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The Twelfth Conference of the International Society for Fluoride Research will convene at the Colonial Gateway Inn (not the Hilton) 6307 Gulf Blvd., St. Petersburg Beach, Florida 33706, May 16-18, 1982. The rate per room is $30.00 for single or double occupancy plus 6% tax, $4.00 in addition for a third person. Kindly make your reservation direct with the hotel. Toll-free telephone number: 1-800-237-8918.

The Program Committee is soliciting abstracts (up to 300 words) of papers to be presented at the conference dealing with any phase of fluoride research. Kindly send abstracts to the Society's office, P.O. Box 692, Warren, Michigan 48090, prior to February 15, 1982.

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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.

FLUORIDE is listed in

Current Contents Agricultural
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EDITORIAL

OSTEOMALACIA IN FLUORIDE TOXICITY

In endemic hydrofluorosis, the clinical manifestations show a certain degree of variability in contrast to industrial fluorosis. Interactions between nutrition, environment, the dose and duration of fluoride exposure determine the nature of its clinical picture. In addition, the effect of fluoride in drinking water is modified by the other constituents in water. Moreover, irrigation activities by impounding large resources of surface water tend to modify the characteristics of the subsoil and thereby influence fluoride levels of water.

Chronic exposure to fluoride is known to produce new bone formation in the form of exostoses and of calcifications of ligaments, muscular attachments, interosseous membranes, tendons, and arteries. Thus osteosclerosis has been the well-documented form of the skeletal phase of fluoride toxicity. However, earlier epidemiological studies did not take into consideration the influence of other trace elements in the diets of the afflicted persons.

During the past two decades skeletal fluorosis in endemic areas of India has been clearly documented to occur either predominantly in sclerotic form or as a combined osteoporotic, osteomalacic and osteosclerotic form.

When fluoride enters the bones, a basic change in the chemical composition occurs which, by definition, is osteomalacia. It is therefore understandable that in fluorosis there is a certain amount of osteomalacia. Krishnamachari and Kamala Krishnaswamy observed in 1973 in some endemic regions of India, genu valgum as a manifestation of fluoride toxicity among population groups in whom dietary calcium is low (1). Genu valgum as a crippling form of fluoride toxicity occurs at a relatively younger age in contrast to the classical osteophytotic and osteosclerotic form of skeletal fluorosis reported by Jolly and his associates from Punjab where the dietary intake of calcium is as high as 1000 mg per day (2). In addition to humans with genu valgum, dogs living in an endemic area are also susceptible to genu valgum and genu varum (3).

Endemic genu valgum reported from India, has certain distinctive epidemiological and clinical characteristics, such as a predominantly male involvement, its occurrence in adolescence, evidence of secondary hyperparathyroidism and radiologically discernible osteoporosis (4). There is evidence of hormonal changes in children with genu valgum such as elevated levels of circulating immunoreactive parathyroid hormone (5), increased growth hormone levels (6) and decreased thyrocalcitonin (7). There is also an increased \(^{47}\)Ca turnover in these children (8) with increased accretion as well as resorption rate. In addition to radiological evidence of osteoporosis, there is biochemical evidence of bone collagen destruction in the form of elevated urinary hydroxyproline excretion (9).

The available studies indicate a coexistence of osteomalacia and hyperparathyroidism. Further studies are, however, needed to determine
Editorial

which change precedes the other. Fluoride is considered beneficial in senile osteoporosis when administered in conjunction with large doses of calcium. It is interesting however, to observe osteoporosis at a young age among population groups whose diets are deficient in calcium as a consequence of consuming habitually large amounts of fluoride.

Another example of osteomalacia related to endemic fluorosis was reported by Jackson and co-workers in areas where drinking water is high in fluoride (10). They observed rickets-like changes in inadequately nourished black children who were economically poor. Such changes did not occur among age-matched white children residing in the same environment. Griffiths, et al. recently observed evidence of osteomalacia in monkeys on a low calcium diet which received high doses of fluoride for 60 months (11). In contrast, monkeys fed high fluoride and adequate calcium diets developed osteosclerosis. A third group kept on a low calcium diet developed osteoporosis. According to Kaj Roholm in his monumental work on fluoride intoxication, upon feeding small amounts of fluoride, growth of bone was stimulated (osteosclerosis) but large doses led to osteomalacia with uncalcified osteoid (12). In an experimental study on two dogs fed cryolite and sodium fluoride, at the end of over 500 days, Roholm observed essentially "a kind of osteomalacia".

In recent years, Teotia et al. described in humans residing in endemic areas, some uncommon radiological features in fluorosis including osteomalacia and neo-osseous porosis (13). The interactions between fluoride, hormones, bone and nutrient intakes seem to be based on intricate mechanisms which are determined possibly by several physiological factors such as age and sex. The sequence of these interactions may be difficult to study in humans.

Therefore ample evidence indicates that osteomalacia and a rickets-like picture should be considered one of the manifestations of chronic fluoride toxicity. Whether, under specified conditions, fluoride will cause osteomalacia in all species, or whether species specificity exists for such an effect and whether dietary or other manipulations can prevent the occurrence of such changes are questions to be answered.

In recent years there has been some awareness regarding the possibility that fluoride is involved in altering Vitamin D metabolism. Preliminary studies in this direction are available from India which show that 25 Hydroxy D3 levels in subjects with genu valgum are not decreased (14). In depth studies, however, are needed including data on 1, 25, Dihydroxy D3 estimations in the serum of subjects with osteomalacia and rickets in fluorosis areas.

Bibliography


K.A.V.R. Krishnamachari

**********

FLUORIDE BRIEF

The authors used sulfur hexafluoride gas instead of air on 57 patients with detached retina. After 6 months the retina was re-attached in 64.9%, in 61.4% visual acuity was stabilized at 20/60 or better. Side effects on the first postoperative day, were progression of mild cataract changes in 27% phakic patients and elevation of intraocular pressure in 26%. Central retinal artery occlusion occurred in four patients with elevated pressure.

FLUORIDE-INDUCED ACTIVATION AND INHIBITION OF GRANULOCYTE FUNCTIONS
(An Overview)

by

J.G.R. Elferink
Leiden, The Netherlands

SUMMARY: Exposure of rabbit peritoneal granulocytes to fluoride (10-20 mM), followed by addition of Ca\(^{++}\)-ions, results in the release of lysosomal enzymes by exocytosis. Fluoride induces production of superoxide (O\(_2^-\)) as a consequence of stimulation of oxidative metabolism in rabbit peritoneal and human peripheral granulocytes; in the former cell type Ca\(^{++}\) must be absent, in the latter cell type Ca\(^{++}\) is required for activation. Both exocytosis and superoxide production are characterized by a pH-dependent lag time between the addition of fluoride and its observed effect. The pH dependence suggests that fluoride must penetrate into the cell to be effective; probably the inner side of the plasma membrane is affected. Fluoride, under certain conditions, may also inhibit exocytosis and superoxide production; inhibition occurs later than activation and has the same pH dependence. Apparently the observed effect of fluoride on granulocytes is due to a combination of opposite reactions. There is a stimulatory and an inhibitory effect; the magnitude of each is determined by the intracellular concentration of fluoride.

Introduction

The main function of the granulocyte (neutrophil granulocyte, polymorphonuclear leukocyte) is the phagocytosis and destruction of invading microorganisms. During phagocytosis, some processes in the granulocyte are activated, which enables this cell to destroy the phagocytosed organism. One of these processes is degranulation. In the granulocyte a large number of granules are present, containing many digestive enzymes. During degranulation the membranes of these granules fuse with the membrane which surrounds the phagocytosed particle, so that degrading enzymes come into contact with this particle. Granule membranes fuse also with the plasma membrane of the granulocyte, and the contents of these granules are then liberated extracellularly. This form of degranulation is called exocytosis. Exocytosis can easily be measured as the release of cytoplasmic enzymes.

Another process which is activated during phagocytosis is a form of oxidative metabolism described as a "respiratory burst". The activation of a membrane-bound oxidase results in an increased uptake of oxygen, which is converted into superoxide (O\(_2^-\)). This superoxide is subsequently con-

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verted into other oxygen metabolites.

The extracellular release of granule constituents by exocytosis, and the generation of oxygen metabolites are of importance in a number of pathological conditions apart from the defense against microorganisms. Some of the granule constituents have inflammatory properties, exocytosis therefore may result in inflammation (1). The proteases present in the granules, by attacking extracellular substrates, produce tissue damage. In some cases this gives rise to inflammatory disease such as rheumatoid arthritis (2). There are indications that oxygen metabolites may play a role in certain forms of cancer (3).

**Figure 1**

Activation of Granulocyte
Granulocytes

It is possible to activate the granulocyte functions mentioned above in the absence of phagocytosis (Fig. 1). A number of non-particulate agents, such as ionophore A23187, phorbol myristate acetate, chemotactic peptide, complement components and fluoride, all can, under appropriate conditions, induce exocytosis and stimulate oxidative metabolism. Exocytosis and the respiratory burst thus can be studied in the absence of phagocytosis.

Induction of Exocytosis by Fluoride

Exocytosis is the secretion of granule-associated components in the external medium without release of cytoplasmic components (Fig. 1). In the granulocyte at least two types of granules are present: zureophilic granules or lysosomes, containing a large number of hydrolytic and proteolytic enzymes, and specific granules. By measuring the enzymes lysozyme, which is present in both types of granules, 8-glucuronidase, which is only present in the zureophilic granules, and lactate dehydrogenase(LDH), which is located exclusively in the cytoplasm, one can estimate the extent of exocytosis. Exocytosis has been mainly studied in two types of granulocytes: human granulocytes from peripheral blood, and peritoneal granulocytes from rabbits or guinea pigs. With regard to some actions, i.e. the effect of fluoride, there are considerable differences between these two types of cells (2, 5-7).

In rabbit peritoneal granulocytes, fluoride strongly induces exocytosis; up to 80% of the granule-associated enzymes are liberated, without release of LDH.

Both types of granules are involved, as can be deduced from the release of both lysozyme and 8-glucuronidase (Fig. 2). Fluoride-induced exocytosis in granulocytes has several characteristics (7):

a) Exocytosis only occurs in the presence of extracellular Ca++. To prevent the formation of CaF2 the following experimental procedure was chosen. Exocytosis was induced in two steps: first the granulocytes were exposed to fluoride (preincubation); then the fluoride-containing medium was removed and a Ca++ containing medium was added. No exocytosis occurred during preincubation; after the addition of Ca++ the release of enzymes occurred rapidly. Other divalent metal ions (Mg++, Sr++, Ba++) were without effect.

b) Relatively high concentrations of fluoride are necessary. Concentrations higher than 3 mM fluoride are required to obtain exocytosis upon subsequent addition of Ca++.

c) The effect of fluoride is highly dependent on the pH of the medium. The preincubation period is characterized by a certain lag: if the cells are separated from the fluoride-containing medium at a time shorter than that lag time then no exocytosis occurs upon subsequent addition of Ca++-containing medium (Fig. 2). This lag time becomes shorter when the preincubation pH is lowered, and becomes longer when the pH is increased.

Though the mechanism of exocytosis in general and of fluoride-induced
Figure 2
Induction of Exocytosis by F⁻

Granulocytes preincubated at 37°C (pH 7.2), cells centrifuged and F⁻ containing medium, which did not contain significant amounts of enzymes was discarded. Subsequently Ca²⁺ containing medium was added, followed by incubation for 30 min at 37°C. Mixture centrifuged and the enzymes liberated were measured in the supernatant.

- O - lysozyme release; - □ - β-glucuronidase release; - △ - LDH release. (Reproduced from Elferink, et al. [7]).

Exocytosis in particular is far from clear, some conclusions appear to be valid. Exocytosis in diverse types of secretory cells is a Ca²⁺-requiring process (8). An increase in cytosolic free Ca²⁺ is believed to be necessary and sufficient to initiate the events leading to exocytosis. Fluoride probably changes the membrane of rabbit granulocytes in such a way that Ca²⁺, when added extracellularly, can penetrate into the cell, after which exocytosis occurs. The pH-dependence of the effect of fluoride indicates that fluoride penetrates into the cell after which it induces a change in Ca²⁺-permeability of the membrane. Lowering of the pH facilitates fluoride penetration. This suggests that fluoride passes the membrane as HF. This weak acid crosses the hydrophobic core of the membrane more easily than the fluoride ion.

Whereas fluoride-induced exocytosis has been observed in both rabbit and guinea pig peritoneal granulocytes, fluoride does not readily induce exocytosis in human granulocytes. Under some conditions, however, i.e. the presence of cytochalasin B, exocytosis may occur, albeit to a small extent (9). Recently Clark (10) found that mycophenoloxidase was secreted by human granulocytes after treatment with fluoride.
Granulocytes

Two other cell types in which exocytosis has been thoroughly studied are platelets, where exocytosis is part of the clotting reaction, and mast cells, where the exocytotic release of histamine and other products are the cause of allergic reactions. In these cell types (11-14), too fluoride is able to induce exocytosis. The conditions as regards Ca++ requirement, fluoride concentration and pH-dependence for fluoride-induced exocytosis in all three cell types are similar.

Cytolysis of Granulocytes by Fluoride in the Presence of Ca++

Cytolysis as well as exocytosis occurs when rabbit granulocytes are exposed to a medium in which both fluoride (20 mM) and Ca++ (1mM) are present; this is evident from the release of LDH (7). The cytolysis induced in this way is a complex phenomenon. The results with granulocytes are difficult to reproduce as well as hard to evaluate because exocytosis and lysis occur simultaneously. For this reason the cytolytic effect of fluoride and calcium was studied in erythrocytes. These cells can lyse but do not give exocytosis. Fluoride with Ca++-induced hemolysis appears to have the same characteristics as lysis induced by certain types of crystals(15); namely potentiation of lysis with positively charged divalent metal ions, and inhibition by negatively charged compounds. It is reasonable to suppose that calcium fluoride crystals are the cytolytic agent. In the absence of cells no crystallisation is apparent in the supersaturated solution, but under certain conditions crystallization may be promoted by components of the cell membrane.

Stimulation of Oxidative Metabolism by Fluoride

Stimulation of oxidative metabolism, i.e. the "respiratory burst", in granulocytes results in a chain of reactions. There is an increase of oxygen uptake, glucose consumption via the hexosemonophosphate shunt, superoxide (O2-) production and the generation of other oxygen metabolites (hydrogen peroxide (H2 O2), hydroxyl radical (OH-) and singlet oxygen (^O2), and enhanced chemiluminescence. Production of superoxide is one of the most striking features of the stimulation of oxidative metabolism; this can be measured easily as cytochrome c reduction. O2- production is thought to be the consequence of the activation of a membrane bound enzyme, an oxidase which catalyzes the reduction of molecular O2 to O2-(16). The O2- thus formed can be transformed in the other oxygen metabolites.

Most agents which induce exocytosis, such as phagocytosable particles, ionophore A23187, chemotactic peptide and phorbol myristate acetate, also stimulate oxidative metabolism. It is therefore not surprising that fluoride has this ability as well.

Fluoride stimulates oxidative metabolism as has been demonstrated by an enhanced hexose monophosphate shunt activity (17), the production of superoxide (6, 18) and the generation of chemiluminescence (5, 19). Other halide ions are not active in this respect. As is the case in exocytosis, stimulation of oxidative metabolisms requires relatively high concentrations of fluoride. These is no effect below ± 5 mM fluoride; most exper-
Fluoride induces superoxide production in human peripheral and in rabbit peritoneal granulocytes. There is, however, one very prominent difference. For human granulocytes the presence of Ca\(^{++}\) in the medium is required for activation; other divalent cations are not effective (6). In rabbit granulocytes fluoride induces \(O_2^-\) generation in the absence of Ca\(^{++}\); the presence of Ca\(^{++}\) is inhibitory for \(O_2^-\) production (Table 1) (18). This inhibition is specific for Ca\(^{++}\), for Mg\(^{++}\) has little or no inhibitory potency. These observations with rabbit granulocytes do not mean that no Ca\(^{++}\) is required. As with phorbol myristate acetate, which also stimulates in the absence of extracellular Ca\(^{++}\), the Ca\(^{++}\) ion may be derived from intracellular stores (20). An interesting observation has recently been reported by Lew and Stossel (21), who studied inside-out vesicles from membranes of macrophages, containing an \(O_2^-\) generating oxidase. They found that Ca\(^{++}\) in micromolar concentrations inhibited the \(O_2^-\) generating activity of these vesicles, whereas this sensitivity did not exist in intact macrophages. It may therefore be, that regulation of intracellular cytosolic Ca\(^{++}\), which in its turn regulates oxidase activity, is different in human as compared to rabbit granulocytes.

As for exocytosis, the pH of the medium has a strong influence on the effect of fluoride (18). There is a delay between the addition of fluoride to the cells and the onset of \(O_2^-\) production; this delay becomes shorter when the pH is lowered (Fig. 3). A lowering of the external pH also lowers the fluoride concentration required to induce \(O_2^-\) production. Probably here too, as in exocytosis, fluoride has to penetrate the cell to activate \(O_2^-\) generation. This conclusion is supported by the observation that after washing the \(O_2^-\) production continues, at least in rabbit granulocytes (Table 1). In human granulocytes the fluoride effect is reversible by washing (6).

The foregoing observations permit us to draw certain conclusions with regard to the spatial organization of the \(O_2^-\)-generating oxidase in rabbit granulocytes. Fluoride exerts its effect from the interior of the cell. Many investigators believe that this oxidase is located on the outer side of the cytoplasmic membrane (16, 22). The results with fluoride indicate that then the enzyme has a transmembranal organization: it either spans the membrane itself, or it is part of a larger transmembrane structural entity. Alternatively, it may be supposed that the oxidase is located on the granules, as has been suggested by some investigators (23).

**Inhibition of Granulocyte Functions by Fluoride**

Apart from the stimulating granulocyte functions, fluoride also possesses the ability to inhibit the same functions (7, 10, 18, 24). When granulocytes are exposed to fluoride (20 mM), exocytosis occurs upon addition of Ca\(^{++}\). When the preincubation time is longer than 20 minutes, a gradual decrease of exocytotic response is observed. A comparable observation is observed for \(O_2^-\) production. Prolonged exposure of granulocytes to fluoride results in decrease of \(O_2^-\) generation. The inhibitory effect
Granulocytes

Figure 3

Effect of pH and F⁻ Concentration During Incubation on O₂⁻ Production

In a control experiment the effect obtained was not due to a pH dependence of the O₂⁻ generating oxidase, but to another phenomenon, namely fluoride penetration into the cells. (Reproduced from Elferink [18]).

- □ - pH 5.8; - O - pH 7.2; - Δ - pH 7.8

is, in the same way as the stimulatory effect-dependent on pH and fluoride concentration: lowering of the pH causes inhibition at shorter exposure times and at lower fluoride concentration.

In both exocytosis and O₂⁻ generation glycolysis plays an important role. Glycolysis is only for the exocytosis, the main source of energy. Energy for the "respiratory burst" is derived from the hexose monophosphate shunt. Inhibitors of glycolysis inhibit both exocytosis (25) and superoxide release (26, 27). Fluoride is a known inhibitor of glycolysis, acting on the enzyme enolase. A reasonable explanation of fluoride-induced inhibition is therefore that fluoride interferes with glycolysis. Apparently this interference occurs at a higher intracellular concentration than required for activation.

There is another reaction in the granulocyte which is affected by fluoride (10, 28). The granulocyte can iodinate proteins extracellularly, upon stimulation with fluoride. This process requires the production of H₂O₂, and the secretion of myeloperoxidase. High concentrations of fluoride result in an inhibition of protein iodination. Clark (10) has sug-
suggested that fluoride induces \( \text{H}_2\text{O}_2 \) production in human granulocytes via the respiratory burst, as well as myeloperoxidase secretion by exocytosis. His results are in contrast to negative results in obtaining exocytosis with this type of cells obtained by other investigators (5-7). Clark suggests that high concentrations of fluoride inhibit either exocytosis or the metabolic burst, or both, by interference with glycolytic energy metabolism.

\[ \text{Table 1} \]

<table>
<thead>
<tr>
<th>Effect of Extracellular Ca(^{++}) and Mg(^{++}) on F(^{-}) Induced Superoxide Production</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{O}_2^- \text{ Production} )</td>
</tr>
<tr>
<td>(n moles ( \text{O}_2^-/5 \times 10^6 \text{ PMN}'s ))</td>
</tr>
<tr>
<td>Additions</td>
</tr>
<tr>
<td>none</td>
</tr>
<tr>
<td>1 mM EDTA</td>
</tr>
<tr>
<td>1 mM EDTA, 20 mM F(^-)</td>
</tr>
<tr>
<td>1 mM Ca(^{++})</td>
</tr>
<tr>
<td>1 mM Ca(^{++}), 20 mM F(^-)</td>
</tr>
<tr>
<td>1 mM Mg(^{++})</td>
</tr>
<tr>
<td>1 mM Mg(^{++}), 20 mM F(^-)</td>
</tr>
</tbody>
</table>

Columns A and B represent different experimental procedures to measure \( \text{O}_2^- \) production; however, cytochrome c is used in both to measure \( \text{O}_2^- \).

A: cells incubated for 30 min at 37°C with F\(^-\) and reagents indicated; cytochrome c is present simultaneously.

B: cells preincubated with F\(^-\) for 20 min at 37°C; then the cells were centrifuged and the supernatant discarded. Next new medium, containing the reagents and cytochrome c (but no fluoride) were added, followed by incubation for 30 min at 37°C. In this procedure thus the residual \( \text{O}_2^- \) production was measured.

**Concluding Remarks**

Fluoride stimulates a number of granulocyte functions. Though the concentrations required are higher than will ever be attained in vivo, fluoride is a useful tool in elucidating the molecular basis of exocytosis and of oxidative metabolism in granulocytes.

The effects of fluoride on granulocytes are determined by two opposing properties: a stimulatory effect and an inhibitory effect. The result depends on the intracellular concentration of fluoride. Fluoride slowly passes the membrane in a pH-dependent manner (7, 18); at a relatively low intracellular concentration there is activation while inhibi-
tion occurs when the fluoride concentration increases beyond a certain value. The nature of the stimulating effect is still obscure. The inhibitory effect may be due to inhibition of glycolysis, because glycolysis plays a role in all functions considered (25-27). In high concentrations fluoride also has a number of other actions. One of these is stimulation of adenylate cyclase (29), resulting in the production of cyclic AMP (cAMP), an important modulator of cell functions, also in the granulocyte. This phenomenon cannot be the basis of the stimulatory effect of fluoride because cAMP inhibits exocytosis in granulocytes. The inhibitory effect of fluoride may, however, be due to this phenomenon.

The occurrence of a pH-dependent lag-time between the addition of fluoride and the effect observed suggests that fluoride first passes the membrane and exerts its effect from the cell interior, probably at the inside of the plasma membrane. With regard to the point of attack, and to the fluoride concentration required, there is a strong resemblance between exocytosis and O$_2^-$ generation. There is, however, a clear difference as concerns the Ca$^{++}$-requirement for fluoride-induced activation (6, 18). Without extracellular Ca$^{++}$ no exocytosis occurs in rabbit granulocytes, whereas the absence of extracellular Ca$^{++}$ is required for O$_2^-$ generation in the same cell type. Though exocytosis and O$_2^-$ generation (with other activating agents) mostly occur simultaneously, the results of the experiments with fluoride clearly show that these processes can proceed independently (18). The nature of this difference in Ca$^{++}$ requirement in O$_2^-$ generation and exocytosis, and the difference in Ca$^{++}$-requirement for O$_2^-$ generation between human and rabbit granulocytes deserves further study.

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**********
USE OF PINE FOLIAGE AS AN INDICATOR OF FLUORIDE ACCUMULATION FROM UF₆ TECHNOLOGIES

by

F.C. Taylor, J.J. Beauchamp, and P.D. Parr
Oak Ridge, Tennessee

SUMMARY: The accumulation of fluoride in loblolly pine needles near the Oak Ridge Gaseous Diffusion Plant (ORGDP) and the nuclear weapons component production plant (Y-12) at Oak Ridge, Tennessee was investigated. Since loblolly pines retain foliage spanning two or three growing seasons, and fluoride continues to accumulate in live foliage, it was felt that this species could be used as an indicator of air quality for the current as well as previous growth periods. The data consisted of fluoride concentrations in current needles (1979) and old needles (1978-77) from samples collected at 34 locations near the two plant sites. The statistical analysis consisted of 1) a comparison of the fluoride concentrations from the old and new needles within the control group, to consider uptake from non-UF₆ fuel cycle sources, and 2) comparison of the fluoride concentration at the different locations with the control location. The paired-sample t-test and an extension of Dunnett's procedure to make multiple comparisons with a control were applied to 1) compare the difference in fluoride concentrations over the different seasons, and 2) group the different locations together according to fluoride concentration. The results of these analyses have been helpful in showing the potential of loblolly pine foliage as an environmental indicator to assess longterm additive fluoride effects.

Introduction

For the past several decades the Department of Energy (DOE) has maintained contractor-operated uranium enrichment (UF₆) and nuclear weapon components production and engineering facilities at the Oak Ridge, Tennessee site. Environmental monitoring programs at each facility routinely sample air and vegetation (fescue grass and pine foliage) to provide effluent control information and to determine compliance with applicable standards. Although scrubbers and other pollution abatement technologies are used to reduce atmospheric emissions, each operation potentially influences the air quality of the area.

A major environmental residual from these nuclear fuel cycle facilities has been atmospheric fluoride. Fluoride emissions from an operating UF₆ facility have been characterized as intermittent, low-level with peak concentrations randomly distributed during the year (1). Fluoride (as

HF) is among the most phytotoxic gases of the major air pollutants (2), and can damage vegetation at a concentration (\(<1 \mu g/m^3\)) that is several orders of magnitude less than air concentrations of other pollutants (\(SO_2, O_3\)) causing similar damage (3). Fluorides can accumulate in living plants resulting in visible injury once threshold levels have been exceeded. For example, light to moderate foliar injury occurs in conifers with fluoride accumulations of 20 to 30 \(\mu g/g\) (4,5). Fluoride concentrations in foliage have been used as indicators of air quality, injury, and to delineate the area impacted by fluoride-emitting industries.

Environmental monitoring of vegetation (grass and pine needles) at the DOE enrichment facility consists of semi-annual grab samples collected in January and July. Pine needles have been analyzed without regard to season (year) of growth initiation. Consequently, assessments of damage potential are based on foliar concentrations depicting short-term considerations only. Since fluoride is a cumulative toxicant, a more appropriate sampling design should utilize more frequent collecting and include foliage which has retained metabolic activity for more than one year. As loblolly pines retain foliage for three growing seasons, we felt that the species could be used as an indicator of air quality to assess long-term additive effects. During the spring of 1978 loblolly pine needles from isolated trees in the environs of the enrichment plant displayed visible injury (10-30\% tip burn) to an unknown pollutant. Fluoride concentrations were determined for whole needles, necrotic tips, and corresponding controls. Fluoride concentrations for whole needles with injury were nine times greater than controls ranging from 25.60.6 to 2.91.2 \(\mu g/g\), respectively. Additional analysis of affected needle parts were similar with concentrations from tips (67.913.1 \(\mu g/g\)) exceeding controls (5.111.7 \(\mu g/g\)) by a factor of thirteen. These results implicated fluoride as a possible causal pollutant and suggested that a more detailed survey be conducted.

Methods

Loblolly pine (\(Pinus taeda\) L.) was selected as an indicator species because it is widely distributed over the reservation, is moderately sensitive to fluorides, and retains metabolically active foliage up to three years. At locations where loblolly was not present, either short-leaf (\(Pinus echinata\) Mill.) or Virginia pine (\(Pinus virginiana\) Mill.) was collected from mid-canopies of mature trees at 20 locations near the Y-12 plant and 14 locations near the ORGDP (Fig. 1). Control samples (three locations) were collected remote from any known fluoride source. An additional site was sampled to compare fluoride levels between species at a single location. In the laboratory, foliage was segregated according to current needle biomass (1979 growing season) and old needle biomass (1978-1977) and subsequently prepared for elemental analysis. Washed and unwashed needles were processed to determine percent particulate and gaseous (fixed) fluorides among some samples.

Samples were oven-dried (80°C) for 24 to 48 hrs. ground in a Wiley mill to pass through a 20-mesh screen, and analyzed potentiometrically by selective ion electrode. The sample aliquots were digested in potassium hydroxide after which phosphoric acid was added to eliminate interference
by complexing agents. Each sample was measured by standard addition techniques and the total fluoride content calculated from the change in potential (5). Foliar fluoride concentrations (µg/g) were determined from the dry weights of the samples.

Results and Discussions

Foliage from controls was segregated as old needles (1978-1977 needle growth) and new needles (1979 needle growth) and analyzed for fluoride content (Table 1). Old needles had ~20% more fluoride than new needles ranging from a maximum of 4.8±0.5 among old needles to a minimum of 3.9±0.1 µg/g among new needles (Table 1). In a test of significance (paired "t") the difference was determined to be significant (P<0.05). Similar differences have been observed between one-and-two-year old foliage among ponderosa pines (7) and among several species of boreal conifers (8). Since loblolly did not occur at all selected sampling locations, a comparison was made between the three pine species growing at a single plot. By analysis of variance it was determined that the fluoride concentrations between the different species were not statistically different. However, as in the case of the loblolly controls, fluoride concentrations of old needles (1978-1977) were significantly higher (P<0.05) than among new needles (1979). This observation has implications for the use of pine foliage in environmental monitoring since a composite sample (all needles) would introduce a negative bias for longterm fluoride accumulation.
Table 1

Mean F⁻ Concentration in Loblolly Pine Foliage Remote From Any Known
F⁺ Source (N = 3 for each mean)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.7±0.3</td>
<td>3.9±0.1</td>
</tr>
<tr>
<td>2</td>
<td>4.5±0.2</td>
<td>4.0±0.2</td>
</tr>
<tr>
<td>3</td>
<td>4.8±0.5</td>
<td>4.1±0.2</td>
</tr>
</tbody>
</table>

Pine foliage in the environs of the two DOE facilities was inventoried for fluoride content. The ORGDP site is influenced by a predominant southwest wind. Plots (Fig. 1a) were located downwind of the facility with access from perimeter roads which primarily serve work traffic. Fluoride concentrations in old foliage (1978 season) were significantly greater than in the new needles (1979 season) or controls. Old foliage from all plots contained elevated fluoride concentrations with maximum levels (~15 μg/g) at locations downwind of the process buildings. Some visible damage (10-20% tip-burn) was present, but the foliar fluoride concentrations were not sufficient to produce the effect. The plots with the highest concentrations were within 500 m of a 270 million BTU/hr. coal-fired steam plant, suggesting that emissions from that facility (SO₂), combined with intermittent fluoride releases, were responsible for the injury. The Y-12 site (Fig. 1b) is located in a valley with predominant ridges north and south of the process buildings. Gaseous emissions from the plant would be directed eastward by prevailing winds or contained in the valley near the plant area. The results of the Y-12 inventory are similar to those of the ORGDP area, with the old foliage (1978 season) containing significantly more fluoride than the 1979 needles or control needles. Plots 5 and 6 had the highest concentration, 41.3±4.5 ppm and 27.5±3.6 μg/g, respectively. These plots are nearest to the gaseous effluent discharge streams. At the two locations with elevated fluoride concentrations (>20 μg/g) there was a corresponding 30% visible injury (tipburn).

By comparing ratios of exposed needles to control needles (1978 needle biomass) it is possible to establish a threshold value to identify longterm additive effects from the UF₆ operations. Since the distribution of many elemental concentrations in biota is lognormal, it implies that the log-concentration has a normal distribution. The comparison of the fluoride concentrations among the old needles and new needles can be contrasted in terms of the difference between the means of the transformed distributions, i.e., ln (conc. in old needles) - ln (conc. in new needles) = ln (conc. in old/conc. in new). As the original concentrations are assumed to be lognormally distributed, the above difference would follow a normal distribution. Therefore, the usual statistical tools available
### Table 2
#### Y-12 Plant Area

<table>
<thead>
<tr>
<th>Plot Location</th>
<th>N</th>
<th>Differences Between Contaminated and Control Sites</th>
<th>Fluoride (µg/g) ±1 SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Old</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>1.534</td>
<td>41.3 ± 4.5</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>1.228</td>
<td>27.5 ± 3.6</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>0.555</td>
<td>11.5 ± 2.5</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>0.554</td>
<td>15.1 ± 0.9</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>0.550</td>
<td>12.4 ± 1.6</td>
</tr>
<tr>
<td>14</td>
<td>3</td>
<td>0.429</td>
<td>10.1 ± 0.9</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>0.423</td>
<td>8.8 ± 1.1</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>0.399</td>
<td>13.8 ± 1.6</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>0.319</td>
<td>7.3 ± 0.8</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>0.315</td>
<td>6.9 ± 0.8</td>
</tr>
<tr>
<td>11</td>
<td>3</td>
<td>0.310</td>
<td>7.8 ± 0.8</td>
</tr>
<tr>
<td>19</td>
<td>3</td>
<td>0.307</td>
<td>7.2 ± 0.7</td>
</tr>
<tr>
<td>17</td>
<td>1</td>
<td>0.238</td>
<td>7.8</td>
</tr>
<tr>
<td>18</td>
<td>1</td>
<td>0.202</td>
<td>8.2</td>
</tr>
<tr>
<td>20</td>
<td>3</td>
<td>0.199</td>
<td>6.1 ± 0.5</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>0.170</td>
<td>6.8 ± 0.5</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>0.030</td>
<td>12.2 ± 1.4</td>
</tr>
<tr>
<td>16</td>
<td>1</td>
<td>0.029</td>
<td>4.9</td>
</tr>
<tr>
<td>13</td>
<td>3</td>
<td>-0.013</td>
<td>5.3 ± 0.5</td>
</tr>
<tr>
<td>15</td>
<td>1</td>
<td>-0.028</td>
<td>5.4</td>
</tr>
</tbody>
</table>

* Differences in transformed ratios of concentrations (old vs new) at each site and control sites which are less than the calculated critical differences (0.338 where N=3 and 0.534 where N=1) indicate the absence of cumulative F⁻ from UF₆ facilities.

### Table 3
#### ORCDP Plant Area

<table>
<thead>
<tr>
<th>Plot Location</th>
<th>N</th>
<th>Differences Between Contaminated and Control Sites</th>
<th>Fluoride (µg/g) ±1 SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Old</td>
</tr>
<tr>
<td>14</td>
<td>3</td>
<td>0.763</td>
<td>15.0 ± 0.9</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>0.678</td>
<td>15.2 ± 5.8</td>
</tr>
<tr>
<td>11</td>
<td>3</td>
<td>0.658</td>
<td>11.3 ± 1.4</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>0.637</td>
<td>11.7 ± 0.7</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>0.557</td>
<td>11.2 ± 1.6</td>
</tr>
<tr>
<td>13</td>
<td>3</td>
<td>0.449</td>
<td>15.4 ± 0.4</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>0.410</td>
<td>8.6 ± 0.4</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>0.317</td>
<td>9.1 ± 0.6</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>0.295</td>
<td>7.5 ± 0.5</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>0.269</td>
<td>8.2 ± 0.3</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>0.256</td>
<td>7.5 ± 1.3</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>0.145</td>
<td>7.5 ± 0.5</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>0.141</td>
<td>6.4 ± 0.2</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>0.043</td>
<td>6.1 ± 0.4</td>
</tr>
</tbody>
</table>

* Differences in transformed ratios of concentrations (old vs new) at each site and control sites which are less than calculated critical difference (0.396) indicate the absence of cumulative fluoride from UF₆ facilities.
for estimating and testing parameters of this distribution are applicable (e.g., t-test).

From the measured fluoride concentrations in the needles from each site (ORGDP and Y-12), the mean concentration (using log-transformation) was calculated. A primary concern for comparing concentrations among old and new foliage is to determine if fluoride residuals from the two facilities contribute significantly to long term foliar burdens. The comparison was achieved by using the transformed ratio of the concentration in the old and new needles from the observations at each site and comparing the results with the corresponding ratio from the control site. Dunnett (9) proposed a multiple comparison procedure for comparing several means with a control and expanded it in a more recent treatment (10). The purpose of the procedure is to construct appropriate confidence statements about the differences between the "contaminated" and "control" sites such that the resulting confidence intervals have the property that the probability of all the confidence statements being simultaneously correct is equal to a specified value, \(100(1-\alpha)\%\), where \(\alpha\) is the level of significance. The steps in this procedure are similar to those of most multiple comparison procedures:

1. The mean square error (MSE), with \(v\) degrees of freedom, is computed from the one-way analysis of variance using all the plots to be compared.
2. A critical difference is calculated for each pair of sites to be compared and equals \(t\sqrt{\text{MSE}(n_c^{-1}+n_t^{-1})}\), where \(t\) is a modified \(t\)-value, \(n_c\) is the control site sample size and \(n_t\) is the treatment site sample size.
3. The observed difference is declared to be significant (i.e., \(>0\)) if the observed difference is greater than the critical difference.

Since the interest is primarily whether the UF6 facilities have contributed significantly to the long-term accumulation of fluoride (i.e., the control site mean concentration is significantly less than the contaminated sites' mean concentrations), only one-sided comparisons were performed. This procedure was repeated for the Y-12 and ORGDP site observations compared with controls.

By comparing the ratios of fluoride concentration in old vs new foliage to similar ratios among controls, it was possible to identify areas accumulating fluorides from intermittent releases to establish a confidence statement about the differences between impacted sites and controls and to illustrate longterm additive effects in the absence of visible injury. Among the twenty plots in the Y-12 area, eight locations provided evidence of longterm (2 years) accumulation (Table 2). Concentrations at plots 5 and 6 were 7 to 10 times greater than background and were comparable to threshold levels for injury as suggested by other workers (4, 11). Washed and unwashed needles (>70% necrosis) collected from a location between plots 5 and 6 were processed to determine particulate and gaseous (fixed) fluoride. The water soluble fraction suggested that 16 to 36% of total fluoride was due to particulates. Among samples col-
lected near ORGDP, 50% of all locations depicted accumulations above controls (Table 3). Concentrations determined at all ORGDP locations were less than the threshold for foliar necrosis. Those plots with concentration ratios (old vs new) above the calculated critical difference were downwind of the diffusion plant in a predominant northeast to east wind dispersion pattern.

Periodic grab samples (biannual grass collections) typical of present monitoring procedures at DOE facilities, or even pine foliage reflecting fluoride accumulation during one growing season as illustrated in this study (1979 season), are inadequate to assess fluoride effects. Since fluoride is an accumulative toxicant and the foliage of pines is persistent for up to three years, injury may not be manifest until after several years exposure as illustrated in the old needles (1978-77 seasons). Results of this study suggest that a detailed analysis of pine foliage according to the year of needle initiation has potential as an environmental indicator to assess longterm, additive (2-3 years) fluoride effects and should be incorporated in monitoring procedures. Where pines are not a component of the native vegetation, indicator plots with selected species can be established and maintained for routine sampling.

Bibliography


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EVALUATION OF EXPOSURE TO FLUORIDE AND ITS COMPOUNDS ON CHILDREN LIVING IN THE AREA OF ALUMINUM PLANTS

by

A. Rydzewska, A. Kaniewski, Z. Piejsierowicz, M. Chmielnik, and F. Cyplik
Poznan, Poland

SUMMARY: To evaluate the degree of exposure to fluoride and its compounds on the children residing in the vicinity of an aluminum smelter, concentrations of fluoride in urine and tooth enamel were measured and radiograms of hands and forearms were made. The urinary fluoride levels were 30% higher than in the control group. Likewise, levels of fluorides in enamel were higher in the exposed group. No radiological changes in the bones were found.

Introduction

Aluminum smelting creates problems related to air pollution by fluorides involving the health of populations. It may conceivably interfere with growth in children. We therefore investigated the effect of fluoride emission on children residing near an aluminum factory.

Material and Methods

Sixty-one children aged 6 - 11 residing in the polluted area since birth were examined (Table 1). In those in whom urinary fluoride concentrations were highest fluoride levels of tooth enamel were measured and bones were examined radiologically. The results were compared with those of a control group of the same age and socio-economic status, but from a typically agricultural area. The urinary assays were determined with a combination ion selective electrode Orion Research model 96-09 and digital pH millivolt meter Mera-Elwro N-517 (1-4). Biopsy of the tooth enamel was made by the method of Brudevold, et al. (5).

Table 1
Age and Sex in the Survey Groups

<table>
<thead>
<tr>
<th>Survey Group</th>
<th>Number of Examined Children</th>
<th>Age Years</th>
<th>X ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum</td>
<td>Male 15, Female 16</td>
<td>31</td>
<td>97/12±1/12</td>
</tr>
<tr>
<td>Smelter Area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Male 12, Female 23</td>
<td>35</td>
<td>97/12±1/12</td>
</tr>
</tbody>
</table>

From the Institute of Social Medicine, Institute of Radiology, Institute of Stomatology, Medical Academy, Poznan, Poland. Presented at the 11th I.S.F.R. conference, April 8-10, 1981, Dresden, G.D.R.
The fluoride concentration of the tooth enamel was determined by the same instruments that were used for urine, the weight of the enamel was calculated on the basis of calcium concentration. For the calcium assays, we used the Atomic Absorption Spectrophotometer Carl Zeiss Jena model AAS-1 (5,6). The right hand and forearm were chosen for the radiograms in order to avoid irradiation of gonads (7,8). On the basis of radiograms we studied:

1. Qualitative evaluation of bone:

Individuated bones showed osteoporosis, osteosclerosis and correct structure in which fine trabecular and thick trabecular constitution was separated. Besides attention was paid to the outline of the bone whether it was sharp or blurred.

2. Quantitative evaluation of bone:

The thickness of cortex and marrow cavity of the 3rd and 4th metacarpal bones was measured in the narrowest places of the shafts. Also cortex-marrow ratio was calculated.

<table>
<thead>
<tr>
<th>Table 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of Fluoride in Urine mg/l</td>
</tr>
<tr>
<td>Survey Group</td>
</tr>
<tr>
<td>Aluminum Smelter Area</td>
</tr>
<tr>
<td>Control</td>
</tr>
</tbody>
</table>

Results

Concentrations of fluoride in urine from children living near the aluminum smelter were 30% higher than those in the control group, a statistically significant difference (Table 2). Concentrations of fluoride in tooth enamel from the children living in the vicinity of the aluminum smelter were higher than in controls, but the difference was not statistically significant (Table 3).

According to Table 4, in 29.04% of children living in the aluminum smelter area, concentrations of fluoride in tooth enamel exceed 800 µg/g and in the control group, only 12.12% exceed this value.
Table 3
Concentration of F⁻ in Tooth Enamel μg/g

<table>
<thead>
<tr>
<th>Survey Group</th>
<th>Number of Samples</th>
<th>Median</th>
<th>Mean ± S.D.</th>
<th>C.V. (%)</th>
<th>Min. - Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum Smelter Area</td>
<td>31</td>
<td>500.0</td>
<td>610.5 ± 405.8</td>
<td>66.47</td>
<td>142.0 - 2022.4</td>
</tr>
<tr>
<td>Control</td>
<td>33</td>
<td>363.6</td>
<td>450.8 ± 284.9</td>
<td>61.18</td>
<td>105.3 - 1250.0</td>
</tr>
</tbody>
</table>

Table 4
F⁻ Concentrations in Tooth Enamel

<table>
<thead>
<tr>
<th>F⁻ μg/g</th>
<th>Relative Frequency %</th>
<th>Relative Frequency %</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 300</td>
<td>9</td>
<td>29.03</td>
</tr>
<tr>
<td>301-500</td>
<td>6</td>
<td>19.35</td>
</tr>
<tr>
<td>501-800</td>
<td>7</td>
<td>22.58</td>
</tr>
<tr>
<td>801-1200</td>
<td>6</td>
<td>19.36</td>
</tr>
<tr>
<td>&gt;1200</td>
<td>3</td>
<td>9.68</td>
</tr>
</tbody>
</table>

Results of radiological investigations: Thick trabecular structure of bones in the children living near the aluminum smelter was found in 43.5% of cases and, in the control group, in 40% of the cases. Fine trabecular structure of bones in the children living near the aluminum smelter was found in 56% of cases and in 60% of controls. No case of osteoporosis, osteosclerosis and blurred outline of bones was found. Periosteal reaction, as crista interossa, was found in two children living near the aluminum smelter. No statistically significant differences between mean values of thickness of cortex were found (Table 5). Mean values of width of marrow cavity did not differ (Table 6) nor did mean values of cortex-marrow ratio (Table 7) with respect both to 3rd and 4th metacarpal bone.

Discussion
Higher excretion of fluoride in urine than in controls and higher concentrations of fluoride in tooth enamel in the children residing in the neighborhood of the aluminum smelter may be due to exposure of this group to fluoride and its compounds emitted by the aluminum works. They may be especially due to pollution of the air and foodstuffs produced in...
Table 5  Thickness of Cortex

<table>
<thead>
<tr>
<th>Group</th>
<th>Bone</th>
<th>Mean±S.D.</th>
<th>C.V.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum</td>
<td>3</td>
<td>3.11±0.43</td>
<td>13.4%</td>
</tr>
<tr>
<td>Smelter Area</td>
<td>4</td>
<td>2.57±0.43</td>
<td>16.73%</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>2.68±0.40</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Table 6  Width of Marrow Cavity

<table>
<thead>
<tr>
<th>Group</th>
<th>Bone</th>
<th>Mean±S.D.</th>
<th>C.V.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum</td>
<td>3</td>
<td>3.71±0.60</td>
<td>16.1%</td>
</tr>
<tr>
<td>Smelter Area</td>
<td>4</td>
<td>3.36±0.62</td>
<td>18.45%</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>3.07±0.46</td>
<td>14.98%</td>
</tr>
</tbody>
</table>

Table 7  Cortex-Marrow Ratio

<table>
<thead>
<tr>
<th>Group</th>
<th>Bone</th>
<th>Mean±S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum</td>
<td>3</td>
<td>0.92±0.23</td>
</tr>
<tr>
<td>Smelter Area</td>
<td>4</td>
<td>0.78±0.16</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>0.89±0.19</td>
</tr>
</tbody>
</table>

The results of the investigations herewith presented are difficult to compare with results obtained by other authors, whose work is concerned especially with the influence of toothpastes, mineral waters and potable water containing varying concentrations of fluoride (9-14).

Conclusions

1. Concentrations of fluoride in urine and tooth enamel in the children aged 6 - 11 residing in the vicinity of aluminum factories were higher than in controls.

2. Radiological evaluation of hand and forearm bones of these children gave no essential information about the degree of exposure to fluoride and its compounds.

3. In areas with elevated emission of fluoride compounds, endogenous anti-caries prophylaxis with fluoride should not be undertaken.

4. Analysis of the fluoride concentration of potable water, determination of fluoride excreted with urine and detailed stomatological investigations should precede introduction of anti-caries prophylaxis with fluoride.

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Bibliography


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TRACE ELEMENTS IN SERUM AND BONE IN ENDEMIC GENU VALGUM: A MANIFESTATION OF CHRONIC FLUORIDE TOXICITY

by

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SUMMARY: Endemic genu valgum has been identified as a clinical manifestation of fluoride toxicity. Serum levels of copper, zinc, calcium and magnesium were estimated in subjects with endemic skeletal fluorosis, endemic genu valgum and in corresponding age-and sex-matched controls. Bone samples obtained during surgical correction were analyzed for trace minerals in subjects with endemic genu valgum and in controls. No difference was found in serum levels of copper, zinc, calcium and magnesium in the study groups compared to controls. The level of fluoride in bones obtained from the National Institute of Nutrition, Indian Council of Medical Research, Jamai Osmania, Hyderabad, India.
from subjects with endemic genu valgum was twice as high as that found in bones of controls whereas levels of copper and manganese were markedly reduced. The possible significance of low copper values is discussed.

**Introduction**

Endemic genu valgum has been identified as a manifestation of chronic fluoride toxicity in some areas of India (1). This newer manifestation of skeletal fluorosis, seen mostly in adolescents with a marked predilection for males, is characterized by simultaneous occurrence of osteosclerosis of the spine and osteoporosis of the long bones (2). Earlier studies have shown that, in endemic genu valgum, there is secondary hyperparathyroidism (3). According to epidemiological studies prevalence of genu valgum in high fluoride endemic areas was significantly higher (4%) among those whose staple was sorghum, than among those whose staple was rice (1%) (4). Analysis of food grains, habitually consumed by subjects with endemic genu valgum, had suggested that altered trace element metabolism may have an etiological role. Sorghum and pearl millet grown and consumed in endemic areas contained higher amounts of molybdenum than that present in rice. Also, molybdenum content of sorghum and pearl millet grown in fluoride areas was significantly higher than that in sorghum and pearl millet grown in nonfluorotic areas in India (5). In addition, there was a strong negative correlation between the concentration of copper in drinking water and the prevalence of genu valgum in areas endemic for fluorosis (6). In view of the well-known relationship between high molybdenum intake and increased excretion of copper (7), a study was undertaken to determine the content of some relevant trace elements including copper in the serum and bones of subjects with endemic genu valgum.

**Material and Methods**

**Trace elements in serum:** Nine subjects with genu valgum between 16 years and 21 years of age (mean 18 years) and twelve subjects suffering from skeletal fluorosis ranging in age between 40 and 48 years (mean 45 years) drawn from villages where the fluoride content of drinking water ranged from 8 mg to 14 mg per liter were investigated. Fifteen subjects whose average age was 18 years, residing in a control village where the fluoride concentration of drinking water was 1.4 mg per liter, served as a control group for endemic genu valgum subjects. Ten subjects residing in the control village whose average age was 42 years, constituted the control group for subjects with skeletal fluorosis. Blood samples were obtained from these individuals, serum was separated and kept frozen until analyzed for copper, zinc and magnesium concentrations. Serum was appropriately diluted with deionized water before analysis by atomic absorption spectrophotometry (Varian Techtron Model 100). All necessary precautions for trace element analysis were taken. Serum calcium was estimated by a standard chemical method (8).

**Trace elements in bone samples:** Twenty subjects suffering from genu valgum, residing in villages in which the fluoride content of drinking
water ranged from 6 mg to 14 mg per liter were hospitalized for the purpose of surgical correction of the deformity. X-rays of the spine, knee joints, ankle joints, and forearm were taken. All subjects had varying degrees of osteosclerosis of the spine, calcification of interosseous membranes and osteoporosis of the lower end of femora and upper end of tibia. Five consecutive 24-hour urine collections were made and the fluoride content was estimated by means of ion ion analyzer fluoride electrode (94-09). Thirty seven samples of cortical bone were secured during surgical procedure either from the upper end of the tibia or from the lower end of the femur (osteoporotic bones) and preserved at -4°C until analyzed. A group of six age and sex matched subjects who had genu valgum due to reasons other than fluorosis and who had resided since birth in nonfluorotic areas were also subjected to corrective osteotomy during which eight samples of bone were secured from sites similar to those in the endemic genu valgum patients.

Bone samples were weighed and dried to constant weight to obtain the moisture content. Known quantities of dry bone were ashed to constant weight in a muffle furnace at 450°C until complete ashing was achieved. The whole ash was completely dissolved in 6N HCl and this solution was appropriately diluted with deionised water before analysis using an atomic absorption spectrophotometer. In the case of copper and manganese no dilution was necessary whereas the dilution needed in the case of zinc was 1:20 or 1:40, as the case may be, and for magnesium 1:2000. Calcium and phosphorus were estimated by chemical methods. Fluoride in mineral solution was estimated by using ion specific fluoride electrode after appropriate dilution (9).

Results

Extensive osteosclerosis, calcification of ligaments and of interosseous membrane of the forearm were seen in all subjects of skeletal flu-

<table>
<thead>
<tr>
<th>Group</th>
<th>Copper (µg/l)</th>
<th>Zinc (µg/l)</th>
<th>Calcium (mg/l)</th>
<th>Magnesium (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endemic genu valgum (9)</td>
<td>1128±72.2</td>
<td>847±113.8</td>
<td>85.3±3.1</td>
<td>38±2.3</td>
</tr>
<tr>
<td>Controls for ECV (19)</td>
<td>1037±49.5</td>
<td>685±42.0</td>
<td>92 ±1.8</td>
<td>37±1.8</td>
</tr>
<tr>
<td>Skeletal fluorosis (12)</td>
<td>1175±50.9</td>
<td>723±57.3</td>
<td>86 ±2.1</td>
<td>38±1.2</td>
</tr>
<tr>
<td>Controls for S.F. (14)</td>
<td>1075±34.7</td>
<td>765±48.3</td>
<td>93 ±1.5</td>
<td>44±1.6</td>
</tr>
<tr>
<td>Mean ± SEMs; Figures in parenthesis indicate number of subjects investigated.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Endemic genu valgum subjects showed evidence of osteoporosis of the limb bones in addition to osteosclerosis of the spine. The results of analysis of serum samples are presented in Table 1. No significant differences were found between the mean values for serum copper, zinc, magnesium and calcium in the endemic genu valgum group and the age-and sex-matched control group. Similarly the mean serum values for these four parameters did not differ between subjects suffering from osteosclerotic skeletal fluorosis and the corresponding age-and sex-matched control subjects. The scatter of values is shown in Figure 1.

Urinary fluoride in skeletal fluorosis ranged between 8 mg and 22 mg/24 hours while in endemic genu valgum subjects it ranged from 6.8 - 16 mg/24 hours. In contrast, values for control subjects were between 1.2 and 1.8 mg/24 hours.

Results of analysis of bone samples are presented in Table 2. The mean fluoride content of bones in the endemic genu valgum group was twice as high as the mean value observed in the control samples (P < 0.001).
Table 2
Biochemical Analysis of Bone Samples Obtained During Surgical Correction

<table>
<thead>
<tr>
<th>Group</th>
<th>Values Dry wt</th>
<th>% ash</th>
<th>Zn</th>
<th>Cu</th>
<th>Mg</th>
<th>Mn</th>
<th>F</th>
<th>Ca</th>
<th>P</th>
<th>Ca/P</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endemic genu valgum</td>
<td>Mean</td>
<td>1.42</td>
<td>41.8</td>
<td>81.0</td>
<td>1.3**</td>
<td>3281* 0.6**</td>
<td>7283**</td>
<td>188</td>
<td>76.5</td>
<td>2.4</td>
</tr>
<tr>
<td>(37)</td>
<td>SE</td>
<td>0.11</td>
<td>1.94</td>
<td>4.05</td>
<td>0.08</td>
<td>164</td>
<td>0.04</td>
<td>416</td>
<td>11.16</td>
<td>3.73</td>
</tr>
<tr>
<td>Nonflorotic controls</td>
<td>Mean</td>
<td>0.90</td>
<td>49.9</td>
<td>96.8</td>
<td>3.7</td>
<td>4768</td>
<td>1.2</td>
<td>3190</td>
<td>232.8</td>
<td>85.7</td>
</tr>
<tr>
<td>(8)</td>
<td>SE</td>
<td>0.21</td>
<td>5.34</td>
<td>11.2</td>
<td>0.80</td>
<td>1275</td>
<td>0.30</td>
<td>705</td>
<td>39.2</td>
<td>7.66</td>
</tr>
</tbody>
</table>

Values are mean and Sems. Figures in parenthesis indicate number of samples analyzed. *P 0.05; **P 0.001.

The concentrations of both calcium and phosphorus were lower in the endemic genu valgum group but not statistically different from control values. The Ca:P ratio was not different from that observed in the control group. The ash content was lower by 20% in the endemic genu valgum group. Whereas the difference between the two groups in the values for zinc was not significant, mean values for copper (P < 0.001) and manganese (P < 0.001) were significantly lower in the endemic genu valgum group, namely 1/3rd and 1/2 of the respective control values. Magnesium was also less in the endemic genu valgum group (P < 0.05).

Discussion

Subjects with endemic genu valgum had significantly larger amounts of fluoride in their bones than the controls. This, along with the observation that they excreted large quantities of fluoride in urine, confirms that they had been suffering from chronic fluoride toxicity. In addition to radiological evidence of osteoporosis, the chemical analysis of bones of subjects with endemic genu valgum showed evidence of reduced inorganic mineral mass per unit dry weight of the bone, but a normal Ca:P ratio. The most significant observation in the study is that the copper content of osteoporotic bone samples obtained from the lower limbs in endemic genu valgum subjects was significantly lower than that seen in the controls. Whether this is a specific attribute of bone in subjects of endemic genu valgum has yet to be investigated.

Serum levels of copper were however normal in subjects with endemic genu valgum. It is possible that homeostasis mechanisms may have been responsible for this.
Copper deficiency had been reported to lead to osteoporosis in cattle which habitually grazed on copper-deficient pastures or on pastures which contained high molybdenum (10). Also, changes similar to those seen in rickets have been described in dogs exposed to high environmental molybdenum which is known to lead to secondary copper deficiency (11). Interestingly, ricket-like changes (genu valgum and genu varum) were recently identified in 3 dogs residing from birth in the same villages where the subjects of endemic genu valgum investigated in this study had been residing (12). This suggests that the same environmental factors may have been responsible for the pathogenesis of genu valgum in both humans and dogs in the area investigated.

Copper is known to have a role in the maturation of body collagen including that of bone. Copper deficiency in human infants has, in fact, been reported to cause osteoporosis of bones in addition to anemia. Isolated copper deficiency in adulthood however appears to be rare but secondary copper deficiency may occur under some circumstances. Earlier reports indicated that sorghum grown in areas endemic for fluorosis and genu valgum had high concentrations of molybdenum (5).

It had also been reported earlier that high molybdenum intake in humans was associated with increased loss of copper from the body (13). It is not known whether these factors have played a role in the observed low copper values in osteoporotic bones of subjects with genu valgum. Also, it is not known whether in the face of increased fluoride ingestion, the effects of molybdenum on copper is exaggerated. Earlier observation that an inverse correlation exists between the prevalence of genu valgum in a village and the copper content of water used for drinking and irrigation therefore assumes importance in the fluorotic areas in Southern India. The observation that endemic genu valgum develops in a community only during the rapidly growing phase of adolescence indicates the possibility that inadequacy of some important factors during the critical period of rapid bone growth is responsible for the genesis of genu valgum. Whether the high content of fluoride coupled with copper deficiency in the target tissue namely bone, plays such a role needs to be further investigated.

Bibliography

FLUORIDE LEVEL IN WHOLE BLOOD AND URINE DURING D-PENICILLAMINE THERAPY OF RHEUMATOID ARTHRITIS

by

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SUMMARY: In 10 female patients with classical rheumatoid arthritis, D-Penicillamine (DPA) was administered in a daily dose of 150-750 mg for 4 months. The only additional antiphlogistic treatment was Indomethacine (100-150 mg daily). At the beginning and during the trial, levels of fluoride in urine and in total blood were determined by means of the fluoride ion selective electrode. The blood level had increased toward the end of the experiment (after 4 months). This increase was associated with a slightly higher urinary fluoride elimination. The implications on DNA-metabolism are discussed.

Introduction

Previous investigations have shown, that DPA-therapy may affect the mineral metabolism (1), mainly because of the ability of this compound to form a chelate binding with divalent cations. An influence of DPA on the replicative DNA-synthesis was also confirmed (2). Since the DNA-polymerases require Zn++, we assume a correlation between increased Zn++excretion during the DPA-therapy and the Zn++ deficiency of the DNA-polymerases(3). Fluoride ions cannot form a chelate binding with DPA, but correlations within the mineral metabolism of cells can cause a change in the concentration of the fluoride ions. In in-vitro tests, a concentration of 0.05 M fluoride ions can cause an inhibition of the DNA-polymerase reaction up to 90% (4).

From the Institut Für Biologie, Seibersdorf and Medizinische (Rheuma) Abteilung im Krankenhaus der Stadt Wien-Lainz, Austria. Presented at the 11th I.S.F.R. Conference, April 8-10, 1981, Dresden, G.D.R.

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At the beginning of this investigation we analyzed the fluoride level in blood and urine during DPA therapy. We desired to determine a possible influence, because fluoride ions which have a high affinity to the electrons and are physiologically more active than the other anorganic anions, can influence enzymatic processes in very low concentrations.

**Material and Method**

As a F⁻-selective electrode, a Coleman-electrode and as a reference electrode, an Orion electrode (No. 90-02) were used. The potential difference was measured by a Digital-pH-meter (Orion, Model 701) with expanded mV-range.

For the calibrating curve and sample preparation the following chemicals were used:

- Sodium fluoride (NaF) p.A., Merck No. 6449
- Trisodiumcitrichydrate (C₆H₅Na₃.2H₂O) p.A., Merck No. 6448
- Perchloric Acid (HClO₄) 60%, p.A. Merck No. 518
- Hydrochloric acid (HCl) min. 37%, p.A., Merck No. 317
- NaF-standard solution: 211.1 mg NaF filled with one liter H₂O distilled (100 ppm F⁻)
- Citratebuffer: 59.0 g Tri-Sodiumcitrate 2-hydrate + 8.5 ml HClO₄ filled with 250 ml H₂O distilled, pH = 5.5

**Blood and Urine:** To 10 women with classical rheumatoid arthritis (cP) according to ARA-criteria doses from 150 to 750 mg per day (Artamin⁰, capsules of 150 or 250 mg from Biochemie) were given. The mean age of the patients was 60.5 years and the mean duration of the disease was 4.5 years. They received during the 1st month, 150 mg Artamin⁰ per day; 250 mg in the 2nd, 375 mg the 3rd, (1-2 capsules of 250 mg alternatingly per day) and 500-750 mg per day in the 4th month.

Only Indocid⁰ 100-150 mg per day was administered as a supplement. It was started 3 months prior to the initiation of the investigation. None of the patients were afflicted with any other diseases. Blood samples were taken at the beginning and after 1, 2, 7, 14, and 21 days and after the 2nd and 4th month; 24-hour urine specimens were analyzed and compared with a control sample taken at the beginning, after the 1st, 2nd, 7th, 14th, and 21st day and after 2 and 4 months.

**Calibration Curve:** A calibration curve was made by adding 5 ml of various suitable dilutions of NaF concentrations + 10 ml citratebuffer to samples (5). All measurements were made with the F⁻-selective electrode in polyethylene tubes. A constant response of the electrode to the F⁻ concentrations was reached approximately after 30 minutes. The room temperature was always about the same, making it unnecessary to use a thermostat to keep the tubes at the same temperature. The calibration curve of blood shows a concentration between 0.015 - 10.0 μg F⁻/ml and the calibration curve of urine, between 0.02 - 10.0 μg F⁻/ml in a semi logarithmic plot.

**Sample Preparation and Measurement:** The F⁻ content of urine samples was analyzed according to the method of Tusi (6,7). Five milliliters of
urine was added to 10 ml of citrate buffer and measured directly. To samples with a very low F concentration (<0.1 μg/ml) a defined amount of F⁻ was added with a micropipette and the analysis was repeated, then the original F content was calculated. A comparison of these double measurements was satisfactory. This showed also that, because of the relatively high concentration of the citrate buffer, the ionic strength of urine samples and of the standards, used for the calibrating curves, was about the same. Besides an evaluation of the precision of the analysis can be made, which lies at about ±10% like the reproducibility found in some tests.

Blood samples were analyzed according to the method of Singer (8). We used the same buffer system plus 10 ml distilled water as in the urine tests instead of acetate buffer. The same calibrating curves as for the urine samples were used.

Results

Samples of blood and urine taken prior to the initiation of the therapy showed F levels in the normal range according to literature (9). The values of urine samples varied on the 1st, 2nd, 7th, and 21st day. After 2 months they were about 229.0 μg/24-hr, after the 4th month they had increased to 684.7 μg/24-hr. (Figure 1). The blood level of fluoride during the DPA therapy (Figure 2) was between 0.08 μg/ml on the 7th day and 0.34 μg/ml (in the fourth month). Hamilton (10) found 0.019 μg/ml in whole blood, Hartmann (11) on the contrary found 0.44 μg/ml. Table 2, Figure 2, show that the F level in blood was increased at the end of the DPA therapy.
Blood $F^-$ in Penicillamine Therapy

Discussion

The $F^-$ concentration in blood and urine increased slightly towards the end of the experimental period of the DPA therapy; during the first 7 days the decline was not statistically significant. Both body fluids show a similar trend, therefore these changes could be influenced by a cellular or an intracellular pool of fluoride. An imbalance of DNA excision repair in peripheral lymphocytes was detected (12) in cP patients in earlier investigations. On the other hand DPA treatment does not affect unscheduled DNA synthesis. Only the replicatory DNA synthesis is inhibited (2). This could be a positive process concerning the tumor-like hypertrophy of marginal synovial cells and the inhibition of lymphoblast development, with a pronounced increase of antibody synthesis in this disease.

The observed changes could be important by influencing the pathogenesis of cP by DPA, because even small changes in the concentration of fluoride lead to either positive or negative effects. ATP synthesis, which is a central factor in the energy providing system, as well as the synthesis of other precursors of nucleid acids are extremely sensitive to fluoride ions.

The regulatory mechanism in the human body seems to hold the NaF content in blood within narrow limits (13).

The excretion of fluoride in urine and the fluoride content in drinking water were in direct correlation (14). The mean normal ranged between 0.3 ppm and 1 ppm fluoride. The fluoride level in blood plasma of control persons, whose drinking water contained 0.15 - 2.5 ppm fluoride was between 0.14 - 0.19 ppm. When drinking water contained more than 5.4 ppm fluoride, the fluoride plasma concentration increased to 0.26 ppm.

NaF therapy of osteoporosis does not influence the DNA metabolism of the peripheral lymphocytes, even at high doses (15). On the other hand, in vitro cells incubated at a concentration of 0.4 $\mu$g/ml, immediately showed a clear influence to the DNA excision repair and to the phosphorylation of the DNA precursors. At a concentration of 1.0 $\mu$g/ml the DNA, RNA and protein synthesis is significantly inhibited and there are changes in the monophosphokinase-step of the nucleotid-phosphorilization (3).

When mice receive 0.4 $\mu$g/ml fluoride in drinking water an inhibition of the analyzed synthesis only occurred after ten weeks of the test but the DNA repair was not affected. Only after concentration, the spleen cells of mice showed DNA repair inhibition after 10 weeks (3).

To summarize, it can be said that of foremost interest is the increase in the fluoride ion concentration in blood. However it cannot be said whether the effect on the cP is positive or negative.
Bibliography


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FLUORIDE TRANSPORT DURING REGULAR HEMDIALYSIS PROGRAM WITH FLUORIDATED WATER

by

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SUMMARY: Hemodialysis by fluoridated dialysate is known to cause a drastic elevation especially of venous but also of arterial plasma fluoride levels because of penetration of fluoride through the semipermeable membrane in the dialysis apparatus. The effects of longterm hemodialysis on fluoride kinetics have been studied hardly at all, although bone tissue toxicity may be the most prominent adverse effect of a longterm dialysis program. Because fluoride is accumulated in the bone, it is probable that calcified tissues in the course of longterm hemodialysis become slowly saturated. This should result in a gradually reduced transfer of fluoride during consecutive hemodialysis, as some earlier findings have suggested.

From the Department of Pharmacology and Department of Nephrology, University of Kuopio, Finland.
In sixteen hemodialysis patients, the ionic arterial and venous plasma fluoride levels were measured before, during and after hemodialysis by means of the fluoride electrode.

The results showed a strong direct correlation between arterial plasma fluoride levels before and during each hemodialysis. On the other hand earlier findings (and equally well the present ones) indicate that the predialysis plasma fluoride levels increase gradually in the course of time which the patient participates in the regular hemodialysis program. The present calculations therefore clearly indicate that fluoride transfer during hemodialysis is strongly dependent on arterial plasma fluoride concentrations, which determine the size of concentration gradient favorable for fluoride transfer. Because the concentration gradient seems to diminish very clearly in the course of regular hemodialysis treatments, also the amount of transferred fluoride is reduced. According to the present calculations it seems probable that after about two years of regular hemodialysis treatments only little if any of the reduced amount of transferred fluoride is accumulated in the bone tissue.

Introduction

Since the first valid finding by Taves et al. in 1968, it has been repeatedly demonstrated that human ionic plasma or serum fluoride concentrations increase considerably during hemodialysis, if the dialysate is fluoridated to 1 ppm (1,2,3,4). Usually the venous blood fluoride levels increase more rapidly than arterial, because fluoride easily permeates the semipermeable membrane in the dialysis apparatus and thus enters the bloodstream. The fluoride concentration in venous blood generally achieves a level of about 2/3 of the dialysate inlet fluoride concentration. In arterial blood the slower increase in fluoride levels results from its distribution into tissues, especially into bones, which clears part of the plasma fluoride transferred through the dialysis membrane (5,6,7).

It has also been suggested that the arterial blood fluoride concentration during hemodialysis has a tendency to increase to the higher level, the longer the patient has been involved in the regular hemodialysis program (3). Therefore it can be expected that, in the course of longterm hemodialysis, the patients gradually store less fluoride in their skeletons because of the smaller concentration gradient. Only few calculations have been made concerning fluoride transfer to the patients in the course of hemodialysis and the changes in fluoride retention may have been partially masked due to variability of the hemodialysis equipment used in the studies (1,3). Therefore the aim of the present study was to detect changes, especially in arterial plasma fluoride levels, during regular hemodialysis in order to observe trends in fluoride retention in a more universal way by reducing interindividual variations of the results by a special approach.
The subjects of the study were 16 patients hemodialyzed with fluoride dialysate at the University Central Hospital of Kuopio, Finland. The fluoride concentration in the drinking water in the city of Kuopio is about 1.0 - 1.2 ppm. Twelve of the individuals suffered from chronic renal failure and 4 were acute patients needing only a few hemodialyses. The diagnosis and the duration of regular hemodialysis for each patient are given in Table 1.

The hemodialysis apparatus was Gambro AK 5, a flat plate dialyzer equipped with cuprophone membrane. The dialysate flow was 600 ml/min. dialysate contained 135 m mol/1 sodium, 2 m mol/1 potassium, 1.75 m mol/1 calcium, 0.5 m mol/1 magnesium, 106.5 m mol/1 chloride, 35 m mol/1 acetate and a mean of 1.18 ppm fluoride. Moreover, all chronic patients used aluminum gel 20 - 40 ml with meals. No vitamin D was given. The usual duration of each dialysis was 3 hours performed 3 times per week. In other words, each patient received hemodialysis treatment for 9 hours weekly.

The blood and dialysate fluoride concentrations were measured with a Radiometer fluoride electrode (F 1052) and a calomel reference electrode connected to a digital mV-meter each manufactured by Radiometer Company (Copenhagen, Denmark). The buffer for the adjustment of the pH to about 5 was 5M acetate - 0.5 M citrate-buffer. (The buffer was made by dissolving 246.1 g sodium acetate and 149 g sodium citrate dihydrate in about 600 ml of deionized water. Then 114 ml strong acetic acid was added, whereafter the solution was diluted to one liter.) For each measurement, 1 ml of sample or standard was pipetted in a specially manufactured container and 100 µl of buffer was added. During the measurement, the sample was continually stirred with a magnetic rod. The electrode potential was considered stabilized when the digital reading (0.1 mV accuracy) remained constant for at least one minute. Each sample was measured at least twice, if the sample volume was sufficient. The recovery varied between limits of 99.3 - 105.8% with the mean value of 102.3%, when the fluoride concentration of the sample ranged from 0.010 - 0.100 µg/ml. At the lower concentration range of 0.002 - 0.008 µg/ml the recoveries were only 78.3 - 102.3% with the mean value of 89.4%.

Results

The principal mean values ± S.D. are presented in Table 2 or ± S.E. Figure 1 of twenty dialyses performed for ten patients indicated in Table 1. The pattern of the increase in venous and arterial plasma fluoride concentrations is similar to previous findings. The mean venous plasma fluoride level is approximately 2/3 of the mean dialysate fluoride concentration which was 1.18 ± 0.188 µg/ml (±S.D.). After three hours of hemodialysis, the mean arterial plasma fluoride concentration is also close to a plateau level.

Figure 2 illustrates the relation of predialysis arterial plasma fluoride concentration to the period of time which each patient has been involved with regular hemodialysis. The regression analysis for 29 samples
from the 16 patients yielded: slope = 1.78, intercept = 0.078 µg/ml, 4 = 0.718. The correlation coefficient differs highly significantly from zero (p < 0.001).

In order to calculate the effect of the regular hemodialysis on the increase rate of arterial plasma fluoride during hemodialysis the following analysis was performed on the same 20 hemodialyses which are presented in Table 2 and Figure 1: Each available predialysis arterial plasma fluoride concentration was plotted against the corresponding arterial plasma fluoride concentration at 5 min., 60 min., 120 min., and 180 min. during, and 60 min. and 120 min. after hemodialysis. Figure 3 illustrates these six plots. The results of the regression and correlation analyses for Figure 3 are presented in Table 3. Each regression coefficient obtained during the course of hemodialysis is not far from the value 1. This indicates that the arterial plasma fluoride concentration increases at a rather steady rate, which is only slightly modified by the predialysis arterial plasma fluoride levels. In venous plasma fluoride no significant similar correlations could be detected.

To facilitate the further calculations Figure 4 shows the arterial plasma fluoride concentrations for 4 hypothetical average patients with 0 µg/ml (patient 1), 0.100 µg/ml (patient 2), 0.200 µg/ml (patient 3) and 0.300 µg/ml (patient 4) before the start of the dialysis. The graphs have been obtained by using the four arbitrary predialysis arterial plasma fluoride concentrations and solving the regression equations for each six time points in Table 3. By using the mean venous plasma flu-
Table 1

<table>
<thead>
<tr>
<th>Code</th>
<th>Age</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Chronic Nephritis NUD</td>
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<td>Renal Dysplasia</td>
</tr>
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</tr>
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<td>K.H.</td>
<td>42</td>
<td>Acute Tubular Necrosis</td>
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<tr>
<td>S.V.</td>
<td>58</td>
<td>Chronic Nephritis NUD</td>
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</table>
| S.P. | 38  | Chronic Pyelonephr., Renal Hypoplasia S.
| H.R. | 30  | Acute Intoxication                     |
| V.E. | 46  | Diabetic Nephropathy                   |
| L.R. | 25  | Chronic Glomerulonephr.                |
| K.T. | 41  | Chronic Glomerulonephr.                |
| P.J. | 71  | Acute Interstitial Nephritis           |
| P.A. | 47  | Renal Polycystic Degeneration          |
| R.P. | 31  | Acute Intoxication                     |
| P.U. | 33  | Rheumatoid Arthritis, Amyloidosis      |
| K.A. | 39  | Diabetic Nephropathy                   |

All patients, except four acute cases, used aluminum-gel regularly.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Venous (µg/ml±S.D.)</th>
<th>Arterial (µg/ml±S.D.)</th>
</tr>
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<tbody>
<tr>
<td>Before Dialysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.158±0.091</td>
<td></td>
</tr>
<tr>
<td>During Dialysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 min.</td>
<td>0.832±0.134</td>
<td>0.252±0.120</td>
</tr>
<tr>
<td>60 min.</td>
<td>0.838±0.122</td>
<td>0.422±0.119</td>
</tr>
<tr>
<td>120 min.</td>
<td>0.865±0.114</td>
<td>0.568±0.161</td>
</tr>
<tr>
<td>180 min.</td>
<td>0.883±0.110</td>
<td>0.621±0.135</td>
</tr>
<tr>
<td>After Dialysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 min.</td>
<td>0.538±0.129</td>
<td></td>
</tr>
<tr>
<td>120 min.</td>
<td>0.488±0.139</td>
<td></td>
</tr>
</tbody>
</table>

Orn concentration from Table 2 it was possible to calculate the approximate amount of fluoride transferred through the cuprophane membrane for each average patient (venous-arterial blood fluoride concentration multiplied by blood flow rate). The results are given in Table 4. When the predialysis arterial plasma fluoride concentration of the four average patients increases from 0-0.300 µg/ml the transferred fluoride dose through the dialysis membrane markedly decreases in good inverse correlation with predialysis plasma fluoride levels. The average patient 1 obtains 20.0 mg fluoride/hemodialysis and patient 4 only 9.3 mg. According to Figure 2 the four average patients have been participating in the hemodialysis program for 0, 12, 69 and 125 weeks respectively.

Discussion

The findings in Figure 1 and Table 2 clearly demonstrate the similarity of the present results with the earlier ones (1, 2). The mean venous and arterial plasma fluoride concentrations in Figure 1 follow the usual pattern published previously (1, 2). Only the venous plasma fluoride level at 5 min. demonstrates that the increase rate of the fluoride concentration in venous blood is faster than previously expected, providing the membrane permeability is similar. Otherwise no significant differences can be shown, keeping in mind that the mean dialysate fluoride level of the present study was 1.18 mg/l.

The slope indicating the increase of the predialysis plasma fluoride level in the course of the regular hemodialysis program (Figure 2) is also similar to earlier findings of Posen et al. and Siddiqui et al. (7,4). Therefore it is reasonable to conclude that the calculated results may be applicable more universally than just to the present material.

FLUORIDE
F⁻ Kinase in Hemodialysis

Figure 3
Predialysis Arterial Plasma F⁻ During and After the 20 Dialysis.

Actual numbers are given in Table 3

Table 3
Relation of Predialysis Arterial Plasma F⁻ to Arterial Plasma F⁻ During and After Dialysis (Regression and Correlation Analyses)

<table>
<thead>
<tr>
<th>Predial. Plasma F⁻</th>
<th>Slope (µg/ml)</th>
<th>Intercept</th>
<th>r</th>
<th>n</th>
<th>Significance of r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Att. Plasma F⁻ at</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 min.</td>
<td>1.18</td>
<td>0.049</td>
<td>0.810</td>
<td>17</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>60 min.</td>
<td>1.01</td>
<td>0.254</td>
<td>0.781</td>
<td>18</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>120 min.</td>
<td>0.913</td>
<td>0.389</td>
<td>0.618</td>
<td>17</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>180 min.</td>
<td>0.974</td>
<td>0.451</td>
<td>0.652</td>
<td>17</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>60 min. after dialysis</td>
<td>0.746</td>
<td>0.393</td>
<td>0.554</td>
<td>13</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>120 min. after dialysis</td>
<td>1.17</td>
<td>0.273</td>
<td>0.807</td>
<td>9</td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>

Significance calculated as the difference of the correlation coefficient from zero.
Figure 4
Arterial Plasma F⁻ for Four Hypothetical Patients

Table 4
Fluoride Transferred During Hemodialysis

<table>
<thead>
<tr>
<th></th>
<th>5 min (mg)</th>
<th>60 min (mg)</th>
<th>120 min (mg)</th>
<th>180 min (mg)</th>
<th>Total (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>8.2</td>
<td>6.4</td>
<td>5.4</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>Patient 2</td>
<td>6.9</td>
<td>5.2</td>
<td>4.3</td>
<td>16.4</td>
<td></td>
</tr>
<tr>
<td>Patient 3</td>
<td>5.6</td>
<td>4.1</td>
<td>3.2</td>
<td>12.9</td>
<td></td>
</tr>
<tr>
<td>Patient 4</td>
<td>4.3</td>
<td>2.9</td>
<td>2.1</td>
<td>9.3</td>
<td></td>
</tr>
</tbody>
</table>

F⁻ concentrations for arterial and venous plasma based on Fig. 4 and Table 3 (arterial) and Table 1 (venous). Blood flow was 200 ml/min.

According to Figure 3 and Table 3, the relation of predialysis arterial plasma fluoride levels to the arterial fluoride levels during hemodialysis were statistically significant. The higher the predialysis plasma fluoride concentration, the higher also was the arterial fluoride level during hemodialysis. All four correlation coefficients (Table 3) indicated strongly a solid straight regression between the related factors. It is also apparent that the regression coefficients have a slight tendency to decrease during the course of hemodialysis. At five minutes the regression coefficient was above the value of 1 (1.18) whereas at 60 minutes it was 1.01, at 120 minutes 0.913 and at 180 minutes 0.974, respectively. This decreasing tendency reflects the rapid increase in arterial plasma fluoride concentration in those patients having regular hemodialysis for a prolonged period of time. In patients with only a short history of regular hemodialysis the arterial plasma fluoride levels increase more slowly. This variation probably reflects a difference in bone fluoride concentrations. The individuals dialyzed only a few times probably absorb considerably more fluoride in their skeletons than those hemodialyzed a great number of times. At any rate after three hours of hemodialysis all patients seem to have increased their arterial plasma fluoride levels by approximately 0.450 µg/l above their individual baseline levels.

Because the mean venous plasma fluoride levels have been obtained from the same 20 dialyses as the above-mentioned arterial ones, it was also possible to calculate the amount of fluoride transferred through the...
cuprophane membrane. Especially interesting is the modifying effect of predialysis arterial plasma fluoride concentration on the transport. To reduce the other interindividual variations, the concept of the four average patients was developed. As mentioned, the predialysis arterial fluoride levels were 0, 0.100, 0.200 and 0.300 μg/ml and the fluoride transport during a three hour hemodialysis treatment for these patients was 20.0 mg, 16.4 mg, 12.9 mg, and 9.3 mg respectively. Thus it is demonstrated, that the fluoride dose transported through the hemodialysis membrane is strongly dependent on arterial blood fluoride levels. To test the validity of the present approach, some individual results were also calculated. For instance, in patient K.R. predialysis arterial plasma fluoride levels were 0.240 - 0.261 μg/ml which, according to above calculations yielded 9.5 - 12.4 mg. On the other hand, in patient R.P. with a predialysis arterial plasma fluoride level of 0.011 μg/ml, 27 mg was absorbed during his one hemodialysis, which is clearly more than the average calculation of about 20 mg. Therefore it is possible that the regression lines in Figure 3 might have been curved at their lower ends had more material been available.

Because the baseline level of arterial plasma fluoride concentration increases significantly in the course of hemodialysis (Figure 2), as indicated by the above calculations, it can be expected that fluoride transport through the cuprophane membrane decreases considerably during the course of prolonged therapy. According to Figure 2 the average patient number 4 with the baseline arterial fluoride level of 0 – 300 μg/ml would have been participating in the hemodialysis program for more than two years whereas average patient number 1 would have just started. The corresponding figures of fluoride transport during one hemodialysis for these patients were 9.3 mg and 20.0 mg respectively (Table 3). Equally the above-mentioned patient K.R. had been treated for about 100 weeks and patient R.P. had just started. Therefore it can be concluded that fluoride transport considerably decreases during prolonged regular hemodialysis.

It is also possible to roughly calculate the amount of fluoride retention by using a different approach. According to previous findings, it can be expected that the interstitial fluid fluoride level is about 80% of the arterial plasma fluoride concentration (8) and the intracellular fluid fluoride concentrations are roughly one third of the plasma fluoride level (9). Based on the above assumptions, we calculated that a 70kg patient without kidney function needs approximately 9 mg of fluoride for soft tissue distribution to achieve the increase in ionic plasma fluoride from the baseline level by about 0.450 μg/ml as is the case in all average patients of the present study. This figure is similar to the total amount of transferred fluoride for average patient 4 or patient K.R. If the calculation is approximately correct it seems obvious that, after about two years of regular hemodialysis therapy, fluoride accumulation into the bone is greatly reduced. Most of the transferred fluoride seems to be distributed in soft tissue. However, because we did not measure the actual ionic fluoride concentrations in the soft tissues, no final conclusion can be made regarding a possible steady state situation in bone tissue. This calculation should not be construed as a reason for using fluoridated water in regular hemodialysis program.
FLUORIDE CONTENT OF URINE, BLOOD, NAILS AND HAIR IN ENDEMIC SKELETAL FLUOROSIS

by

Algiers, Algeria

SUMMARY: In 79 patients with skeletal fluorosis in Southern Algeria, a direct correlation was observed between the fluoride concentration of drinking water and the biochemical and radiological parameters. The fluoride content of nails represents a good index of fluoride storage which can be useful in the diagnosis of skeletal fluorosis.

Introduction

In a former publication (1), we reported x-ray and biochemical findings in adults residing in an endemic skeletal fluorosis area near El From the El Oued Hospital and Medical Institute, Algiers, Algeria
Skeletal Fluorosis: F⁻ Content

Oued, South Algeria. In the past, relatively little attention has been paid to the fluoride content of skin, nails, and hair compared to fluoride assays of bone, teeth, aorta, kidneys and bladder (2). The purpose of this paper is to determine a possible correlation between fluoride in drinking water and urinary and plasma fluoride, the fluoride content of nails and hair, and the skeletal changes in the endemic area of El Oued. We proposed to determine a) whether or not the radiological and biochemical changes of skeletal fluorosis correlate with the concentration of fluoride in drinking water which varied from 1 to 4.5 mg/l fluoride inside the endemic zones, and b) whether fluoride assays of nails and hair can serve as a parameter of fluoride storage similar to the use of skeletal radiography (3, 4). The assays would be less injurious than bone biopsy (5).

Materials and Methods

The Subjects: The study was carried out in April 1980 on 98 adults, men and women, 19 of whom had for 30 to 60 years been permanently residing in "low fluoride" Algiers and 79 in the natural fluoride region (1 to 4.5 ppm fluoride). They were divided into four groups. Group 1 (N) in Algiers with 0.2 ppm fluoride in drinking water served as a control. The other three groups resided inside the El Oued endemic zone namely, Group A in communities with 1 to 2 mg/l fluoride in water, Group B with 2 to 3.5mg/l and Group C with 4.5 mg/l. Figure 1 shows the location of the areas A, B and C in the environs of El Oued, a town included in Group A. Its water is relatively pure because it is derived from deep wells ("borings"). We estimated that the mean total daily fluoride uptake from food and water in sedentary subjects in spring ranges between 10 and 20 mg per day, considering that daily water consumption is greater in summer than in winter, that persons with sedentary habits require less water and food and that they consume high fluoride tea and dates.

![Figure 1: Fluorosis Endemic Zone](image)

F⁻ in drinking water in different areas

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January 1982
Biochemical Study: The fluoride content of the 24-hr. drinking water and urine was measured by the specific electrode method (8). Fluoride in blood, nails and hair were assayed by the same method after alkaline mineralization (9).

Skeletal Radiography: Complete skeletal radiography was performed on each patient with the same technique. We followed the classification of Pinet et al. (3) namely 0 when radiologically negative and grade I, II, III to indicate skeletal changes according to their degree of severity.

Results

The comparison of the skeletal x-ray changes between the control(N) group (Algiers) and the A, B, and C groups inside the El Oued endemic zone, is presented in Figure 2. In the control group N, 95% had no skeletal abnormalities but 5% showed minor sclerotic bone changes of unknown origin. Stage I fluorosis was less pronounced in Group A and B than in the control group N (p 0.05) and occurred in group C in only 4.55%. On the other hand the incidence of Stages II and III fluorosis was higher in groups A and B than in the control group N (p 0.05) and reached 54.55% in group C. In the endemic zone, skeletal damage of stages II and III was more frequent in group C compared with A (p 0.01).

Urinary fluoride excretion was considerably higher in the three groups A, B, and C compared to the control (p 0.001) (Fig. 3). This increase was more pronounced in group C than in A (p 0.05). Fluoreemia was markedly elevated in the three endemic groups A, B, C (p 0.001), but there was no difference in the serum fluoride levels between the subjects of C and A in the communities within the El Oued endemic zone. The fluoride.
content of nails (Fig. 4) showed a gradual increase in the A and B patients (p 0.10 or 0.05), but was most significant in group C. In this group, the p value was 0.001 compared with the control group and 0.10 compared with group A. The fluoride content of hair was slightly elevated in groups A, B, and C but did not differ significantly from that of normal subjects.

**Discussion**

In this study we noted a correlation between the concentration of fluoride in drinking water and the degree of skeletal damage and of changes in the fluoride metabolism when compared to the low fluoride control cases in Algiers as well as to those in different areas of the El Oued endemic zone. Even in group A with only 1 to 2 ppm fluoride in water, the elevation of urinary and serum fluoride (Fig. 3) was very pronounced whereas the skeletal changes (Fig. 2) and the fluoride levels of nails (Fig. 4) did not differ materially (p 0.05) from the controls. In a former study we observed that even without skeletal changes, fluoride may have adverse effects, which indicates that biological changes appear prior to damage to the skeleton in a population exposed to fluoride. Skeletal damage and a high fluoride content of nails were substantial in group C. Because of the early development of biological changes in group A, the difference between groups C and A, inside the endemic area, is of greater importance with respect to the development of skeletal damage.

We believe that fluoride assays of nails can serve as a test for fluoride storage (Fig. 4). In group C, the fluoride content of nails appeared to be a highly sensitive parameter compared to the controls. How-
ever the difference between groups C and A was less significant. Herman et al. (2) reported excessive fluoride content of skin, hair and nails in patients with kidney stones. Our results confirm his findings with regard to nails, but not to hair. In subacute fluoride intoxication of rabbits, also, we observed an increase of fluoride in claws but not in hair (10). We consider the fluoride content of nails to be a good indicator for fluoride storage. A 1 to 2 gm sample is sufficient for the assays. Analysis of nails is less injurious than bone biopsy for fluoride and its value might be comparable to that of skeletal radiography.

Bibliography

ABSTRACT

EFFECTS OF HALIDES ON REDUCED NICOTINAMIDE ADENINE DINUCLEOTIDE BINDING PROPERTIES AND CATALYTIC ACTIVITY OF BEEF HEART LACTATE DEHYDROGENASE

by

S.R. Anderson
Corvallis, Oregon


Anion binding sites in beef heart lactate dehydrogenase a nonmetallo-protein enzyme involved in the terminal stages of anaerobic muscle glycolysis, are shown to differ strikingly for fluoride and chloride. Fluoride ion behaves as a competitive inhibitor of the catalytic activity of the enzyme toward pyruvate and enhances binding to the enzyme by the coenzyme NADH (reduced nicotinamide adenine dinucleotide). The author proposes that histidine-195 in the peptide chain of the enzyme, which is believed to hydrogen bond with pyruvate or lactate in the operation of the enzyme, is involved in hydrogen bonding with the highly negative fluoride anion. By contrast, NADH and chloride ion are strongly antagonistic in their interactions with the enzyme. Spectral data clearly demonstrate that chloride binds at sites other than the site(s) occupied by fluoride.

A.W.B.
(Reprints: Department of Biochemistry and Biophysics, Oregon State University, Corvallis, Oregon 97331).

*******

I.R. EVIDENCE FOR A STRONG HYDROGEN BOND IN THE FLUORIDE-URACIL SYSTEM

by

J.H. Clark, and J.S. Taylor
Heslington, York

(Abstracted from J.C.S. Chem. Comm., 466-468, 1981)

A novel 1:1 complex precipitates as a white solid from an 80% dimethyl sulfoxide or NN-dimethylformamide-20% water solution of equimolar amounts of potassium fluoride and uracil, a key component of nucleic acid. Infrared spectral properties of this KF-uracil adduct indicate the presence of a strong asymmetrical NH···F hydrogen bond. The normal N-H stretching frequency at 3050 cm$^{-1}$ in uracil is shifted and split in the complex to three pseudo maxima at ca. 2950, 2600, and 1930 cm$^{-1}$. Although the authors caution that more detailed studies are required, they conclude from their investigation that "The observed ability of fluoride
ABSTRACT

to disrupt the self-association in uracil and to produce strong NH₁₋F⁻ inter-
teractions is obviously of considerable importance to the possible biologi-
ically disruptive action of the fluoride ion.

A.W.B.

(Reprints: Dept. of Chem. Univ. of York, Heslington, York Y01 5DD, U.K.)

**********

COMPARISON OF FLUORIDE BALANCES DURING AMBULATION AND BED REST

by

San Francisco, California


In order to determine differences of fluoride excretion during ambu-
lation and bed rest, six healthy male Caucasian volunteers, 20-45 years of age, were placed on distilled water and a daily conventional diet pre-
pared with distilled water the fluoride content of which averaged 0.36 ±
0.02 mg F/day. One week of equilibration was followed by a 10-week am-
bulatory period and 17 weeks of bed rest. Seven day collections of stools and urine were pooled. The feces were homogenized with a known quantity of distilled water.

The average urinary excretions for the six subjects ranged from 0.38 to 0.90 mg/day during ambulation and from 0.38 to 1.18 during bed rest. The fecal fluoride excretions were low for each subject; they averaged for both periods, between 0.03 to 0.09 mg/day. Urinary fluoride excretion, which varied from one individual to another, averaged 92% whereas fecal was only 8%. The average urinary fluoride excretion was slightly higher than average fluoride intake, presumably because of removal of flu-
oride from bone stores.

(Reprints: School of Dentistry & School of Medicine, University of Calif-
ornia, San Francisco, California 94143).

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Correction: The last paragraph on page 126 and reference 35 on page 128 of R. Ziegelbecker's article "Fluoridated Water and Teeth (14:
123-128, 1981) should be deleted. They were not written by the author and should have been designated editorial comment.
ABSTRACT

THE ACTION OF VARIOUS FLUORIDES ON RAT MAST CELLS: A COMPARATIVE STUDY

by

W. Kazimierczak, and B. Adamas
Cracow and Lodz, Poland

(Abstracted from Archivum Immunologicae et Therapiae Experimentalis, 28: 941-946, 1980).

Pretreatment of mast cells with potassium and lithium fluoride induces release of histamine provided calcium is present.

This study was carried out on mast cells of Wistar rats in two steps. In step 1, cells were incubated for about 15 minutes with various fluorides (potassium fluoride, lithium fluoride and ammonium fluoride), without calcium. In the second step, cells were transferred to new tubes which contained 1 mM calcium for 10 minutes. Following centrifugation, histamine was determined fluorometrically in the supernatant and cell residues. The most potent histamine releaser was potassium fluoride, whereas the action of ammonium fluoride seemed to be cytotoxic.

The release of histamine by potassium and lithium fluoride increased with the increase of the preincubation time. Histamine release was also dependent on the concentration of calcium the optimum of which was around $10^{-3}$. The time course of the calcium-triggered release of histamine from mast cells activated by fluoride, was similar to the secretory process produced by an antigen antibody reaction, or by other mast cell secretagogues. The ability of fluorides to activate mast cell action is parallel to the dissociation constant of the fluoride compound. In other words, histamine release by these compounds seems to be dependent on the availability of fluoride ions for mast cells.

**********

FLUORIDE BRIEF

One to 10 ppm fluoride added to drinking water enhance the activity of carbonic anhydrase in the submandibular salivary gland of rats. With 25 ppm fluoride in drinking water, the fluoride content of the parotid gland increased considerably. It is concluded that fluoride affects the function of the salivary gland.


**********

Volume 15 Number 1
January 1982
ABSTRACT

EFFECT OF FLUORINE COMPOUNDS ON RESPIRATORY CHAIN

by

Z. Machoy
Szczecin, Poland


The author reviews the action of various doses of inorganic and organic fluorine compounds on the respiratory cell chain.

Fluoride inhibits the activity of cytochrome oxidase, succinate dehydrogenase as well as the phosphorylation processes. The inhibition of succinate dehydrogenase is confirmed in vivo on animals.

Fluororganic anesthetics (halothane, methoxyflurane, fluroxane) inhibit the oxidation of NADH-dependent substrates but do not inhibit the succinate oxidation. The mono- and higher-fluorosuccinic acids exhibit an inhibitor effect with soluble succinate dehydrogenase too.

In the final part of the review the effect of fluoride on other terminal oxidases containing copper and on compounds containing heme iron is discussed. The review cites 62 references most of which are written in English.

Author's Abstract

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FLUORIDE BRIEF

A part of the bactericidal action of polymorphonuclear leucocytes is the iodination of protein which involves the covalent bonding of iodide to protein with the formation of such structures as iodotyrosine, indohistidine and sulfenyl iodides. This process is catalyzed by the enzyme myeloperoxidase and requires the participation of H₂O₂.

Fluoride initiates the metabolic burst of polymorphonuclear cells which provides H₂O₂ in the presence of calcium. It also causes degranulation of polymorphonuclear cells.


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