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The Twelfth Conference of the International Society for
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48090, prior to February 15, 1982.

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, irrigational 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 predominently 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 predominently 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

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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 ricketslike 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 D_3 levels in subjects with genu valgum are not decreased (14). In depth studies, however, are needed including data on 1, 25, Dihydroxy D_3 estimations in the serum of subjects with osteomalacia and rickets in fluorosis areas.

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An Epidemiological Study of the Syndrome of Genu Valgum among Residents of Endemic Areas for Fluorosis in Andhra Pradesh. Indian J. Med. Res. 62:1415-24,1974. 5. Sivakumar, B., and Krishnamachari, K.A.V.R.: Circulating Levels of Immunoreactive Parathyroid Hormone in Endemic Genu Valgum. Horm. Metab. Res., 8:317-19,1976. 6. Sivakumar, B.: Circulating Levels of Growth Hormone in Endemic Genu Valgum. Horm. Metab. Res., 9:436-37,1977. 7. Annual Report, National Institute of Nutrition, India, 1978. 8. Narasinga Rao, B.S., Krishnamachari, K.A.V.R., and Vijayasarathy, C.: Ca[47] Turnover in Endemic Fluorosis and Endemic Genu Valgum. Brit. J. Nutr.,41: 7-14,1979.9. Krishnamachari, K.A.V.R.: Endemic Genu Valgum, a New Dimension to the Fluorosis Problem in India, Fluoride, 9:185-200,1976. 10. Jackson, W.P.U.: Further Observations on the Kenhardt Bone Disease and its Relation to Osteoporosis. S.Afr. Med. J. 36:932-36,1962. 11. Griffiths, H.J., Hunt, R.D., Zimmerman, R.E., et al.: The Role of Calcium and Fluoride in Osteoporosis in Rhesus Monkey. Invest. Radiol. 10:263-68,1975. 12. Roholm, K.: Fluorine Intoxication. A Clinical-Hygienic Study. NYT Nordisk Forlag,Arnold Busck, Copenhagen, 1973. 13. Teotia, S.P.S., Teotia, M., Burns, R.R., and Heels, S.: Secondary Hyperparathyroidism Contributing to Bone Disease in Endemic Skeletal Fluorosis. Proc. Symposium Fluorosis, Indian Academy of Geoscience, Hyderabad, India,1974,p.455-65. 14.Raghuramulu, N., and Krishnamachari, K.A.V.R.: Personal communication.

<div align="right">K.A.V.R. Krishnamachari</div>

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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.

Sabates, W.I., Abrams, G.W., Swanson, D.E., and Norton, E.W.: The Use of Intraocular Gases: The Results of the Sulfur Hexafluoride Gas in Retinal Detachment Surgery. Ophthalmology, 88:447-54, 1981.

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

by

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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 exocyto-
sis. 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 lat-
ter cell type Ca^{++} is required for activation. Both exo-
cytosis 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 affect-
ed. Fluoride, under certain conditions, may also inhi-
bit exocytosis and superoxide production; inhibition oc-
curs later than activation and has the same pH depend-
ence. Apparently the observed effect of fluoride on
granulocytes is due to a combination of opposite   re-
actions. There is a stimulatory and an inhibitory ef-
fect; the magnitude of each is determined by the intra-
cellular concentration of fluoride.

Introduction

The main function of the granulocyte (neutrophil granulocyte, polymor-
phonuclear leukocyte) is the phagocytosis and destruction of invading mi-
cro-organisms. During phagocytosis, some processes in the granulocyte are
activated, which enables this cell to destroy the phagocytozed organism.
One of these processes is degranulation. In the granulocyte a large num-
ber of granules are present, containing many digestive enzymes. During
degranulation the membranes of these granules fuse with the membrane which
surrounds the phagocytozed 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 exocyto-
sis. Exocytosis can easily be measured as the release of cytoplasmic en-
zymes.

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 path-
ological conditions apart from the defense against microorganisms. Some
of the granule constituents have inflammatory properties, exocytosis there-
fore 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
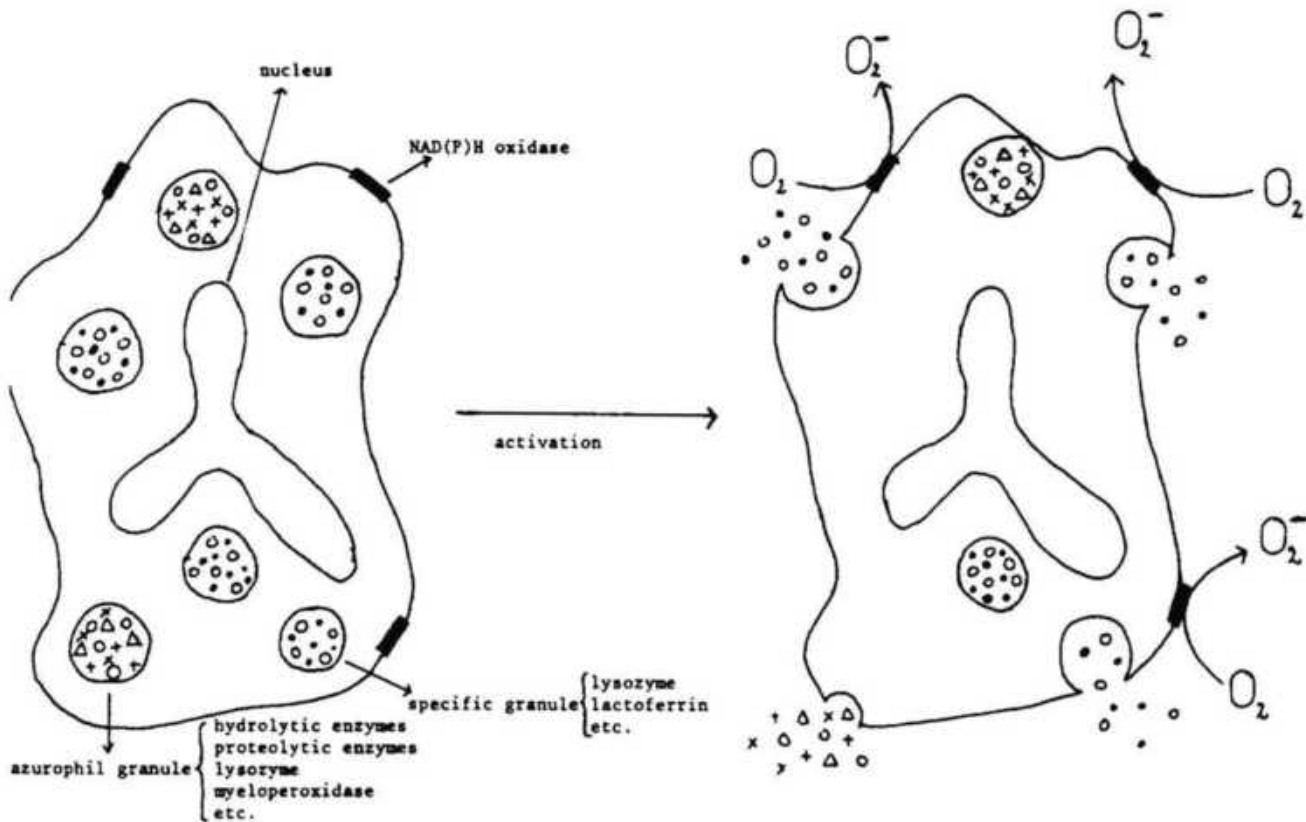
It is possible to activate the granulocyte functions mentioned above in the absence of phagocytosis (Fig. 1). A number of non-particulate agents, such as ionophore A23187, phorbol myristate acetate, chemotactic peptide, complement components and fluoride, all can, under appropriate conditions, induce 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: zurophilic 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, β-glucuronidase, which is only present in the zurophilic 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 β-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 CaF_2 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⁻
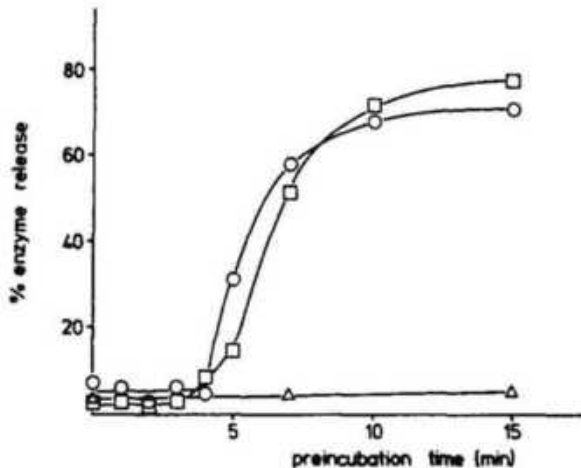
Granulocytes preincubated at
37°C (pH:7.2), cells centri-
fuged and F⁻ containing medium,
which did not contain signifi-
cant amounts of enzymes was dis-
carded. Subsequently Ca^{++} con-
taining medium was added, fol-
lowed by incubation for 30 min
at 37°C. Mixture centrifuged
and the enzymes liberated were
measured in the supernatant.

- 0 - lysozyme release; - ◻ - β-glucuronidase release; - Δ - 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 Ca^{++}-requir-
ing process (8). An increase in cytosolic free Ca^{++} 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 Ca^{++}, when added extracellularly, can penetrate into the cell,
after which exocytosis occurs. The pH-dependence of the effect of fluo-
ride indicates that fluoride penetrates into the cell after which it in-
duces a change in Ca^{++}-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 mem-
brane 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 mycloperoxidase was secreted
by human granulocytes after treatment with fluoride.

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 (O_2^-) production and the generation of other oxygen metabolites (hydrogen peroxide (H_2O_2), hydroxyl radical (OH·) and singlet oxygen ($^1O_2$), 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. O_2^- production is thought to be the consequence of the activation of a membrane bound enzyme, an oxidase which catalyzes the reduction of molecular O_2 to O_2^-(16). The O_2^- 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-

iments have been carried out with 20 mM fluoride.

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

Figure 3

Effect of pH and F⁻ Concentration During Incubation on O_2^- Production
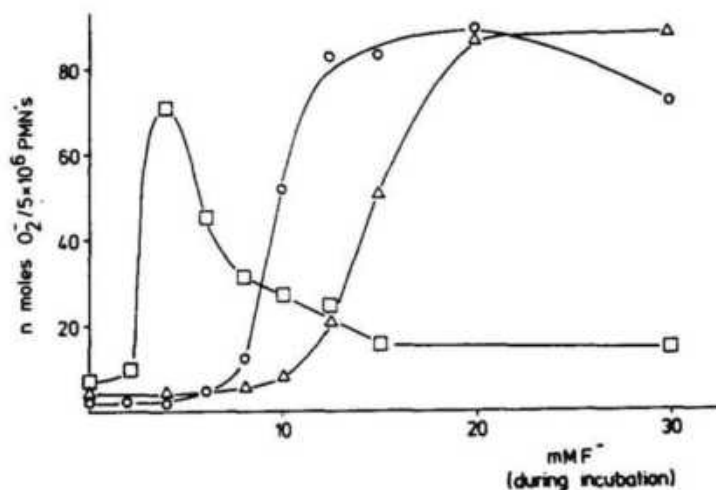
In a control experiment the effect obtained was not due to a pH dependence of the O_2^- generating oxidase, but to another phenomenon, namely fluoride penetration into the cells. (Reproduced from Elferink [18]).

- □ - pH 5.8; - 0 - 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 O_2^- 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 H_2O_2, and the secretion of myeloperoxidase. High concentrations of fluoride result in an inhibition of protein iodination. Clark (10) has sug-

gested that fluoride induces H_2O_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.

Table 1

Effect of Extracellular Ca^{++} and Mg^{++}
on F^- Induced Superoxide Production

| | O_2^- Production | |
| | (n moles O_2^-/5 x 10^6 PMN's) | |
Additions	A	B
none	1.3±1.1	78.8±1.4
1 mM EDTA	2.0±0.7	80.6±2.7
1 mM EDTA, 20 mM F^-	80.5±2.2	51.8±0.7
1 mM Ca^{++}	4.7±0.9	3.8±1.8
1 mM Ca^{++}, 20 mM F^-	26.7±5.0	14.4±4.4
1 mM MG^{++}	4.7±1.5	79.9±3.8
1 mM Mg^{++}, 20 mM F^-	53.6±6.0	51.9±2.0

Columns A and B represent different experimental procedures to measure O_2^- production; however, cytochrome c is used in both to measure 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 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 UF$_6$ TECHNOLOGIES

by

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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 (UF$_6$) 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 longterm 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 25.6±0.6 to 2.9±1.2 $\mu g/g$, respectively. Additional analysis of affected needle parts were similar with concentrations from tips (67.9±13.1 $\mu g/g$) exceeding controls (5.1±1.7 $\mu g/g$) 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

Figure 1

Sampling Sites at Dept. of Energy Uranium Enrichment Facility ORGDP (a)
and the Weapon Components Production and Design Facility Y-12 (b)
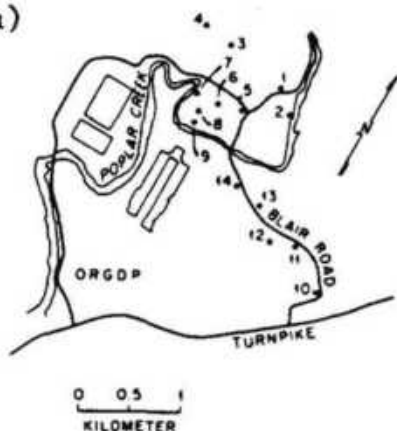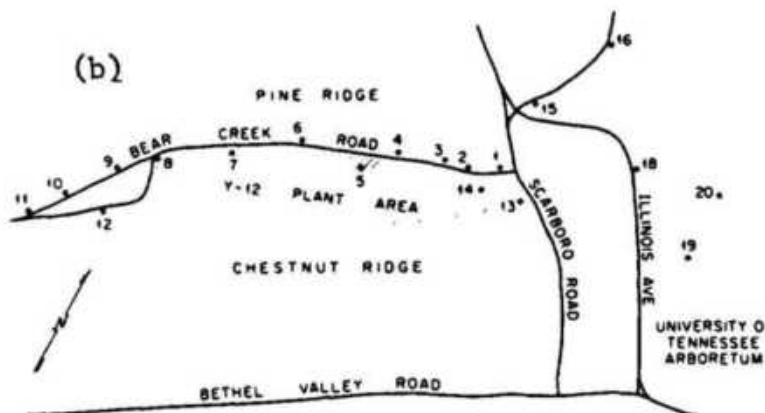
The Y-12 plant is approximately 8 km east of ORGDP

by complexing agents. Each sample was measured by standard addition tech-
niques and the total fluoride content calculated from the change in poten-
tial (6). 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 con-
tent (Table 1). Old needles had ~20% more fluoride than new needles rang-
ing 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 differ-
ences have been observed between one-and-two-year old foliage among ponder-
osa 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 a-
nalysis of variance it was determined that the fluoride concentrations be-
tween 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 fo-
liage 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)

	F⁻ concentration ($\mu gF^-/g \pm 1$ SE)	
Plot No.	Old Needles (1978)	New Needles (1979)
1	4.7±0.3	3.9±0.1
2	4.5±0.2	4.0±0.2
3	4.8±0.5	4.1±0.2

Pine foliage in the environs of the two DOE facilities was invento-
ried 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. Flu-
oride concentrations in old foliage (1978 season) were significantly great-
er than in the new needles (1979 season) or controls. Old foliage from
all plots contained elevated fluoride concentrations with maximum levels
(∿15 ug/g) at locations downwind of the process buildings. Some visible
damage (10-20% tipburn) was present, but the foliar fluoride concentrations
were not sufficient to produce the effect. The plots with the highest con-
centrations were within 500 m of a 270 million BTU/hr. coal-fired steam
plant, suggesting that emissions from that facility (SO_2), 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 signifi-
cantly 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 ug/g, res-
pectively. These plots are nearest to the gaseous effluent discharge
streams. At the two locations with elevated fluoride concentrations (>20
ug/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 UF_6 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 con-
trasted 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 as-
sumed to be lognormally distributed, the above difference would follow a
normal distribution. Therefore, the usual statistical tools available

Comparison of Cumulative F⁻ Concentrations Among Old and New Needles
(Multiple Comparison Procedure (Dunnett, 1955))

Table 2
Y-12 Plant Area

Plot Location	Differences Between Contaminated and Control Sites N		Fluoride (µg/g)±1 SE Old	New
5	3	1.534	41.3±4.5	7.8±1.6
6	3	1.228	27.5±3.6	6.8±0.7
12	3	0.555	11.5±2.5	5.3±0.2
2	3	0.554	15.1±0.9	7.5±0.6
3	3	0.550	12.4±1.6	6.0±0.3
14	3	0.429	10.1±0.9	5.6±0.3
7	3	0.423	8.8±1.1	4.9±0.3
4	3	0.399	13.8±1.6	7.8±0.3
10	3	0.319)*	7.3±0.8	4.5±0.1
9	3	0.315)	6.9±0.8	4.3±0.2
11	3	0.310)	7.8±0.8	4.8±0.2
19	3	0.307)	7.2±0.7	4.5±0.2
17	1	0.238)	7.8	5.2
18	1	0.202)	8.2	5.7
20	3	0.199)	6.1±0.5	4.2±0.1
8	3	0.170)	6.8±0.5	4.9±0.1
1	3	0.030)	12.2±1.4	10.0±0.4
16	1	0.029)	4.9	4.1
13	3	-0.013)	5.3±0.5	4.6±0.1
15	1	-0.028)	5.4	4.7

* 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
ORGDP Plant Area

Plot Location	N	Differences Between Contaminated and Control Sites	Fluoride (µg/g)±1 SE Old	New
14	3	0.763	15.0±0.9	6.0±0.6
12	3	0.678	15.2±5.8	5.8±0.5
11	3	0.658	11.3±1.4	5.0±0.5
9	3	0.637	11.7±0.7	5.3±0.4
8	3	0.557	11.2±1.6	5.4±0.3
13	3	0.449	15.4±0.4	8.5±0.8
1	3	0.410	8.6±0.4	4.9±0.3
3	3	0.317)*	9.1±0.6	5.7±0.2
2	3	0.295)	7.5±0.5	4.8±0.3
7	3	0.269)	8.2±0.3	5.4±0.3
6	3	0.256)	7.5±1.3	4.8±0.1
10	3	0.145)	7.5±0.5	5.5±0.2
5	3	0.141)	6.4±0.2	4.8±0.4
4	3	0.043)	6.1±0.4	5.0±0.2

* - 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 longterm foliar burdens. The com-
parison was achieved by using the transformed ratio of the concentration
in the old and new needles from the observations at each site and com-
paring the results with the corresponding ratio from the control site.
Dunnett (9) proposed a multiple comparison procedure for comparing sev-
eral means with a control and expanded it in a more recent treatment(10).
The purpose of the procedure is to construct appropriate confidence state-
ments 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 α is the level of signifi-
cance. The steps in this procedure are similar to those of most multiple
comparison procedures:

1. The mean square error (MSE), with ν degrees of freedom, is com-
    puted 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 $t\sqrt{MSE(n_c^{-1}+n_t^{-1})}$, where t is a modified t-
    value, n_c is the control site sample size and n_t is the treat-
    ment site sample size.
3. The observed difference is declaired to be significant (i.e.,
    >0) if the observed difference is greater than the critical
    difference.

Since the interest is primarily whether the UF_6 facilities have con-
tributed significantly to the longterm accumulation of fluoride (i.e.,
the control site mean concentration is significantly less than the conta-
minated sites' mean concentrations), only one-sided comparisons were per-
formed. This procedure was repeated for the Y-12 and ORGDP site obser-
vations compared with controls.

By comparing the ratios of fluoride concentration in old vs new fo-
liage to similar ratios among controls, it was possible to identify are-
as accumulating fluorides from intermittent releases to establish a con-
fidence statement about the differences between impacted sites and con-
trols and to illustrate longterm additive effects in the absence of visi-
ble injury. Among the twenty plots in the Y-12 area, eight locations
provided evidence of longterm (2 years) accumulation (Table 2). Concen-
trations 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 work-
ers (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 con-
trols (Table 3). Concentrations determined at all ORGDP locations were
less than the threshold for foliar necrosis. Those plots with concentra-
tion 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 flu-
oride 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 indi-
cator 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 es-
tablished and maintained for routine sampling.

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

by

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SUMMARY: To evaluate the degree of exposure to fluo-
ride and its compounds on the children residing in the
vicinity of an aluminum smelter, concentrations of flu-
oride in urine and tooth enamel were measured and ra-
diograms 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 fluo-
rides involving the health of populations. It may conceivably interfere
with growth in children. We therefore investigated the effect of fluo-
ride 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 concen-
trations 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 digi-
tal pH milivolt 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

Survey Group	Number of Examined Children Male	Female	Total	Age Years $X \pm$ S.D.
Aluminum Smelter Area	15	16	31	$9^{7}/12 \pm 1^{1}/12$
Control	12	23	35	$9^{7}/12 \pm 1^{1}/12$

F⁻ Exposure near Aluminum Plant

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 2

Concentration of Fluoride in Urine mg/l

Survey Group	Number of	Mean ± S.D.	Min. - Max.
Aluminum Smelter Area	61	0.65 ± 0.28	0.16 - 1.78
Control	120	0.50 ± 0.19	0.16 - 1.10

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

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

Table 4

F⁻ Concentrations in Tooth Enamel

F⁻ µg/g		Control Group Relative Frequency %		Relative Frequency %
< 300	9	29.03	10	30.30
301-500	6	19.35	12	36.37
501-800	7	22.58	7	21.21
801-1200	6	19.36	3	9.09
>1200	3	9.68	1	3.03

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

Group	Bone	Mean±S.D.	C.V.%
Aluminum Smelter	3	3.11±0.43	13.4%
Area	4	2.57±0.43	16.73
Control	3	3.12±0.40	13.4
	4	2.68±0.40	0.43

Table 6

Width of Marrow Cavity

Group	Bone	Mean±S.D.	C.V.%
Aluminum Smelter	3	3.71±0.60	16.1
Area	4	3.36±0.62	18.45
Control	3	3.63±0.39	10.74
	4	3.07±0.46	14.98

Table 7

Cortex-Marrow Ratio

Group	Bone	Mean±S.D.
Aluminum Smelter	3	0.92±0.23
Area	4	0.78±0.16
Control	3	0.87±0.16
	4	0.89±0.19

this area, in view of the fact that analysis of potable water in both groups showed no differences in their fluoride content. In both, the fluoride level was about 0.2 mg/liter.

The radiological investigations did not give essential information about the degree of exposure to fluoride and its compounds. They seem to be of little value in estimating the influence upon human health of an environment polluted with fluoride compounds.

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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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 lev-els of copper, zinc, calcium and magnesium were esti-mated in subjects with endemic skeletal fluorosis, en-demic genu valgum and in corresponding age-and sex-matched controls. Bone samples obtained during surgi-cal correction were analyzed for trace minerals in sub-jects with endemic genu valgum and in controls. No dif-ference was found in serum levels of copper, zinc, cal-cium and magnesium in the study groups compared to controls. The level of fluoride in bones obtained

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from subjects with endemic genu valgum was twice as
high as that found in bones of controls whereas lev-
els of copper and manganese were markedly reduced. The
possible significance of low copper values is discuss-
ed.

Introduction

Endemic genu valgum has been identified as a manifestation of chron-
ic fluoride toxicity in some areas of India (1). This newer manifesta-
tion of skeletal fluorosis, seen mostly in adolescents with a marked pre-
dilection for males, is characterized by simultaneous occurrence of os-
teosclerosis of the spine and osteoporosis of the long bones (2). Earlier
studies have shown that, in endemic genu valgum, there is secondary hyper-
parathyroidism (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 meta-
bolism 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 mil-
let grown in fluorosis areas was significantly higher than that in sorg-
hum and pearl millet grown in nonfluorotic areas in India (5). In addi-
tion, there was a strong negative correlation between the concentration
of copper in drinking water and the prevalence of genu valgum in areas en-
demic 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 includ-
ing 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 con-
trol group for subjects with skeletal fluorosis. Blood samples were ob-
tained from these individuals, serum was separated and kept frozen until
analyzed for copper, zinc and magnesium concentrations. Serum was appro-
priately 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 iron 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
nonfluoretic 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 1

Trace Elements in Serum in Fluorosis and in Endemic Genu Valgum

Group	Copper (µg/1)	Zinc (µg/1)	Calcium (mg/1)	Magnesium (mg/1)
Endemic genu valgum (9)	1128±72.2	847±113.8	85.3±3.1	38±2.3
Controls for EGV (19)	1037±49.5	685±42.0	92 ±1.8	37±1.8
Skeletal fluorosis (12)	1175±50.9	723±57.3	86 ±2.1	38±1.2
Controls for S.F. (14)	1075±34.7	765±48.3	93 ±1.5	44±1.6

Mean ± SEMS; Figures in parenthesis indicate number of subjects
investigated.

Figure 1

Distribution of Copper, Zinc, Magnesium and Calcium in Sera of Fluorosis and Control Groups
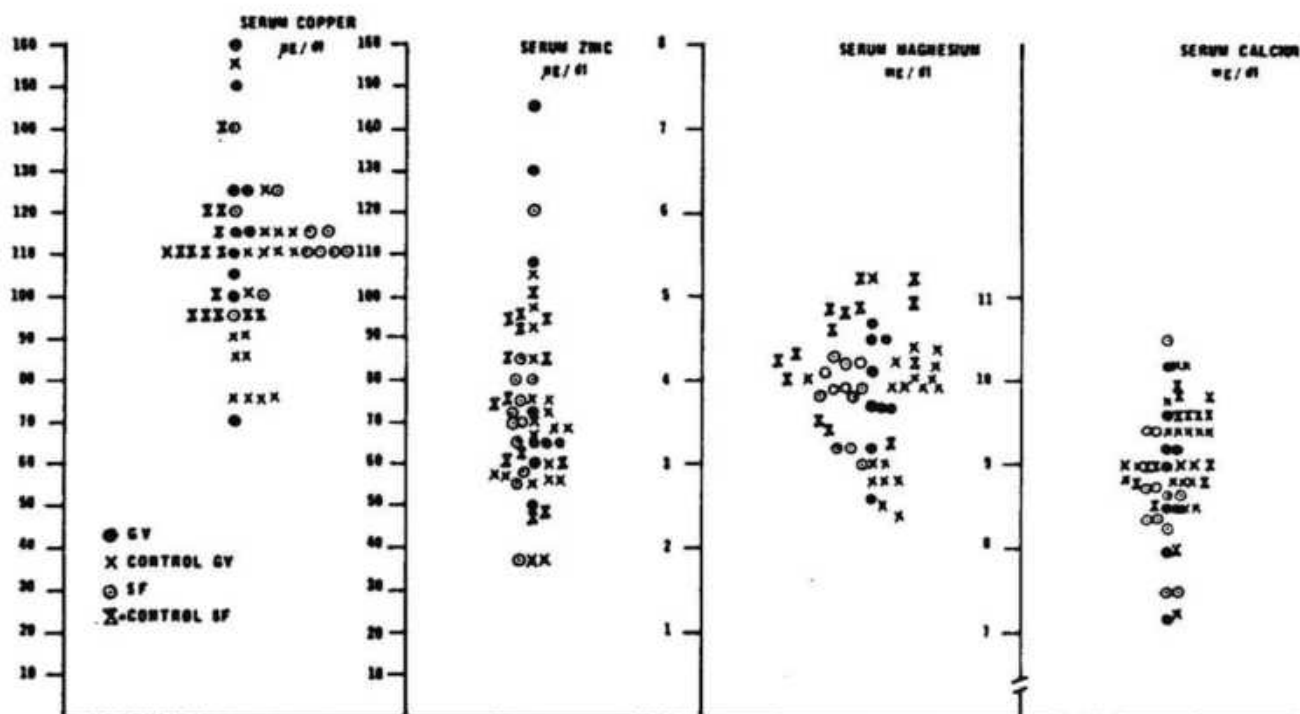
orosis. Endemic genu valgum subjects showed evidence of osteoporosis of the limb bones in addition to osteosclerosis of the spine. The results of analysis of serum samples are presented in Table 1. No significant differences were found between the mean values for serum copper, zinc, magnesium and calcium 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 22mg/ 24 hours while in endemic genu valgum subjects it ranged from 6.8 - 16mg/ 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

Group	Values	Dry wt (g)	% ash	Zn	Cu	Mg	Mn	F	Ca	P	Ca/P
						µg/g/dry wt.			Mg/g dry wt.		
Endemic genu valgum (37)	Mean	1.42	41.8	81.0	1.3**	3281*	0.6**	7283**	188	76.5	2.4
	SE	0.11	1.94	4.05	0.08	164	0.04	416	11.16	3.73	0.09
Nonfluo-rotic con trols (8)	Mean	0.90	49.9	96.8	3.7	4768	1.2	3190	232.8	85.7	2.6
	SE	0.21	5.34	11.2	0.80	1275	0.30	705	39.2	7.66	0.39

Values are mean and Ses. 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 signficantly 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 homeostatis 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.

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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 Z^{++} 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).

At the beginning of this investigation we analyzed the fluoride lev-
el in blood and urine during DPA therapy. We desired to determine a pos-
sible 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 dif-
ference was measured by a Digital-pH-meter (Orion, Model 701) with expand-
ed mV-range.

For the calibrating curve and sample preparation the following chem-
icals were used:
Sodium fluoride (NaF) p.A., Merck No. 6449
Trisodiumcitrichydrate ($C_6H_5Na_3.2H_2O$) p.A., Merck No. 6448
Perchloric Acid (HClO$_4$) 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 H$_2$0 distil-
led (100 ppm F$^-$)
Citratebuffer: 59.0 g Tri-Sodiumcitrate 2-hydrate + 8.5 ml HClO$_4$
filled with 250 ml H$_2$0 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 (Artamin^R
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 4.5years.
They received during the 1st month,150 mg Artamin^R 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 Indocid^R 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 tem-
perature was always about the same, making it unnecessary to use a ther-
mostat 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 ca-
libration curve of urine, between 0.02 - 10.0 μg F$^-$/ml in a semi logarith-
mic plot.

Sample Preparation and Measurement: The F$^-$ content of urine samples
was analyzed according to the method of Tusl (6,7). Five milliliters of

urine was added to 10 ml of citrate buffer and measured directly. To sam-
ples 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 ori-
ginal F⁻ content was calculated. A comparison of these double measure-
ments was satisfactory. This showed also that,because of the relatively
high concentration of the citrate buffer,the ionic strength of urine sam-
ples 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 the-
rapy 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 in-
creased 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 0.31 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.

Figure 1

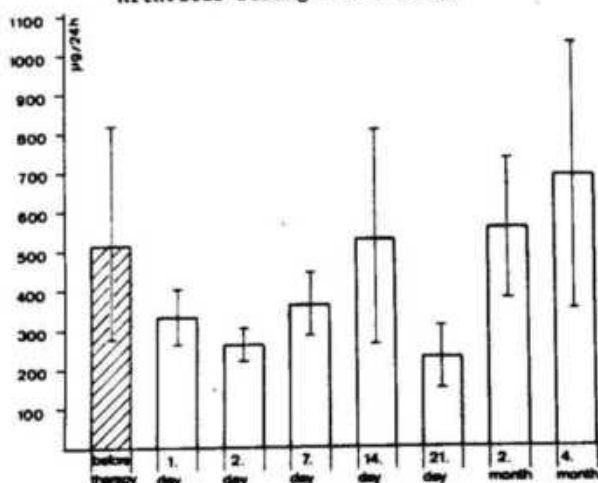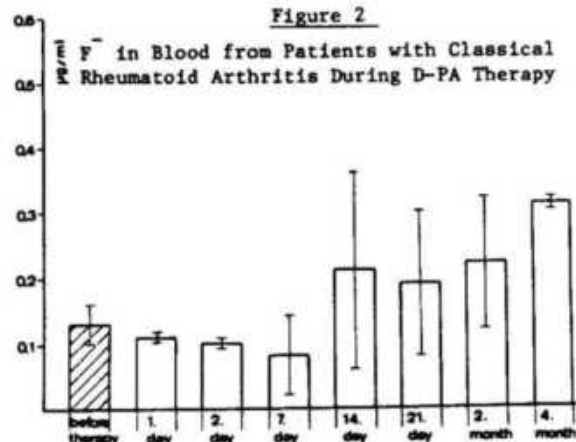Urinary F⁻ from Patients with Classical Rheumatoid
Arthritis During D-PA Therapy

Figure 2

F⁻ in Blood from Patients with Classical
Rheumatoid Arthritis During D-PA 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 exci-
sion repair in perpheral 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 inhibit-
ed (2). This could be a positive process concerning the tumor-like hyper-
trophy of marginal synovial cells and the inhibition of lymphoblast de-
velopment, with a pronounced increase of antibody synthesis in this dis-
ease.

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

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

The excretion of fluoride in urine and the fluoride content in drink-
ing water were in direct correlation (14). The mean normal ranged be-
tween 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 0.26ppm.

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 µg/ml, immedi-
ately showed a clear influence to the DNA excision repair and to the phos-
phorilization of the DNA precursors. At a concentration of 1.0 µg/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 µg/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 in-
crease in the fluoride ion concentration in blood. However it cannot be
said whether the effect on the cP is positive or negative.

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FLUORIDE TRANSPORT DURING REGULAR HEMODIALYSIS 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 al-
so of arterial plasma fluoride levels because of penetra-
tion of fluoride through the semipermeable membrane in
the dialysis apparatus. The effects of longterm hemodial-
ysis 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 flu-
oride is accumulated in the bone, it is probable that cal-
cified tissues in the course of longterm hemodialysis be-
come slowly saturated. This should result in a gradually
reduced transfer of fluoride during consecutive hemodialy-
sis, as some earlier findings have suggested.

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sity 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 be-
tween 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 calcula-
tions 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. Ac-
cording 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 concen-
trations 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 dis-
tribution 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 concentra-
tion during hemodialysis has a tendency to increase to the higher level,
the longer the patient has been involved in the regular hemodialysis pro-
gram (3). Therefore it can be expected that, in the course of longterm
hemodialysis, the patients gradually store less fluoride in their skele-
tons 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 par-
tially 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 he-
modialysis in order to observe trends in fluoride retention in a more uni-
versal way by reducing interindividual variations of the results by a spe-
cial approach.

Materials and Methods

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 cuprophane membrane. The dialysate flow was 600 ml/min..dialysate contained 135 m mol/l sodium, 2 m mol/l potassium, 1.75 m mol/l calcium, 0.5 m mol/l magnesium, 106.5 m mol/l chloride, 35 m mol/l 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 citratedihydrate 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

Figure 1

Mean Arterial and Venous Plasma F⁻
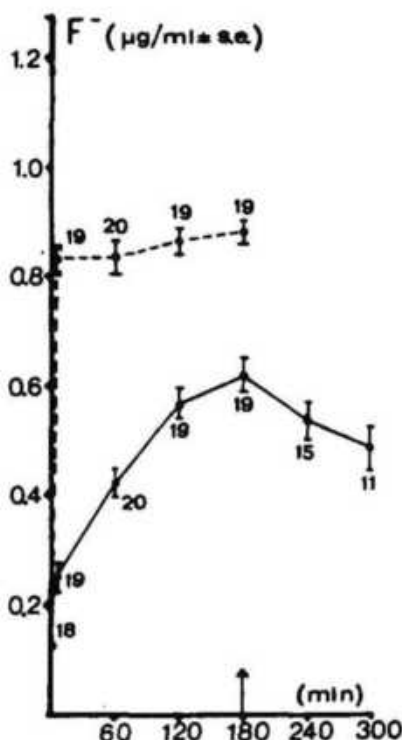
Number of samples indicated for each time point. (---)=venous plasma, (—)=arterial plasma.

Figure 2

Relation of Predialysis
Arterial Plasma F⁻

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

Patients Included in the Study

Code	Age	Diagnosis
K.I.	23	Chronic Nephritis NUD
K.R.	19	Renal Dysplacia
H.M.	37	Diabetic Nephropathy
S.H.	58	Chronic Nephritis NUD
K.H.	42	Acute Tubular Necrosis
S.V.	58	Chronic Nephritis NUD
S.P.	38	Chronic Pyelonephr., Renal Hypoplasia Sin.
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

F^- in Venous and Arterial Plasma (Mean Values in 10 Patients)

|	Venous ($\mu g/ml \pm S.D.$)	Arterial ($\mu g/ml \pm S.D.$)
Before Dialysis		0.158 ± 0.091
During Dialysis		
5 min.	0.832 ± 0.134	0.252 ± 0.120
60 min.	0.838 ± 0.122	0.422 ± 0.119
120 min.	0.865 ± 0.114	0.568 ± 0.161
180 min.	0.883 ± 0.110	0.621 ± 0.135
After Dialysis		
60 min.		0.538 ± 0.129
120 min.		0.488 ± 0.139

oride concentrations 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 ug/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.

Figure 3

Predialysis Arterial Plasma F⁻ During and After the 20 Dialysis
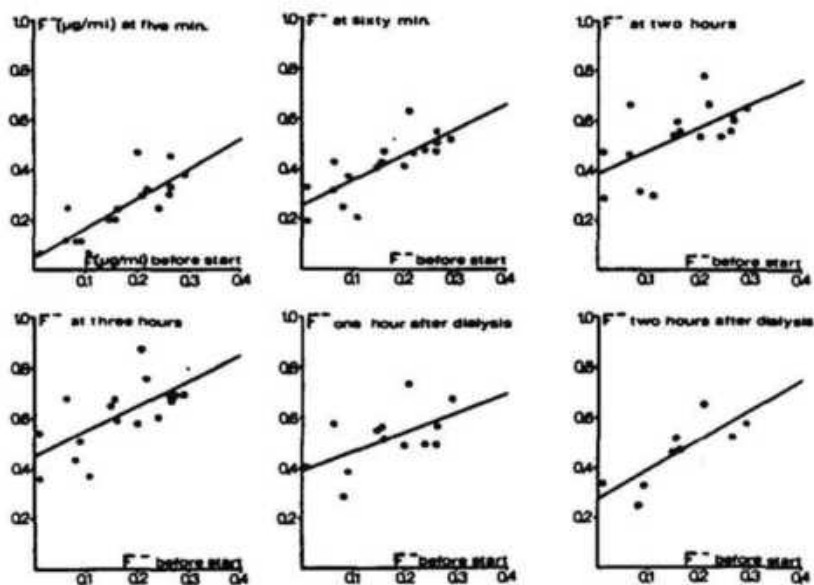
Actual numbers are given in Table 3

Table 3

Relation of Predialysis Arterial Plasma F^- to Arterial Plasma F^-
During and After Dialysis (Regression and Correlation Analyses)

Predial. Plasma F^- / Art. Plasma F^- at	Slope	Intercept (µg/ml)	r	n	Significance of r
5 min.	1.18	0.049	0.810	17	$p < 0.001$
60 min.	1.01	0.254	0.781	18	$p < 0.001$
120 min.	0.913	0.389	0.618	17	$p < 0.01$
180 min.	0.974	0.451	0.652	17	$p < 0.01$
60 min. after dialysis	0.746	0.393	0.554	13	$p < 0.05$
120 min. after dialysis	1.17	0.273	0.807	9	$p < 0.01$

Significance calculated as the difference
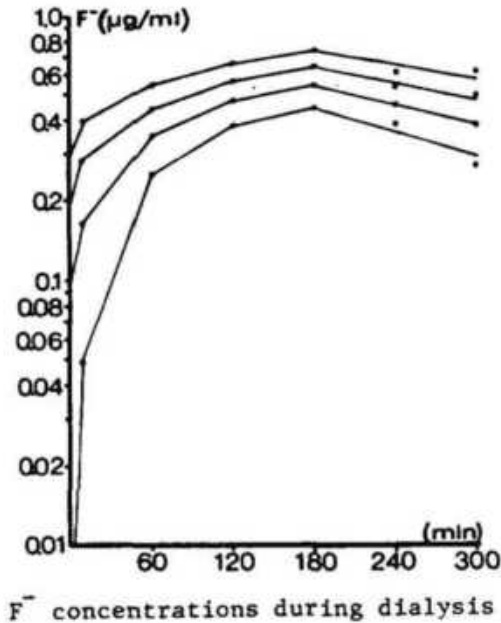of the correlation coefficient from zero.

Figure 4

Arterial Plasma F⁻ for Four Hypothetical Patients

F⁻ concentrations during dialysis

Table 4

Fluoride Transferred During Hemodialysis

	5 – 60 min. (mg)	60 – 120 min. (mg)	120 – 180 min. (mg)	Total mg
Patient 1	8.2	6.4	5.4	20.0
Patient 2	6.9	5.2	4.3	16.4
Patient 3	5.6	4.1	3.2	12.9
Patient 4	4.3	2.9	2.1	9.3

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 ug/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.

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FLUORIDE CONTENT OF URINE, BLOOD, NAILS AND HAIR IN ENDEMIC SKELETAL FLUOROSIS

by

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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

Oued, South Algeria. In the past, relatively little attention has been
paid to the fluoride content of skin, nails, and hair compared to fluo-
ride assays of bone, teeth, aorta, kidneys and bladder (2).   The pur-
pose of this paper is to determine a possible correlation between flu-
oride in drinking water and urinary and plasma fluoride, the fluoride con-
tent 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 in-
side 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 skel-
etal 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 wa-
ter is relatively pure because it is derived from deep wells ("borings").
We estimated that the mean total daily fluoride uptake from food and wa-
ter in sedentary subjects in spring ranges between 10 and 20 mg per day,
considering that daily water consumption is greater in summer than in win-
ter, that persons with sedentary habits require less water and food and
that they consume high fluoride tea and dates.

<div align="center">

Figure 1

Fluorosis Endemic Zone

</div>
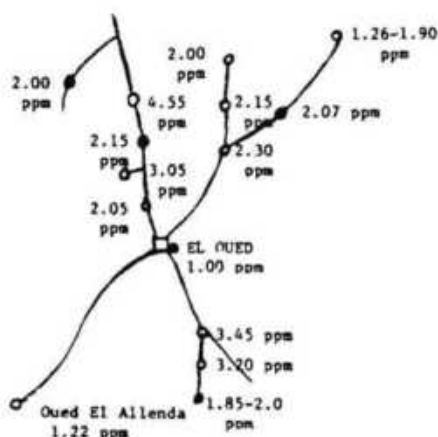
F⁻ in drinking water in different areas

Biochemical Study: The fluoride content of the 24-hr. drinking wa-
ter and urine was measured by the specific electrode method (8).   Fluo-
ride in blood, nails and hair were assayed by the same method after alka-
line 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 skel-

Figure 2
Skeletal Changes
(% of normal 0)
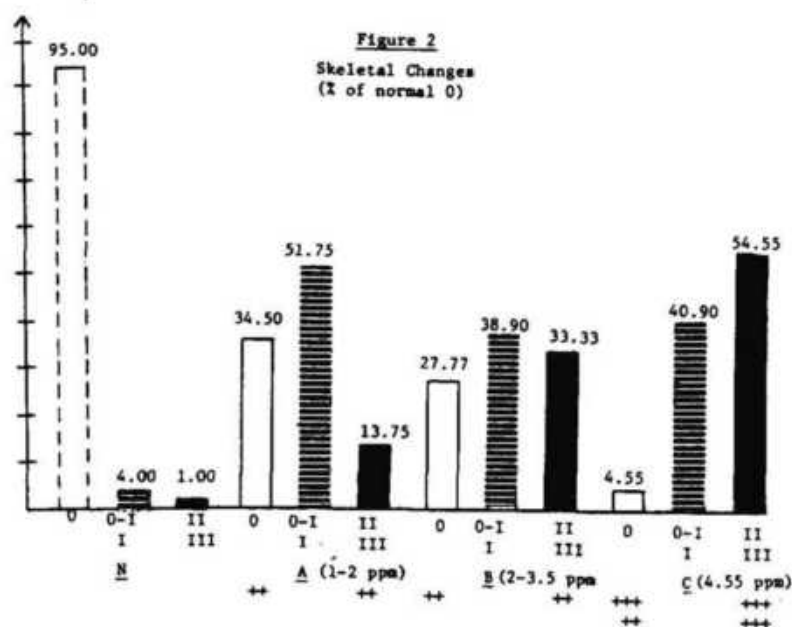
etal abnormalities but 5% showed minor sclerotic bone changes of unknown
origin. Stage I fluorosis was less pronounced in Group A and B than in
the control group N (p 0.05) and occurred in group C in only 4.55%.   On
the other hand the incidence of Stages II and III fluorosis was higher in
groups A and 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 in-
crease was more pronounced in group C than in A (p 0.05). Fluoremia 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.

Figure 3
Fluoruria and Fluoremia
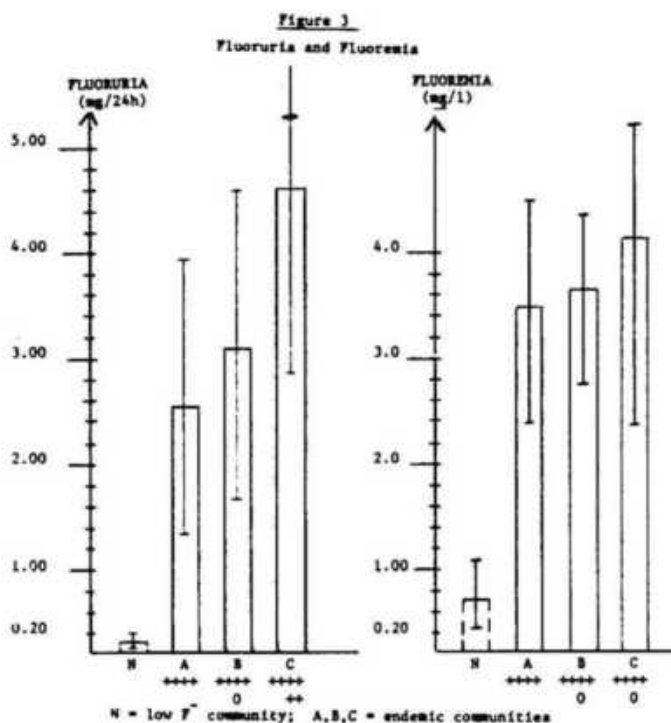
content of nails (Fig. 4) showed a gradual increase in the A and B patients (p 0.10 or 0.05), but was most significant in group C. In this group, the p value was 0.001 compared with the control group and 0.10 compared with group A. The fluoride content of hair was slightly elevated in groups A, B, and 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 biochemical 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-

Figure 4
F⁻ in Nails and Hair (ppm)
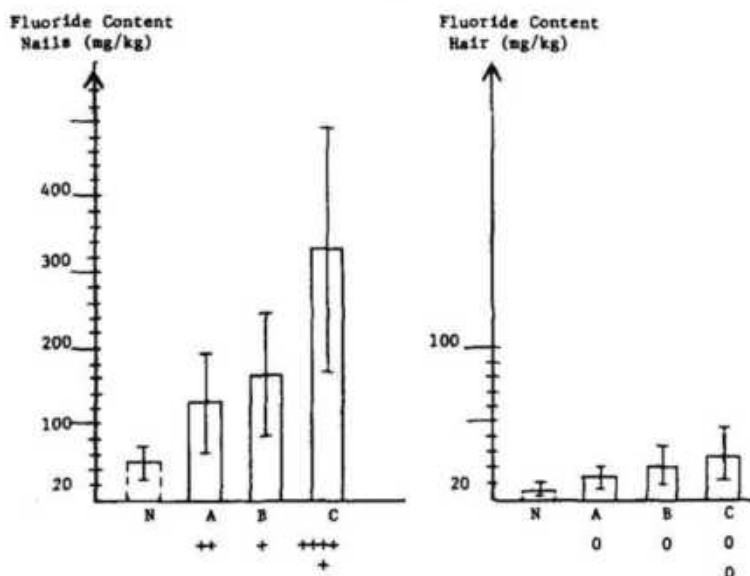
ever the difference between groups C and A was less significant. Herman et al. (2) reported excessive fluoride content of skin, hair and nails in patients with kidney stones. Our results confirm his findings with regard to nails, but not to hair. In subacute fluoride intoxication of rabbits, also, we 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.

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

by

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Anion binding sites in beef heart lactate dehydrogenase a nonmetallo-
protein enzyme involved in the terminal stages of anaerobic muscle glycol-
ysis, 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 in-
teractions with the enzyme. Spectral data clearly demonstrate that chlo-
ride binds at sites other than the site(s) occupied by fluoride.

A.W.B.

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

by

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

A novel 1:1 complex precipitates as a white solid from an 80% dime-
thyl sulfoxide or NN-dimethylformamide-20% water solution of equimolar
amounts of potassium fluoride and uracil, a key component of ribonucleic
acid. Infrared spectral properties of this KF-uracil adduct indicate the
presence of a strong asymmetrical $NH \cdot \cdot F^-$ 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}. Al-
though the authors caution that more detailed studies are required, they
conclude from their investigation that "The observed ability of fluoride

to disrupt the self-association in uracil and to produce strong NH··F⁻ in-
teractions is obviously of considerable importance to the possible biolog-
ically   disruptive action of the fluoride ion."

                                                    A.W.B.

(Reprints: Dept. of Chem. Univ. of York, Heslington, York YO1 5DD, U.K.)

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COMPARISON OF FLUORIDE BALANCES DURING AMBULATION AND BED REST

by

U.R. Maheshwari, L. Leybin, H.C. Hodge, et al.
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 excre-
tion, 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.

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

by

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

Pretreatment of mast cells with potassium and lithium fluoride in-
duces 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 cal-
cium.   In the second step,cells were transferred to new tubes which con-
tained 1 mM calcium for 10 minutes. Following centrifugation, histamine
was determined fluorometrically in the supernatent 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 secreta-
gogues. The ability of fluorides to activate mast cell action is parallel
to the disassociation constant of the fluoride compound. In other words,
histamine release by these compounds seems to be dependent on the avail-
ability of fluoride ions for mast cells.

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FLUORIDE BRIEF

One to 10 ppm fluoride added to drinking water enhance the activi-
ty 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.

Boros, I.; Keszler, P., and Zelles, T.: Functional Changes
in the Salivary Glands of Rats after Sodium Fluoride Treat-
ment. Acta Physiologica Academiae Scientiarum Hungaricae,
53:155, 1979.

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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 bacteriocidal 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 H_2O_2.

Fluoride initiates the metabolic burst of polymorphonuclear cells which provides H_2O_2 in the presence of calcium. It also causes degranulation of polymorphonuclear cells.

Clark, R.A.: Neutrophil Iodination Reaction Induced by Fluoride: Implications for Degranulation and Metabolic Activation. Blood, 57:913-921, 1981.

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