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EDITORIAL

FLUORIDE IN SOIL

The fluoride content of soil is of considerable importance in the assessment of the biological role of the halogen. To a large extent soil fluoride determines its levels in water, vegetation, domestic and wild animals, and indirectly in humans, whose food is derived from the above sources. It is, therefore, not surprising that biologists have devoted considerable attention to the determination of fluoride in soil.

Data on the fluoride content of soil have shown variations ranging as widely as from near 0 up to 184,000 ppm. The latter value was recorded near a fluor spar mine in Great Britain (1). In an endemic fluorosis area of India Jolly et al. reported an average of 241.2 ppm (2). The overflow of a pond collecting the waste water from a phosphate fertilizer factory resulted in the accumulation of 93 to 384 ppm fluoride in the surrounding acreage (3). Hani observed fluoride levels of the order of 0.096 ppm in acid soil and 0.33 in limed soil at Liebefeld near Bern, Switzerland (this issue page 20).

Treatment by fluoride-bearing phosphate fertilizers and pollution from industrial sources account to a large extent for such wide fluctuations. Another major source which is not man-made is volcanic eruptions (4). Fertilization adds from 8 to 20 kg/ha/year to soil (5). Macuch et al. observed fluoride depositions of an average of 10.7 kg/ha/year near a Czechoslovakian aluminum factory (6).

The surface of soil undergoes a constant purification process which is largely dependent on weather conditions, mainly on the degree of precipitation, i.e. duration and intensity of a dry or rainy season (6). Seepage into deeper layers and run-off with surface water produce major changes in the fluoride profile of soil which, according to Oelschlager, amounts to some 6% of the yearly fluoride increment deposited by fluoride-containing phosphate fertilizers (5). Intensity and direction of prevailing winds, the topography of the land and the presence of vegetation also influence the deposition of fluoride on soil.

Robinson and Edgington noted greater fluoride accumulation in soil with increasing depth (7). At the surface they found 200 ppm and at a depth of 9 to 14 cm 1300 ppm. This trend is reversed in a polluted area. For instance, near a Scottish aluminum smelter 1010 ppm was recorded at the surface (up to a depth of 1 inch) in contrast to 161 ppm at a depth of 11 to 15 inches (8).

The current study by Hani shows that fluoride concentrates in colloidal material which explains why clay is much richer in fluoride.
than sandy soils. In a New Jersey agricultural soil, Bear (9) found up to 409 ppm in clay as contrasted to 29 ppm in sandy soil. His mean of 188 ppm fluoride in 16 soils was higher than that of all other trace elements with the exception of manganese.

Near an Ohio aluminum smelter soil assays by McClenahen (10), sponsored by the aluminum corporation, revealed fluoride levels of the order of 304 + 48 to 379 + 92 ppm. He confirmed that greater accumulation takes place at the surface than in layers up to 30 cm deep. He selected the 14 test sites according to the amount of fluoride present in pasture grass and hay.

In carrying out soil tests for fluoride the selection of the test sites and conditions at the time of sampling are the key to the proper assessment for comparative studies (11). The distance from the contaminated source and the direction of prevailing winds are major considerations. Van Hook found a range of 415 to 1840 ppm fluoride within 1 mile of a Montana chemical factory and from 265 to 830 within 4 miles (12). At a distance of seven miles downwind of an aluminum smelter at Fort Williams, Scotland, the soil concentration of fluoride was still four times that of normal controls, whereas at three miles in the opposite direction it approached the control levels (8).

A detailed description of the topography of the area is also required. In low land surrounded by protective hills, less contamination can be expected than in mountainous regions; however, the chance of surface washout is greater on hills and mountains than in valleys. Also important is the kind and extent of vegetation, since trees, shrubs, and grass surrounding a test site attract gaseous and particulate fluorides and act as a sink, protecting nearby soil from contamination.

Weather conditions can alter the fluoride levels in soil considerably. If, for instance, there is heavy precipitation shortly before sampling the values of fluoride in the soil will be lower than on dry days. Obviously the extent of activity of the industrial source immediately prior to sampling must also be taken into account.

As already indicated, data on the total composition of the soil are significant since the uptake of fluoride in plants is determined markedly by the amount and kind of other minerals present. The acidity of the soil and, particularly, its content of calcium and aluminum to which fluoride has a strong affinity affects its biological action on plants as demonstrated by Hanl. High levels of boron in the soil account for greater fluoride uptake (13).

In relating fluoride in soil to its uptake in plants, Johnson pointed out that the amount of fluoride in soil does not parallel
that taken up by plants, although he observed a direct correlation of
the water-soluble fluoride in the soil to fluoride in plants (1).

It is, therefore, clear that soil assays for fluoride are of
limited value unless all supplementary factors are taken into account.

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G.L.W.

***************
INCORPORATION OF A DIETARY FLUORIDE SUPPLEMENT INTO BONES AND DEVELOPING TEETH OF THE PIG

by

R.L. Speirs
London, England

SUMMARY: The aim of this study was to determine (a) the stage in the development of enamel at which fluoride is incorporated most readily and (b) the availability of fluoride when added to water or milk for deposition into mineralized tissue. The dentition of the young domestic pig provides a spectrum of phases of enamel formation. It is possible to supplement the diet with fluoride for a short period after which one can relate the amount of fluoride taken up by enamel during this period to the stage of mineralization.

Litter-mate pigs aged 3 and 9 months were fed a stock diet (8 ppm F\(^-\)) for 6 weeks. Subsequently milk or water containing 16 ppm F\(^-\) was added. The supplement provided about 0.5 mg F/kg body wt/day. These animals and controls were then sacrificed. Their unerupted permanent teeth were extracted. Enamel and dentine were separated in sections of teeth. Density of enamel particles was measured by flotation. Fluoride was determined in enamel of different density fractions and the mean fluoride and mean density of enamel from each tooth were calculated. Fluoride was also measured in surface enamel, in dentine and in the mandible.

The dietary fluoride intake was reflected in all the tissues examined. The amounts of fluoride incorporated from milk and water were similar. In bulk enamel there was a peak fluoride level corresponding to a density of 2.2 - 2.3 g/cm\(^3\) after which there was a steady fall as maturation continued although in surface enamel the F\(^-\) levels increased.

In communities in which fluoridated water is not available it is a common practice to give fluoride to children in tablets or less frequently as fluoridated milk, fruit drinks or in domestic salt. The questions arise: When is the most effective time in the development of the permanent teeth for the incorporation of fluoride? Is the first

year at school too late to begin ingestion of fluoride for protection of the most caries-susceptible teeth, first permanent molars? Can fluoride treatment be restricted to certain short periods? Until recently no direct evidence provided answers to these questions. Some clinical observations made over 30 years ago in this country (1) and more recently in Holland (2) suggest that just before and at the time of eruption is the critical period but this has not been tested experimentally.

Objectives

To study such a problem it is necessary to have a model as it is impracticable to investigate this in children. Our earlier work (3) showed that the dentition of the young domestic pig provides a spectrum of stages in enamel formation. There is a close similarity in the profile of tooth development in a 7 month old pig and a 6 year old child (Fig. 1). The aim of this study was to supplement the diet of pigs with fluoride for a short period and then to relate the amount of fluoride taken up in the enamel of each unerupted tooth during this period to the particular stage reached in mineralization.

By including animals aged 3 and 9 months it was considered that both early and late stages of enamel formation and maturation would be covered. Bone and dentine were also analyzed. Since bone is more metabolically reactive than enamel, it can serve as a guide to the availability of ingested fluoride. It was not feasible to carry out balance studies on food, urine, and feces.

Figure 1a
Schematic Representation of the Lower Dentitions of a Pig about 7-Months Old

The permanent teeth are labelled. Only the first molar is erupted.
Litter-mate pigs aged 3 months were fed a nutritionally adequate stock ration containing 8 mg F/kg for 6 weeks. Two of the pigs were given a fluoride supplement in the form of sodium fluoride added to milk or water at a final concentration of 16 mg F⁻¹/₁ (16 ppm F⁻¹). These solutions, mixed with the food, supplied an additional 0.5 mg F/kg body weight daily. At 4 1/2 months of age, these pigs were sacrificed. The same protocol was repeated with three more litter-mates aged 9 months. Extra fluoride was again given to two of these for 6 weeks. The experimental animals were killed when 10 1/2 months old. Almost fluoride-free drinking water was supplied ad libitum.

The permanent teeth, which were all unerupted except for the first molar, were dissected out. Those on one side were sectioned and, under the dissecting microscope, the enamel was stripped away from the dentine. Enamel particles from single teeth were pooled, air-dried, then separated into density fractions by flotation in mixtures of dibutylphosphate and n-butyl phthalate over the density range 2.0 to 3.0 g/cm³. The fluoride concentrations in these fractions were determined by the Orion-specific ion electrode after preliminary diffusion with 60 percent perchloric acid.

In this manner the mean fluoride level in bulk (whole thickness) enamel was measured and related to the mean enamel density. Fluoride was also determined in dentine and in bone removed from 6 identical sites in the mandibles. Teeth from the opposite side were used for determining the fluoride concentration in the outer 150 microns of the enamel thickness. Surface enamel was carefully removed by means of a
dental hand-piece with a diamond bur. The depth of the layers was calculated from the weight of enamel and area of the tooth surfaces which was abraded.

Results

The mean fluoride levels in bone, dentine and enamel reflect the fluoride intake in both the young and old pigs (Table 1). In the young pigs there is a tendency for more fluoride to be incorporated from the fluoride supplement in water than in milk, but the older pigs show the reverse trend. The fluoride content of the mandible increased with age but no change was found in dentine. In enamel the mean fluoride concentrations decreased with age but the uptake from the supplements appears to be similar in both age groups. However, since comparisons are being made between a 9 month old control and 10 1/2 month old experimental pigs and since there would have been a further small fall in the fluoride concentration in the teeth of the control pig over the 6 week period, it can be concluded that the uptake of fluoride was probably slightly greater in the teeth of the older pigs. Within one animal considerable variation in the fluoride levels in the different regions of the mandible was noted, a fact attributable to the variations in bone structure and vascularity, the patterns of growth, and metabolic activity (4). Likewise in enamel there was a large variation in fluoride concentration among different teeth. This variation paralleled differences in enamel density and confirmed our earlier findings (3). The fluoride concentrations in dentine were remarkably constant.

Table 1
Mean Fluoride Concentrations (ppm) in Air-Dried Tissues of Pigs

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Water</th>
<th>Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone</td>
<td>247.9 (8)</td>
<td>308.9 (8)</td>
<td>271.4 (8)</td>
</tr>
<tr>
<td>Dentine</td>
<td>275.0 (5)</td>
<td>351.2 (5)</td>
<td>339.6 (5)</td>
</tr>
<tr>
<td>Enamel</td>
<td>146.8 (5)</td>
<td>217.4 (5)</td>
<td>205.2 (5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Water</th>
<th>Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone</td>
<td>305.5 (6)</td>
<td>415.7 (6)</td>
<td>521.0 (6)</td>
</tr>
<tr>
<td>Dentine</td>
<td>252.6 (7)</td>
<td>318.6 (7)</td>
<td>384.9 (7)</td>
</tr>
<tr>
<td>Enamel</td>
<td>100.0 (9)</td>
<td>150.3 (9)</td>
<td>170.8 (9)</td>
</tr>
</tbody>
</table>

Statistical significance

<table>
<thead>
<tr>
<th></th>
<th>1 vs 2</th>
<th>2 vs 3</th>
<th>1 vs 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dentine</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Enamel</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ Control pig was only 9 months old.
Statistical analysis by Duncan's New Multiple Range Test applied to Analysis of Variance.
* P < 0.05
from one tooth to another.

A detailed analysis of the enamel findings is presented in Figure 2. The scatter of points for any one pig precludes calculation of linear regression lines over the full density range but lines have been drawn to give an indication of the probable relationship between fluoride concentration and density. It appears that a peak in the fluoride levels is consistently reached when the enamel density is about 2.3 g/cm³; above that there is a steady fall so that the final concentrations are less than half the maximum. These observations were made on the teeth of the older pigs. The results from the younger group are more difficult to interpret as there were fewer teeth for analysis and most of the enamel which was obtained was of low density. As seen in Figure 2 they did provide confirmation of the increase in fluoride as the density increased from 2.0 to 2.2 g/cm³.

From the results it is seen that the intake of fluoride in the diet is reflected at all stages in development of enamel with some preferential incorporation into enamel having a density of about 2.2 - 2.3 g/cm³. This is confirmed when fluoride uptake is compared for enamel samples of different mean densities (Table 2). The stage of enamel mineralization is clearly more important than the age of the animals in dictating the amount of fluoride which is deposited in developing teeth. Obviously these factors are interrelated in any one tooth.

<table>
<thead>
<tr>
<th>Table 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean F⁻ Increment (ppm) in Bulk Enamel of Similar Densities in Pigs of Two Age Groups Resulting from Supplementation of the Diet with F⁻ in Water (W) and Milk (M) for Six Weeks</td>
</tr>
<tr>
<td>Mean Density of Enamel</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>2.10</td>
</tr>
<tr>
<td>2.20</td>
</tr>
<tr>
<td>2.30</td>
</tr>
<tr>
<td>2.75</td>
</tr>
</tbody>
</table>

As maturation proceeds the mean fluoride concentrations in enamel generally decrease (Fig. 2) but when the fluoride levels in different density fractions from the same tooth were examined it was found that there were often inconsistencies in this pattern; the most dense samples sometimes contained higher concentrations than were to be expected from the graphs in Figure 2. It was postulated that maturation is made up of two processes as far as fluoride is concerned, namely a progressive loss of fluoride from the whole thickness of enamel, but in addition an associated deposition of fluoride at the tooth surface in unerupted fully mature enamel. When surface fluoride was determined, and the concentration in the outer 50 microns calculated, it became clear that this ex-
Figure 2

Mean Density of Enamel and Mean Fluoride Concentrations (ppm F) in Permanent Teeth of Control Pigs Aged 3 (●) and 9 Months (○) and Pigs Aged 3 and 10 1/2 Months which Received a Fluoride Supplement in Water (▲ and △), and in Milk (■ and □).

Most of the points represent the mean value for the enamel of one unerupted tooth but where several distinct density fractions were obtained in a tooth these were plotted.
planation was essentially correct (Fig. 3). However, the results showed

Figure 3
Fluoride Concentrations in the Outer 50 Microns of the Enamel
Surface and the Mean Density of Bulk Enamel from Unerupted Teeth of
Control Pigs Aged 9 Months (O) and Pigs Aged 10 1/2 Months
Given Fluoride in Water (●) and Milk (■)

two features of particular interest. First, the surface accumulation
takes place much earlier in maturation than had been predicted and sec-
ond, the concentration of fluoride at the surface is related to the di-
etary intake. In the immature enamel with a density of less than 2.3
g/cm³, the surface figures correspond to those obtained from analysis
on whole-thickness enamel (Fig. 2).

Discussion

These results agree with previous findings (3) showing that as
maturation proceeds, that is, as water and protein are progressively re-
placed by mineral in the partially mineralized enamel matrix, fluoride
is also removed. In addition they show that a peak value is reached
during the early phase of maturation when the enamel has a density of
about 2.2 – 2.3 g/cm³ and that this is influenced by the amount of fluo-
ride in the diet.

There is no theoretical reason to suggest that the "loss" of
fluoride is attributable entirely to dilution of a small amount of fluo-
ride-rich material by fluoride deficient mineral as maturation progresses
and as the crystals increase in volume. Incidentally, the decrease in
fluoride content is still seen when the level is calculated in terms of
volume (µgF/cm³ tissue) rather than on the more customary weight basis
\(\text{F}^-\) Uptake in Bones, Teeth

(\(\mu\text{gF/g}\)). Admittedly, the incorporation of fluoride into hydroxyapatite crystals by surface exchange or surface adsorption might result in a small apparent decrease in fluoride since as the crystal grows in size the surface area becomes smaller relative to the weight of the mineral. But this is not the only way by which fluoride is taken up during the formation of mineralized tissues—it can also be incorporated within the hydroxyapatite as it forms. There is no evidence to suggest that the 'loss' of fluoride takes place because less fluoride is available systematically for incorporation into enamel at the later stages of its formation on account of the animal becoming older. It has been pointed out that the decrease in fluoride concentration in maturing enamel is observed in teeth of pigs of quite different ages.

At the dosage used here, fluoride is probably incorporated into developing enamel at all stages. It is unlikely that the fluoride in almost fully mineralized enamel (density about 2.8 g/cm\(^3\)) of the fluoride-supplemented pigs is there because it entered more immature enamel some 6 weeks earlier at the onset of the fluoride feeding, and that despite some loss later, much of it has remained during maturation. We have observed that the rate of maturation is somewhat variable in different teeth. In teeth in which enamel mineralization was nearing completion (the first incisor, canine, and second molar) the changes in density were only from about 2.72 to 2.93 g/cm\(^3\); the enamel was therefore quite well mineralized when the extra fluoride was supplied in the diet. This rate of change in density was incidentally much greater than in teeth with immature enamel. It seems more probable that there is some incorporation in the relatively well mineralized enamel. Studies on incisors of rats exposed to an atmosphere containing HF support this view (5) as does the data in Figure 3. If enough fluoride is acquired by the surface of almost completely mature enamel it will probably be reflected in higher levels when bulk enamel is analyzed.

The concept of labile fluoride in developing enamel is not specific to the pig. It was first reported in the continuously growing rat incisor (6,7) then in the deciduous bovine incisor (7,9) and has also been observed in the deciduous human dentition (5,10). The nature and function of the fluoride which has become concentrated in the early phase of maturation are not known. Fluoride is capable of catalyzing crystal formation and influencing crystal morphology (11,12). It might also act on the ameloblasts and affect the removal of the proteins in the matrix; this might explain how small increases in fluoride intake produce detectable changes in enamel (mottling) before any other tissues.

A somewhat paradoxical situation therefore exists during maturation. Fluoride is removed from the thickness of the mineralizing enamel only to be recycled and become concentrated at the surface. This leads to a net deficit in the fluoride concentration of bulk enamel.

One of the most interesting findings to emerge from this work

FLUORIDE
is that fluoride in milk and water is equally available for incorporation into hard tissues at the concentrations used (16 ppm F\textsuperscript{−}). This is in agreement with results obtained in rats which were fed lower levels of fluoride, namely 4 (13) and 10 ppm (14). However, in human subjects who ingested much larger amounts (30 mg F\textsuperscript{−}) the absorption of fluoride from milk was delayed and lower peak levels of fluoride in plasma were observed (15). We have no explanation to offer for the superiority of milk over water as a vehicle for fluoride in the older pigs (Table 1). The weight gain in these pigs was very similar.

In conclusion it must be mentioned that the final concentration of fluoride in mature enamel may not necessarily be the best or only criterion on which to predict the effectiveness of fluoride in improving resistance to caries. Fluoride probably acts in several ways. Supporting this view indirectly are the findings of the current study; high concentrations of labile fluoride in developing enamel might have one effect while the later incorporation of fluoride in surface enamel might have another. The demonstration of this last phase corroborates the evidence from clinical studies and we are reminded of their conclusions: "F\textsuperscript{−} could not have conferred any protection on the first permanent molars until shortly before their eruption" (1), and "...it does not seem necessary that the extra fluoride is present during the entire tooth formation" (2).

Acknowledgements

I am indebted to the Borrow Dental Milk Foundation for assistance towards the cost of this study. The excellent technical help given by Ann Levicount and John Houseman is also acknowledged. Professor R.W. Fearnhead kindly organized the purchase and maintenance of the pigs.

Bibliography


Discussion

Prof. Burgstahler: You indicated that the daily supplement of fluoride in the experimental animals was 0.5 mg/kg of body weight. What was the fluoride intake per kg of body weight in the controls?

Prof. Speirs: The diet contained 8 mg of fluoride per kg, and the young pigs consumed about 2.5 kg of food per day, so they were getting about 20 mg of fluoride per day from the food. The young pigs weighed about 40 kg, thus intake from food was about 0.5 mg/kg of body weight.

Prof. Teotia: In endemic areas [of India], where the water is alkaline and soft, more fluoride is incorporated from the water than where water is hard and not alkaline. Milk contains 100-200 mg of calcium per 100 ml whereas water, which is usually neutral, contains as little as 8 mg of calcium per 100 ml. Yet, it seems that incorporation of fluoride into the dentine of the animals was about equal from the water and milk. I would like to know the alkalinity of the milk and whether the availability of the fluoride in it was, in any way, reflected in the uptake by the dentine.

Prof. Speirs: In our experiments concerning the availability of fluoride added to milk, we were surprised to find that 85 percent of the added fluoride seemed to be free. Only about 15 percent was not dialyzable and was precipitated or lost by high-speed centrifugation. I am not sure why we are getting the apparent reversal [in uptake] between the younger and older pigs.

Volume 11 Number 1 January, 1978 ************
ACONITATE HYDRATASE ACTIVITY AND CITRATE CONTENT OF HEART AND KIDNEY IN FLUORIDE AFFECTED COWS

by

G.W. Miller, M.N. Egyed and J.L. Shupe
Logan, Utah

SUMMARY: Heart and kidney tissues from Holstein, Hereford and cross bred beef cattle suffering from chronic fluoride toxicosis were analyzed for citric acid content and aconitate hydratase (citrate (isocitrate) hydro-lyase, E.C. 4.2.1.3) activity and the results were compared with those obtained with tissues from healthy cattle. Citric acid concentration was decreased 54 – 60% in kidney and heart of fluoride affected cattle. Aconitate hydratase activity in heart tissue showed an increase of about 25% in the heart tissue and a decrease of about 52% in the kidneys. The possible mode of action of these findings is discussed.

Introduction

Previous studies (1) indicated that Agropyron cristatum L. Gaerth (crested wheat grass) collected from an area high in atmospheric fluoride contained apparent trace amounts of fluoroacetate and fluorocitrate in addition to high levels of inorganic fluoride. Animals grazing on this vegetation exhibited chronic fluoride toxicosis. Although it is known that a significant increase of fluoride concentration in the bone ash of humans, bovines, laboratory rodents, and chickens resulted in reduction in citrate concentration (2–5), this finding was not confirmed in fluoride-affected cattle when citric acid was analyzed in bones on a dry fat-free basis (6). As some of the soft tissues from animals suffering severe osteofluorosis appeared to contain trace amounts of organically bound fluoride (7), it was deemed of interest to measure in these preliminary studies the citric acid content and aconitate hydratase activity in soft tissues of fluoride-affected cows as compared with the same parameters in soft tissues of healthy cows.

Materials and Methods

Soft tissues (hearts and kidneys) from 4 healthy cows served as controls. Tissues from twelve 5 to 12 1/2 year old cows from an area containing industrial fluoride-air pollution and exhibiting chronic fluoride toxicosis were obtained immediately after sacrificing the animals. Tissue extracts for citric acid determination were prepared as outlined

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by Buffa and Peters (8). The tissues were kept in an ice bath and extracts for citrate analyses were made within 2 hours after the death of animals. Citric acid was determined by the colorimetric method of Taylor (9) and the values are given in µg per gram wet tissue. Crude aconitate hydratase was extracted as follows: Twenty g of the fresh tissue was ground with 60 ml of cold citrate buffer (4 x 10^-3M, pH 4.7) in a Waring blender for 2 min., and the slurry was passed through four layers of cheesecloth and centrifuged at 20,000 x g for 20 min. The supernatant was suitably diluted with 0.1M phosphate buffer (pH 7.4) and used immediately. The spectrophotometric assay procedures were the same as described by Hsu and Miller (10). Protein was determined by the method of Lowry et al. (11). The specific activity was given as optical density Δ/min/mg of wet tissue.

Results

Citric acid concentration of the normal animals ranged from 21 to 49 micrograms per gram wet tissue (average 39.1) for hearts and 23 to 55 micrograms per gram (average 40.2) for kidneys. Concentrations of citric acid in tissues from fluoride-affected animals ranged from 9 to 30 micrograms per gram (average 15.7) for hearts and 8 to 34 micrograms (average 18.4) for kidneys. This represents a 60 to 54% decrease respectively as compared with control values (Table 1). The aconitate hydratase activity of the heart and kidney tissues exhibited a different pattern of change. Whereas the enzyme activity of the kidney tissue extract from the fluoride-affected animals decreased an average of 50%, that of the heart increased 25% over the controls (Table 2).

Table 1
Citric Acid Concentration of Soft Tissue

<table>
<thead>
<tr>
<th>Animals</th>
<th>Number Tested</th>
<th>Citric Acid (µg/g wet Tissue)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Heart</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>39.1 ± 10.0</td>
</tr>
<tr>
<td>Fluoride affected</td>
<td>12</td>
<td>15.7 ± 5.9</td>
</tr>
</tbody>
</table>

*Sample mean and standard deviation.

Table 2
Aconitate Hydratase Activity of Soft Tissues

<table>
<thead>
<tr>
<th>Animals</th>
<th>Number Tested</th>
<th>Aconitate Hydratase Activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>O.D. increase at 240 mp/min/mg protein</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heart</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>0.66 ± 0.126</td>
</tr>
<tr>
<td>Fluoride affected</td>
<td>12</td>
<td>0.829 ± 0.183</td>
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</table>

*Sample mean and standard deviation
Discussion

Shupe et al. (12) studied the metabolism of inorganic fluoride in dairy cattle and found that only insignificant amounts of fluoride were retained in soft tissues. The fluoride content of kidneys was somewhat higher than other soft tissues, which mainly reflects the route of elimination of fluoride from the body (13). No gross or histologic changes due to fluoride were found in the soft tissues of cows ingesting as high as 109 ppm of fluoride. In the present study it was found that the citric acid concentration in the soft tissues of fluoride-affected animals was significantly decreased (54 to 60%), a finding similar to the pattern seen in bones of various animal species when exposed to high levels of fluoride (2,3,4,5). We feel that the similarity of pattern is not enough to prove the existence of identical mechanism, i.e., the competition between fluoride and citrate in bones. Long-term ingestion of excessive fluoride might reduce the availability of citrate in soft tissues, thus decreasing concentration, similarly to bones.

The elevated activity of aconitic hydratase in the heart and decreased activity in the kidneys seems controversial. Since fluorocitric acid is a strong inhibitor of aconitic hydratase both in vitro and in vivo (14), the decreased enzyme activity in kidney tissue might indicate its presence. This suggestion is supported by the failure of an earlier attempt to demonstrate its presence in the heart by gas chromatography, while it was detected in kidney of fluoride-affected animals (7). In our present study, the effects and detection of organically bound fluoride (fluorocitric acid) in soft tissues was not investigated. Therefore, it is difficult to interpret our findings related to the decreased and increased aconitate hydratase activities in various soft tissue of fluoride-affected cows. A further problem arises in the interpretation of decreased citrate levels in the soft tissues (hearts and kidneys) of fluoride-affected cows which should be higher than control values if fluorocitrate were present at least in the kidney. Since the kinetics of fluorocitrate formation in animals is unknown at present, further investigations should be done to elucidate the exact mechanism of aconitase-citrate-fluorocitrate and inorganic fluoride interrelationships in cattle affected by ingestion of excessive fluorides.

Acknowledgments

The authors wish to thank Mrs. Sally Cotter, Mrs. Gertrude Hsieh, Dr. M.H. Yu, Mr. D. Wang and Mr. A.E. Olson for their assistance. Some financial support was provided by the National Air Pollution Control Administration, Consumer Protection and Environmental Health Service, Public Health Service, Grant AP 00276-06, and the Agricultural Experiment Station, Utah State University.
Bibliography


***************
INTERACTIONS BY FLUORIDE WITH A MINERAL SOIL CONTAINING ILLITE AND ALTERATIONS OF MAIZE PLANTS GROWN IN THIS SOIL

by

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SUMMARY: The changes in soil following addition of sodium fluoride and their effect on plants were investigated. Fluoride dissolves aluminum-organic matter out of the Liebefeld soil. More of these compounds go into solution from acid soil than from limed soil. Titration of the soil extracts showed that the dissolved organic matter belongs to the low molecular weight fraction (\(< 1000\)).

The fluoride and the aluminum compounds are taken up by maize plants. Plants grown in extracts of acid soil with higher concentrations of aluminum and fluoride undergo more damage than plants grown in limed soil. This lower plant yield in acid soil becomes noticeable after addition of 200 ppm of fluoride of which 24 ppm are water-soluble. The fixation of the added fluoride not exceeding 500 ppm can be described by the Langmuir isothermal. This relation only applies to the acid soil.

Illite, the main component of the clay fraction of the Liebefeld soil, reacts solely with a pH 4.7 acid solution of sodium fluoride at a temperature of 50°C. After an initial exchange of surface OH-groups against fluoride the crystal lattice of the illite is gradually decomposed forming cryolite and amorphous silica.

Introduction

Soil may contain considerable fluorine, but most of it is bound tightly in silicate- and phosphate minerals and therefore only little of the total fluorine is soluble in water. Normally the values of water-soluble fluorine range between 0.3 and 0.5 ppm whereas the total fluorine levels fluctuate between 10 and 1000 ppm (1).

In the neighborhood of aluminum plants and brickyards the soil

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may contain several hundred ppm of fluorine due to emission of fluoride gases or dust. Much more disturbing is the simultaneous increase of water-soluble fluorine which rose to 25 ppm in the Möhlin-Riburg area (2).

Therefore we decided to add fluoride to one of our test soils in the form of sodium fluoride and to investigate, whether and how the artificially introduced fluoride is fixed in the soil, what changes in the solid soil constituent are thereby produced and how plants respond to these fluoride supplements.

Aside from the question of fluoride fixation in soils one property of the fluorine ion has to be considered carefully, namely the tendency to form complexes with aluminum and to a lesser extent with iron. By this reaction, the solid soil constituents may be changed, due to the fact that either the aluminum is being dissolved out of the aluminum silicates or that the aluminum ion bridge in the clay-organic matter complex is attacked.

It is the aim of this work to investigate in detail the reactions mentioned in order to obtain a better insight into the composition of a solution equilibrated with a fluoride-contaminated soil. The dissolved particles in this solution are directly accessible to the plant roots and can cause damage by the assimilation of one or several of these particles.

**Results and Discussion**

**Dissolution of Aluminum-Organic Matter Complexes:** We studied a sandy loam from Liebefeld with a pH of 5.3. By liming a pH of 7.3 was obtained. The main portion of the clay fraction consisted of illite.

As can be seen in Table 1, fluoride dissolves aluminum, iron and organic matter out of this soil whereby the solubility is considerably reduced through liming. Obviously metal-organic matter (OM) complexes are brought into solution. The assumption that the aluminum is the main metal in these complexes is supported by the analytical composition of the solution and by the differential thermal analytical investigation of the soil after the influence of fluoride. In the fluoride-treated soil an exothermic peak, originally present at 410°C, has almost completely disappeared. This temperature is, according to Schnitzer (3), characteristic for the burning of organic matter bound to aluminum.

The infrared spectra of the freeze-dried extracts show a binding of the metal to the COO⁻ groups of the organic matter. Moreover it is recognized by a band near 600 cm⁻¹ that some of the fluoride ions in the acid soil extract form an aluminumfluoro-complex.

An estimate of the molecular weight of the dissolved organic
Table 1
Changes in the Liebefeld Soil (acid and limed) and in Maize Plants Grown in this Soil after the Addition of Fluoride in the Range from 100 to 1000 ppm

<table>
<thead>
<tr>
<th>Acid Soil</th>
<th>Soil Extract</th>
<th>Plant Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>F' Added</td>
<td>µg/ml</td>
<td>ppm</td>
</tr>
<tr>
<td>(ppm)</td>
<td></td>
<td>Al</td>
</tr>
<tr>
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<td>8.59</td>
<td>0.437</td>
</tr>
<tr>
<td>100</td>
<td>10.2</td>
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<tr>
<td>300</td>
<td>19.5</td>
<td>0.925</td>
</tr>
<tr>
<td>400</td>
<td>27.0</td>
<td>1.52</td>
</tr>
<tr>
<td>500</td>
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</tr>
<tr>
<td>750</td>
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<td>1000</td>
<td>56.4</td>
<td>2.44</td>
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</table>

Limed Soil

<table>
<thead>
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<th>Soil Extract</th>
<th>Plant</th>
<th>Plant Yield</th>
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</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>0</td>
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<tr>
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<td>3.2</td>
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</tr>
<tr>
<td>1000</td>
<td>21.5</td>
<td>1.31</td>
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</tbody>
</table>

matter is possible by titrating the soil extracts with 0.02 N NaOH. The consumption of alkali corresponds with a molecular weight of 700 provided we adopt the findings of Schnitzer (4), that 6 COOH - and 2 phenolic-OH-groups are present in one molecule of organic matter. On this basis the dissolved organic matter belongs to the low molecular weight fraction.

The changes in the soil solution brought about by the addition of fluoride are likewise reflected in the maize plant. The values in Table 1 illustrate that increasing levels of fluoride in the soil cause both the levels of fluoride and those of aluminum to rise in the plant. One must conclude that the aluminum-organic matter complexes are taken up by the plant. On the other hand, the iron content in the plant remains more or less constant, although its level in the soil solution is much lower than that of aluminum. The lesser increase of the aluminum content of the plants grown in the limed soil is in direct relation to the composition of the soil solution.

The assimilation of aluminum and fluoride is parallel to the damage to the plant which manifests itself by a lower production of dry matter (Fig. 1). However, the complex composition of the soil solution makes it impossible to estimate whether this damage is caused sole-
Figure 1
Effect of Fluoride Added to Liebefeld Soil on Growth of Maize Plants

Fixation of Fluoride: To estimate the toxicity to a plant of fluoride in soil, it is important to know the fluoride binding capacity of a soil. Several soil components exhibit fluoride fixing properties. According to different authors (6,7,8) the adsorption on amorphous oxides, halloysite and kaolinite is mainly responsible for the fixation of fluoride in acid soils. In alkaline soils, the fixation is essentially governed by the presence of calcium-phosphates and calciumcarbonate.

The fluoride adsorption in the acid Liebefeld soil can be determined up to an addition of 500 ppm of fluoride by the Langmuir isotherm. The adsorption maximum of 38.5 mg of fluoride per 100 g of soil is calculated from the slope of the linear Langmuir plot. With higher additions of fluoride a deviation from this linear equation is observed.

The fixation process in the limed soil cannot be described by the Langmuir isotherm. It is assumed that the fluoride is rapidly adsorbed on the added lime, followed by a migration into deeper layers leading to the formation of a CaF₂ nucleus on the lime particles. This reaction is mainly responsible for the enhanced fixation in the limed soil.
soil. The values of the water-soluble fluoride in this soil are on the average two to three times lower than in the acid soil (Table 1).

The Reaction of Illite with Fluoride: Since the behavior of illite (the main component of the clay fraction of the Liebefeld soil) in a fluoride-containing solution is not well known, experiments were made to investigate this reaction. It is known that the kaolinite crystal lattice is disrupted in the presence of fluoride, leading to the formation of sodium fluorosilicate and cryolite at pH 7. At this pH, only cryolite is formed as a solid phase (9).

A standard illite (Fithian, Illinois) which has been treated in the usual way so as to destroy the organic matter and the iron oxides was subjected to the same conditions as described by Semmens (9). Contrary to the kaolinite, illite did not react with a neutral solution of sodium fluoride. Even after 240 hours at a temperature of 50°C no changes in the composition of the solution were observed. Only an acid sodium fluoride solution of pH 4.7 gradually loses the fluoride in contact with the illite. Figure 2 represents the percentage weight gain per 100 mg of illite compared to the loss of concentration of fluoride from solution to the solid phase, expressed in milliequivalents per 100 mg of illite.

**Figure 2**
*Plot of P (percentage weight gain per 100 mg of illite) Against Loss of Concentration of Fluoride (mequiv./100 mg) for the Reaction Between Illite and an Acid Sodium Fluoride Solution*

Contact 0.4 m NaF-Solution
pH = 4.7 Reaction Temp. 50°C

Two types of reaction can be recognized from the different slopes of the curve. Up to one hour of reaction time, the slope is best explained by an exchange reaction of the type

\[
\text{Al}_2(\text{OH})_2(\text{Si}_2\text{O}_5)_2 + \text{F}^- \rightleftharpoons \text{Al}_2(\text{OH,F})(\text{Si}_2\text{O}_5)_2 + \text{OH}^-
\]

whereas the following linear graph with a steeper slope suggests a reaction in which sodium fluoroaluminate (cryolite) is formed:

\[
\text{Al}_2(\text{OH})_2(\text{Si}_2\text{O}_5)_2 + 6 \text{Na}^+ + 12 \text{F}^- + 6 \text{H}_3\text{O}^+ \\
2 \text{Na}_3\text{AlF}_6 + 3 \text{Si}(\text{OH})_4 + \text{SiO}_2 + 4 \text{H}_2\text{O}
\]
The formation of cryolite is confirmed by X-ray diffraction method. Colloidal silica is recognized as small spherical particles on electron microscope photographs (Fig. 3).

Figure 3
Electron Microscope Photographs of Illite (carbon replica)

A: Before the reaction with fluoride

B: 240 hours in a sodium fluoride solution (0.4 g-mol/l) of pH 4.7 at 50°C

The clay fraction, separated from the Liebefeld soil, shows the same behavior as the standard illite in a fluoride solution. Therefore, it is very unlikely that a fixation mechanism, involving the formation of cryolite, is of any importance under the conditions existing in the soil.
The reason for the greater stability of illite compared to kaolinite in a neutral sodium fluoride solution may be found in the structural differences. In the illite, which is a 2:1 mineral, the octahedral layers of aluminum are tightly packed between the tetrahedral layers of silicon whereas in the kaolinite, which is a 1:1 mineral, the AlO₆ - octahedra are bound one-sided to the next tetrahedral layer simply by hydrogen bridges.

Bibliography


Discussion

Dr. Flühler: I would like to call attention to the fact that it appears that fluoride is very mobile and leaches out very slowly. In some ecosystems we may well witness in the future an increase of fluoride in ground water.

**************
LONG-TERM EFFECTS OF FLUORIDE ADMINISTRATION - AN EXPERIMENTAL STUDY

ii) EFFECT ON SERUM PROTEINS

by

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Patiala, India

SUMMARY: Thirty rabbits were given subcutaneously daily for 12 months sodium fluoride in doses ranging from 0.5 mg/kg to 5 mg/kg and the serum electrophoretic patterns were studied. There was a significant fall in the total serum proteins. An inversion of the albumin/globulin ratio was found, namely a decrease of albumin and a rise of globulin.

The effects of sodium fluoride on total serum proteins has been studied by Majumdar and Ray (1) in calves and hill-bulls. Azar et al. (2) reported the total serum proteins in eight fluorotic patients from the Persian Gulf. However, no work on the differential protein patterns is available to the authors. The current communication presents the effect of sodium fluoride on total serum proteins and on the albumin globulin ratio.

Thirty 10 to 12 week old rabbits were divided into five groups. Group A which served as control, was injected with distilled water without fluoride; group B received 0.5 mg sodium fluoride per kg body weight subcutaneously daily for twelve months; group C, 1 mg/kg; group D, 2 mg/kg, and group E 5 mg/kg sodium fluoride. The estimates of the serum proteins were made by the standard Biuret method of King and Wootton (3) and the differential proteins by paper electrophoresis (4). These determinations were made after 1, 2, 3, and 12 months.

Results

Tables 1 to 5 show the total serum protein in the five groups. These tables reveal a distinct trend for the total serum proteins to fall in the rabbits that were given sodium fluoride. The decrease is statistically related to the amount of sodium fluoride injected. In group B after 12 months, there is a reduction of 0.1 percent; in group C, 1.3 percent; in group D, 1.5 percent; and in group E, the reduction in serum proteins amounted to 1.6 percent. These findings are in agreement with the observations of Majumdar and Ray (5) in calves and bulls which were treated with fluorides.

From the Civil Hospital, Jullundar (R.K.), and the Government Medical College, Department of Anatomy, Patiala, India 147001.
### Table 1
Total Serum Proteins in Control Group A

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Initial (gm%)</th>
<th>Months 1</th>
<th>Months 2</th>
<th>Months 3</th>
<th>Months 12</th>
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<td>6.3</td>
<td>6.4</td>
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Mean: 5.7
Standard Deviation: 0.1

### Table 2
Total Serum Protein in Group B (0.5 mg/kg NaF)

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Initial (gm%)</th>
<th>Months 1</th>
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Mean: 5.6
Standard Deviation: 0.3

### Table 3
Total Serum Protein in Group C (1 mg/kg)

<table>
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<th>Rabbit No.</th>
<th>Initial (gm%)</th>
<th>Months 1</th>
<th>Months 2</th>
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Mean: 5.6
Standard Deviation: 0.1

### Table 4
Total Serum Protein in Group D (2 mg/kg)

<table>
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<th>Rabbit No.</th>
<th>Initial (gm%)</th>
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Mean: 5.6
Standard Deviation: 0.3

### Table 5
Total Serum Protein in Group E (5 mg/kg)

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<th>Rabbit No.</th>
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<tr>
<td>29</td>
<td>5.6</td>
<td>5.5</td>
<td>5.2</td>
<td>5.0</td>
<td>4.0</td>
</tr>
<tr>
<td>30</td>
<td>6.0</td>
<td>5.8</td>
<td>5.7</td>
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</tbody>
</table>

Mean: 5.6
Standard Deviation: 0.3
The differential protein patterns are presented in Figure 2. These data reveal a lower percentage of albumin in the rabbits which received fluoride as compared to the control groups. On the other hand, there was a distinct rise in the serum gamma globulin fraction in the fluoride rabbits. In the control group the mean values of the gamma fraction ranged from 14.16 to 27.27 during the twelve month period (maximum 35.96, minimum 24.23 percent). In group E, the rabbits receiving 5 mg/kg fluoride, the gamma proteins increased from 17.70% to 30.57% after twelve months.

Figure 2 Effect of Sodium Fluoride Administration on Serum Albumin and Gamma Globulins in Gram Percentage

Discussion

Little work has been done on serum protein electrophoresis in fluorosis with which our data could be compared. Majumdar and Ray (1) found in their study on hill-bulls an initial mean total protein value of 7.8; after 9 months of fluoride administration it fell to 6.9 gm/100 cc. serum. In calves the initial mean total protein value was 7.3 gm/100 cc. which declined after 11 months to 6.5 gm/100 cc. serum. Although the total proteins decreased in their series, they concluded that their level did not differ significantly from normal.

The eight patients with skeletal fluorosis reported by Azar et al. (2) from the Persian Gulf had normal serum proteins. No work on the differential protein pattern is available.

Our data suggest that fluoride tends to impair the function of the liver since an increase in serum globulins with inversion of the albumin/globulin ratio is a common finding in chronic liver disease (6) although even in acute parenchymatous disease of liver normal serum proteins are frequently observed (7). Reversion of the albumin/globulin
ratio in liver diseases is well documented in the literature and confirmed by Gray et al. (8). Currently there is little information on the effect of fluoride on the liver. Tadashi found a significant decrease in glycogen of the liver of rabbits, especially in necrotic areas in acute and chronic fluoride intoxication (9).

In another study we shall present evidence that the rise of gamma globulins is directly proportional to damage to the liver cell which in turn is directly proportional to the dose of sodium fluoride given to animals. Since the liver is the site for catabolism of gamma globulins (10) damage by fluoride ions will interfere with its proper function which leads to the increase of gamma globulins (5,11-14).

Bibliography


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SKELETAL CHANGES IN INDUSTRIAL AND ENDEMIC FLUOROSIS

by

E. Czerwinski and W. Lankosz
Cracow, Poland

SUMMARY: Fluorotic changes in bones and joints were evaluated in 105 aluminum workers and 20 residents of an endemic fluorosis region in India. The age of the workers averaged 51.2 years, and the duration of their exposure 18.2 years. The skeletal changes in the aluminum workers exhibited the same characteristics as those of endemic fluorosis. In industrial fluorosis the changes were less advanced than in endemic fluorosis. Generalized sclerosis, alterations in the bone structure and periosteal reactions are the most typical features of skeletal fluorosis; ossification of the interosseous membranes and muscle attachments, are less characteristic.

The skeletal changes, an inseparable feature of chronic fluoride intoxication, result from the specific affinity of fluoride for hydroxyapatite, the basic substance of bone tissue (1,2,4-7).

Whereas in endemic fluorosis, the diagnostic value of skeletal changes is incontrovertible, in industrial fluorosis the findings may be modified by other factors. Among the employees of an aluminum factory the basic group studied, these factors include vibrations, mechanical overstrain, marked variations in temperature and humidity, etc.

We attempted to determine whether or not specific diagnostic criteria could be recognized in industrial fluorosis. We therefore compared the skeletal changes in a group of aluminum workers with those in patients from an endemic fluorosis area.

Material and Methods

The 105 aluminum factory workers ranged in age from 37 to 69 (average 51.2). They had been exposed to fluoride for 8-24 years (average 18.2). Ninety-seven (92.4%) of them had been working in the electrolysis department. The 20 patients from the endemic fluorosis area were 18 to 50 years old (average 30.7). Their water supplies contained 8.5 to 25.0 ppm fluoride. The patients were examined in the Department

From the Orthopedic Department, Cracow Academy of Medicine, Poland.

of Human Metabolism, Meerut University (Head: Professor S.P.S. Teotia), during the Scientific Expedition of Students of the Cracow Academy of Medicine in 1975 to India.

Orthopedic, radiological and additional examinations were made in all cases, both in the industrial and the endemic fluorosis groups.

Results

The aluminum workers most frequently complained of pains in the lumbar region of the spine, less often of pains in the large joints, forearms and lower legs. On examination various degrees of limitation in the mobility of the spine and joints were found. In the patients

![Figure 1](Lumbar Spine in (A) Aluminum Worker (B) Endemic Fluorosis)

with endemic fluorosis these changes were of a similar character except that the localization differed since the cervical region of the spine was often affected. On the basis of the clinical symptoms, it is not possible to differentiate fluorotic changes from other bone and joint diseases. Typical fluorotic changes may be evident on radiological examination (4-7).

In the aluminum workers, the most frequent changes in the spine were exostoses and ossification of the ligaments (Fig. 1). These changes did not differ in appearance from those seen in spondylarthritis or vertebral ankylosing hyperostosis. Radiograms of the pelvis very often showed ossification of the muscle attachments to the iliac crest and to the ramus of the ischiatic bone. Generalized osteosclerosis and alterations in bone structure were much less common than in the endemic fluorosis group (Fig. 2).

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Ossification of muscle attachments less marked osteosclerosis in (A). Osteosclerosis and increased trabeculation in (B).

The changes most often seen in the long bones of the aluminum workers included ossification of the interosseous membrane, thickening of the cortical bone, and obliteration of the medullary cavity. Perioosteal reactions and generalized osteosclerosis were rarely observed. In the endemic group these changes were usually more pronounced, but were absent in some (Fig. 3). It should be emphasized that ossification of the interosseous membrane or of muscle attachments are often found in manual workers who have not been exposed to fluoride compounds. Hence these changes cannot be regarded as typical of industrial fluorosis (3).

Ossification of muscle attachments, thickened cortical bone, obliteration of medullary cavity, patchy condensation in the bone structure in (A). Advanced ossification of interosseous membrane in (B).
Discussion

In evaluating our observations we must consider that the exposure to fluoride in the aluminum workers averaged 18.2 years as compared to 30.7 years for the residents in the endemic area. Furthermore, the exposure of the workers was intermittent whereas the intake of fluoride in the endemic fluorosis cases was continuous. Therefore, one would expect the disease to be farther advanced in the endemic cases. In the aluminum workers, on the other hand, inhalation of fluoride played a major role whereas among the residents of the endemic area the alimentary canal was the main port of entry. How much these factors affected the total amount of fluoride adsorbed and its resulting changes is difficult to assess.

Acknowledgements

We would like to express our cordial thanks to Professor S.P.S. Teotia and to Dr. M. Teotia for their kind help which enabled us to carry out this study.

Bibliography


Discussion

Dr. Teotia: This is the first paper comparing endemic and industrial fluorosis. Osseous calcification develops early in the disease and therefore is its earliest diagnostic feature.
ELECTROMYOGRAPHIC STUDIES IN ENDEMIC SKELETAL FLUOROSIS

by

M. Veera Raghava Reddy, D. Raja Reddy, S.B. Ramulu, and D.S. Mani
Andhra Pradesh, India

SUMMARY: In thirty-six cases (32 males, 4 females) with advanced fluorosis electromyograms were taken and motor nerve conduction was measured. In 19 patients the findings were abnormal. The motor nerve conduction in fluorosis was found to be within normal limits unless compressed by bony spurs. A fairly good correlation occurred between the electromyographical findings and those of clinical neurological examination. The findings of this electrophysiological study of endemic fluorosis are not in accord with the concept of fluorotic myopathy reported by others. Unequivocal evidence of neurogenic atrophy in the EMG leads us to conclude that the traditional concept of compression myeloradiculopathy of fluorosis is correct.

The neurological manifestations of skeletal fluorosis leading to myelopathy and radiculopathy are considered to be mechanical by nature (1). The reported non-skeletal toxic effects of high levels of fluoride in experimental animals have stimulated investigations of the involvement of skeletal muscle and of other organs in human fluorosis (2,3,4). Another interesting fact which has drawn attention to this subject is the effect of fluoride on the adenyl cyclase system of the cell membrane of the skeletal muscle. Reports by Kaul and Susheela (2,4) and Franke (3) indicate that myopathy occurs in human fluorosis as well as in experimental animals, although the experimental model used by Kaul and Susheela is not comparable to human fluorosis. Our own preliminary electrophysiological observations have shown no evidence of myopathy, but, on the contrary, were indicative of neurogenic atrophy. Because of the limited number of hospitalized fluorotic patients, we carried out a field survey in a highly endemic fluorosis area. For this purpose we selected the village of Yellareddyguda of Nalgonda district of Andhra Pradesh, where the fluoride content of well water is one of the highest in India (5).

Materials and Methods

Thirty-six cases ranging widely in age with skeletal and den-
tal fluorosis of varying degrees were chosen for study. Since the purpose of this study was to prove the possible validity of primary skeletal muscle involvement in fluorosis, we deliberately studied the most advanced cases. This also explains the preponderance of males over females whose average duration of stay in the endemic area is much shorter than that of males. All cases were studied neurologically and electromyographically which included sampling of both proximal and distal groups of muscles in the upper and lower extremities. We used the portable MS4 Medelec EMG machine with concentric needle electrodes. Moreover, we recorded motor nerve conduction velocities in the median and lateral popliteal nerves.

Results

Altogether 32 males and 4 females were included in this series. Their age distribution is shown in Figure 1. Fourteen cases were in the 5th decade of life, 7 in the 4th, 6 in the 3rd, and 5 in the 6th decade. There was one patient each in other decades. The functional disability of the patients is recorded in Table 1. Grade-I had spinal

Table 1

<table>
<thead>
<tr>
<th>Grades</th>
<th>No. of Cases</th>
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<tbody>
<tr>
<td>I</td>
<td>31</td>
</tr>
<tr>
<td>II</td>
<td>4</td>
</tr>
<tr>
<td>III</td>
<td>1</td>
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</table>

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and appendicular bony deformities but were ambulatory; 31 fell into this category. The four cases in grade-II needed support to walk. The one individual in grade-III was totally bedridden. Neurological examination was negative in 20 cases and abnormal in 16. Lower motor neuron type defects were seen in 7 individuals, upper motor neuron type defects in 4, and in 5 cases both were combined (Table 2). None of the patients had any sensory deficit, 2 had sphincter disfunction.

In 17 cases the electromyographic studies were within normal range and abnormal in 19 (Table 3). Five out of the 20 cases with normal neurological condition had abnormal EMG whereas in 4 out of 16 cases with neurological abnormalities EMG was normal (Table 4). In all cases

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Neurological Examination</th>
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<tr>
<td></td>
<td>No. of Cases</td>
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<tr>
<td>1. Normal</td>
<td>20</td>
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<tr>
<td>2. Abnormal</td>
<td>16</td>
</tr>
<tr>
<td>3. Lower motor Neuron deficit</td>
<td>7</td>
</tr>
<tr>
<td>4. Upper motor Neuron deficit</td>
<td>4</td>
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<td>5. Combined defects</td>
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<table>
<thead>
<tr>
<th>Table 3</th>
<th>Electromyographic Findings</th>
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<tr>
<td></td>
<td>No. of Cases</td>
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<tr>
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<tr>
<td>Abnormal</td>
<td>19</td>
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<thead>
<tr>
<th>Table 4</th>
<th>Analysis of Abnormal E.M.G. Findings in 19 Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.A.</td>
<td>M.A.P.D.</td>
</tr>
<tr>
<td>No. of Cases</td>
<td>15</td>
</tr>
</tbody>
</table>

S.A.=Spontaneous activity; M.A.P.D.=Mean action potential duration; M.A.P.A.=Mean action potential amplitude; I.P.=Interference pattern.

where the mean action potential amplitude was increased, the duration of the mean action potential was also increased, but in some cases only the duration increased without any changes in the amplitude. The EMG findings conform with the pattern of neurogenic atrophy and revealed no
evidence of myopathy in any of the cases. The proximal muscle groups were more involved than the distal ones (Table 5). Changes in the upper extremities were more pronounced than those in the lower. These variations might have occurred because of the anatomical difference between cervical and lumbar canals.

Table 5
Abnormal E.M.G. Break-Up of 19 Cases

<table>
<thead>
<tr>
<th>Muscles of</th>
<th>Upper Extremity</th>
<th>Lower Extremity</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Proximal</td>
<td>Distal</td>
</tr>
<tr>
<td>No. of Cases</td>
<td>17</td>
<td>11</td>
</tr>
</tbody>
</table>

The nerve conduction velocities are shown in Table 6. The terminal latency in the median nerve varied between 1.7 and 3.7 milliseconds and in the common peroneal nerve between 4.5 to 5.7 milliseconds. Only

Table 6
Nerve Conduction Velocities

<table>
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<tr>
<th>Median Nerve</th>
<th>Below 40</th>
<th>40-50</th>
<th>50-60</th>
<th>60-70</th>
<th>Over 70 Milliseconds</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Cases</td>
<td>1</td>
<td>3</td>
<td>22</td>
<td>9</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Common Peroneal Nerve</th>
<th>Below 40</th>
<th>40-50</th>
<th>50-60</th>
<th>60-70</th>
<th>Over 70 Milliseconds</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Cases</td>
<td>2</td>
<td>21</td>
<td>12</td>
<td>1</td>
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</tbody>
</table>

in three cases were the nerve conduction velocities below 40 meters per second; in the majority of cases this ranged between 40 to 60 milliseconds. In almost all cases the compound action potentials were within normal range. In our experience nerve conduction is not affected by fluorosis unless peripheral nerves are compressed by exostotic spurs in limbs as seen in a single patient whose median nerve was compressed by a spur near the elbow from the lower end of the humerus. Median nerve conduction in this case was 31 meters per second.

Bibliography


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MECHANICAL PROPERTIES AND DENSITY OF BONE IN A CASE OF SEVERE ENDEMIC FLUOROSIS

by

F.G. Evans and J.L. Wood
Ann Arbor, Michigan


The mechanical properties of 25 standardized specimens of compact bone from a case with advanced fluorosis, a 45-year-old man, were compared with similar specimens of non-fluorotic bone. The patient, who had been residing in an endemic area of India, had been bedridden for nearly five years of his life. In both dry and wet specimens the fluorotic bone exhibited reduced tensile strength and strain, but the compressive strength and strain were increased. The fluorotic specimens absorbed less energy to failure in tension (3.53 kg-cm/cm³) than the non-fluorotic specimens (8.10 kg-cm/cm³). However, in compression, more energy to failure was absorbed by the fluorotic (34.33 kg-cm/cm³) than the non-fluorotic bone (17.59 kg-cm/cm³). Fluorotic specimens also had a lower modulus of elasticity (1,362 kg/mm²) than the non-fluorotic specimens (2,178 kg/mm²). The compressive properties exceeded tensile properties. Drying increased the tensile and compressive strength and modulus but decreased tensile and compressive strength and energy absorbed. The wet fluorotic specimens had lower tensile but higher compressive properties. The average density of the dry fluorosed specimens was 2.01 g/cm³ compared with 1.84 g/cm³ for the non-fluorotic bone.

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CYTOGENETIC INVESTIGATIONS ON LEUCOCYTES OF CATTLE INTOXICATED WITH FLUORIDE

by

A. Leonard, G.H. Deknudt, G. Decat and E.D. Leonard
Brussels, Belgium

(Abstracted from Toxicology, 7:239-242, 1976)

The authors studied leucocytes of eight cows grazing near a fluoride-emitting factory that displayed signs of chronic fluoride poisoning, such as osteosclerosis and dental fluorosis. For each animal they analyzed 100 cells, selected on the basis of the quality of chromo-
somal spreading, for the presence of structural chromatid and chromosome aberrations. The blood samples from two animals that were not exposed to fluoride served as controls and 200 cells were examined for each of them. Among the intoxicated cows, they found 5.3% structural abnormalities, mainly chromatid aberrations as compared with 4.5% in the control animals. Statistical analysis, however, showed no significant difference ($X^2 = 0.432$) between the two groups.

No data on the extent of poisoning and on the amount of exposure to fluoride of the diseased animals were presented. The authors suggested that "lymphocytes bearing chromosome aberrations may have been eliminated since cattle lymphocytes have been shown to be extremely sensitive to such a selection process."

**************

DISTRIBUTION OF SOIL FLUORIDES NEAR AN AIRBORNE FLUORIDE SOURCE

by

J.R. McIlenahen
Wooster, Ohio

(Abstracted from J. Environ. Qual., 5:472-475, 1976)

Fluoride concentrations in soils of the United States are generally in the range of $< 100 \mu g/g$ to $> 1000 \mu g/g$. The author examined the levels of total soil fluoride near the site of an aluminum manufacturing facility in order to determine: 1) the possible influence of an important airborne fluoride source on geographical soil fluoride distribution; 2) seasonal and annual trends in total soil fluoride concentrations as related to the fluoride source; and 3) the distribution of fluoride in the soil profile in relation to the fluoride source. The study was supported by the Ormet Corporation of Hannibal, Ohio.

During the two years in which the soil data were collected the aluminum production averaged 218,000 metric tons per year. The area under study is situated on the Ohio river where ridges of the Ohio valley rise 150 to 200 m above the river. Soil samples were derived from pastures or grassland at 14 sites in the environs of the fluoride source. The sites were selected from data on the amounts of fluorides present in pasture grass and hay. In the forage the high and low fluoride sites averaged 46 to 16 $\mu g/g$ fluoride, respectively, during the 2 years of the study. The kind of soil was described as deep, well-drained silt loam common on upper slopes and ridgetops.

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At each of the 14 locations, 6 to 8 soil cores at different depths up to 30 cm were extracted with a standard soil sampler in spring and early fall of 1973 and 1974. A sulfuric acid steam distillation fluoride specific ion electrode technique was developed for the total soil fluoride determinations.

At the depth of 0 to 5 cm, the fluoride level ranged from $311 \pm 56$ to $371 \pm 88$ ppm; at 5 to 15 cm, from $304 \pm 48$ to $357 \pm 80$ ppm; at 15 to 30 cm depth, it ranged from $315 \pm 53$ to $379 \pm 92$ ppm. The highest levels were found in spring 1973. The total soil fluoride differed from one location to another and at different sampling depths. Seasonal trends also accounted for considerable differences between the high and low atmospheric fluoride sites. In the less polluted areas, the fluoride concentrations in soil increased with depth. However, this profile was inverted in the high airborne fluoride sites with the most superficial soil showing larger amounts of fluoride than at lower levels. The fluoride content of the soil decreased with increasing distance from the factory. No data were available at distances greater than 10 km.

The authors found no correlation between the average soil surface fluoride levels and the average fluoride levels in pasture. They conclude that the polluting source increased the fluoride content of nearby soils and changed its distribution in the soil. Its fluoride content decreased with increasing depth and as the distance from the source lengthened. At 3 km northeast of the source, the nearest agricultural land in this direction, the gain of fluoride amounted to as much as 180 µg/g in surface soil. This increase is believed to have less impact on forage fluoride than direct absorption of the halogen from the air. (For further data on this subject see the editorial on pages 1-3.)

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FLUORIDES - USE WITH CAUTION

by

L.E. Church
Washington, D.C.

(Abstracted from J. Maryland State Dent. Assoc., 19:106, 1976)

A three-year-old male child was given prophylactic fluoride treatment to the teeth by a dental technician with a mixture of a 4%
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Stannous fluoride solution and pumice. This mixture was applied to the teeth with a cotton swab. Shortly thereafter the pumice adhering to the teeth was removed with a cotton swab dipped in the fluoride solution. The child was instructed to rinse his mouth with the 4% stannous fluoride solution. Within five minutes the child vomited, developed a convulsive seizure and shock. He was promptly admitted to the intensive care unit of a pediatric ward where it was determined that he had swallowed a 1/2 cup of fluoride solution. He died approximately three hours after the topical application of the solution.

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FLUORIDE KINETICS AFTER ENFLURANE ANESTHESIA IN HEALTHY AND ANEPHRICTIC PATIENTS AND IN PATIENTS WITH POOR RENAL FUNCTION

by

R. Carter, M. Heerd and S. Acchiardo
Memphis, Tennessee


Enflurane, a methylethyl ether, is metabolized in part, to inorganic fluoride not unlike methoxyflurane. Both anesthetics have been reported to induce renal failure. Fluoride ion values above the 50 μM (.95 ppm) level are believed to cause subclinical renal toxicity. The authors tried to establish the role of the kidney in determining peak inorganic fluoride levels, particularly whether or not impaired renal function might prolong the duration of elevated serum inorganic fluoride.

They studied three groups of patients, group I, 16 healthy individuals; group II, 6 patients on hemodialysis with an unusually low kidney function, namely a creatinine clearance of less than 5 ml per minute; and group III, 18 patients without kidneys. In all three groups of patients the anesthesia was induced with sodium thiopental (4 mg/kg). Enflurane was administered by calibrated flow-compensated vaporizers with nitrous oxide oxygen at a flow rate of 5 L/min in a semiclosed system with carbon dioxide absorption. On group I a variety of operations were performed, group II underwent either placement of bovine heterograft or subtotal parathyroidectomy, and procedures on group III patients varied from bovine heterograft, multiple extraction of teeth, subtotal parathyroidectomy, and nephrectomy. Surgery lasted from 1 to 3 hours in all three groups.

Serum inorganic fluoride levels were measured pre-operatively

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and at 1.5, 3, 5, 7, 10, and 24 hours after initiation of exposure to enflurane. Renal fluoride and creatinine clearances were determined on two successive 2 hour periods following the termination of the surgery and of anesthesia in 10 control patients. Twenty-four hour urine samples were collected for these determinations in 5 of the 6 patients of group II with creatinine clearances of less than 5 ml/min. Serum inorganic fluoride and creatinine concentrations were obtained at the midpoint of each urine collection for calculation of fluoride ion and creatinine clearances in these two groups.

The patients with low creatinine clearances showed significantly higher pre-enflurane serum inorganic fluoride. Their mean maximum serum inorganic fluoride was 19.41 µM (.37 ppm) compared with 13.36 µM (.24 ppm) among the anephric patients and 13.16 in the normal controls. In one individual with low creatinine clearance the peak serum inorganic fluoride amounted to 46.31 µM (.88 ppm) three hours after the start of the enflurane administration which is less than the 50 µM (.95 ppm) at which subclinical renal toxicity has been reported.

The fluoride ion concentration in serum fell rapidly after termination of the anesthesia even in the patients without kidneys. This is presumed to be due to uptake of fluoride by bones. It was also noted that patients with a low creatinine clearance had a low clearance of fluoride ion. The authors reported a positive correlation between age and serum inorganic fluoride concentrations in 24 hours. They stressed the importance of uptake of the free fluoride by bone which they hold responsible for the rapid fall of the inorganic fluoride ion concentration in serum, even in patients without kidneys or with poor renal function.

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THE EFFECT OF MAGNESIUM AND FLUORIDE ON NEPHROCALCINOSIS AND AORTIC CALCIFICATION IN RATS GIVEN HIGH SUCROSE DIETS WITH ADDED PHOSPHATES

by

H. Luoma, T. Nujuja, Y. Collan, and P. Nummikoski
Helsinki, Finland


This study was prompted by the authors' objective "to find additives to reduce the cardiogenic properties of sucrose or sucrose-containing foods" which have minimal or no adverse effect on the human

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organism. In some rural districts in Finland with low concentrations of fluoride and magnesium in drinking water the incidence of cardiovascular diseases in humans was much higher than in districts with high concentrations of the two elements in water. Previous studies also indicated that excessive intake of phosphorus and a low magnesium content of the diet are conducive to ectopic mineralization of some internal organs. The authors purported to determine whether a combination of magnesium, orthophosphate and fluoride might reduce the incidence of dental caries without giving rise to calcifications in other organs, especially in the kidneys.

Two sets of experiments were carried out on 90 and 44 Osborne-Mendel rats, respectively. Both groups received a high (67% and 66%) sucrose diet designed to induce dental caries. In group I the total magnesium content was 490 ppm, phosphorus 2800 ppm, and calcium 4300 ppm. The diet of group II contained 460 ppm magnesium, 7600 ppm phosphorus, and 8300 ppm calcium. Magnesium (20 ppm), fluoride (10 ppm), phosphate mixture (2%), and the combinations magnesium-phosphates and magnesium-phosphates-fluoride were added in the first experiment. The second group received a 2% phosphate mixture, magnesium plus phosphate (40 ppm), magnesium plus phosphates plus fluoride (15 ppm).

The diet of group I produced renal calculi (mainly in the tubules of the loops of Henle) as well as dental caries. Fluoride added to this diet reduced the severity of the nephrocalcinosis whereas added magnesia failed to do so. Addition of both magnesium and fluoride slightly reduced the formation of kidney stones. However, in the group of experimental rats which had fewer kidney stones, they were larger and more numerous than in controls. The control rats which received solely the stock diet were free of kidney stones.

In group II, addition of phosphate alone increased significantly the renal calcium content which was reduced by addition of a combination of magnesium and fluoride. Added phosphate also significantly increased the calcium level of the aorta but the combination of magnesium and fluoride countered this effect.

Thus the experiments confirmed that high phosphates promote nephrocalcinosis in "low magnesium" rats which is countered by low (10 to 15 ppm) concentrations of dietary fluoride alone. However, a fluoride concentration of about 50 ppm was toxic. Addition of magnesium also induced a slight elevation of kidney potassium.

The authors concluded that the combination of fluoride and magnesium tends to inhibit ectopic mineralization. They explained that fluoride may maintain the plasma magnesium which in turn protects the kidneys and heart from calcification.

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