pictures to illustrate undecalcified bone with histo-morphological evaluation.

In summary Prof. H. Jesserer (Vienna) outlined the following directives for a broader application of NaF-treatment of osteoporosis in hospitals:

Currently fluoride is the sole agent which stimulates new bone formation in osteoporosis. For one to one and a half years, doses of 50-80 mg NaF daily should be given orally followed by low dose therapy (total dose: 20 to 30 g NaF).

The addition of calcium and vitamin D is declined. Treatment with sodium fluoride is indicated in every case of osteoporosis that manifests symptoms, not merely when fractures occur.

The diagnosis of osteoporosis should be established histologically and biochemically. Side effects involving the gastrointestinal tract are counteracted with enteric-coated tablets, for example “Ossin” of the CHEMIPHARM Co. Occurrence of arthritis, especially in the lower extremities calls for a reduction in the dose. Liver and kidney damage is not expected.

In children and young women (especially during pregnancy), in individuals with liver and kidney damage the treatment is contraindicated.

Causes of failure of the NaF-therapy are disturbances in resorp-
tion of fluoride as for instance in cases without free gastric acid or in already fully "burned out" osteoporosis.

A control of intake of fluoride is recommended by means of fluoride analysis in urine. In order to evaluate the results objectively the following procedures are recommended: roentgenograms (after one half to one year, iliac crest biopsies, densitometries, clinical finding, assays of serum alkaline phosphatase, of urinary excretion of hydroxyproline and of serum calcium. During the treatment the blood picture, the liver and kidney functions should be kept under surveillance.

**CORRECTION**

In the paper "Metabolism of Fluoroacetate by Lettuce" by P. F. V. Ward (FLUORIDE, 6:194-202) two sentences were inadvertently typed incorrectly:

The sentence starting at the end of the twelfth line on page 196 should read: "Radioactivity was found in many classes of compounds in 14C-acetate treated plants such as lipids, organic acids and amino acids, but only in the water soluble fraction of 14C-fluoroacetate treated plants."

The sentence starting in the middle of line 43 on page 201 should read: "This is not unlikely for Acacia georgianae, a plant known to biosynthesize fluoroacetate, but it is surprising for the other plants."

**CORRESPONDENCE**

To the Editor:

May I point to several errors in the October 1973 editorial entitled "Fluoroacetate Toxicity" (FLUORIDE, pages 187 and 188) which reported the Organic Fluoride Symposium held at Oxford University in April, 1973:

J. S. C. Marais (J. Vet. Sci. Anim. Ind., 20:67-73, 1944), not Sir Rudolph Peters, was the first to identify fluoroacetate in gifblaar (p. 187).

In the fifth paragraph, p. 187, regarding the synthesis in the human organism of organofluorides from inorganic fluoride, no data indicate that fluoroacetate is formed from inorganic fluoride in the animal body. That any animal organism, including humans, is able to convert inorganic fluorides to the organic form is doubtful.

On the same page reference is made to the work of Ward and Hugkisson. They showed that fluoroacetate is present in lettuce in microgram quantities when lettuce is treated with fluoroacetate.

In experiments in our laboratory we found the presence of trace quantities of fluoroorganic compounds in the kidney of only one horse. It was not detected in organs from other animals that were obtained from the same area.

Signed Gene W. Miller
EFFECTS OF FLUORIDE ON CALCIUM METABOLISM IN OSTEOPOROSIS

by

S. H. Cohn, C. S. Dombrowski, W. Hauser, and H. L. Atkins
Long Island, New York


In order to quantify the changes in the skeletal metabolism and the bone mass resulting from administration of fluoride extending over a period of 2 to 7 months, the authors employed three techniques. 1: Compartmental analysis based on 47Ca kinetic tracer data, a technique previously established to determine values for a number of parameters of calcium metabolism; 2: A calcium balance study; 3: An in vivo neutron activation analysis technique for the determination of total body levels in calcium as well as in sodium, chlorine, phosphorus, and nitrogen, for the purpose of establishing the total amounts of these elements present in the body during the administration of fluoride.

Methods

Eight patients aged 50 to 86, with various degrees of osteoporosis, were studied. They had no gastrointestinal disorders nor any other unusual dietary history, nor had they received any treatment with hormones.

The calcium balance studies were carried out on a metabolic ward over a 10-day period following two weeks of equilibration with a constant diet of 539 mg calcium and 838 mg phosphorus daily. The patients received 20 mg per day supplementary fluoride in enteric-coated capsules three times daily with meals. After two months, the balance studies were repeated. Five patients continued to take the fluoride supplements for an additional five months. Sample diets and 24 hour urine and stool were analyzed for calcium and phosphorus by the atomic absorption techniques.

The 47Ca tracer kinetic studies were done by intravenous injections with 20 μCi, high specific activity 47CaCl2. Blood samples were collected at 1 and 6 hours daily for 10 days. Calcium 47 was measured on all 24 hour samples of urine and stool with standard counting techniques. The patients were counted daily in the Brookhaven whole-body counter for direct measurements of retention of 47Ca.

The compartmental analysis was made according to the Berman...
simulation, analysis and modelling (SAAM) program (modified for the CDC-6600). The sizes of the compartments and the flow rates between the compartments were calculated before and after the period of fluoride administration.

The in vivo neutron activation analysis was made for calcium, sodium, chloride, phosphorus and nitrogen by whole body in vivo neutron activation techniques before, during and after the fluoride treatment.

Results

The kinetic study indicated no significant change in the size of the exchangeable calcium pools or in the excretion rate following administration of sodium fluoride. There was a slight increase in $^{47}$calcium retention as a result of fluoride supplementation. After 2 to 7 months of treatment, no significant increase in total body calcium, as measured by the above in vivo neutron activation technique, was noted.

From these results on the 8 individuals, the authors concluded that fluoride is not an effective treatment for osteoporosis. Because of the very slow response to fluoride they considered the possibility that considerably longer periods of treatment may be required to adequately assess the efficacy of this treatment.

During the whole experimental period, the levels of calcium and phosphorus in the plasma remained within normal range. There was an increase in alkaline phosphatase activity in most patients on the fluoride supplement which the authors attributed to a regeneration of new bone induced by fluoride.

The incidence of spontaneous fractures did not decrease during the seven months' period of treatment and subjectively the administration of fluoride failed to evoke a positive response of the kind which is noted following treatment with high calcium. X-rays did not reveal a significant change in the bone density.

GLW

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SKELETAL CHANGES IN CHRONIC INDUSTRIAL FLUOROSIS OF COWS

by

E. Lasarov, B. Tatarov, G. Dimitrov, L. Kantschev, and K. Kovatschev
Sofia, Bulgarian

(Abstracted from Wiener Tierärztliche Monatschrift, 59:258-263, 1972)

This study concerns six cows, aged 6 to 12, with marked dental fluorosis on pasture in the vicinity of a phosphate fertilizer factory which were studied clinically and at autopsy. Chemical and histological investigations were carried out on the middle portion of the diaphysis, metatarsal and metacarpal bones, and on the border between epiphysis and diaphysis.

Clinically the cows exhibited marked dental fluorosis (grade 4 to 5 according to Dean) and exostoses on the metatarsal and metacarpal bones. Some of the animals had been limping.

At autopsy, the mandible was enlarged; its surface was rough and covered with exostoses. The ribs showed localized areas of thickening and the metacarpal and metatarsal bones were thickened. The dorsal metacarpal and metatarsal arteries were encased in a canal surrounded by bony proliferations. On the joint surfaces of these bones osteophytes were noted. The tendons were calcified at the areas of their insertion; the interosseous muscles were completely ossified.

The chemical analysis revealed that the fluoride level of the bone ash had reached 7984 ppm. It was highest in the spongiosa, followed next in order by the compacta and by exostoses. In the control animals, the maximum value of fluoride was 1,018 ppm.

Histologically the essential changes of the bones were the hyperplastic, ossified periostitis of the metacarpal and metatarsal bones, a spotty reduction of the compacta which simulated the spongyous tissue, and a destructive process involving the zones surrounding the Haversian and the Volkmann canals of the compacta in which detritus had been deposited. The exostoses were markedly porotic. In the spongiosa of the above-mentioned bones, the trabeculae were atrophic and showed sharply contrasting demarcations. Well-formed osteons were found rarely in the spongiosa. The marrow canal of long bones was considerably narrowed.

The authors assumed that much of the localized thickening of the ribs can be attributed to spontaneous or traumatic fractures resulting from a de-

From the Institut Superieur d'Agriculture "Georgie Dimitrov" 8, bouj. Dragan Zankov, Sofia, Bulgarian.
increased resistance of the fluorotic bone tissue. In the areas of the diaphysis and epiphysis localized osteoporosis, osteodystrophy and subsequent mineralization took place. In fluorosis, the destructive process of the bone prevails over the remodelling of the bone which leads to osteoporosis. The hyperplastic ossifying periostitis is considered to be the expression of the tendency of the bone to compensate for its decreased stability.

J. F.

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EFFECTS OF EXCESS CALCIUM ON A FLUORIDE-MAGNESIUM INTERRELATIONSHIP IN CHICKS

by

J. C. Rogler and H. E. Parker
West Lafayette, Indiana

(Abstacted from the Journal of Nutrition, 102:1699-1708, 1972)

The authors had shown in previous studies that feeding chicks high levels of both fluoride and magnesium resulted in a greater depression of growth than by feeding fluoride alone. The combination of the two elements also produced a characteristic leg weakness and reduced bone ash which was not observed in chicks fed either fluoride or magnesium alone. They also had established that increase of phosphorus or calcium in the diet benefited, but did not completely correct, certain symptoms induced by the high fluoride magnesium supplements. The current study was designed to investigate the mechanism by which dietary calcium exerted its beneficial effect.

Method

A total of 320 one day old White Mountain male chicks were used.

From the Departments of Animal Sciences and Biochemistry, Purdue University, West Lafayette, Indiana.
for 8 experimental treatments on ten chicks each. Calcium was administered in amounts of 1% and 1.67%, fluoride at 0.005 to 0.085% (50 and 850 ppm) and magnesium at 0.07 and 0.47%. The increase of 0.67% in the high calcium diet is equivalent, on a gram molecule basis, to 0.4% of magnesium which is the increment used in the high magnesium diet. Fluoride was given as sodium fluoride, magnesium as magnesium carbonate and calcium as calcium carbonate. One tenth ppm of selenium was added to all diets; phosphorus levels remained constant at 0.9%. Sodium intake also remained constant.

The fluoride of the plasma and bone were determined by the use of the fluoride electrode; magnesium and calcium of plasma and bone by the atomic absorption spectrophotometry and phosphorus by the AOAC molybdovanadate procedure.

Results

Growth Rate: High (0.4% magnesium) supplements to the diet alone did not influence growth rate but high fluoride (0.08%) alone did reduce the rate of growth; a combination of fluoride and magnesium induced an even more pronounced reduction in growth rate.

An increase in calcium from 1.0% to 1.67% improved the growth of chicks which had been fed fluoride alone or fluoride plus magnesium. The increase in dietary calcium had no effect on the control chicks nor on those fed magnesium alone.

Bone Ash: Fluoride alone increased bone ash significantly. Administration of magnesium induced a slight reduction in bone ash. The combination of both fluoride and magnesium led to a marked reduction in bone ash whereas excess calcium increased bone ash only slightly in the chicks which had been on the high magnesium and on the combined magnesium and fluoride supplement.

Plasma Fluoride: Dietary fluoride greatly elevated the fluoride levels of plasma, whereas both calcium and magnesium drastically reduced the fluoride level of plasma as well as that of bones in chicks on the high fluoride diet. The authors noted that chicks, fed diets, containing sodium fluoride, consumed excessive water and their excreta was very watery, a condition which was ameliorated by addition of calcium and magnesium to the diet.

Plasma Magnesium: The high magnesium diet approximately doubled the plasma magnesium. The high dietary calcium reduced the plasma magnesium levels in chicks fed the high magnesium diet. This effect was not as consistent or of as great a magnitude when calcium was added to the high fluoride, high magnesium diets.
It appeared that the deleterious effects on bone calcification by the combination fluoride plus magnesium was not overcome by increasing calcium intake.

The authors concluded that the beneficial effects of excess calcium on the fluoride-magnesium interrelationship was primarily due to reduced absorption of fluoride. They felt that the harmful effects of elevated dietary fluoride and magnesium on bone calcification cannot be overcome by increasing the calcium in the diet.

* * * *

FLUORIDE CONTENT OF NORTH INDIAN FOODS

by

R. S. Nanda
Oklahoma City, Oklahoma


In the area near Lucknow, India, 18% of 16,565 children had dental fluorosis at the low fluoride level of 0.4 ppm in domestic water supplies. This fact prompted the author to make an extensive study on the fluoride content of North Indian food. Determinations were made of the total daily intake of fluoride and of vitamin C by children aged 0 to 8 in endemic and in non-endemic areas of rural Lucknow. The respective foods were also analyzed for calcium, magnesium, phosphate, because these minerals affect fluoride metabolism in the human body. The study was sponsored by the Division of Dental Health, U.S. Public Health Service under the aegis of the Indian Council of Medical Research, New Delhi.

From the Division of Orthodontics and Pedodontics, Dental College and Hospital Lucknow-3 India, now Professor of Dentistry, University of Oklahoma Medical Center, Oklahoma City, Oklahoma.
After drying and ashing the food items at 600°C for 10 to 12 hours, fluoride determinations were made by the use of multicellular polypropylene diffusion trays and a Klett-Summerson photoelectric colorimeter (Model 8003) by the method of Nicholson.

Results: The results of this study revealed a distinct difference in the fluoride content of uncooked and cooked foods.

In Uncooked Vegetables the fluoride content was low. It ranged from 0.2 to 1.5 ppm with potatoes showing the highest values (1.5 ppm). Coriander leaves, peas, green mint, maize were high in phosphates; green chillies, green peas, and cabbage were high in vitamin C. Similarly, raw fruit contained low fluoride levels in the range of 0.1 to 0.4 ppm. In general the calcium, magnesium, and vitamin C content of raw fruit was high. Vitamin C was particularly abundant in guava, high in citrus fruit, mango and in bor (or ber) karonda, Kaitha and Kamrakh.

Nearly all Spices and Condiments showed high levels of fluoride and also of calcium, magnesium, and phosphate. For instance red pepper reached 10.7 ppm of fluoride, coriander powder (Coriandrum sativum) 8.3, red pepper 8.07 and big cardamom (Amomum subulatum) 14.4, and small cardamom (Elletaria cardamomum) 8.3.

The fluoride content of Salt varied widely with black rock showing the highest amount (17.6 ppm) compared to crude table salt (0.88 ppm).

Fluorides in Nuts contained 0.6 to 3.8 ppm with salted cashew nuts showing the highest level. Nuts were also high in calcium and magnesium.

The fluoride content was distinctly higher in Cooked Foods than in unprocessed food. Products made of wheat flour contained 0.5 to 2.4 ppm of fluoride. Especially high were the fluoride levels in food items which were fried and subsequently seasoned with salts, spices and cooking oils. The phosphate content of most wheat products was also high, namely more than 200 mg%. Roti, a flat bread made from various cereals, contained from 0.6 to 1.6 ppm fluoride. Somewhat lower levels of fluoride namely 0.4 to 0.7 ppm were found in preparations made from rice. Values of the order of 1.4 ppm were found in processed food items such as rice pulao to which oils, salt and spices had been added. Similarly high levels ranging up to 3.8 ppm were also noted in pulses (a high protein cereal) and legumes. Most pulses are also high in magnesium and phosphates.

In other cooked vegetables smaller amounts of fluorides and other elements were identified. Whereas uncooked pumpkin contained only 0.5 to 0.9 ppm, the fluoride content of cooked pumpkin rose to 2.5 ppm. Fluoride in potato curry may range from 0.8 to 1.8 ppm in green beans 0.6 to 1.6. Both are likewise high in magnesium and calcium. High fluoride spices and
condiments play an important role in the total amount of fluoride contained in foods. For example, some people eat their flat bread (Roti) with salt, red pepper, and onions.

Of much interest are the levels of fluoride in fish preparations which were relatively low namely 1.0 to 1.9 ppm as compared to the data available in the literature. These values however are higher than those of other cooked meat products such as mutton where fluoride ranged between 0.6 to 1 ppm.

The lowest content of fluoride and magnesium was found in fats and edible oils; but they were rich in calcium and phosphates. Similarly milk and milk products contained little magnesium and fluoride, but high levels of calcium and phosphate.

Large amounts of fluoride were found in "sweet preparations" made from Bengal gram and gur (Jaggery) with a fluoride content of 2.4 ppm. These preparations were conspicuous for their low content of magnesium. Betel nuts contained between 7 and 12 ppm, chewing tobacco between 3.1 to 38.0, Dal Moth, a salty snack prepared from legumes, up to 4.2 and Karhi, a cooked paste of Yoghurt and gram flour up to 2.7 ppm.

Indian tea leaves are also high in fluoride but tea effusions prepared for drinking contained only up to 3 ppm.

In pointing to the variation in fluoride levels in cooked and uncooked foods the authors attributed the higher fluoride content in cooked food to fluoride-containing water with which they are prepared and to salt which is used more freely in hot climates than elsewhere. Specific foods which raise the fluoride content of meals are Bengal gram, a legume containing 3 ppm and gur. In gur, the high fluoride content is due to the use of lime in bleaching and in preparing gur from sugar cane juice, which in itself is low in fluoride. Lime contains fluoride of the order of 23.5 ppm.

In order to determine the daily consumption of fluoride through food in children, the author conducted a dietary study on 500 rural Lucknow children aged 5 to 8. Their total daily fluoride intake was 2.1 mg with 1.3 being contributed by cooked and uncooked food and 2/3 by water.

The authors noted that in general, if a food item is high in fluoride, the respective calcium and magnesium levels are also high.

GLW

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INORGANIC FLUORIDE POISONING:
TREATMENT BY HEMODIALYSIS

by

L. Berman, D. Taves, S. Mitra, and K. Newmark
Cleveland, Ohio and Rochester, New York

The authors reported the case of an 18 year-old man who ingested roach powder containing an unknown amount of sodium fluoride. His initial blood level of 500 μM per liter (9.5 ppm) was one of the highest ever recorded, normal fasting levels being less than 1.0 μM.

The symptoms were low blood pressure, bloody mucous, gastric secretions and hypercalcemia as well as a prolonged QT interval in the electrocardiogram.

The patient was given hemodialysis for eleven hours with an RSP machine with 1.5 m² coil and non-fluoridated water in the dialysis bath. After eleven hours the whole-blood fluoride/serum fluoride had fallen to 15 μM. The fluoride clearance during the analysis was 143 and 84 ml per minute. The concentration of whole blood fluoride/serum fluoride averaged 0.85. The authors determined that approximately 30% of the fluoride was removed from the blood by dialysis.

They recommended hemodialysis as an effective and potentially lifesaving treatment in acute inorganic fluoride poisoning. They emphasized that the bath should be made with non-fluoridated water, with calcium and magnesium adjusted to the patient's needs.

From the Mt. Sinai Hospital of Cleveland, Ohio and University of Rochester, New York.

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