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The International Society for Fluoride Research will hold its Eighth Conference in London, England, May 29-31, 1977. Further details will appear in subsequent issues. The Program Committee is soliciting abstracts (up to 300 words) of papers to be presented at the conference dealing with any phase of fluoride research. Kindly send abstracts to the Society's office, P.O. Box 692, Warren, MI 48090. The deadline for the abstracts will be January 1, 1977.

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## EDITORIAL

## INDUSTRIAL FLUOROSIS

Since Roholm (1) reported skeletal fluorosis in cryolite workers only sporadic data have appeared in the medical literature on industrial fluorosis. In 1936 Speder (2) presented 7 cases from the phosphate mines of Morocco. Wilkie (3) described ostesclerosis in two Yorkshire workers engaged in the manufacture of $AlF_3$ and HF respectively. Reports issued from France on patients who had been working in the North-African phosphate mines and in other fluoride-emitting facilities (4-6).

In the United States Bishop (8) recorded detailed X-ray findings in a 48 year old colored man who had been employed in a fertilizer factory for 18 years. Evans (9) presented X-ray findings on 74 men who had been exposed to HF and "other chemicals used as refrigerants" for an average of 2.7 years. He found "no increased fibrosis" from one examination to another, but the X-ray films to illustrate this point showed in most cases exaggerated lung markings.

In 1963 Derriberry et al. (10) carried out laboratory and clinical studies on 74 workers exposed to high fluoride concentrations as reflected by an increase in their urinary excretion throughout their employment at the Tennessee Valley Authority fertilizer plant at Wilson Dam. They found no difference in their urinary fluoride levels compared with those of 67 other employees who had been less exposed but noted minimal or questionable degrees of excessive bone density in 23% of the exposed group, and a higher incidence of albuminuria, of abnormal respiratory disease and muscular-skeletal conditions in the exposed than in the "unexposed" group.

A high incidence of respiratory diseases was also encountered by Bruusgard (11) in workers of a Norwegian aluminum smelter, by Novratil et al. (12) in a Czechoslovakian superphosphate factory, and by Midttun (13) in 55 workers in a Norwegian aluminum smelter. In Holland (14) 20 to 30% of the workers of an aluminum smelter developed dyspnea within the first 3 years of work.

Recently research on industrial fluorosis has been greatly stimulated by the excellent work of Franke et al. (15) an orthopedic surgeon who established the diagnosis of skeletal fluorosis in 3 autopsied cases through histological studies and microanalytical determinations of fluoride in the iliac crest. In Switzerland, Schlegel (16) observed 61 cases employed in an aluminum smelter 4 of which were in the advanced stage. Their major symptoms were arthritic changes in the joints, especially in the spine.

Another recent survey extending over a 5 year period by the medical staff of the Aluminum Company of America designed to prevent excessive fluoride absorption in workers concluded that no serious fluoride-related damage occurred among their workers (see p. 220). They essayed 52,000 urine samples of workers for fluoride and compared the values before and after conclusion of the work-shift. They also analyzed urine for albumen, sugar ketones and occult blood in 96 workers with 10 to 43 years occupational exposure to fluoride. Skeletal x-rays failed to reveal evidence of fluoride-related bone changes. In one of their smelters the authors were able to correlate "post-shift" urinary excretion with inappropriate work practices and inadequate hygienic conditions. Improvement of this condition was reflected in the urinary "post-shift" values.

The question arises whether or not in these and similar studies statistical evaluation of laboratory procedures such as urinary fluoride excretion, fluoride assays of the iliac crest bone, or chest X-rays are sufficient to establish that the health of workers had not been damaged by fluoride, without careful correlation of these findings with clinical data. For instance, in the ALCOA study full case histories and clinical data on patients who excreted large amounts of fluoride in the urine would be desirable. The highest amount in one worker was 13.3 mg. Furthermore, the absence of protein in the urine does not necessarily rule out adverse effects on the kidneys. Available data indicate that certain enzyme activities in the kidneys of primates are impaired by fluoride even if the routine urine examination shows no abnormalities whatsoever (17).

Be that as it may, the ALCOA report indicates that improved working conditions, protective measures for workers and attempts at controlling atmospheric pollution may have reduced the formerly widely prevalent occurrence of fluorosis in industrial facilities.

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# EFFECTS OF FLUORIDE ON BLOOD PLATELETS
(An Overview)

by

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SUMMARY: Studies of the effect of sodium fluoride on the function and metabolism of blood platelets are reviewed. The results indicate that fluoride penetrates the platelet plasma membrane slowly at physiologic pH. An effect on the energy metabolism (the metabolic ATP level) is visible before the other effects are observed, suggesting that the induction of these effects demands higher intracellular concentrations of fluoride. The platelet activities shown to be induced by fluoride in excess of 10 mM (190 ppm) concentration are: platelet shape change, aggregation, adhesion to surfaces and secretion of stored compounds. The secretion is optimal between 5 and 10 min and shows a 3 to 5 min.lag. Below 10 mM concentration no induction takes place. The secretion is energy dependent in that it is inhibited when a mitochondrial blocker is added. Lowering of pH results in induction of secretion from platelets at a lower concentration of fluoride, or a faster induction at the higher concentration, with optimal release the first 3 min. The use of fluoride as inducer of secretion allows the study of secretion in acid incubation medium down to pH 5.3.

## Introduction

An extensive study is in progress investigating the different functions of the blood platelets and their role in thrombosis and hemostatis.

The well defined functions are:

Retraction of a fibrin clot (1, 2)
Adhesion to glass and other foreign surfaces (2, 3)
Aggregation of one platelet to another (2, 4)
Secretion of compounds from storage organelles in the platelet (2, 5, 6)
Uptake of serotonin (7, 8)

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In addition much interest has concentrated recently on the study of the initial shape change (light absorption changes), which may or may not include swelling of the platelets (2, 9, 10).

In the initial survey of factors influencing platelet functions a number of metabolic inhibitors were tested singly, among them NaF.

Fluoride did not prevent platelet aggregation (11) or clumping (12) at concentrations between 0.25 and 2.5 mM. 8-40 mM NaF induced platelet aggregation in heparinized plasma (13). 25 mM NaF resulted first in decrease and then in increase in platelet adhesion to glass (14) whereas other workers reported an increase at lower concentrations and an inhibition of spreading on glass (15) at higher concentrations. 2.5 mM NaF had a slight and 10 mM a strong inhibitory effect on the retraction of platelet-fibrin clots (16). All these studies were done with human platelets.

Weissbach and Redfield (17, 18) reported that the uptake of serotonin by dog platelets was inhibited 28% by $10^{-4}$ M and 90% by $10^{-3}$ M fluoride at pH 5.7, whereas at pH 7.4 $10^{-3}$M produced no effect in contrast to 80% inhibition in saline and 89% in plasma by $10^{-2}$M. They also reported (17)without drawing any conclusions, an increased release of serotonin at pH 5.7 with 2 mM fluoride above that seen by long time incubation in saline. This finding and its consequences for the interpretation of fluoride's effect on the uptake of serotonin by platelets was overlooked by subsequent workers.

Fantl (19) studied the effect of 10-60 mM fluoride on light absorbance through suspensions of marsupial and mammalian blood platelets. The medium was either citrated or EDTA-anticoagulated plasma or saline. Under isosmolar conditions 1-2.3 mM fluoride caused a slow decrease in absorbance in plasma, whereas higher concentrations showed a 5 min lag, followed by a sharper drop which reached about 40% at 15 min incubation. In the other incubation media the effect was similar, although maximal results were obtained only in the citrated medium. That the effect appeared in the presence of calcium chelators was taken as indication that fluoride did not owe its effect to the known ability of ADP to induce changes in light transmission. 5 mM N-ethylmaleimide prevented the fluoride-induced changes.

The fluoride-induced swelling was not affected by 7 hours storage at 4° C, conditions which normally resulted in a drastic reduction in platelet activity. The fluoride effect was temperature dependent, and non-existant at 5.5° C. The author also reported a fluoride-induced shortening in recalcification time at 37° C, indicating liberation of platelet factor 3, a phospholipid clotting factor thought to appear by exposure of previously protected

sites on the outer membrane of the platelet (20).

The author suggested that fluoride reacted with a structurally important SH group in the platelet, resulting in altered permeability and swelling. It has been shown that at most only part of the change in light absorption is caused by swelling, since a major part results from the change of the platelet appearance from a discoid smooth to a spherical pseudopod-rich structure (21).

## The Blood Platelet Release Reaction

The platelet release reaction is defined as the secretion of stored compounds from storage organelles inside the platelet to the platelet exterior without the secretion coming into contact with the platelet cytoplasm (5, 6). Probably the most interesting compounds in this respect are the adenine nucleotides, which exist in two forms, one which is readily labeled when the platelets are incubated with radioactive phosphate or adenine (the metabolic pool) and one which remains virtually unlabeled after incubation for several hours at 37° C (the non-metabolic pool). The labeling experiments make it possible to study the release reaction and the changes in nucleotide metabolism in parallel (5).

A fast secretion from platelets has been obtained with a number of compounds (thrombin, latex particles, collagen, etc.(5)). It has been demonstrated that the slow release induced by other compounds e.g. fluoride (22, 23, 24) and immune complexes (25) is also a true secretory process and should be included in the definition of the platelet release reaction (6). The released material can be divided into at least two categories according to the type of release: 1) compounds which can be released by a weak release induction (e.g. through the ADP-mediated release or with very low concentrations of thrombin (release I); 2) compounds which are released to a significant degree only when strong release induction is imposed; this group consists mainly of certain lysosomal enzymes (release II) (26, 27).

Three criteria have been used by this author to establish whether the material found extracellularly after exposure of the platelet to a specific compound (e.g. NaF) is secreted:

1. The material is not released in parallel with a cytoplasmic marker.

2. The reaction is inhibited by the combination of blockers of respiration and glycolysis.

3. Different compounds of the same release category are released in parallel.

The released compounds which have been most thoroughly studied are: "non-metabolic" adenine nucleotides (22, 28); preabsorbed serotonin (24); and calcium (23). All three compounds have been shown to be released by the same time course. Fig. 1 shows the time course obtained with adenine nucleotides when release is induced with 10 mM NaF at pH 7.4. The release (secretion) from human platelets is not dependent upon the presence of $Ca^{++}$, in analogy with the fast release induced by thrombin (22). It has not yet been established whether or to which degree lysosomal enzymes are released with fluoride. Buckingham and Maynert (29) have described release of serotonin, $K^+$ and amino acids after one hour exposure to 10 mM fluoride. The significance and category of the $K^+$ and amino acid release has not been established as yet.

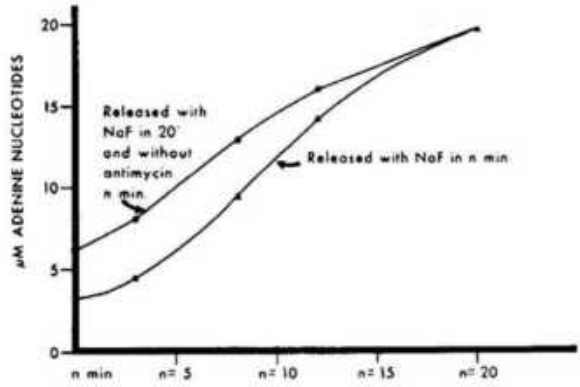
Buckingham and Maynert also showed that the release of serotonin induced by fluoride (27% of the platelets' store of serotonin - preabsorbed radioactive serotonin was not applied in these studies) was completely suppressed when the temperature was reduced from 37° C to 0° C, indicating an energy-dependent reaction. When the time course for release of adenine nucleotides was determined, it was established that addition of antimycin during incubation with fluoride (antimycin blocked mitochondrial respiration while the presence of fluoride blocked glycolysis) had the same effect as shortening of the incubation time with fluoride, i.e. energy metabolism was needed during the whole release reaction, including the lag period (24) (Fig. 1).

No more than 13% of total lactate dehydrogenase was recovered extracellularly after the exposure of the platelets to fluoride for 20 min at 37° C. This represented only a small increase over that found after 20 min without release inducer (23).

The slowness of the release with fluoride was a puzzling phenomenon which did not fit with the efficiency of the inducer in releasing at least as much of the stored compounds as thrombin. We speculated that the low level of available energy in the presence of a glycolytic inhibitor might cause the slowness. However, with thrombin the presence of another glycolytic inhibitor, deoxyglucose, had only a partial inhibitory effect on the amount of release, and none on the time course (22). This suggested that the answer must be sought in another direction. While most release inducers act on the outer membrane, it is the working hypothesis for the action of calcium ionophores that they have to be transported through the membrane in order to liberate the non-granulestored calcium into the platelet cytoplasm and thus trigger the release reaction (30).

Figure 1

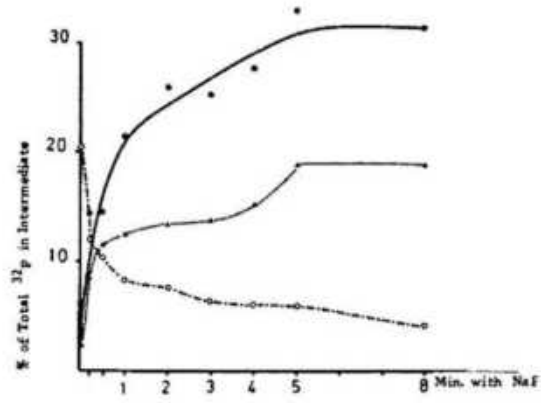
Effect of Metabolic Blockers on Time Curve for Secretion




Platelets incubated for 20 min at 37°C in tris-buffered saline of pH 7.4 with 0.15 mM EDTA. Lower curve: 10 mM NaF added at beginning of incubation or after 8, 12 or 17 min incubation. Upper curve: Fluoride added at beginning of incubation and antimucin (60 n g/ml) added together with fluoride, or after 3, 8 or 12 min uncubation. Adenine nucleotides in the supernatant determined from the spectrum at 260 nm as described (24).

Figure 2

Distribution of Radioactivity between Phosphorus Containing Metabolic Intermediates when Platelets are Incubated with NaF (32)




Platelet-rich plasma incubated with $^{32}P_i$ for two hours; platelets isolated and washed. Suspension of platelets in buffered saline (25 mM tris, HCl of pH 7.4) incubated at 37°C with 10mM NaF.

——— $^{32}$p-fructose 1, 6-diphosphate
▲.....▲ $^{32}$p-Phosphoglyceric acid
○-.-.-.-○ $^{32}$p-ATP

} per cent of total radioactivity of trichloroacetic acid extract (28).

The first point to establish in order to obtain an understanding of the mechanism of release induction is whether the induction takes place from the outside or inside of the plasma membrane. Two ways of assessing this are: 1) to study whether the inhibition of glycolysis measured as decrease in the platelet level of metabolic ATP is related to the release induction as the former must be assumed to be caused by internally situated fluoride ; 2) to study whether a lowered pH will result in more efficient release induction, from the assumption that at a lower pH more of the fluoride exists in a non-ionic form, so that it will penetrate the platelet membrane easier. Other studies have indicated that this is the case, in that a lower concentration of fluoride is needed to impose the same effect (e.g. contraction of vascular smooth muscle (31)). Weissbach and Redfield (17,18) showed that fluoride inhibition of serotonin "uptake" was more effective when pH was reduced from 7.4 to 5.7, presumably because the uptake was counteracted by the fluoride-induced release; such a release was demonstrated during long time incubation with 1mM fluoride (17). A systematic study shows that both pH and fluoride concentration influence the efficiency of the release reaction (Table 1).

Table 1

$F^-$ as Release Inducer Increased Efficiency at lower pH

| Fluoride Concentration | pH | Incubation Time 3 min | 10 min | 20 min |
|---|---|---|---|---|
| 1 mM | 7.9 | 0 | 0 | 0 |
| 10 mM | 7.9 | 13.5 | 63.0 | 79.0 |
| 1 mM | 6.0 | 5.0 | 36.0 | 55.0 |
| 10 mM | 6.0 | 90.0 | 91.5 | 96.5 |
| 2 mM | 5.3 | 60.0 | 71.5 | 73.5 |

Washed platelets were incubated at 37° in 0.13 M NaCl containing 10 mM Na citrate of pH 6.0 or 5.3, and with 25mM tris, HCl added to the pH 6 citrate in the samples of pH 7.9. 2.5 μM imipramine was present to prevent reabsorption of serotonin.

The fate of the metabolic ATP after exposure of whole platelets to fluoride has been studied with platelets preincubated with inorganic $^{32}P^-$ labeled phosphate or $^{14}C$-labeled adenine (16, 24, 28). The recipients of the label in $^{32}P$-ATP are mainly fructose 1, 6-diphosphate and 3-phosphoglycerate (Fig. 2). This results in a trapping of intracellular phosphate in an "inactive" form (32), as can be seen in the steep drop in $P_i$ from 40% of total $^{32}P$-radioactivity after exposure to 2.5 mM fluoride for 35 min to 16% after exposure to 10 mM NaF (16). The fall in metabolic ATP after one min incubation with a specified concentration of NaF is highly pH-dependent (Table 2), indicating

Table 2

Effect of Different pH and $F^-$ Concentrations on Release and on Metabolic Parameters

| Min incubation with NaF | 0 | 1 | 3 | 5 | 10 | Conditions pH | Conditions mM NaF |
|---|---|---|---|---|---|---|---|
| % $^{14}C$ in | | | | | | | |
| ATP | 60.7 | 58.4 | 32.4 | 16.2 | 10.9 | 7.9 | 10 |
| IMP | 3.2 | 5.1 | 19.2 | 19.6 | 4.2 | | |
| Inosine + hypoxanthine | 14.5 | 15.2 | 25.5 | 42.0 | 60.2 | | |
| % $^{3}H$-serotonin in supernatant | 7.4 | 8.0 | 13.8 | 26.3 | 63.1 | | |
| Adenylate Energy Charge | .842 | .842 | .734 | .603 | .514 | | |
| % $^{14}C$ in | | | | | | | |
| ATP | 59.2 | 40.6 | 29.2 | 21.0 | 9.5 | 6.0 | 1 |
| IMP | 2.2 | 4.8 | 7.5 | 10.1 | 3.8 | | |
| Inosine + hypoxanthine | 19.4 | 25.1 | 35.6 | 44.5 | 58.4 | | |
| % $^{3}H$-serotonin in supernatant | 4.5 | 3.0 | 4.1 | 9.7 | 34.3 | | |
| Adenylate Energy Charge | .859 | .728 | .676 | .624 | .460 | | |
| % $^{14}C$ in | | | | | | | |
| ATP | 62.1 | 17.4 | 7.5 | 5.2 | 4.2 | 6.0 | 10 |
| IMP | 2.9 | 6.3 | 17.7 | 20.8 | 13.9 | | |
| Inosine + hypoxanthine | 18.9 | 22.4 | 29.0 | 40.3 | 58.3 | | |
| % $^{3}H$-serotonin in supernatant | 7.9 | 67.3 | 90.3 | 92.3 | 91.5 | | |
| Adenylate Energy Charge | .878 | .407 | .292 | .299 | .315 | | |

Platelets were prelabeled with $^{14}C$-adenine and $^{3}H$-serotonin. Incubation conditions as explained in Table 1. Samples were taken from total incubate and from supernatant after incubation for determination of % released serotonin and of distribution of $^{14}C$-labeled nucleotide metabolites.

that the transport through the plasma membrane is rate limiting for the inhibitory effect, as for the release induction. The metabolic effect seems however to precede the release induction. Whether there is direct effect on the enzyme system which regulates the breakdown of metabolic ATP, or whether fluoride's effect on the different stages of the nucleotide metabolism is secondary to structural and other changes accompanying the fluoride-induced release reaction and the lowered energy level, is unclear. When pH and NaF concentrations are varied, it becomes evident that there is at least no time relationship between the hypoxanthine production and the release reaction, since the former sometimes precedes and sometimes follows after the latter. Platelets seem to have undergone great changes (big vacuoles and a swollen appearance) which prevail after the fluoride-induced reaction, in contrast to those seen with thrombin, which seem to be reversible, except for the disappearance of granules (33,13). The difference may result from a difference in available energy for the restoration of the platelet structure, assuming that energy is needed to counteract the apparent swelling of the platelets which ac-

companies the release reaction.

A transient accumulation of IMP which can also be seen with thrombin is much more marked with fluoride. A transient change of similar magnitude (comprising almost 50% of total $^{14}C$ incorporated into platelet ATP) has only been demonstrated when platelets are exposed to $H_2O_2$ (34). There is however no indication that this compound is involved in the fluoride-induced reactions.

## Mechanism of the Fluoride Induction of Platelet Activity

Rysanek et al. (13) suggest that fluoride induces aggregation through its interference with general platelet metabolism. This seems unlikely since the added inhibition of metabolism obtained with antimycin effectively blocks the fluoride-induced reactions (Fig. 1) (22).

Lysates of human platelets showed a 7 to 26 fold increase in adenylate cyclase activity with 10 mM NaF, versus 3 to 7 fold increase with prostaglandin $E_1$, whereas 200 units thrombin and 2 $\mu$g serotonin per ml inhibited the cyclase activity 76% and 67% respectively (35). Enhanced cAMP in platelets has been shown to inhibit platelet functions (36). There is therefore no obvious link between the fluoride effect in platelet lysates and the fluoride-induced activation of platelet function. Most references indicate that extra-cellular fluoride does not activate adenylate cyclase in the cell (37). Hashimoto et al. (38) claim that they get a continuous strong rise in cAMP label in intact platelets prelabeled with $^{14}C$-adenine after exposure to 10 mM fluoride. The exposure to fluoride takes place in plasma, where this author has found that fluoride does induce leakage (39). Besides, their chromatographic system does not include IMP as marker, so that there is a possibility that at least part of the label is in this compound. Vigdahl et al. (40) reported that fluoride does not activate cyclase in intact platelets. However, they do not report the length of incubation. If we assume that the lack of effect found by most workers is genuine, and if fluoride has to be transported into the platelet in order to exert its effect, then intact platelets must have an intracellular compartmentation which prevents exposure of the adenylate cyclase to the intruding fluoride. This argumentation would be expected to be applicable to other cell types as well. The fluoride effect on exposed adenylate cyclase may however be related to its inducer effect. Layne et al. (41, 42) have shown that fluoride acts as a phospho-protein phosphatase, and that adenylate cyclase is inhibited when phosphorylated, suggesting that the activation takes place through dephosphorylation. If the activating principle for the platelet release reaction is in a phosphorylated inactive form in the resting state, then dephosphorylation might be the triggering effect of fluoride. In contrast, a specific inhibition of esterases by fluoride has been demonstrat-

ed in monocytes (43). Data by Macdonald (44) suggests that there are two independent "adenylate cyclase" components in brain cortex, one which is enhanced 6 fold with NaF and activated by low [Ca++] or [Mg++] and one which is completely Ca++ - dependent and inhibited by 6 mM fluoride. Solubilization of the cortex homogenate with Triton x-100 resulted in a preparation which was not activated by fluoride, but 100% inhibited by ethylene glycol-bis ($\beta$-aminoethyl ether) N, N'-tetraacetic acid (EGTA). This presents a structurally-linked interaction between the biological effect of fluoride and Ca++ and thus a possible missing link between the fluoride effect and the induction of secretion, a process which in most cell systems is triggered by either extra- or intracellular calcium (45). Bergey (31) has shown that fluoride in itself does not activate smooth muscle contraction in the absence of available calcium, and suggests that it depends upon, and probably works through, the liberation of stored calcium in the muscle. Thus, there might be a near relationship between the induction of platelet function by fluoride and by calcium ionophores. The suggested ionophore effect may be caused by biochemical changes through the action of fluoride (such as dephosphorylation of the inactive form of an enzyme) or by fluoride becoming part of a structural organization which thereby is converted to an ionophore.

## Applications

Even though the induction of platelet function requires high concentrations of fluoride, it is possible that long time exposure will result in a significant uptake also from a medium with lower NaF . The results obtained *in vitro* might therefore have some relevance to the *in vivo* situation. When pH decreases below the physiologic level, the sensitivity of cells to lower fluoride concentrations is shown to increase greatly, if one can generalize from the findings with platelets and smooth muscle cells. The pH milieu in the mouth, for instance, can at times be much more acid than that in the blood stream. The responsiveness of platelets to fluoride is of extra interest because the major effect of fluoride on cell constituents apart from the inhibitory effect on enolase, namely the effect on adenylate cyclase, could not be demonstrated in intact cells. Therefore, if the effect of fluoride on a whole cell system should be examined, the platelet seems a very good candidate.

The specific study of platelet functions has been to a certain degree hampered by the fact that induction of platelet function was very much reduced below pH 6. 5. In whole cell systems a drastic altering of conditions, including extracellular pH, is very important for the elucidation of cell processes. Fluoride also gives us the tool for studying platelet functions when platelet energy metabolism is seriously impaired, thus helping in determining which role an intact energy metabolism has in the functioning of the platelet. This has the added advantage of helping us find the right conditions for preservation and resto-

ration of blood platelets, a blood component which because of its greater complexity and intensive metabolism is more shortlived and susceptible to alterations in the environment than is the red cell.

Finally, by acting from the interior of the platelet (as all data suggest it does) fluoride may have a more direct effect on the secretory mechanism than those release inducers which act from the outside of the cell. By cutting down on intermediary steps fluoride would therefore provide us with a more direct approach to the study of the platelet's secretory mechanism.

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# ENDEMIC GENU VALGUM
# A NEW DIMENSION TO THE FLUOROSIS PROBLEM IN INDIA

by

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SUMMARY: In a detailed clinical study of over 80 subjects, all of whom had evidence of skeletal fluorosis, a clinical syndrome characterized by genu valgum and osteoporosis of long bones was revealed among residents of an endemic fluorosis area in South India. The syndrome occurred mostly among young male adults and adolescents belonging to the poor socio-economic groups. This disease is not only crippling but also has socio-economic implications.

Epidemiological studies carried out in over 300 villages revealed that this manifestation of fluoride toxicity has emerged as a new dimension to the problem of fluorosis in this country. Large reservoirs in these areas may have been etiologically related to this syndrome.

The close similarity between "Endemic Genu Valgum of South India" and "Kenhardt bone disease of Africa" suggests that environmental alterations are involved in the development of this syndrome. A tentative hypothesis to explain the pathogenesis of this syndrome will be presented and discussed.

Endemic fluorosis had been a public health problem in many States of India. The first description of the disease by Shortt and co-workers (1) was based on the studies carried out in parts of Andhra Pradesh (formerly Madras State), in southern India. Subsequent investigations (2-6) carried out from time to time in the country have brought to light the existence of large belts of endemic areas in Andhra Pradesh and Punjab. More recently, it has been shown that fluorosis exists in endemic form in parts of Uttar Pradesh (7), Rajasthan (8), Tamil Nadu (9) and Karnataka (10). The classical features of endemic fluorosis are dental and skeletal changes characterized by osteosclerosis of the spine, pelvis

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and other bones, calcification of interosseous membrane, of ligaments and muscular attachments. Studies carried out more recently by us at the National Institute of Nutrition, Hyderabad, India, have focussed attention on the existence of a new clinical entity, genu-valgum, among subjects with fluorosis (11-15). The salient features of "Endemic Genu Valgum" are presented in this communication.

## Endemic Genu Valgum as Related to Endemic Fluorosis

The cardinal feature of this syndrome is the occurrence of genu valgum and osteoporosis of the bones of the lower extremities in subjects suffering from endemic fluorosis. Thus, the peculiarity of this syndrome is the association of osteosclerosis of the spine, on the one hand and extensive osteoporosis of the limb bones, on the other. Genu valgum makes its appearance among children around 8-10 years of age residing in endemic fluorosis villages. The deformity develops slowly and progresses insidiously over many years, before the fullfledged clinical picture is established. The disease is painless, the deformity usually bilateral. The distance between the two medical malleoli when measured while the child is standing upright with the knees gently touching each other, gives a measure of the degree of genu valgum. In advanced cases, the deformity restricts physical movements and impedes walking. When extreme, the lower extremities are so distorted that the knees actually cross over while the subject walks. Obvious shortening of stature occurs within a few years. More males are affected than females with a sex ratio of over 10:1. In some families more than one member is affected. It is not only a physical but also a social and economic handicap. Several instances of broken family ties have been brought to our notice during field studies. The typical clinical picture is presented in Figure 1.

## Radiological Features

Antero-posterior views of the cervical and lumbo-dorsal regions of the spine were taken in more than forty subjects afflicted with the syndrome. All but two had evidence of sclerosis of the spine. Spinal ligaments were calcified and "bamboo spine" was seen in some of them. Calcification of interosseous membrane of the forearm was observed in all cases studied. In addition, sclerosis of humerus, scapulae, ribs, forearm bones and pelvis and calcification of muscular attachments were seen in some subjects. The most striking radiological feature, however, was marked osteoporosis of the lower end of the femur and of the upper ends of the tibia and fibula and rarefaction of the metacarpal bones. In some cases, however, lower ends of radius and ulna were also rarefied. The typical radiological appearances of the knees, forearm and spine are presented in Figures 2 and 3.

Fig. 1

Typical Endemic Genu Valgum

Fig. 2

X-ray Spine of Subject with Endemic Genu Valgum

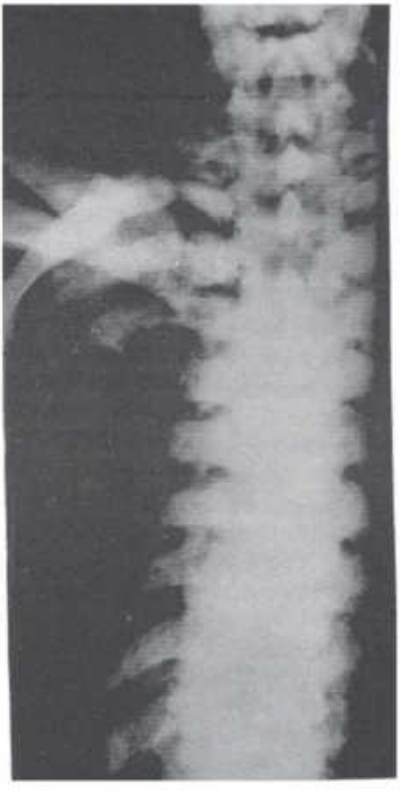


Fig. 3

Interosseous Membrane Calcification. Same Subject.

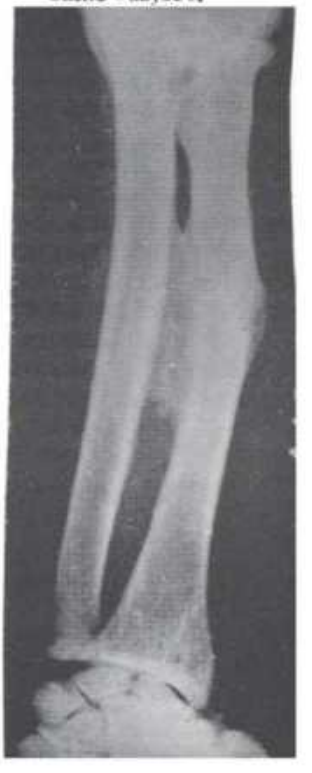
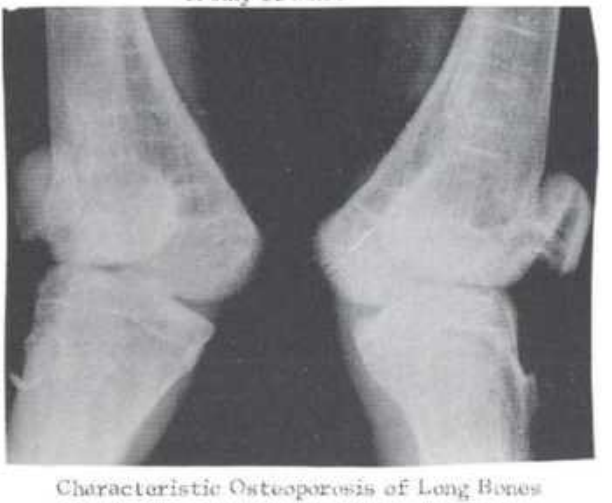


Fig. 4

X-Ray of Knee



Characteristic Osteoporosis of Long Bones

The metacarpals were rarefied with coarse and sometimes cystic trabecular markings. Subcortical loss of metacarpal bone was seen in some skiagrams; the cortex was thinned with reduced cortico-medullary ratio in some cases. Multiple horizontal lines of trabeculae, suggestive of repeated arrests of osteoblastic activity, were seen in the tibial and femoral radiographs.

## Biochemical Data

Levels of serum calcium, phosphorus and alkaline phosphatase activity were within normal limits in established cases.

Urinary hydroxyproline was assessed in four subjects afflicted with the syndrome and in three normal control subjects of the same socio-economic group and of the same age and sex. Total and free hydroxyproline levels were estimated and the bound form of hydroxyproline was calculated from these values. The results are presented in Table 1. As can be seen, the total urinary hydroxyproline was in-

Table I

Mean Urinary Hydroxyproline in Endemic Genu Valgum (mg/24 hrs)

| Group | Subject | Total | Free | Bound |
|---|---|---|---|---|
| Endemic Genu Valgum | 1 | 122.0 (62.5-158.6) | 28.4 (2.2-89.5) | 93.7 (60.3-144.2) |
| | 2 | 85.1 (43.6-130.2) | 40.7 (4.3-77.7) | 55. (23.9 71.5) |
| | 3 | 89.6 (84.8-99.5) | 8.6 (3.0-19.0) | 81.6 (74.8-94.1) |
| | 4 | 121.9 (119.4-141.8) | 8.3 (4.4-12.2) | 113.6 (100.1-133.5) |
| Control | 1 | 23.8 (6.3-45.2) | 8.7 (6.3-13.8) | 12.5 (4.5-31.4) |
| | 2 | 30.3 (24.040.7) | 6.2 (4.6-8.1) | 24.1 (18.1-32.6) |
| | 3 | 44.4 (36.7-51.0) | 6.7 (5.1-8.0) | 37.7 (31.6-44.0) |

creased in this syndrome, indicating active breakdown of collagen. Earlier studies from our Institute had clearly shown that in classi-

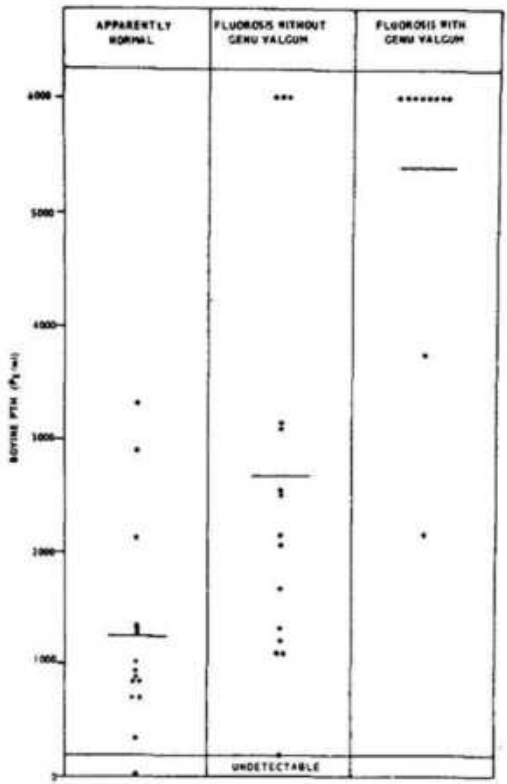
cal endemic fluorosis without genu valgum, total urinary hydroxyproline excretion remained normal although changes in hydroxyproline peptides were observed (16).

## Circulating Parathyroid Hormone and Thyrocalcitonin

The radiological changes in subjects with genu valgum suggested a possible increase in activity of the parathyroid glands. Teotia and Teotia (17) had shown that, in classical endemic fluorosis, circulating levels of parathyroid hormone (PTH) are significantly elevated. Circulating levels of PTH and thyrocalcitonin were, therefore, estimated. Immuno-reactive PTH was estimated in 15 subjects with endemic fluorosis, in 10 subjects with endemic genu valgum and in 15 apparently normal, non-fluorotic subjects drawn from the poor socio-economic population groups. A double-antibody procedure was used (18). The mean concentration of PTH was appreciably higher in subjects with endemic fluorosis (2.67 ng/ml) as compared to that of controls (1.24 ng/ml). Significantly, however, in subjects with genu valgum, levels of circulating PTH were higher (5.39ng/ml) than those encountered in fluorosis without genu valgum (Fig. 5).

Fig. 5

Parathyroid Hormone Levels in Endemic Genu Valgum




Plasma calcitonin levels were estimated by means of a double-antibody radioimmunoassay. The minimum level of the hormone detectable by this method is 125 pg/ml. In 3 out of 6 control subjects, levels of the hormones were in the detectable range, whereas only 1 of 15 subjects with fluorosis with or without genu valgum had detectable levels of the hormone. These studies suggest that some of the radiological changes seen in subjects with genu valgum may be attributed to elevated levels of circulating PTH

without a compensatory elevation of thyrocalcitonin.

After establishing the clinical, radiological and some biochemical features of this new syndrome, a series of epidemiological studies were carried out to assess the magnitude of this problem and to understand the etiological factors related to it.

## Epidemiological Studies

Andhra Pradesh: Over 150 villages affected with fluorosis were surveyed. The location of these villages is shown in Figure 6 . A great majority of these villages are situated within a radius of 50-75 miles from the Nagarjunasagar Dam and its canals, a large water reservoir constructed less than 20 years ago. The residents of these villages, mostly agricultural laborers, are permanent residents of these villages

Fig. 6

Map of Andhra Pradesh, India
The Dam and the Affected Areas

having resided there since birth. The fluoride content of drinking water in these villages ranged from 3 to 13 ppm. In the majority of villages, 70-90% of the residents had dental mottling. Some of the villages had been thoroughly investigated by earlier workers before 1950, and genu valgum had not been found. During the current study, the classical features of fluorosis such as kyphosis, exostoses, radiological evidence of interosseous membrane calcification and sclerosis of bones were seen among the elderly residents of these villages whereas genu valgum was widely prevalent exclusively among the younger age groups.

The prevalence of the syndrome in some villages of Andhra Pradesh is presented in Table 2. In one village, an incidence as high as 17 per cent was observed. Over 90 per cent of the subjects affected with genu valgum belonging to the poorest segments of the population manifested several signs of nutritional deficiency (Table 3). Their calcium intake was particularly low. The syndrome was not related to any specific occupation, caste or to the severity of physical exercise. This disease differed distinctly from Vitamin D deficient rachitis for the following reasons:

1. Prevalence of rickets in the age group of 1-3 years in the affected villages was very low and was not different from that observed in the rural areas of the other parts of the State.

2. The age of onset of this syndrome was always beyond 7 years, a period uncommon for vitamin D deficiency in this country.

3. The affected communities were exposed to plenty of sunshine and no radiological or biochemical evidence of rachitis among the affected children was found.

4. The syndrome did not occur in villages free from the problem of fluorosis, where the fluoride content of water was within acceptable limits.

## Recent Emergence of the Problem

As mentioned previously in detailed studies carried out in this part of India by earlier workers this syndrome in relation to fluorosis had not been observed. The emergence of this new dimension to the fluorosis problem in this State constituted an enigma. However, circumstantial evidence that only adolescents and young adults suffered from the syndrome whereas the elderly residents drinking the same water for longer periods of time manifested no deformity, strongly suggested that, in addition to ingestion

Table 2

Percent Incidence of Genu Valgum
in Villages of Andhra Pradesh

| | Village | Population | No. of subjects | Incidence |
|---|---|---|---|---|
| | **PRAKASAM DISTRICT** | | | |
| 1. | Peddaullagallu | 1500 | 60 | 4.0 |
| 2. | Lankojanapally | 500 | 25 | 5.0 |
| 3. | Venkatachalampally | 600 | 30 | 5.0 |
| 4. | Kothapalem | 600 | 10 | 1.7 |
| 5. | Venkatapuram | 500 | 35 | 7.0 |
| 6. | Buchannapalem | 500 | 5 | 1.0 |
| 7. | Rajupalem | 400 | 40 | 10.0 |
| 8. | Gundl asamudram | 1300 | 300 | 2.3 |
| 9. | Kellampally | 300 | 15 | 5.0 |
| 10. | Kellampally village | 1200 | 12 | 1.0 |
| 11. | Baptistpalem | 500 | 5 | 1.0 |
| 12. | Prakashnagar | 300 | 15 | 5.0 |
| | | 8200 | 282 | 3.4 |
| | **NALGONDA DISTRICT (RAMANNA PET TQ.** | | | |
| 1. | Batlapally | 350 | 60 | 17.1 |
| 2. | Vottipally | 1000 | 40 | 4.0 |
| 3. | Yelagapally | 900 | 35 | 3.9 |
| 4. | Shivannagudem | 1500 | 25 | 1.7 |
| 5. | Voilapally | 1500 | 50 | 3.3 |
| 6. | Arregudem | 300 | 25 | 8.3 |
| 7. | Anthampet | 1500 | 40 | 2.6 |
| | | 7050 | 275 | 3.8 |
| | **GUNTUR DISTRICT** | | | |
| 1. | Ummadivaram | 300 | 3 | 1.0 |
| 2. | Nagulavaram | 1200 | 9 | 0.75 |
| 3. | Pittambanda | 800 | 2 | 0.25 |
| 4. | Enugupalem | 500 | 8 | 1.6 |
| 5. | Dhenuvukonda | 500 | Nil | |
| 6. | Vykally | 600 | Nil | 2.0 |
| 7. | Doddavaripalem | 700 | 3 | 0.43 |
| 8. | Perayapalem | 500 | 5 | 1.0 |
| 9. | Gonugantivaripalem | 1100 | 10 | 0.9 |

Table 3

Age-wise Distribution of Genu Valgum, Dental Changes
And Nutritional Deficiencies in One Village

| | 5 Years | | 6 - 15 | | 16 - 39 | | 40 | |
|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F |
| Total Subjects | 30 | 28 | 54 | 41 | 59 | 59 | 38 | 35 |
| Anemia | 4 | 5 | 4 | 8 | 8 | 29 | 4 | 5 |
| B-Complex | | 1 | 5 | 5 | 9 | 12 | 8 | 3 |
| Vitamin A Deficiency | 12 | 4 | 11 | 8 | 2 | | | |
| Genu Valgum | 7 | 2 | 8 | 1 | 22 | 6 | 3 | |
| Dental Changes | | | | | | | | |
| Normal | 10 | 8 | 4 | 2 | 0 | 1 | 0 | 0 |
| Grade 1 | 10 | 10 | 5 | 5 | 9 | 8 | 0 | 5 |
| Grade 2 | 9 | 10 | 32 | 24 | 34 | 24 | 25 | 18 |
| Grade 3 | 3 | 3 | 13 | 10 | 15 | 26 | 14 | 12 |
| Skeletal Changes | 0 | 0 | 0 | 0 | 21 | 12 | 11 | 14 |
| Skeletal Symptoms | 1 | 5 | 3 | 7 | 38 | 51 | 19 | 23 |
| Neurological Signs | 8 | 1 | 5 | 0 | 22 | 7 | 6 | 7 |

of fluoride, some environmental change in recent years had contributed to the genesis of the syndrome. Moreover, in the past, no cases of genu valgum have been reported from Punjab, another State in India, well known for its fluorosis endemicity. In view of the fact that construction of a huge water reservoir was a major environmental change in this part of Andhra Pradesh, further studies were carried out in areas where the problem of fluorosis exists and where dams have been constructed.

## Studies in Other States of Southern India

A systematic epidemiological study was carried out in Coimbatore District of the adjacent State of Tamil Nadu (20), the results of which can be summarized as follows:

1. Fluorosis is a problem in Coimbatore District.

2. Many fluorosis-affected villages are situated in the vicinity of Aliyar Dam, constructed more than ten years ago.

3. Endemic genu valgum was found in 6 of 23 villages situated in the neighborhood of the dam.

4. The clinical and epidemiological features observed were similar to our earlier observations in Andhra Pradesh.

5. Villages situated nearer the dam had greater prevalence of genu valgum (Table 4).

6. The fluoride content in well water in these villages ranged between 1 and 3.5 ppm.

7. The elderly residents had manifestations of classical skeletal fluorosis, confirmed by radiological examination.

8. The incidence of genu valgum was less than 2 per cent, considerably less than in Andhra Pradesh.

Studies carried out in over 50 villages in Karnataka, a third State in Southern India, revealed that skeletal fluorosis was widely prevalent in an area, Mundargi, where the fluoride content of water varied between 3 and 7.6 ppm. The residents of these villages had all the features of skeletal fluorosis seen in Punjab. Genu valgum was not encountered among the population of this area. Interestingly, this area is situated far from any dam (Table 5). In contrast to this, a few cases of genu

Table 4

Analysis of Water Samples for Fluoride Content (ppm)

| S. No. | Source of water sample | Fluoride content ppm | Distance from Dam Km |
|---|---|---|---|
| 1. | Aliyar Dam Reservoir | 0.1 | |
| 2. | Angalakurichi* | 3.3 | 6 |
| 3. | Vedasandur | 1.2 | 8 |
| 4. | Thorayur* | 3.0 | 10 |
| 5. | Arasur well water* | 3.0 | 12 |
| 6. | Kullegaundanur* | 1.9 | 13 |
| 7. | Kambalparthi* | 2.5 | 16 |
| 8. | Poovalamparthi* | 4.0 | 17 |
| 9. | Paramadaiyur | 1.0 | 18 |
| 10. | Kodangipatty* | 3.2 | 19 |
| 11. | Reddiarur | 1.6 | 22 |
| 12. | Arthanaripalayam well water | 1.5 | 23 |
| 13. | Devanurpudur well water | 0.8 | 24 |
| 14. | Kodingyam (near leather factory) | 0.5 | 30 |
| 15. | Jellypatty | 1.7 | |
| 16. | Odayakulam Harijan colony well water | Very small amounts | |
| 17. | Odayakulam Harijan colony pipe water | 0.5 | |
| 18. | Kursanur | 3.2 | |
| 19. | Thirumurthi Dam waters | 0.2 | |

*Indicates occurrence of genu valgum.

valgum were seen in villages nearer to Hospet Dam, where the fluoride levels in the drinking water ranged between 1 and 3 ppm. During these studies, it was also observed that communities exposed to the same level of fluoride where the staple was sorghum or bajra (Pearl millet) had a higher incidence of genu valgum than those which subsisted on rice.

These epidemiological studies strongly suggested that fluoride toxicity had been modified by factors related to recent changes in environment. A systematic analysis of food samples was, therefore, carried out to determine any specific attribute of foods grown in this area. The results are presented in Table 6. As can be seen, the molybdenum content of sorghum and bajra were significantly higher in samples obtained from fluorosis-affected villages than in corresponding samples obtained from villages with no fluorosis (19).

Table 5

Results of Fluoride Analysis in Well Water Samples and its Relation to Genu Valgum

| State | Taluk | No. of Villages Surveyed | Well Water Fluoride Content (ppm) 1.0 | 1-2 | 2-3 | 3-5 | 5-0 | Distance From Dam | Occurrence of Genu Valgum | Range of Fluoride Content (ppm) |
|---|---|---|---|---|---|---|---|---|---|---|
| Karnataka | Koppal | 8 | 2 | 4 | 1 | 1 | | Farther off (Above 50 Kms) | Absent | 0.4 -3.0 |
| | Mundargi | 21 | 3 | 7 | 2 | 5 | 4 | -do- | -do- | 0.8 - 7.6 |
| | Mallapuram | 26 | 14 | 11 | 1 | | | Near Hospet Dam (5-25 Kms) | Present (A few cases) | 0.6 - 2.2 |
| | Hospet | 3 | 3 | | | | | Near Hospet Dam (5 Kms.) | Absent | 0.5 - 0.8 |
| Tamilnadu | Pollachi | 14 | 2 | 6 | 1 | 5 | | Near Aliyar Dam (5-30 Kms.) | Present (Ranging from 2 cases to 15 cases per village, i.e., 0.2 - 1.6%) | 0.5 - 4.0 |

Table 6

Comparative Data on Molybdenum, Copper and Zinc Content of Foodgrains (Rice, Sorghum and Bajra) from Normal and Fluorosis Areas

| | | No. of Samples | Molybdenum | Copper | Zinc |
|---|---|---|---|---|---|
| | | | mg/100g | | |
| Rice (Brown) | Normal | 8 | 0.93 ± 0.052 | 3.67 ± 0.314 | 15.5 ± 0.78 |
| | Fluorosis | 39 | 0.89 + 0.042 | 4.64 + 0.310 | 16.4 + 0.56 |
| Sorghum | Normal | 47 | 0.75 + 0.039 | 4.03 + 0.202 | 10.7 + 1.12 |
| | Fluorosis | 52 | 1.20 + 0.057*** | 4.80 + 0.222 | 19.4 + 0.76 |
| Bajra | Normal | 15 | 0.78 + 0.040 | 6.60 + 0.748 | 39.1 + 6.99 |
| | Fluorosis | | 1.22 + 0.066*** | 5.67 + 0.268 | 22.4 + 1.06 |

(All values are mean ± S.E.)
***P 0.001

## Trace Metal Content in Water

Representative water samples were obtained from 44 fluorosis-affected villages belonging to the three States studied. Trace metal analysis was carried out by means of Atomic Absorption Spectrophotometer. The results are presented in Table 7. None of the villages with a fluoride content of less than 1 ppm had cases of genu valgum. The occurrence of genu valgum was twice as frequent in villages where the fluoride content of water was above 3 ppm as in those where fluoride levels ranged between 1 and 3 ppm. In about 70 per cent of villages affected with genu valgum, very low levels of copper were found in the water samples; most were below 0.01 ppm. No cases of genu valgum were found in villages with a copper content in water of more than 0.1 ppm in spite of high fluoride levels.

Likewise, a similar trend was observed in the case of zinc. About 45 per cent of the villages with zinc values in water below 0.1 ppm had cases of genu valgum. However, no association between the occurrence of genu valgum and magnesium content of water was observed.

## Possible Prevention

Our studies also showed that surface water in the irrigation canals of the dams, carried very low levels of fluoride in spite of passing through many miles of fluorosis area (Table 8). In view of the observation that the village canals invariably contained several times less fluoride than the village well water, it seems reasonable that one of the ways to prevent the problem of endemic genu valgum is to utilize the water of the irrigation canals - which is relatively low in fluoride - for drinking purposes.

## Discussion

It is obvious from these observations that the syndrome of endemic genu valgum has emerged as a new dimension to the fluorosis problem in India. Circumstantial evidence strongly suggests a relationship between construction of large water reservoirs in the vicinity of fluorosis areas in parts of India and genesis of this syndrome. The clinical picture, epidemiological features and radiological nature of this syndrome are similar to those of "Kenhardt Bone Disease" described by Jackson (21). Furthermore, the community in which Kenhardt bone disease was prevalent, had been an endemic fluorosis area and was situated in the vicinity of a dam, Rooieburg Dam. However, it is not clear why

Table 7

Trace Metal Content of Well Water Samples in Relation to Genu Valgum

| | Concentration (ppm) | Fluoride Content (ppm) | | |
|---|---|---|---|---|
| | | 1 | 1.1-3.0 | 3.1 |
| | 0.01 (16) | - | 4* | 7* |
| Copper (44) | 0.01 - 0.1 (19) | - | 1* | 4* |
| | 0.1 (9) | - | - | - |
| | 0.1 (14) | - | 1* | 8* |
| Zinc (44) | 0.1 - 0.3 (23) | - | 2* | 3* |
| | 0.3 (7) | | 2* | 0 |
| | 10 (2) | - | - | - |
| Magnesium (44) | 10 - 30 (32) | - | 5* | 6* |
| | 30 (10) | - | - | 5* |
| Total Villages Studied | | 4 | 23 | 17 |
| Villages Affected with Genu Valgum- | | | 5 | 11 |

Figures in parentheses indicate number of villages surveyed.

*Number of villages affected with genu valgum.

Table 8

Source of Water Sample Analyzed

| | ppm | | ppm |
|---|---|---|---|
| 1. Nagarjunasagar Main Reservoir | 0.75 | 15. P.M. 30th mile | 0.782 C |
| 2. Chejerla | 0.957 | 16. Ummadivaram village | 3.005 V |
| 3. Addanki Branch canal 5th mile | 0.714 | 17. -do- NSC.W. | 1.274 C |
| 4. -do- 10th mile | 0.234 | 18. Nagularam village | 1.515 V |
| 5. -do-- 15th mile | 0.602 | 19. -do- NSC.W. | 1.054 C |
| 6. -do- 20th mile | 0.571 | 20. Pittambanda village | 4.934 V |
| 7. -do- 25th mile | 0.6312 | 21. Enugupalem village | 8.619 V |
| 8. -do- 30th mile | 0.389 | 22. Enugupalam NSC.W. | 2.963 C |
| 9. Pittambanda Major | 1.027 | 23. Denuvukonda | 1.602 V |
| 10. P.M. 5th mile | 1.069 | 24. Vykallu village | 10.2605 V |
| 11. P.M. 10th mile | 1.970 | 25. Perayapalem village | 1.294 V |
| 12. P.M. 15th mile | 3.0042 | 26. Doddavaram village | 1.316 V |
| 13. P.M. 20th mile | 0.529 | 27. Govunugantivari palem | 6.952 V |
| 14. P.M. 25th mile | 0.389 | | |

V = Village C = Canal

this syndrome is not prevalent in Punjab, which is also near a dam. Since the food habits, environment and soil conditions of Punjab are very much different from those of the Southern Indian States, the need for further studies with regard to the role of ecological factors in modifying fluoride toxicity is apparent.

It is well known that construction of large water reservoirs bring about changes in the subsoil water level (22). This can lead to alterations in the pH of the soil. It is also known that changes in the alkalinity of soil play an important role in the uptake of trace elements by plants. Whether such changes in parts of India in recent years have modified the fluoride-induced manifestations requires further study.

The high molybdenum content in the staple sorghum contrasts strikingly to that of rice. Whether prolonged consumption of high amounts of molybdenum modifies fluoride toxicity needs further elucidation, since interaction between molybdenum and fluoride is well known. Deosthale and Gopalan (23) from our Institute have shown that ingestion of large amounts of dietary molybdenum results in increased elimination of copper through the urine. In view of the known role attributable to copper in collagen metabolism, it would be interesting to study this community in greater detail so that the relationship, if any, between high molybdenum ingestion and the genesis of the syndrome of endemic genu valgum can be ascertained.

The pathogenesis of genu valgum in fluorosis appears to be complicated. Whereas increase in levels of parathyroid hormone can explain some of the radiological features observed, it does not seem to be the only factor in the genesis of this syndrome. Although low intake of calcium may bring about changes in the level of parathyroid hormone, it cannot explain many of the epidemiological features observed. The reason for sex difference in the occurrence of the syndrome also is not clear. Studies are now in progress aimed at answering some of these questions.

Acknowledgements

The authors express their gratitude to Dr. C. Gopalan, Director-General, Indian Council of Medical Research, and to Dr. S. G. Srikantia, Director, National Institute of Nutrition, for their constant encouragement and guidance during the conduct of these studies.

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## Discussion

Dr. Waldbott: The transverse lines in X rays of bones in your slides show similarities to Pb poisoning. Are you going to study entry of other major elements such as Pb, Cd and Sr into the bone? You concentrated on Mo. Did you analyze the water from the dam in this area?

Dr. Krishnamachari: We analyzed water from the dam for fluoride content. It was less than 1 ppm. Well water in the region contained over 1 ppm fluoride and up to 10 ppm. The Ca in the diet of individuals in this region was very low which may be the major explanation for the occurrence of osteomalacia and other bone disorders in conjunction with high fluoride. Soil and changes caused by the dam may be a factor in relation to micronutrients found in the soil.

Dr. Jolly: What was the critical level of fluoride in the blood?

Answer: The blood fluoride in continuous equilibrium should be 1-2 μM (0.19-0.38 ppm); above 10 is critical.

Dr. Sinclair: Are the only changes in enzymes those of high alkaline phosphatase and low succinic dehydrogenase? We know that cyclic AMP increases with fluoride. Are there any studies on this?

Dr. Jolly: We have not studied these enzymes and currently are not set up to do so. It is probable that fluoride effects are at the enzymic level, but we do not have facilities for such studies at the present time.

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# FLUORIDE IN SPANISH BOTTLED WATERS

by

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SUMMARY: Samples of twelve bottled waters which are widely distributed in Spain were analyzed for their fluoride content. Twenty four hour urine specimens of individuals who had taken controlled doses of these waters upon analysis for fluoride revealed that four of them greatly exceeded the internationally recommended "permissible limits".

## Introduction

In view of the high fluoride values indicated on the labels of some of the brands of "mineromedicinal" waters, 12 kinds of mineral waters were analyzed using the selective electrode technique (1,2). As indicated in Table 1, most samples contained slightly higher levels of fluoride than indicated on the labels (Table 1).

Table 1

| Definition on the Label | mg $F^-$/liter (Label) | mg $F^-$/liter (Analysis) |
|---|---|---|
| A - Natural mineral water | 0.1 | < 0.8 |
| B - Natural table water | 4.9 | 9.8 |
| C - Mineromedicinal water | Traces | 2.9 |
| D - Mineromedicinal water | not specified | < 0.8 |
| E - Mineral table water | " " | Undetected * |
| F - Natural mineral water | " " | " |
| G - Mineral water | " " | " |
| H - Natural mineral table water | " " | 0.8 |
| I - Natural water | 3.5 | 8.6 |
| J - Mineromedicinal table water | 9.5 | 12.2 |
| K | 0.9 | 1 |
| L | 1.2 | < 0.8 |

* The smallest amount recorded by the analysis was 0.1 ppm of fluoride.

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Because of these surprising results, urine specimens of individuals who were administered controlled doses of water high in fluoride (J) were analyzed and the results are shown in Table 2. The water in Santander is low in fluoride (<0.1 ppm).

Table 2

| Volunteer | Initial Urinary Fluoride Content mg/liter | Volume Dose in Liters | Time Days | Maximum Fluoride Value Reached mg/liter |
|---|---|---|---|---|
| 1 | 0.4 | 1 | 1 | 6 |
| 2 | 0.6 | 5.5 | 5 | 8.7 |
| 3 | 0.2 | 4 | 3 | 7.7 |
| 4 | 0.8 | 6 | 5 | 8.6 |

It is evident from this table that volunteer No. 1 exceeded Elkins limit (3) and volunteers No. 2 and 4 exceeded Largent's limit of maximum safety (8 mgs/liter) (4).

The individual who consumed J water (12.2 ppm) exclusively for approximately 3 years (except on rare occasions) warrants special attention. His urine analyzed at different intervals during the day yielded the following fluoride values:

| Time of Day | mg $F^-$/liter |
|---|---|
| 19.30 | 7.4 |
| 23.30 | 13.2 |
| 4.15 | 12.7 |
| 7.30 | 6.2 |

Such large amounts of fluoride excreted in the urine are not unexpected in view of the high fluoride content of the water.

Mineromedicinal waters are widely consumed in Spain and bottled water (e.g. the J water) is available in any restaurant or supermarket. No data on fluorosis have been reported on individuals who consume this water but it is not unlikely that such cases exist, especially among children since in Spain children customarily drink "mineromedicinal" water during their early years. It may be necessary to control the sale of fluoridated mineral waters in the dining rooms of factories which use fluoride compounds.

We therefore suggest that the effects of bottled water on the consumer population should be investigated and proper precautions taken for prevention of untoward effects. As another precaution, water with a high fluoride content should be sold on prescription only. Furthermore, it should be established whether or not the mineral waters containing fluoride have any beneficial effect. In the bibliography that we consulted, only toxic effects were reported from water containing more than 1 ppm fluoride (5).

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# THE UPTAKE OF SODIUM MONOFLUOROACETATE BY PLANTS AND ITS PHYSIOLOGICAL EFFECTS

by

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SUMMARY: The uptake and physiological effects of sodium fluoroacetate and sodium fluoride on a number of plant species are compared. Both ionized fluoride and fluoroacetate were taken up by Helianthus annuus although the patterns of distribution differed. Fluoride accumulated mainly in the root but a small percentage was translocated to the shoot causing premature senescence of the leaves. Fluoroacetate, however, was translocated to the shoot with little accumulation in the root. On a μgF/ml basis, the fluoroacetate solutions were more toxic reducing dry weight and producing leaf necrosis. However, fluoroacetate did not have the rapid effect on the water balance of the plants shown by fluoride. The significance of the metabolism of fluoroacetate is discussed in relation to these physiological studies.

## Introduction

Fluoroacetate and other organic fluoride compounds occur naturally in a number of tropical species such as Dichapetalum cymosum (Hook) Engl. and Acacia georginae (F. M. Bailey)(1). The presence of fluoroacetate in these species has led to the view that these plants are able to metabolize inorganic fluoride to fluoroacetate and other fluorocarbon compounds. The synthesis of the C-F bond has been reported in laboratory studies with A. georginae (2, 3 and 4). Other work suggests that the synthesis of organofluorides may be a more general property of plants occurring in common pasture and agricultural species (5, 6 and 7). However, the situation is far from clear. For example, the synthesis of organofluorides could not be demonstrated by Hall (8) in experimental A. georginae and Weinstein (4) could not induce C-F bond synthesis in crop plants exposed to atmospheric fluorides. Also, the presence of fluoroacetate has been demonstrated in tropical soil surrounding various "toxic" species (9). This might indicate that the fluoroacetate is elaborated in the soil by microorganisms and then taken up by the plant as was suggested by Aldous (10).

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Presented at the 7th Conference of the I.S.F.R., Feb. 8-10, 1976, Zandvoort, Holland.

Fluoroacetate and related longer-chain organofluoride compounds are extremely toxic to animals, including man. The classical theory of the toxicity is that postulated by Peters (11) where the compound undergoes "lethal synthesis" to fluorocitrate which blocks the enzyme aconitate hydratase of the tricarboxylic acid cycle. The effects of fluoroacetate on plants, however, are not well documented. This paper is concerned with some of these effects, particularly with growth and water relations. Comparison is made with inorganic fluoride whenever possible.

## Materials and Methods

All seeds were germinated in Petri dishes on filter paper moistened with deionized water and the seedlings transplanted into sand culture (12). Culture solution was given alternately to fluoride or fluoroacetate solutions; the sand was washed with deionized water between treatments. Inert polystyrene "mono" containers were used as pots in all experiments. The treatments were set out in random block design and conducted in a growth cabinet. In water culture calcium only must be removed from the culture solution to prevent precipitation of fluoride (13). Thus, in short term experiments on water relations, calcium nitrate was replaced by sodium nitrate when fluoride (or fluoroacetate) was present.

Total fluoride was analyzed by ashing followed by potentiometric determination; inorganic fluoride by direct acid extraction and potentiometric determination (13, 14).

## Results

Helianthus annuus seedlings were treated with either 200 µgF/ml as sodium fluoride or 100 µgF/ml as sodium fluoroacetate for four weeks. There were at least five replicates of each treatment. The plants were harvested and the tissues of the replicates analyzed separately. The total fluoride content is shown in Table 1.

Uptake of sodium fluoride and fluoroacetate was substantial. However, the distribution and the sites of accumulation differed. When given as fluoroacetate, very high concentrations of fluoride are reached in the leaves and cotyledons. Exposure to inorganic fluoride caused greatest accumulation in the roots with much lower concentrations occurring in the leaves. The total amount of fluoride in the roots and leaves gives a clearer indication of this difference in portioning of fluoride.

Fluoroacetate showed a much greater toxicity than ionized fluoride

Table 1

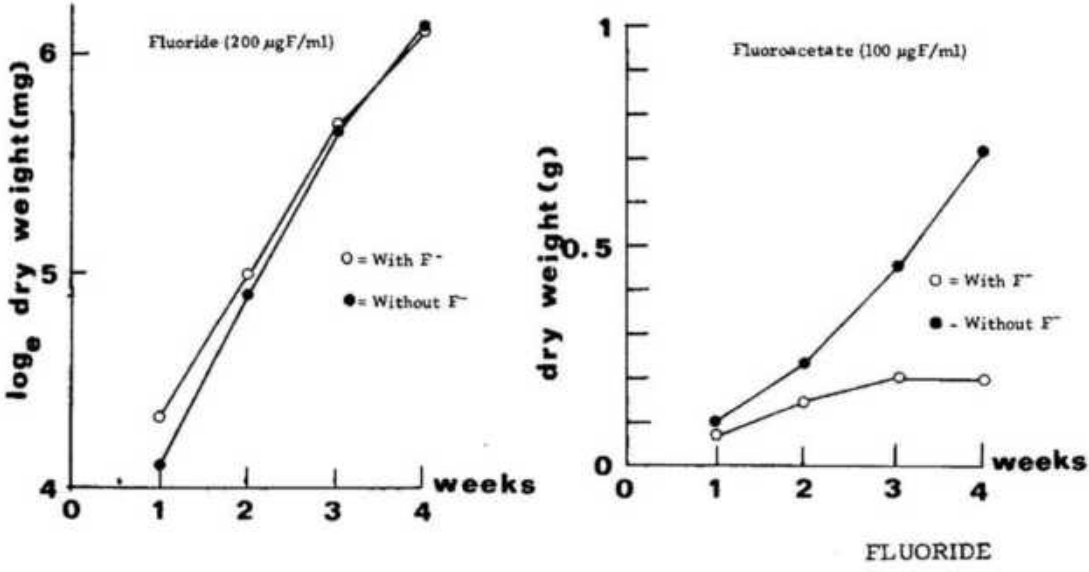
Total fluoride in Hilianthus annuus after four weeks' treatment with sodium fluoride or sodium monofluoroacetate

| | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | Fluoride | | Fluoroacetate | | Control | |
| | Concentration (μgF/g) | Total (μgF) | Concentration (μgF/g) | Total (μgF) | Concentration (μgF/g) | Total (μgF) |
| Root | 3215 | 390.3 | 592 | 19.2 | 4.3 | - |
| Stem | 16 | - | 623 | - | 0 | - |
| Cotyledons | 237 | - | 1673 | - | 3.2 | - |
| Leaves | 112 | 19.8 | 1326 | 109.8 | 7.2 | - |

to H. annuus. No significant differences in total dry weight were noticed over a four week treatment period with 200 μgF/ml as sodium fluoride (Fig. 1). However, there were significant differences in leaf growth with premature senescence of the leaves and equivalent leaves did not attain the same dry weight. Very little growth was achieved by the plants treated with 100 μgF/ml as sodium fluoroacetate for four weeks (Fig. 2). These plants showed chlorosis of the leaves after two weeks and, with continuing treat-

Figures 1 and 2

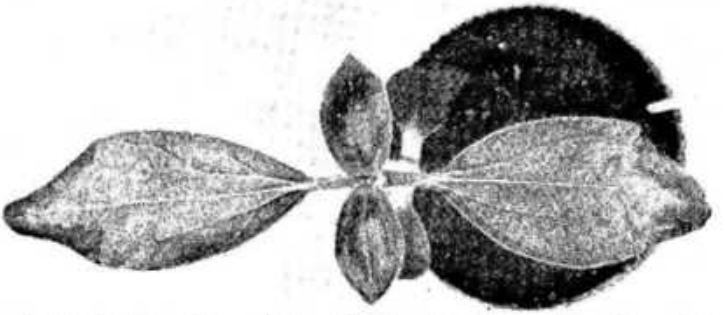
Changes in Dry Weight of H. annuus



ment, developed a red-brown necrosis at the tip which was clearly delimited from the rest of the leaf (Fig. 3). Root growth seemed to be particularly sensitive to fluoroacetate. This can be seen from the decrease in root/shoot ratio during the treatment as compared to the control (Fig. 4).

Figure 3

Helianthus annuus seedling showing necrosis of the first leaf tips after sodium fluoroacetate treatment (100 µgF/ml).



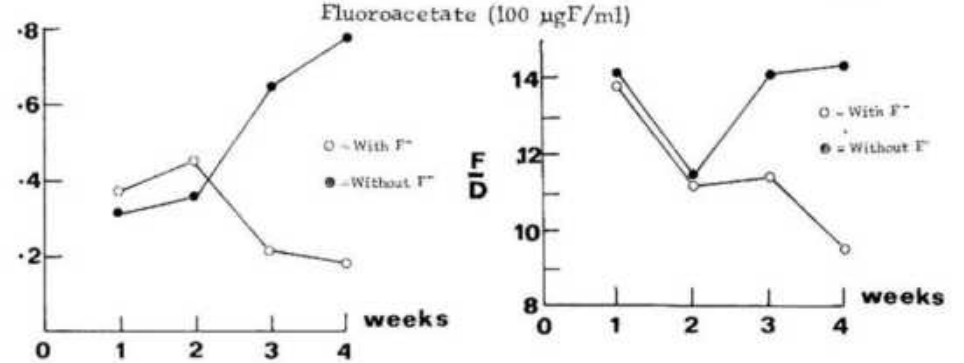
In another experiment the effects of inorganic fluoride and fluoroacetate were compared, on the growth of Lolium perenne L., a grass, and Achillea millefolium L., a dicotyledon. These species were chosen because of their fast growth rates and small seed weight which gives an early dependence on the external nutrient environment. These data are more easily interpreted by plotting the relative growth rates (mg/g/week), between week 2 and 7, with the various treatments as a percentage of the controls. Fig. 5

Figure 4

Root/Shoot ratio (R/S) of H. annuus

Figure 5

Fresh/Dry weight (F/D) of H. annuus

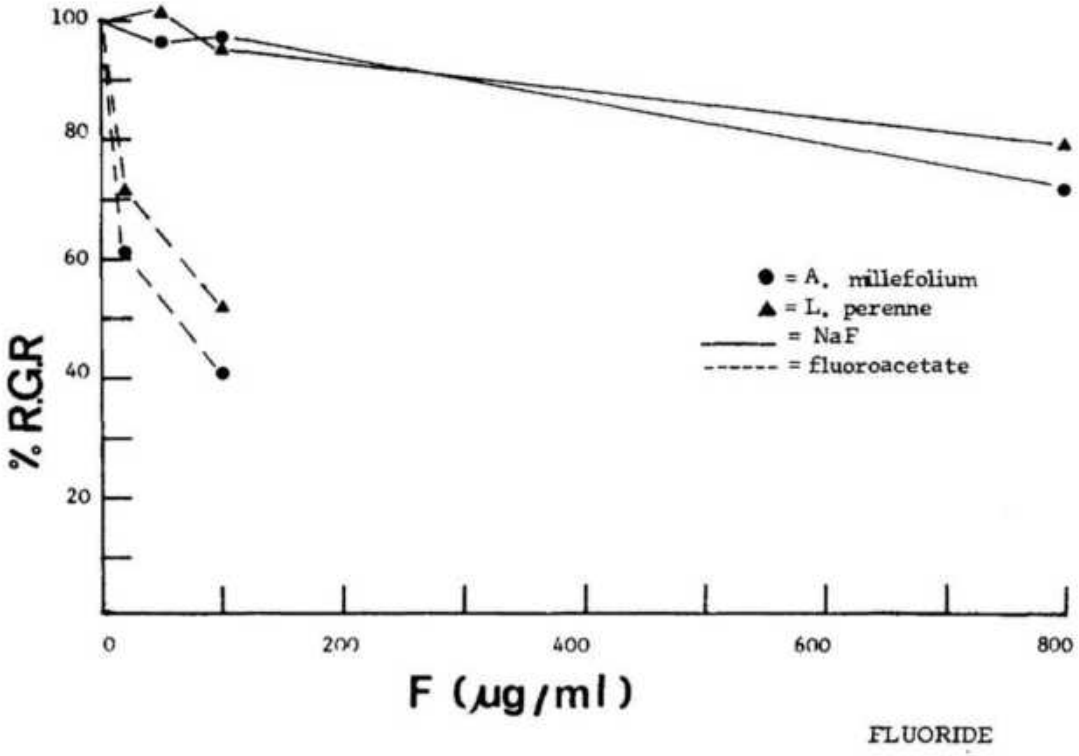


shows these data for A. millefolium and L. perenne. Again fluoroacetate was much more toxic to both species than inorganic fluoride confirming the results obtained with H. annuus.

In view of the changes in fresh weight/dry weight ratio in H. annuus treated with fluoroacetate, its short term effects on transpiration were examined. In the first experiment Glycine max plants in calcium-free culture solution containing 200 µgF/ml as sodium fluoride or 100 µgF/ml as sodium fluoroacetate were used. The transpiration was measured as the total water loss from the system by weighing the containers at set intervals. There were five replicates of each treatment. As the plants differed in their initial transpiration rates (expressed per unit area) the results were expressed as a percentage of the initial rate as well as a percentage of the control (Fig. 6). Both treatments caused the initial rise in the transpiration. The fluoride treatment caused a decrease of 50% after 22 hours and wilting after only 4 hours.

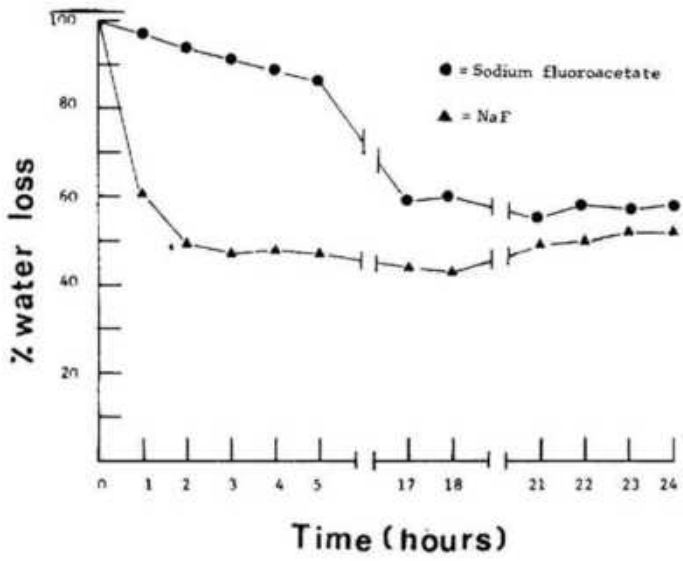
Figure 6

Relative growth rates, as percentages of the control



The rapid uptake of fluoroacetate to the aerial parts suggested that its influence on the water balance might have been on water loss from the leaves. This aspect was investigated using rootless H. annuus seedlings, initially grown in sand, harvested, the roots cut off under water and the stems, still under water, inserted into 20 ml polythene bottles sealed with silicone rubber. Using a syringe the bathing solution was adjusted to 25 µgF/ml as sodium fluoride or fluoroacetate. There were four replicates of each treatment and the results are shown in Fig. 7. Both fluoride and fluoroacetate reduced water loss but there was a lag in the fluoroacetate effect.

Figure 7
Water loss, expressed as % of control, of rootless H. annuus seedlings




## Discussion

The uptake of fluoroacetate by seedlings of H. annuus was substantial and there was rapid translocation into the leaves. The rapid uptake of aliphatic fluoride compounds has been noted by other workers (15, 16). However, Hilton (17) and Hall (8) presented data which suggested that in cotton and in experimental A. georginae respectively, the fluoroacetate was retained in the root with little translocation. The situation, however, is confused by the defluorination of these compounds.

In Hall's work nearly all the fluoroacetate was defluorinated and present as inorganic fluoride in the root which would not be translocated to the shoot. H. annuus also defluorinated fluoroacetate (Table 2).

TABLE 2
Total and inorganic fluoride (μgF/g) in four week old H. annuus after treatment with sodium fluoroacetate (100 μgF/ml)

| | Total | Inorganic | Inorganic/organic |
|---|---|---|---|
| Roots | 592 | 69 | .12 |
| Stem | 623 | 265 | .43 |
| Cotyledons | 1673 | 1340 | .80 |
| Leaves | 1326 | 959 | .72 |

The low percentage of inorganic fluoride in the roots suggests that the major site of defluorination is in the shoot and not in the root. The defluorination by plants of exogenously supplied fluoroacetate has been reported for other species (18, 19) and the site of defluorination may determine its translocation and toxicity.

The severe inhibition of growth by fluoroacetate shown in this study is in contrast to other work (19, 20 ) where ionized fluoride was more toxic. Inhibition of plant growth by fluoroacetate could have been due to a metabolic effect similar to that in animals where it is converted to fluorocitrate via fluoroacetyl CoA. It is interesting to note that if fluoroacetyl CoA was formed in vivo this could, in germinating H. annuus which has fatty reserves, enter the glyoxalate cycle via malate synthase giving 3-fluoromalate (21, 22 ). By competitively inhibiting malate dehydrogenase 3-fluoromalate would also block the T. C. A. cycle (23) with similar drastic effects as fluorocitrate.

Fluoroacetate treatment caused the leaves to become necrotic. The foliar lesions were similar to those caused by inorganic fluoride. Thus, it is possible that the toxicity of fluoroacetate, particularly in the shoot, is due to its metabolism to fluoride. It is paradoxical that higher concentrations of inorganic fluoride can occur in the shoots when the element is given to the roots as fluoroacetate than when given to the roots as inorganic fluoride. No evidence was found in this work for the biosynthesis of organofluorides from inorganic fluoride.

## Acknowledgements

The author is grateful for the financial support of the Wellcome Foundation and a grant-in-aid from the Royal Society during the course of this work.

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## Discussion

Dr. Sinclair: Didn't Sir Rudolf Peters show that fluorocitrate is present in lettuce?

Dr. Cooke: Peter Ward showed metabolism of FAc in lettuce and the probable appearance of fluorocitrate from FAc. We did not detect FAc but did not use sensitive methods for detection such as gas chromatography.

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# THE ROLE OF CALCIUM AND FLUORIDE IN OSTEOPOROSIS IN RHESUS MONKEYS *

by

H. J. Griffiths, R. D. Hunt, R. E. Zimmerman, H. Finberg, and J. Cuttino
Boston, Massachusetts

(Abstracted from the Invest. Radiol., 10:263-68, 1975)

The authors studied the effect of fluoride on bone metabolism in rhesus monkeys with varying dietary intake of calcium fluoride. Thirty-one female rhesus monkeys, about two years of age, were divided into four subgroups and were fed purified diets containing varying levels of calcium and fluoride for a period of 60 months. Six animals (group I) constituted the normal controls. Groups I and II received about 2 grams of calcium per day which is currently accepted adequate for rhesus monkeys; groups III and IV were given only 0.3 grams of calcium per day.

Radiographs of skulls, vertebral column and limbs were taken every three months. After 48 months on the diet, bone mineral was analyzed by photon absorptiometry with a $^{125}I$ point source on the cortical bone of the left arm at the midpoint of the shaft of the radius and ulna. This performance was repeated every three months over the course of one year. Tetracycline labeling and fluorescent staining were made on iliac crest and on two vertebrae of the tail after 54 months. Calcification fronts were determined by measuring the distance of the two fluorescent labels in undecalcified tissue sections. On each biopsy 30 to 50 measurements were made. The thickness of osteoid seams was determined by measuring the total area occupied by osteoid with a planimeter and by dividing this figure by the total perimeter of osteoid seams. The resultant figure was termed the index of osteoid thickness. The x-rays of the lungs were examined for the trabecular patterns, cortical widths, epiphyseal fusion and radiographic density.

## Results

In group I the epiphyseal fusion was considered normal. Group II showed a definite increase in overall density but the cortices and epiphyseal fusion were within normal range. Group III manifested a uniform decrease in density with thin cortices and accelerated epiphyseal fusion. Group IV exhibited thin cortices with increased density, irregular tra-

*See page 169, July 1976

becular patterns, indicative of osteomalacia with wide flared epiphyseal plates (equivalent to rickets) and also abnormal trabecular patterns.

Scanning revealed a marked decrease in bone mineral and reduced widths of bones in group III. Group IV exhibited normal bone minerals but the width of the bones was slightly less than in group I. However, in group IV the bone appeared sclerotic and the osteoid seams were greatly thickened. In group IV also the distance between the two fluorescent labels was significantly narrower than in groups I, II, and III.

These results indicate that a low calcium diet leads to development of osteoporosis with decreased radiographic density, decreased bone mineral content, and a decrease in width with no increase in osteoid, i.e. reduced bone mass or osteoporosis. Addition of fluoride to a diet containing a normal amount of calcium does not increase the width of bone, nor its bone mineral content, although it increased radiographic density. It appears that the addition of fluoride to a low calcium diet prevents osteoporosis. However, in these animals fluoride interfered with mineralization of osteoids, which resulted in marked thickening of osteoid seams as seen in osteomalacia. Fluoride seems to alter osteoid so that it will calcify only in the presence of adequate dietary calcium but simple calcium deficiency without added fluoride does not result in similarly altered osteoid.

The authors concluded that, under conditions of their study, fluoride reduced the metabolic activity of both osteoblast and osteoclast.

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## Symposium on Fluorosis in Hyderabad

The Indian Academy of Geoscience announces publication of the Proceedings of its October 1974 interdisciplinary Symposium on Fluorosis. It focuses attention on the effect of fluoride on humans and livestock and on the control of fluorosis. The volume of about 400 pages, Rexine bound with 67 plates is divided into 5 sections: Geology, mineralogy & geochemistry of fluoride-bearing minerals; Role of fluorides in agriculture; Incidence of fluorides in surface and ground waters and defluoridation of water; Treatment of fluorosis in domestic animals; Human fluorosis. The book is available c/o Geological Survey of India, 1-7-155, Bakaram, Hyderabad 500048. The cost is 150 Rupees, including postage.

# PREVENTION OF BONY FLUOROSIS IN ALUMINUM SMELTER WORKERS

by

B. D. Dinman, W. J. Bovard, T. B. Bonney, J. M. Cohen, and M. O. Colwell
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(Abstracted from J. Occup. Med. Parts 1-4, 18: 7-25, 1976)

The authors set forth a program for prevention of excessive fluoride absorption in aluminum smelter workers on the basis of urinary fluoride excretion and X-ray examinations of workers. During a five-day period semi-annual sampling of urinary fluoride was carried out on approximately 6,500 workers in eight smelters of the Aluminum Company of America.

In the first portion of the four part study analyses of the air to which 12 workers were exposed for seven consecutive days throughout the eight hour shift for particulate and gaseous fluoride were correlated with daily 24 hour urinary fluoride. Because of wide variations in fluoride uptake it was concluded that the actual body fluoride loading potential cannot be adequately determined for individual workers on the basis of fluoride concentrations in air, but in an occupational group the urinary fluoride two to three days after cessation of work-related exposure reflects the bony burden of fluoride.

The second study pertained to 147 workers employed in the pre-bake reduction process. Daily urine collections were made after work for seven days and specimens were also collected before the worker returned to his first day of a new shift following an interval of 48 to 72 hours off work. Variations between individual samplings were such that no regression line could be constructed after day 3 and no conclusion concering the state of fluoride absorption of any individual could be made. The authors believe that the extreme variability after day 3 due to differences in exposure associated either with the job or smelter location occurring during the last few hours of their work day invalidated any conclusions regarding the fluoride absorption state of any individual. They concluded that the use of post-shift urinary fluoride concentrations as an indicator of exposure should be limited to groups of workers rather than to individuals. After the third day urinary fluoride sampling is an adequate indicator for fluoride exposure.

The third study involved 52,000 urinary fluoride determinations collected over five years. The pre-shift urinary fluoride concentrations appears to increase less rapidly than the post-shift concentration. In over 16,000 urinary tests for protein no correlation between urinary fluoride concentrations and the presence of albuminuria was established.

The fourth study presented the X-ray findings on 56 aluminum smelter workers with 10 to 43 years' occupational exposure who had previously been studied both medically and by means of X-rays. These so-called anodemen, whose job is to remove and replace carbon anodes from the molten aluminum-cryolite baths as well as to tap the pots to obtain the finished molten metal were the most exposed individuals in the smelters. "Post-shift" concentrations in samples of the urine ranged widely namely between 3.9 and 13.3 mg/l with a mean of 7.7 mg/l. The "pre-shift" concentrations ranged from 0.5 to 3.4 mg/l with an average of 2.24 mg/l. Comparison of X-ray films taken during 1960 to 1966 and in 1974 failed to reveal any evidence of skeletal fluorosis.

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## LANGZEITERGEBNISSE NACH KOLLEKTIVEN MUNDSPÜLUNGEN MIT NATRIUM FLUORID LÖSUNG IN DER REPUBLIK KUBA *

by

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Leipzig, DDR and Habana, Cuba

(Abstracted from Zahn-, Mund-u. Kieferheilk. 62: 683-689, 1974)

Since 1969, more than 900,000 children throughout the Republic of Cuba have been subjected to systematic brushing of teeth and mouth washing with a 0.2% sodium fluoride solution. In pre-school children, the teeth were brushed twice monthly with tooth brush and cleaned with sodium fluoride solution. The mouths of pre-school and school children were rinsed with the fluoride solution about 16 times throughout the year for approximately one minute. After 28 months, a sampling of 25 boys and girls for each group between 6 and 13 years were chosen totaling 400 examinations. The teeth of these children were compared with those of a neighboring city where the natural fluoride content in drinking water was 0.1 ppm. For the second sampling 42 months following the beginning of the program only 10 and 11 year old children - who had been 6 and 7 years old at the beginning of the experiment - were selected. Twenty-five boys and girls in each group were compared with a similar group of children as controls.

After 28 months (approximately 33 rinsings with sodium fluoride

*See page 169, July 1976

solution) the DMF index in the fluoride-treated children was 18.4% less than in the controls. In the second group among 10 and 11 year old children after 42 months with 55 rinsings the difference was 35.0%. The author acknowledged that factors other than applications of sodium fluoride may have contributed to the prevention of caries in the fluoride-treated groups.

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## EFFECT OF FLUORIDE ON UPTAKE AND LOSS OF FLUORIDE IN SUPERFICIAL ENAMEL IN VIVO

by

G. Ahrens
Hamburg, Germany

(Abstracted from Caries Res., 10: 85-95, 1976)

In order to study the effect of fluoride tablets on superficial tooth enamel the authors biopsied 436 sections of human enamel about 2 to 3 mm in size from 112 caries-free teeth. The sections were fixed in the oral cavities of 5 dental students, 22 to 25 years of age, with small acrylic appliances which could be worn buccally or lingually near the lower second premolars. The holders had a small open box in which as many as 6 enamel sections were fixed with sticky wax; thus the enamel was exposed to the oral fluid, was kept free of wax and was not extruding from the surface of the appliance. During the experimental exposure, a control section was stored in a moist chamber. The fluoride content of the superficial enamel was determined at varied intervals.

Daily administration of one tablet containing 1 mg $F^-$ as NaF caused a fluoride uptake of about 50 ppm/day in the outer enamel. There was a rapid loss of fluoride when the tablets were discontinued after 6 days; after 10 days the initial low concentration was reached. A single tablet caused a fluoride increment of 147 ppm within 30 min after complete dissolution. This contrasts with 409 ppm, the fluoride value after topical application of amine fluoride under identical conditions. Daily brushing of the enamel sections reduced uptake of fluoride to a certain degree.

It was concluded that, in spite of high initial fluoride uptake, no stable binding between fluoride and enamel during this limited period of time took place.

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## NORMAL VALUES OF FLUORIDE FROM A DEFINED REGION OF THE HUMAN ILIAC CREST

by

B. Shellman and A. Zober
Erlangen, DBR

(Abstracted from Int. Arch. Occup. Environ. Hlth. 35: 233-244, 1975)

The authors assayed the fluoride concentration of the human iliac crest (dried bone) of 100 cadavers of both sexes using the ion sensitive electrode. A linear relationship between fluoride content and age was statistically positive. The values ranged from a low of 69 ppm in a girl aged 3 1/2 years who died of meningitis to 1740 ppm in a 85 year old male who succumbed to pulmunary embolism. In contrast to other authors, Shellman et al. found no leveling off of fluoride concentrations after age 50.

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## RELATIONSHIPS OF HUMAN PLASMA FLUORIDE AND BONE FLUORIDE TO AGE *

by

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M. B. Anstey, and M. H. Waziri
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(Abstracted from the Calcif. Tiss. Res. 16: 335-338, 1974)

There is evidence that fluoride levels in plasma correlate with the fluoride content in bones. The authors determined whether or not fluoride in plasma and bones might correlate with age.

In 41 inpatients at the University Hospital, Iowa City, 36 of whom had been residing in fluoridated communities plasma fluoride was determined in the fasting stage by the fluoride ion selective electrode. For bone fluoride, the iliac crests on 20 autopsied cases were examined 19

*See page 169, July 1976

of whom had been residing in fluoridated communities; the length of their residence was not known. The results were expressed as ppm of fluoride per wet weight of bone.

In plasma, fluoride readings ranged from 0.019 to 0.112 ppm with a mean of 0.047 ppm; the ages of the individuals studied were 17 to 82 with a mean of 52 years. Between the two factors a positive linear correlation of 0.53 was obtained with coefficient of determination amounting to 28%.

In the iliac crest, the fluoride values ranged from 1295 ppm to 5745 ppm with a mean of 2824 ppm for ages 21 to 77 years (mean of 50). A positive linear correlation of 0.67 was obtained between fluoride concentration and age and the coefficient of determination was 45 percent. Thus this study demonstrates a significant correlation between bone fluoride and plasma fluoride levels with age.

AUTHOR'S INDEX

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