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Due to unforseen circumstances the date of the Fifth I.S.F.R. Conference had to be postponed until April 8 to 11, 1973. For details regarding hotel reservations and conference hall see next issue. Abstracts of 250 words in English in triplicate should be mailed prior to October 15, 1972, to the Secretary, International Society for Fluoride Research, Box 692, Warren, Michigan 48090.

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FLUORIDE is listed in
Current Contents Agricultural
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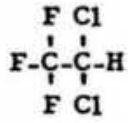
EDITORIAL

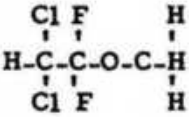
HALOTHANE HEPATITIS AND METHOXYFLURANE NEPHROPATHY

Fluorine, when introduced into anesthetics, increases their potency. This fact has led to the development of a number of preparations which have proven to be of great value to anesthetists. Probably the most widely employed anesthetic of this kind is halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) which was introduced in 1953.



In the early 1960's, cases of liver damage following halothane anesthesia were described for the first time. By 1966, the conservative estimate by the National Halothane Study of the incidence of deaths from liver damage following halothane anesthesia was 1 in 10,000 (1).

Another widely used fluoride compound is methoxyflurane (2,2-dichloro-1,1-difluoroethyl methyl ether) from which liver damage (2)



and particularly damage to the kidney function are being reported in increasing numbers. The current issue of FLUORIDE contains abstracts of two such recent accounts.

The data gleaned from the toxic effects of the two anesthetics reflect, to some extent, the general picture of fluoride intoxication by other organic compounds - particularly hydrocarbons which are being used pharmaceutically - as well as by the free fluoride ion itself. The belief has been widespread that the carbon-fluorine bond in organic fluorine compounds, especially in drugs, is so tight that damage to the human body is unlikely once the agent is incorporated into the system. Indeed it appears that neither one of the two anesthetics by themselves are chemically reactive or that they cause injury to tissue cells. However, studies on animals have established that degradation of these compounds occurs once they are absorbed and that serious damage can be anticipated from their metabolites.

According to Rosenberg (page 106) the manner in which halothane is metabolized in the body is highly complex. Intermediate metabolites, trifluoroethanol and trifluoroacetaldehyde hydrate were found to be hepatotoxic in mice. Trifluoroacetic acid_the end-product of halothane metabolism_is released from the storage depots, namely fatty tissues, and is found in excess in the urine. Fatty changes in the liver and a decline in its glycogen content— features of

halothane poisoning – are probably related to the presence of trifluoroacetate. The same kind of damage has been observed by Egyed (3) in his experimental studies on fluoroacetate intoxication. Rosenberg noted that poisoning occurs mostly in allergic individuals, in females, and in individuals who have been anesthetized repeatedly with halothane. Obese persons seem to be particularly susceptible to halothane poisoning. He postulates that either an allergic type of sensitivity or an auto-immunization process is involved.

A different kind of damage is encountered in methoxyflurane intoxication, the incidence of which - according to recent literature - appears to be climbing steadily. Here the kidneys are the major target organs. Polyuria - a feature of fluoride intoxication - occurs promptly, sometimes while the patient is still in the recovery room following surgery. After 4 to 10 days excessive urinary output gives way to oliguria which, in some cases, is permanent and requires drastic measures such as continued hemodialysis or kidney transplantation (4). The blood urea nitrogen rises, the creatinine and sodium excretion is reduced: the pattern is that of typical acute renal failure. The disease can be recognized in its early polyuric stage when the polyuria fails to respond to pitressin, the pituitary hormone which controls urinary output.

Methoxyflurane intoxication is characterized by increased excretion of oxalic acid and an elevation of the free fluoride ion in the blood. Other possible metabolites of methoxyflurane are chloride ions, methoxydifluoracetic acid and hydroxydifluoracetic acid.

Mazze and co-workers (5) demonstrated the presence of markedly increased quantities of inorganic fluoride and oxalic acid in the serum and urine of all patients following methoxyflurane anesthesia. He noted a close correlation between the amount of metabolites and the degree of renal dysfunction. Fluoride and oxalate are both nephrotoxic.

In another report Mazze et al. (6) strongly indict the fluoride ion as the nephrotoxic agent with oxalic acid as a contributing toxic factor. The type of renal damage and the clinical signs differ from those of oxalic acid intoxication. Mazze believes that the fluoride ion inhibits sodium reabsorption in the renal medulla.

The acetic acid derivatives are not eliminated by exhalation. Organic fluorine metabolites are unstable and are likely to be the immediate source of inorganic fluoride. The clearance of organic metabolites is very low according to Taves et al. (7), and the serum concentration very high. This fact provides a basis for prolonged elevation of inorganic fluoride.

The above-mentioned experiences shatter the long accepted theory that because of the tight fluorine-carbon bone, fluorine-containing anesthetics are harmless after incorporation into the body. In fact, it appears that the free fluoride ion found in situ, is not readily detoxified in the human organism.

Monitoring of plasma fluoride and citric acid levels in patients with prolonged anesthesia of any fluoride product has been suggested in order to recognize kidney damage in its early stage so that prompt measures for its control can be instituted.

To prevent polyuria the amount of anesthesia, particularly for obese patients or patients with impaired kidney function should be limited. Protection might be provided by increasing the urine flow rate in order to dilute the fluoride in the kidney and hence reduce direct toxic effects. A patient with unexplained polyuria after methoxyflurane anesthesia should not receive this agent again.

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HALOTHANE HEPATITIS CAUSED BY HALOTHANE METABOLITES

by

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SUMMARY: Data on hepatitis due to halothane, the widely used anesthetic, a polyfluorinated hydrocarbon, are being reviewed. According to conservative estimates by the National Halothane Study, halothane anesthesia induces approximately one death in 10,000 cases.

The author's experiments in mice and rats revealed that trifluoroacetate is the end metabolite of halothane and trifluoroethanol and trifluoroacetaldehyde hydrate. They are hepatotoxic to mice but necrosis of liver cells was not seen even after multiple injections. The injury was probably caused through binding of trifluoroacetaldehyde or trifluoroacetate to $-NH_2$ and -SH groups of peptides or proteins. After multiple exposures to halothane, increasing amounts of unidentified metabolites are retained in the liver of mice. Trifluoroacetylethanolamine has recently been identified in the urine of man following injections of halothane, indicating binding to phosphatides in membrane structures. The metabolites of halothane probably act as antigens or haptens, which cause an auto-immune reaction rather than a hypersensitive process.

The development of chemical warfare agents during World War II undoubtedly activated the study of fluorinated hydrocarbons. Whereas most of the polyfluorinated hydrocarbons were found to be inert biologically, other fluoro-compounds, especially the monofluoroacetates, which were used as rat poisons, are known to be toxic. In general, the chemical behavior of monofluorinated gases is little different from that of the corresponding unsubstituted gases in contrast to other monohalogenated acids. The process of fluorination with corresponding shortening and strengthening of interatomic bonds appears to confer an increasing nonreactivity of the molecules. The carbon-fluorine bond is very stable, particularly in the fluoroacetic acid group of the compounds.

Whereas the action of fluorinated compounds varies in different animals, they usually attack the central nervous system causing convulsions and respiratory

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Presented at the Fourth Annual Conference of I. S. F. R., The Hague, 10/24-27/71.

arrest. Another effect common to many of these compounds, a progressive depression of the central nervous system with or without respiratory or cardiac failure led Robbins (1) in 1946 to examine the anesthetic properties of some 46 fluorinated compounds. Among other things, he discovered that introduction of another halogen into a fluorinated compound markedly increased the anesthetic potency. In 1953 fluroxene (trifluoroethyl vinyl ether) was the first agent to be tested in clinical practice (2) and in 1956 Raventos (3) demonstrated the clinical usefulness of halothane (2-bromo-2-chloro-1,1,1-trifluoroethane). Halothane has, since that time, become the most widely used volatile anesthetic throughout the world. It has also been considered one of the safest. The side effects which seemed to be few were overshadowed by the enthusiasm over this new excellent drug.

However, in the early 1960's, more and more cases of postoperative liver injury after halothane anesthesia were observed (4, 5). The question of hepatic damage following exposure to halothane is by no means settled. Single cases are no longer reported in the world literature, but prior to 1968 about 400 cases were documented. The National Halothane Study (6) in 1966 estimated the overall low death rate from massive hapatic necrosis following halothane anesthesia to be 1 in 10,000. One might ask whether such an entity as halothane hepatitis really exists in view of many nonanesthetic factors which can be shown to contribute independently to hepatic necrosis. On the other hand, e.g. methoxyflurane, another useful anesthetic, has also been shown to be associated with hepatic necrosis in some patients(7).

Certain features of the disease clearly separate halothane jaundice from "toxic hepatitis" due to direct liver poisons: The disease cannot be reproduced in animals (8,9), is independent of dosage and occurs sporadically with higher frequency in females and in allergic patients. It has a variable latent period which is too long for liver poisons (10). More than two thirds of the cases develop after multiple exposures, and attacks of increasing severity follow if a sensitized patient is given another dose of halothane (10). Histologic appearance in the liver favors drug hypersensitivity but,in some cases, this type of hepatitis cannot be readily distinguished from viral hepatitis. On the other hand, in support of a nonviral origin (11), Australia antigens are not recognized in halothane "hepatitis".

Halothane itself is not thought to be chemically reactive and is not known to act as a hapten (12). It is therefore probably not responsible for the sensitization. Thus, interest in the possible role of halothane metabolites as causative factors has been stimulated.

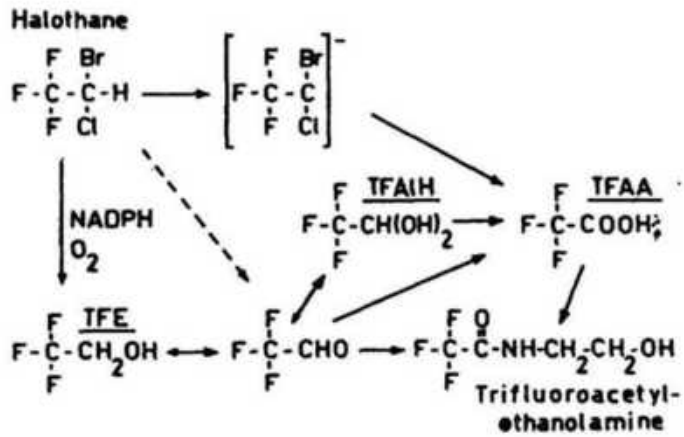
Cohen (13) in 1969 has shown that, in autoradiographic studies in mice, certain nonvolatile metabolic products of halothane are retained in the body and that, following repeated weekly injection of halothane, a markedly increased level of these materials develops. This increase in concentration suggests an independent mechanism such as stimulation of enzyme induction (12).

The metabolism of halothane is by no means clear (Fig. 1). The end product which appear in the urine, is trifluoroacetic acid. Recently Cohen (14) has identified trifluoroacetylethanolamine in the urine of a patient who received ^{14}C-halothane.

The origin of this ethanolamine is probably the phosphatides in different membrane structures of the cells. The trifluoroacetylethanolamine-molecule is small enough to be filtered through the kidneys in man, but several unidentified trifluoro-peptides and proteins have been shown to accumulate in the liver or circulate in blood (15).

Fig. 1

Probable Metabolic Pathways of Halothane



Experimental Data

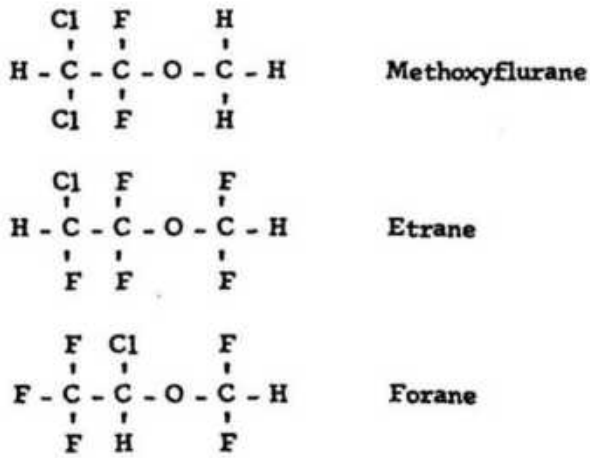
We have studied trifluoroacetate and the probable intermediate metabolites, trifluoroethanol and trifluoroacetaldehyde hydrate in mice and rats. Trifluoroacetaldehyde is the suggested causative agent of toxicity, and the harmful effects are probably caused by blocking thiole or amino groups of different compounds (15,16,17,18). These metabolites were direct hepatotoxins. They caused dose dependent fatty changes, but no necrosis even after repeated administration (19). Trifluoroethanol and trifluoroacetaldehyde hydrate induced a significant decrease in glycogen of the livers.

Discussion

Trifluoroacetic acid continues to be excreted in urine for a period of about two weeks after halothane anesthesia (20). The toxic agent may, therefore, be released slowly from storage depots in fatty tissues and gain entrance to the liver, where it damages the membranes which have already been attacked by the intermediate metabolites produced in the liver itself. It, therefore, seems to be some kind of an auto-immunization rather than a hypersensitivity reaction. The antimitochondrial antibodies sometimes seen in the sera of patients with halothane jaundice (11) probably indicates that metabolic products of halothane have combined with mitochondrial proteins altering their immunological structure.

Fig. 2

The Molecular Structures of the Fluorinated Anesthetic Ethers



Halothane is currently the most widely used volatile anesthetic in the world. Hundreds of thousands of patients, not to mention the anesthesia personel, are being sensitized to it each year. Whether this fact will sooner or later stop the use of this valuable anesthetic is hard to say.

The development of new fluorinated anesthetics is continuously under way, and the tendency is definitely towards metabolically inert anesthetics. Methoxyflurane (2, 2-dichloro-1, 1-difluoroethyl methyl ether) (Fig. 2), introduced prior to 1960, has been shown to be nephrotoxic in some patients, probably due to liberation of fluoride from its molecule during its metabolism (21). The newest fluorinated ethers, ethrane (2-chloro-1, 1, 2-trifluoroethyl difluoromethyl ether) and forane (1-chloro-2, 2, 2-trifluoroethyl difluoromethyl ether), are promising as anesthetics. Preliminary reports indicate that they are metabolized only to a very low extent.

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Discussion

J. R. Marier: Do you know of any controlled study in which obese patients have been shown to be more sensitive to Halothane and Pentrane than others?

Dr. Rosenberg: No, but the drug is stored mainly in fatty tissue.

Dr. G. L. Waldbott: Are gall bladder, liver and kidney diseases contraindications to the use of Halothane or Pentrane anesthesia? Is it not true that some patients develop unexplained jaundice and fever several days after surgery when halothane has been used?

Dr. Rosenberg: Yes, this is true. Over 400 cases of Halothane deaths were reported before 1968 - mostly due to hepatic necrosis. No further count has come to my attention since that time. Maybe the newer anesthetics, Ethrane and Forane, will be safer.

DETERMINATION OF FLUORIDE STANDARDS FOR VEGETATION AND ANIMALS

by

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SUMMARY: In determining fluoride emission standards with respect to possible damage to vegetation, essentially better results are obtained if fluoride in gaseous form is taken into account exclusively. On the other hand, in evaluating damage to animals, fluoride uptake in gaseous form through respiration is negligible compared with fluoride uptake through forage, through soil and through flydust. In establishing emission standards, the fact that vegetation and animals differ in their ability to take up fluoride, and that its availability varies widely, should be taken into account.

There are two methods of determining fluoride in the atmosphere, namely:

A. The analysis for fluoride in its gaseous form and

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B. analysis of solid particles such as flyash and soil for their fluoride content.

The purpose of this presentation is to determine which of the two possibilities provides the best results for evaluating damage to vegetation and to animals and for establishing standards.

I. Vegetation

Our own studies, as well as those of others, indicate that atmospheric fluoride in gaseous form is the principal cause for damage to vegetation as the result of fluoride emission. For this reason, we applied relatively thick layers of soil as well as flyash derived from an enamel factory and an aluminum smelter to the leaves of apple, pear, sweet and sour cherry trees. The soil contained 1.5 g F/kg and the two kinds of flyash, 12 and 132 g F/kg respectively. The studies were carried out in open fields. Some of the days during the course of the experiment were sunny, but on the rainy days portions of the soil and flyash were washed off. On such occasions, the applications of soil and flyash to the surface of the tree leaves were repeated. This procedure was pursued over many weeks. No visible damage such as brown necrosis appeared as the result of the applications.

Our studies demonstrated that the amount of fluoride present in the solid particles is of little importance and does not provide a clue concerning damage to vegetation. Therefore, emission standards cannot be established on the basis of the amount of fluoride present in solid dust particles. The quantities of fluoride thus determined are subject to wide variations. Therefore they either fail to comply with the aim of emission measurements or do so only to a small extent.

Brandt (1) established tolerance values on the basis of the resistance of certain plants to fluoride and of the fluoride contents of various species. In determining fluoride damage to animals, the degree of individual tolerance and the fluoride levels in forage are of considerable assistance. With respect to possible damage to vegetation, however, they are not reliable. Brandt considers 50 ppm of fluoride the maximum tolerance values for very sensitive species of plants, 50 to 200 ppm for less sensitive ones, and above 200 ppm for resistent species. As is generally known, numerous factors such as intensity of light and its effect on assimilation, the time of day the plant specimens are collected, the moisture of the air and many other factors have a critical effect upon the extent of damage to vegetation.

Most significant is the amount of water present in the soil. In an area near an aluminum smelter and a superphosphate factory in the Rhône Valley where a drought occurred for several weeks prior to the harvest, damage to apricot leaves was approximately 80%. However during the following year, after irrigation had been initiated, the damage to the leaves was reduced to approximately 5% although the fluoride content of the leaves was twice as high as in the

previous year. The adjoining apricot orchard which had not been irrigated showed 70 to 80% damage to leaves in spite of the fact that the fluoride levels in the leaves were only half as high. It is likely that through adequate uptake of water larger amounts of calcium and magnesium had entered the leaves simultaneously with fluoride and that these elements had combined with fluoride to form less soluble compounds. Under conditions of plentiful water supply, larger amounts of fluoride enter the leaves because their stomata remain open for longer periods.

II. Animals

a) FLUORIDE UPTAKE THROUGH RESPIRATION: In animals, fluoride uptake through respiration is very limited even near fluoride-emitting factories compared to the uptake of fluoride through forage. Assuming that the fluoride content of the inspired air were large enough to cause material damage to vegetation and to animals, as for instance a daily uptake of 50 $\mu g/m^3$, only about 0.001 mg/kg per day would be taken up through respiration. Since grown cattle inhale about 100 liter/minute and if this amount of fluoride were completely utilized, this amount would be equivalent to a forage ration of approximately 0.05 ppm F in dry substance. In comparison, raw forage such as grass, alfalfa and clover contain approximately 100 to 200 times more fluoride in a non-polluted area. Therefore it would make no difference whether the determination of the emission factor were carried out according to method A or B.

b) FLUORIDE UPTAKE THROUGH FLUORIDE "in" FORAGE: Under normal conditions fluoride uptake by vegetation from the soil is low. In fluoride emission areas, vegetation absorbs most fluoride through the leaves. However, the determining factor for measuring emission values is the amount of fluoride which occurs in gaseous form near fluoride-emitting factories.

c) FLUORIDE UPTAKE THROUGH PARTICULATES "on" FORAGE: Soil and fluoride, emitted with flyash near factories, is attached to raw forage such as grass, clover, alfalfa, etc. Since the amount of fluoride in soil is approximately 20 to 150 times as high as the fluoride content of forage and that of flyash is more than 1,000 times as high, the question arises whether or not the solid particles should be taken into account when measuring emissions. For this reason it was necessary to determine how fluoride in the form of solid particles is metabolized. Therefore we administered a nearly identical supplement of fluoride namely 72 ppm in the form of soil, sodium fluoride, and flyash from an aluminum factory smelter. Since more than 95% of the fluoride retained in the body is stored in hard tissue, we assayed - among other organs - a large number of hard tissue specimens. We found that fluoride uptake from soil is 0.7 times, from flyash 1.4 times and from NaF 3.0 times as high in the experimental animals as in those which received the fluoride supplement. We were able to evaluate the trend of this elevation of fluoride in hard tissue samples by studying 16 head of beef cattle which had been fed, following their 98th day of life, silage of beet sugar leaves for 441 days at a mean daily magnitude of 15 to 20 kg/day. The uptake of fluoride from soil contained in this silage was about equal to fluoride uptake in the nine steers. However, the water solubility

of the fluoride contained in the silage was only about 1/10 of that in the feed of the nine oxen.

These data demonstrate that the utilization of fluoride derived from soil in ruminant animals is highly variable depending upon the origin of the soil. The fluoride from soil and flydust is more or less available although at lower magnitudes than fluoride contained in forage. However, if for establishing emmission standards, particulate fluoride in addition to gaseous fluoride is being considered, values can be obtained which are not in accord with the possible damage to animals. Thus damage to health might be more extensive with a relatively low gaseous emission than with a high level depending upon the proportion of fluoride in gaseous and particulate form.

As already stated, determination of the fluoride content of forage represents a useful guide for the extent of damage to animals. As a maximum value for fluoride, 40 ppm per day is being currently considered for high productive cattle. This value, however, is not only reached, but occasionally substantially exceeded, in non-polluted areas, because of the top permissible fluoride levels in phosphate containing feed. In pastures, beetfields, and other forage producing areas, fluoride values of between 45 and 61 ppm in forage (dry substance) are found. However, since the utilization of the fluoride contained in phosphates is approximately 50 to 65% and that of fluoride contained in raw forage amounts to about 70 to 80%, the EWG (governmental) decision constitutes the low tolerance levels for high productive cattle. Fortunately, through installation of effective wash and filtering equipment in fluoride emission industries, damage to cattle has been considerably reduced during the past decade. However, if these measures are not utilized, acute damage may ensue because, for physical and economic reasons, complete elimination of fluoride emission is impossible. Therefore, it is imperative that in areas near fluoride-emitting factories, fluoride phosphates should be used which are as free of fluoride as possible.

For the uptake of fluoride contained "in" forage only the assays of gaseous fluorides gives correct results. For uptake of fluoride which is "on" forage the same method is preferable. The availability and the retention of fluoride derived from soil and flyash is lower than that of sodium fluoride and of fluoride contained in feed supplements.

The determination of particulate fluoride can be carried out advantageously by the tetrachlorcarbon method. An even better method consists in washing the rough forage grass and clover and then determining the fluoride level in the sample, in the distilled water which was used for washing, and in the sediment.

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HYPERACTIVITY OF THE PARATHYROID GLANDS IN ENDEMIC OSTEOFLUOROSIS

by

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SUMMARY: A study on patients with endemic skeletal fluorosis provided evidence that hyperactivity of the parathyroid glands are a frequent consequence of skeletal fluorosis. The classical biochemical findings in skeletal fluorosis are normal, or higher than normal, serum levels of calcium; lower than normal or normal serum phosphorus; high phosphate clearances; low tubular absorption of phosphates and elevated serum alkaline phosphatase. These values presumably reflect the bone resorptive and the renal phosphaturic actions of parathyroid hormone and the stimulation of osteoblastic cells respectively. The calcification of interosseous membranes, subperiosteal phalangeal resorption and the loss of lamina dura were the diagnostic radiological findings seen in each patient. Histological proof of osteoclastic resorption of bone trabeculae was available in the bone biopsy examination of each patient.

The hyperactivity of the parathyroid glands in skeletal fluorosis, in the presence of decreased solubility of the bone mineral fluoroapatite, suggests that it is a compensatory attempt to maintain normal extracellular ionized calcium equilibrium.

Introduction

The first comprehensive account of skeletal fluorosis was made by Roholm in 1937 (1). In animal studies, he demonstrated an increase in the cortical and trabecular bones, a cell-rich periosteum and an abundance of osteoid lining the bone surfaces. The compact bone frequently showed osteoclastic resorption cavities which contained fibrous tissue. Subsequently Kellner (2) found similar changes in dogs. DeSenarclens (3) in his experimental study laid emphasis on the resorption cavities found in cortical bone. In his thesis, he included a description of an osteoclastoma which affected the mandible of one of his fluorotic goats. He further

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discovered that whereas resorption of the bone was a marked feature of experimental fluorosis in rabbits and sheep, this was not the case with rats. Bauer (4) also reported rapid bone formation, wide osteoid seams and extensive resorption in dogs. Rockert and Sunzel (5) and Rockert (6) reported that the small demineralized areas which appeared in the vertebrae, femur and mandible of fluorosed rats eventually developed into large resorption cavities when the experimental period exceeded a year.

During the past four years we observed,not infrequently in patients with endemic skeletal fluorosis, radiological bone changes such as coarse cystic trabeculations and sub-periosteal phalangeal resorptions.

These observations coupled with the reports in the literature of resorption cavities in experimental fluorosis have suggested to us a possible hyperactivity of the parathyroid glands in patients with skeletal fluorosis. The current study, therefore, was undertaken to investigate the pathogenetic role of the parathyroids in patients with skeletal fluorosis.

Methods

Six out of the 16 patients of proven endemic skeletal fluorosis who have been attending our metabolic clinic showed radiological changes suggestive of hyperfunction of the parathyroid glands. The investigations, therefore, were confined exclusively to these six cases of endemic skeletal fluorosis, who were admitted to the metabolic wards. They belonged to a poor socio-economic strata and had resided since birth in the district of Rai Bereli, U. P., India, an endemic fluorosis area.

The criteria for the diagnosis of endemic skeletal fluorosis included: 1) The endemic nature of the disease; 2) the characteristic clinical picture; 3) the high fluoride content in the drinking water; 4) increased urinary excretion of fluoride; 5) increased retention of calcium; 6) excess calcium and fluoride in bone ash; and 7) positive radiological and histopathological changes.

Laboratory investigations performed in each case included plasma calcium, plasma phosphorus, plasma alkaline phosphatase, creatinine clearance, phosphate clearance, tubular reabsorption of phosphate, urinary calcium, urinary fluoride, chemical analysis of the bone ash for calcium, phosphorus and fluoride. The fecal excretion of fat was estimated in each patient on a six day collection.

Skeletal and dental roentgenograms and the histology of the biopsied iliac crest bone were studied. The fluoride content of the bone, drinking water and urine samples was determined spectrophotometrically by means of the zirconium dye complex as recommended by the Indian Council of Medical Research (7).

Results

Radiological Changes: Diagnostic radiological features of skeletal fluorosis were observed in each patient. They included osteosclerosis, particularly of

the spine, pelvis and thorax, irregularly outlined osteophytes, periosteal bone formation, irregular exostoses, calcification of ligaments, of the interosseous membrane and of muscular attachments.

The roentgenologic findings characteristic of hyperparathyroidism, were present in all cases namely: 1) the subperiosteal resorption in phalanges and digital tufts; 2) erosions or loss of the lamina dura; 3) coarse and cystic trabeculations; metaphysial erosions, and thinning of the cortex, particularly in the pelvis, knees and hands; and 4) generalized resorption of the bones.

Histological Data: The undecalcified and decalcified sections of the bone specimens obtained from the iliac crest by open biopsy were studied in each case. The compact bone showed poorly formed Haversian systems and disordered lamellar orientation. Irregular deposits of osteoid tissue were seen among the bone trabeculae which were thickened at places and appeared to contain an excess of calcium.

Osteoclastic resorption and irregular erosions at the edges of the bone trabeculae were observed in all six cases.

Fluoride in Drinking Water: Forty samples of drinking water were analyzed from four wells located at different places in the endemic area. They contained 0.5 to 10.35 ppm of fluoride.

Results

Six out of 16 patients of proven endemic skeletal fluorosis had evidence of hyperparathyroidism. All were symptomatic and had the characteristic clinical features of stiffness, back pain, painful and restricted movement at the spine and joints. The laboratory investigations (Table 1) showed an elevated plasma alkaline phosphatase, increased phosphatase clearance, decreased tubular reabsorption of phosphate, increased urinary fluoride and low urinary calcium as constant findings in each patient. The chemical composition of the bone obtained by open iliac crest biopsy showed an elevated content of calcium and fluoride (Table 2).

The most common radiological changes seen included dense bones, subperiosteal erosions in phalanges and digital tufts, erosion or loss of lamina dura. The metaphyses were widened and showed rarefactions with irregular erosions, coarse cystic trabeculations and thinning of the cortex (Fig. 1 to 5).

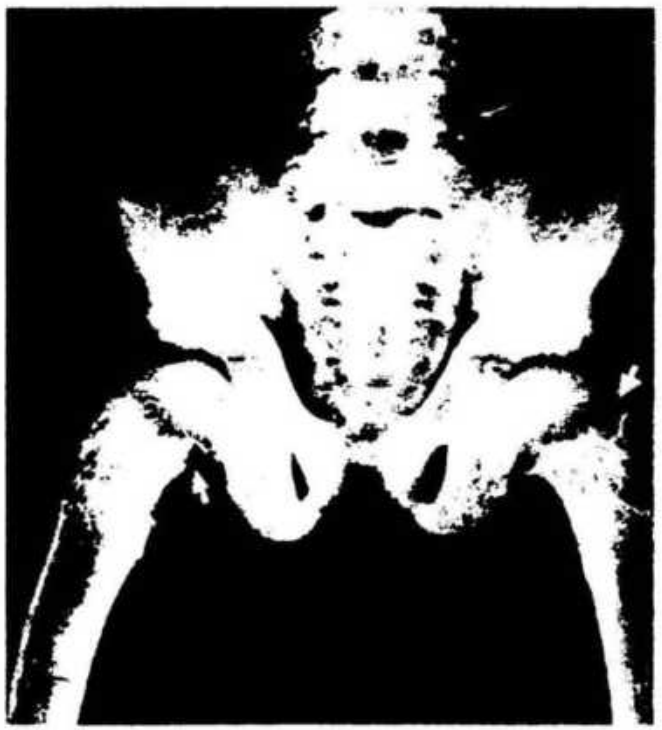
Discussion

The histological proof for the hyperactivity of the parathyroid glands was obtained in the iliac crest biopsies of all patients. Osteoclastic erosions of the bone trabeculae and fibrous invasion of the marrow were the characteristic changes.

In all patients the radiological picture of the bone lesions was a combination of fluorosis and hyperparathyroidism. None of the cases exhibited evidence of vitamin D deficiency or renal disease (Table 1 and 2). The possible interference of

Fig. 1

X-ray of Pelvis (Case 2)



Dense bones with irregular erosions in the femoral neck and metaphyses indicative of hyperparathyroidism secondary to the fluorosis.

Fig. 2

X-ray of Hand (Case 4)

Dense epiphyses, subperiosteal phalangeal erosions, resorption of digital tufts, thinning of the cortex and coarse cystic trabeculations indicative of hyperparathyroidism secondary to the fluorosis.

Fig. 3

X-ray of Foot (Case 5)

Increased density with coarsened trabeculations in the metatarsal bones. Thinned cortex and cystic expansion of the first metatarsal.

Fig. 4

X-ray of Arms (Case 4)

Thinning of cortex, coarse trabeculations, subperiosteal erosions (↑).

Fig. 5

Undecalcified Iliac Crest (Biopsy)

Excess of Osteoid (OS) coverage with osteoclastic resorption (arrow) of the bone trabeculae, characteristic of secondary hyperparathyroidism.

fluoride with the absorption of Vitamin D and calcium from the bowels through enzyme inhibition and a failure to break down fat was excluded as all patients showed the fecal fat output within the normal range (1.8 to 3.6 g/day). That the calcium absorption from the bowels is always increased in patients with endemic skeletal fluorosis has been reported by us in a previous publication (8).

Reports in the literature on parathyroid hyperfunction in skeletal fluorosis are sparse. Jolly et al. (9) studied the parathyroid functions by estimating serum calcium, inorganic phosphorus and alkaline phosphatase and carrying out phosphate-clearance and calcium-deprivation tests. They failed to detect significant changes in the parathyroid functions as revealed by these tests. They were of the opinion that the skeletal changes take place so slowly (extending over 20 years or more) that they are not reflected in the conventional parathyroid tests. Bernstein and Cohen(10)reported parathyroid hyperplasia during surgery in three of their osteoporosis patients who received fluoride for six months to a year. After six months' fluoride administration, one of their patients showed an increase in urinary phosphate with a reduced tubular reabsorption (T. R. P.), histological bone changes suggestive of hyperparathyroidism; microcyst formation and osteoclastic resorption - and a suggestive positive selenium-75 methionine parathyroid scan. Parathyroid hyperplasia was discovered during surgery and the right upper parathyroid gland which was 50% larger than normal,was removed. Subsequently, two other patients on long-term fluoride therapy were found to have "mild" parathyroid hyperplasis during surgery. Davies et al. (11), in his studies of parathyroid adenomas, reported marked dental fluorosis in one of his cases. Teotia et al. (8) reported radiological changes suggestive of hyperparathyroidism secondary to fluorosis in an 11 year old patient with endemic skeletal fluorosis.

TABLE 1

Laboratory Data* in Patients with Endemic Skeletal Fluorosis Associated with Hyperactivity of the Parathyroid Gland

Patient No.	Age	Sex	Plasma Calcium mg/100 ml	Plasma Phosphorus mg/100 ml	Plasma Alkaline Phosphatase K.A. Units	Fecal Fat g/day
1	53	M	9.2	4.3	80	1.8
2	11	M	11.2	3.0	65	2.2
3	13	M	11.0	3.1	32	2.8
4	15	M	11.3	3.4	46	3.6
5	10	M	9.3	4.8	28	2.5
6	18	M	9.9	4.0	36	3.4
Normal			9 - 11	3.5-6	3 - 16	0.5

Patient No.	Creatinine Clearance	Phosphate Clearance	Tubular Reabsorption of Phosphate %	Urinary Calcium mg/day	Urinary Fluoride ppm/day
1	126	20	75	65	4.8
2	145	28	65	78	4.6
3	133	26	82	50	3.6
4	118	30	72	60	3.0
5	125	22	84	35	3.5
6	116	18	75	54	3.7
Normal	110-130	14-18	88-94	80-200	0.5

*Fluoride content of drinking water: 10.35 ppm.

TABLE 2

Bone Composition (Ilium) per 100 grams dry Fat Free Bone Ash*

Patient No.	Calcium (g)	Phosphorus (g)	Fluoride (mg)
1	13.2	5.3	585
2	12.0	5.2	320
3	13.5	4.8	488
4	12.7	5.0	365
5	12.4	5.2	278
6	11.9	5.1	425

Control figures obtained from a person residing in a nonfluoride area: Calcium 10.8 g; phosphorus 4.9 g; and fluoride 30 mg.

In a thorough experimental study, Faccini and Care (12) observed overactivity of the parathyroid glands in a fluorotic sheep. They demonstrated this feature by an electron-microscopic study of the parathyroid glands and by a concomitant immuno-assay of the amount of circulating parathyroid hormone which was five times higher than resting and control levels. The serum calcium had remained within normal limits. Faccini (13) in his studies on growing rabbits further reported that fluoride, probably by producing a more stable mineral system, i.e. fluoroapatite, reduces the resorption of fluoride-containing bone, with a resultant increase in the resorption of normal non-fluoride containing bone. Pierce (14) reported enlarged parathyroid glands in the presence of diminished bone resorption in osteopetrosis in rabbits.

The demonstrable hyperactivity of the parathyroid glands in fluorotic rabbits and sheep in the presence of decreased bone resorption suggests that it could be a compensatory phenomenon to maintain the serum calcium at a constant level.

In our experience, parathyroid hyperfunction accompanies endemic skeletal fluorosis more frequently in children than in adults (Table 1). In children, a higher proportion (up to 72%) of the absorbed fluoride is retained in the body compared with the retention of 28.3% in adults (15). This could be the reason for the higher incidence of hyperparathyroidism in children than in adults. The decreased solubility of the bone salt after incorporation of the fluoride ion, alters the equilibration of calcium ions between bone and blood. This is probably more pronounced in children in whom the mechanisms for calcium homeostasis are labile and need prompt adjustments.

Selye (16), Gaillard (17), Flanagan (18) and Sledge (19), in their experimental studies reported increased periosteal bone deposition associated with increased resorption of bone substance following the administration of parathyroid hormone. Evidence that fluoride-containing bone from other species can resist resorption

has been provided by Havivi and Guggenhein (20). These investigators found that fluoride bone from mice had a reduced Ca-45 release, compared with control bone when the animals were given injections of parathyroid extract for two weeks. Berry and Trillwood (21) and Proffit and Ackerman (22) reported a depression of cellular activity by fluoride in tissue culture studies and considered it unlikely that fluoride exerts its effects by direct stimulation of cells. If the fluoride toxicity is not severe enough to cause a generalized cellular depression, a compensatory mechanism mediated through the parathyroids, seems to develop in order to overcome the physical effects of bone apatite crystals and structure.

Furthermore, Nichols et al. (23) reached almost the same conclusion following "in vitro" metabolic studies of bone on a patient with multiple myelomatosis treated with fluoride, who subsequently developed radiological evidence of fluorosis. The metabolic pattern was indistinguishable from that seen in patients with hyperparathyroidism. Other "in vitro" studies showed that the collagen synthesis is increased in hyperparathyroidism. The authors (23) concluded that the new bone formation in skeletal fluorosis was an effect of parathyroid hormone and that in fluoride-induced hyperparathyroidism, increased resorption of bone is blocked and only formation of new bone is stimulated.

Our observations, however, indicate that new bone formation in patients with endemic skeletal fluorosis may result through the direct stimulation of the osteoblastic activity by fluoride and that parathyroid hyperfunction may not always be present (8). This finding is consistent with that of Roholm (1) who reported thickened and cell-rich periosteum in fluorosis.

The mechanism leading to the hyperfunction of the parathyroid glands in skeletal fluorosis is not known. Crystallographic evidence indicates that the apatite crystals in fluorotic bone are larger in size (24) than those of normal bone. This improvement in crystal texture is accompanied by the diminished solubility and mobility of the bone salt. The fluoroapatite crystals, therefore, are more stable and less reactive in surface exchange reactions, since larger crystals offer less surface area for a given weight of bone. It may therefore be assumed that these changes increase the resistance of bone to the actions of the parathyroid hormone and may cause lowering of the plasma calcium which in turn would stimulate the parathyroid activity.

However, it seems unlikely that the reduced resorption of fluoride bone is the sole exciting factor in parathyroid stimulation, particularly in the light of certain animal experiments. Yates et al. (25) presented evidence of parathyroid stimulation on a short-term basis using intraperitoneal lavage in rats. Faccini (26) who performed immuno-assay of parathyroid hormone in sheep, demonstrated a significant increase in circulating hormone levels only a week after starting fluoride administration. Fluoride might also interfere with the calcium equilibrium between bone and serum by accelerating crystal growth or producing a more rapid ion exchange.

Our findings are consistent with the decreased resorption of osteofluorotic bone and a homeostatic elevation of calcium removal rate from bone through

compensatory hyperparathyroidism. Our findings suggest that hyperactivity of the parathyroid glands is a compensatory phenomenon consequent to decreased resorption of the osteofluorotic bone. The homeostatic elevation of calcium removal rate from bone seems to be the most likely mechanism for the development of coarse cystic trabeculation and the soft tissue calcifications, which are common findings in skeletal fluorosis.

Reddy and Srikantia (27) reported that experimentally adequate calcium and Vitamin C in the diet mitigated the toxic effects of fluoride and that monkeys on a low calcium diet develop the most severe skeletal fluorosis as shown by new bone formation, elevated levels of serum alkaline phosphatase and higher amounts of fluoride retained in the skeleton. The ability of high calcium diets to prevent the development of the bone lesions in experimental skeletal fluorosis and our observations of increased parathyroid activity in patients of endemic skeletal fluorosis suggest that high calcium intake prevented bone lesions by suppressing the parathyroid glands which otherwise become overactive secondary to fluoride-induced inhibition of bone resorption. The exact mechanism , however, causing the hyperactivity of the parathyroid glands in endemic skeletal fluorosis is not clear and needs further study.

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FLUORIDE, OSTEOPOROSIS AND NEO-OSSEOUS-POROSIS

by

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SUMMARY: A survey on osteoporosis was carried out in two groups of individuals, one residing in an area of endemic fluorosis where the drinking water contained 8 to 10.5 ppm fluoride, the other in a similar area with a fluoride content of 0.5 to 1.2 ppm in the drinking water.

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In the endemic zone, none of the adults had spinal osteoporosis; in the non-endemic area, seven cases were encountered.

The survey of children revealed the presence of skeletal fluorosis in eight, osteoporotic bone lesions in six and neo-osseous-porosis in two. In the non-endemic area, no cases of bone disease were encountered.

Localized osteoporosis of the joints, coarsened trabeculations, metaphysial rarefactions and sub-periosteal phalangeal erosions in the individuals residing in the endemic areas suggested the possibility of fluoride-induced parathyroid hyperplasia.

From the current study it may be inferred that the long-term administration of fluoride in the treatment of metabolic bone diseases may cause chronic toxic effects on the skeletal system similar to the bone disease caused by the intake of water with a high natural fluoride content.

Osteosclerotic bone changes due to chronic fluorosis are produced in both humans and animals by the inhalation of fluoride vapor, by ingestion of vegetables contaminated with fluoride or by a high fluoride content of drinking water consumed for extended periods of time. Fluoride is found in drinking waters in concentrations varying from 0.05 to 20 ppm (mg per liter). One half of the ingested fluoride is retained in the bone.

The recent increase in literature on the use of fluoride in the treatment of certain metabolic bone diseases, particularly osteoporosis, has prompted the present study to determine the incidence of bone diseases, particularly skeletal fluorosis and osteoporosis, in random samples of individuals who had resided since birth in the endemic and non-endemic fluorosis area in the district of Rai Bareli, Uttar Pradesh, India.

Methods and Materials

Altogether 100 adults and 22 children were studied from each population. They were selected at random. All were males who belonged to the poor socio-economic strata of the Muslim community. The ages of the adults ranged from 45 to 65 years, those of the children from 10 to 14 years. The occupations and the dietary patterns in the two groups were similar.

All individuals were subjected to a thorough clinical evaluation. Each patient was screened radiologically. Matched films of lateral view of the dorsal and lumber spine, pelvis, knees and hands were taken to permit uniform evaluation of the bone disease.

The roentgenograms were correlated with individual histories and clinical examinations. The radiological diagnosis of osteoporosis was based on the findings of biconcave vertebrae, expansion of the intervertebral discs, compressed vertebral fractures, visibility of trabeculation and demineralization of the bone. The diagnosis of skeletal fluorosis was made on the basis of the characteristic radiological findings of osteosclerosis, osteophytosis, periosteal bone formation and calcification of ligaments.

Fluoride in Water Supply

Thirty-six samples of drinking water analyzed from six wells located at different distances in the endemic area showed a fluoride content ranging from 8 to 10.5 ppm. The fluoride content of the drinking water in the non-endemic areas ranged from 0.5 to 1.2 ppm.

Results

The survey on the adult males (Table 1) from the endemic area showed diagnostic radiological findings of skeletal fluorosis in 38%. Fifteen percent of these also had osteoporosis of knee joints with coarse trabeculations; subperiosteal phalangeal erosions were seen in 13%. The latter findings suggest

TABLE 1

Roentgenologic Findings in Adults
(Ages 45 to 65)

a) Endemic Area	Number of Subjects
Total number screened	100
Positive radiological findings (Skeletal fluorosis)	38
Osteosclerosis	38
Osteophytosis	12
Periosteal bone formation	29
Osteoporosis of joints with coarse trabeculations	15
Sub-periosteal phalangeal erosions	13
Calcification of	
Ligaments	38
Interosseous membrane	30
b) Non-Endemic Area	
Total number screened	100
Positive radiological findings	7
Biconcave vertebrae (osteoporosis)	7
Wedged vertebrae	1
Generalized demineralization	1

the possibility of hyperparathyroidism in these cases, resulting from the decreased solubility of the bone mineral.

In the non-endemic group, seven individuals showed definite radiological findings of vertebral osteoporosis with biconcave vertebrae in all, wedging and crush fracture in one and generalized demineralization in one case.

The second group embraced 44 children aged 10 to 14. Among the 22 children living in the endemic area, the positive radiological findings of skeletal fluorosis were seen only in eight. Six of these 8 cases of skeletal fluorosis also exhibited coarse trabeculations in their pelvic knee and spine bones. Two of the fluorosed children further showed gross rarefaction of the growing parts of their bones, particularly the femoral-neck metaphyses, a condition suggestive of neo-osseous-porosis. None of the children residing in the non-endemic areas manifested evidence of bone disease (Table 2).

TABLE 2

Roentgenologic Findings in Children
(Ages 10 to 14)

a) Endemic Area	Number of Subjects
Total number screened	22
Positive radiological findings (Skeletal fluorosis)	8
Osteosclerosis	8
Periosteal bone formation	5
Calcification of ligaments and Interosseous membrane	5
Osteoporosis with coarse trabeculations	6
Neo-osseous-porosis	2
b) Non-Endemic Area	
Total number screened	22
Positive radiological findings (Osteoporosis)	None

Discussion

The absence of vertebral osteoporosis in adult males residing in the endemic area and its presence in seven of the individuals living in the non-endemic area indicates that in areas with a high fluoride content in drinking water, the incidence of osteoporosis is less common than in low fluoride areas.

Leone et al. (1) reported a low incidence of osteoporosis in naturally fluoridated areas. These workers reported roentgenographic evidence of bone changes in 10 to 15% of humans who used water supplies containing excessive fluoride (8 ppm) for long periods. Furthermore they reported (2) that inges-

tion of water containing fluorides up to 8 ppm produced no deleterious bone changes, no functional or systemic effects except for dental mottling.

Our current study revealed an incidence of X-ray evidence of advanced skeletal fluorosis in 38% of the adults who had consumed drinking water with a fluoride content of 8 to 10.5 ppm for long periods. All individuals had such symptoms as backache, joint pains and stiffness of the spine. Eight exhibited crippling fluorosis.

These findings do not support the data by Leone et al. (2). They suggest that drinking water high in fluoride produces deleterious effects on bones and may cause clinical invalidism in many individuals exposed to it (3).

The presence of osteofluorotic and osteoporotic bone changes in the children exposed to high fluoride intake indicates that the deleterious effects of fluoride are most pronounced in growing bones. The osteoporotic changes have probably been due to parathyroid hyperfunction, secondary to the diminished solubility of bone mineral and to interference with the ionized calcium equilibrium in these cases.

All affected children were symptomatic. Three of them showed retardation in their physical growth in addition.

Recently, fluoride has been used in the treatment of certain metabolic bone diseases. Observation of positive calcium balances was interpreted to indicate that a positive bone balance had been achieved and that it was partially fluoride-induced.

Rich and Ensinck (4) reported extraordinary retention of calcium in patients with osteoporosis who had received 60 mg fluoride daily for several months. This finding was not confirmed by others (5, 6, 7). Rich and Ivanovich (8) pointed out that the effect of fluoride may be delayed for 4 to 6 months. Bernstein et al. (9) claimed that, in areas of North Dakota with a high fluoride content of water, the incidence of osteoporosis was low compared with that encountered where water contained little or no fluoride. They suggested that a lifelong ingestion of about 4 ppm of fluoride may be associated not only with better bone density, but also with lesser hardening of the aorta. At the same time, they maintained that the efficacy of fluoride supplementation in a treatment for osteoporosis has not been established (10).

Certain basic metabolic differences in the behavior of bone and teeth should be pointed out. The mottling effect of fluoride on the teeth is not ameliorated by morphologic turnover under subsequent new environmental or metabolic states. However, at the same time, teeth still have a capacity to incorporate large amounts of fluoride through a chemical exchange mechanism with the adjacent fluid environment such as connective tissue, saliva or topical fluoride even after passing the primary developmental stage (11).

Therefore in contrast with bone, this secondary fluoride increment is largely permanent without being subject to subsequent removal through cellular turnover. On the other hand, in osseous structures due to their continuous cellular remodelling, it would seem to be more important to provide repeated long-term administration of fluorides for protection against osteoporosis. One short-term treatment with fluoride would protect the bone surface only temporarily until it is again renewed. Thus to protect the bone from rarefaction, a continuous contact with fluoride would be necessary. Furthermore, it may well be that to protect an adult man's aging bones he should ingest more, rather than less daily fluoride than children.

Thus in view of the continuous bone turnover, the short-term therapeutic administration of fluorides would not produce any lasting protective effects. Under these circumstances the following points require further exploration: If long-term or lifelong administration of fluoride is employed, in what form should the fluoride be administered? What should be its dosage? How could the deleterious effects of such a therapy, particularly if given for a prolonged period, be prevented?

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Discussion

Dr. Jolly: In our series of cases we did not find evidence of hyperparathyroidism secondary to skeletal fluorosis. Although the levels of calcium were shown to be higher than normal, the same changes occur in Paget's disease and in other skeletal diseases. They are not necessarily due to hyperactivity of the parathyroid glands.

Dr. Teotia: I agree that changes in hyperparathyroid function can be secondary to skeletal fluorosis, Paget's disease, rickets, etc.

Dr. Waldbott: The concept of secondary hyperparathyroidism in skeletal fluorosis is not new; the early writers on skeletal fluorosis such as Speder, Charnot, Goldemberg have pointed to the association of hyperparathyroidism with skeletal fluorosis. However, the mechanism has not been adequately investigated.

J. R. Marier: We have encountered secondary hyperparathyroidism due to chronic kidney disease. In our experiments on beagle dogs, with increased intake of fluoride there appeared to be an increase in parathyroid function.

Dr. N. Jenkins: Dr. Jolly has not seen skeletal fluorosis in children whereas Dr. Teotia has. What is the reason for this?

Dr. Jolly: Dr. Teotia has had greater experience with children because his wife happens to be a pediatrician.

Dr. Franke: 1. Why did you not include women in your study? Isn't hyperparathyroidism more common in women than in men? 2. Could the skeletal fluorosis in the children represent hypochondrial dystrophy, which is a congenital hereditary condition?

Dr. Teotia: I happen to treat males exclusively in this way. I can compare them better. I have no information concerning the second question.

Dr. Sinclair: Prof. Jolly, how do you explain increased calcium balance and decreased serum calcium in these cases?

Prof. Jolly: We have no explanation as yet.

BIOLOGICAL-BIOCHEMICAL METHOD FOR THE DIAGNOSIS OF FLUOROACETAMIDE POISONING

I. CITRIC ACID

by

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SUMMARY: Two sheep were given a lethal dose (20 mg/kg) of fluoroacetamide (FAA) orally. After death their tissues (heart, kidney, spleen and muscle) were extracted with water and concentrated by boiling (1 ml=10 g of tissue). The tissue extracts or urine injected into guinea pigs, caused a 2.4 to 8.5 fold increase in the citrate level of the kidneys. The values obtained were compared with those of guinea pig kidney citrate levels following injection of normal sheep tissue extract. The method described here is suggested as a diagnostic criterion of clinical FAA poisoning.

The routine laboratory diagnosis of sodium fluoroacetate (FAC) or fluoroacetamide (FAA) poisoning is fraught with difficulties. Although the detection of FAC or of its metabolite, fluorocitrate (FC) with gas chromatography is an extremely precise method (1), the procedure is time consuming and therefore its use is limited mainly to research purposes. The spectrochemical determination of FAA (2) and the use of an ion-selective fluoride electrode for screening of FAC in bait and biological material (3) in many respects meets the requirements of routine diagnostic procedures. These methods, however, are not specific enough because they do not differentiate between inorganic and organic bound fluorides. Feeding dogs with poisoned bait or meat induces secondary poisoning with characteristic clinical symptoms and biochemical changes, yet the method cannot be recommended for general use (4).

In view of the many diagnostic difficulties, attempts were made to develop a joint biological-biochemical method for supporting the diagnosis of FAA poisoning by utilizing its effect on citrate metabolism and by inducing secondary poisoning in guinea pigs, thus eliminating the dog as host.

Material and Methods

1. Sheep: Two female Awassi sheep were given 20 mg/kg FAA orally,

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Presented at the Fourth Annual Conference of I.S.F.R., The Hague, 10/24-27/71.

TABLE 1

Guinea Pig Kidney Citrate Content Following i. p. Injection of Normal and FAA Poisoned Sheep Tissue Extracts and Urine

	Sheep Tissue			Mean citric acid in guinea pig kidney µg/g	Increase in citric acid conc. in %
	Heart		Storage		
1)	Control		x	50	
2)	"		xx	50	
3)	Poisoned	+	x	180	360
4)	"	++	x	180	360
5)	"	++	xxx	290	580
	Kidney				
1)	Control		x	40	
2)	"		xx	45	
3)	"		xxx	45	
4)	Poisoned	+	x	130	325
5)	"	+	xx	150	333
6)	"	++	x	150	375
7)	"	++	xxx	250	511
	Spleen				
1)	Control		x	45	
2)	"		xx	50	
3)	Poisoned	+	x	170	377
4)	"	+	xx	120	240
	Muscle				
1)	Control		x	40	
2)	Poisoned	+	x	275	687
3)	"	++	x	340	850
	Urine				
1)	Control		xxxx	40	
2)	Poisoned	+	xxxx	120	300
3)	"	++	xxxx	300	750

+: Sheep no. 1; ++: Sheep no. 2
x: stored at room temperature overnight
xx: frozen overnight
xxx: frozen for 6 days
xxxx: stored at 4°C for 6 days

and died 6 to 7 1/2 hours later. Urine samples and tissues were stored under different conditions before aqueous extraction was performed as indicated in Table 1. One control sheep was slaughtered and the urine and tissues were stored under similar conditions.

2. Guinea pigs of approximately 300 g of weight were used. Poisoned and normal sheep tissue extracts and urine samples were injected intraperitoneally into guinea pigs and were sacrificed about 4 to 5 hours after the injection. The citrate concentration of kidneys was estimated without delay.

Biochemistry

Tissue extraction: Minced tissues were mixed with water (1:3) and boiled. The mixture was cooled and left at 4°C overnight, then filtered through muslin and the volume reduced by boiling, so that 1 ml of the final concentrate became equivalent with 10 g of tissue (5). The volume of injected material was 2.5 ml.

Analysis of kidney citrate in guinea pigs: The method of Taylor (6) was used. The arithmetic mean of estimations is recorded in ug/g wet weight of kidney.

Results

The citrate concentration of a guinea pig's kidney following the injection of normal sheep tissues was found to be similar to that found in normal, untreated guinea pigs (7). The injection of poisoned tissues and urine induced a 2.4 to 8.5 fold increase in the citric acid concentration of guinea pig kidney depending on the tissue and storage conditions. The lowest increase was obtained following the injection of spleen tissue and the highest following the injection of muscle extract. A marked increase was found after the injection of poisoned urine (Table 1).

Discussion

The in vivo accumulation of citric acid in various organs in FAA poisoning cannot be utilized for the diagnosis because the concentration of accumulated citrate disappears rapidly after death, especially if the tissues are not refrigerated (5, 8). Our investigation showed that the injection of concentrated poisoned sheep tissue extracts and urine into guinea pigs induced a significant increase of citric acid in their kidneys even if the tissues and urine were stored at room temperature. This finding indicates that the aqueous extract of the tissues contains the toxic agent in sufficient concentration to induce secondary poisoning in guinea pigs. A similar but less dramatic increase of citric acid was found in various organs of guinea pigs, when FAA contaminated surface water, grass or concentrate of chronically poisoned tissues were given orally to guinea pigs (5). In our experiment, urine originating from poisoned sheep without previous extraction induced toxic effects in guinea pigs with elevation of

citric acid in their kidneys. This observation is in agreement with the experiment that Adrian made on himself namely after he consumed a sub-toxic dose of FAC he secreted urine toxic to guinea pigs (9).

Whereas the number of compounds capable of causing an increase of citrate concentration in organs is limited, acetamide and monoacetine - the suggested antidotes for FAC and FAA poisoning - do have such an effect (10. This possibility which should be taken into account whenever circumstantial evidence of poisoning is being investigated.

The combined biological-biochemical method described here was found to be suitable for the supporting diagnosis of field cases of FAA poisoning when carcasses or organs arrived at our laboratory as long as 48 hours after death in hot weather without refrigeration. In a limited number of investigations, the method described here and the method based on the inhibition of aconitase in various organs gave similar results with respect to the laboratory diagnosis of cases of fluoroacetamide poisoning.

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BIOLOGICAL-BIOCHEMICAL METHOD FOR THE DIAGNOSIS OF FLUOROACETAMIDE POISONING

II. CERTAIN ENZYMES AND ELECTROLYTES

by

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SUMMARY: The levels of various serum enzymes, calcium, magnesium and phosphorus, were measured in guinea pigs with fluoroacetamide poisoning.

In a dose study, increases in the levels of isocitric dehydrogenase, lactic dehydrogenase and sorbitol dehydrogenase were proportional to the dose of poison given.

In a time study, increases in the levels of isocitric dehydrogenase, lactic dehydrogenase, sorbitol dehydrogenase, serum glutamate oxalacetate transaminase, serum glutamate pyruvate transaminase and serum inorganic phosphorus were related to the interval following administration of a lethal dose of the poison.

Tissue extracts from fluoroacetamide poisoned sheep injected into guinea pigs caused an elevation of the isocitric dehydrogenase and sorbitol dehydrogenase but not of lactic dehydrogenase levels in the guinea pig sera.

Histological examinations showed toxic degeneration of liver cells in the poisoned guinea pigs.

Introduction

Fluoroacetamide (FAA) is a highly toxic rodenticide; occasionally it also causes accidental poisoning in other animals. It is difficult to identify FAA poisoning conclusively because pathognomonic post-mortem or histopathological changes (1) are lacking. Often the analysis of food or water and a careful anamnesis prove helpful for the diagnosis.

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Some of the biochemical changes found in various animal species include ketonemia, hyperglycemia (2, 3), elevated blood lactate levels, increase in fatty acid content of the blood (4) glycogenolysis (5) and increased levels of citrate in the tissues (3, 6, 7). Customarily the latter constitute the basis for the diagnosis of FAA poisoning at the present time.

The accumulation of citrate is brought about by a "lethal synthesis" in the body of the poisoned animal of fluorocitric acid (FCA) from the FAA. Fluorocitric acid inhibits the enzyme aconitase, which normally metabolizes citrate in the tricarboxylic acid (TCA) cycle (8, 9. 10). An impairment of cellular function ensues due to the reduction of energy supplied by the cycle.

The purpose of this communication is to determine whether or not changes occur in the levels of certain serum enzymes and serum ions in experimental FAA poisoning of guinea pigs.

Material and Methods

1) Sheep: Two female Awassi sheep were given a lethal dose (20 mg/kg) of FAA orally. After 6 to 7 1/2 hours, the sheep died. Their thigh muscle and the kidneys were removed and stored for extraction (20).

A control sheep was slaughtered and the same tissues were taken and stored similarly.

2) Guinea pigs:

a) Dose Study: Guinea pigs weighing from 300 to 400 grams were injected intraperitoneally with various doses (1 to 20 mg/kg) of FAA, and were sacrificed five hours after the injection.

b) Time Study: Guinea pigs of similar weights were given a lethal dose of FAA (20 mg/kg) i. p., and were sacrificed at various times (0 to 5 hours) after the injection.

c) Tissue Extract Experiment: Guinea pigs of similar weights were injected i. p. with 5 cc of tissue extracts (1, 20) from a normal and from a poisoned sheep and were sacrificed 4 to 5 hours later.

Normal guinea pigs were used as controls. Blood was taken and serum prepared from the normal and the experimental guinea pigs as they were sacrificed.

Biochemistry

1) Blood: Blood was collected, allowed to clot, and incubated at 37°C for 1 hour. Serum was obtained by centrifugation at 1000 g for 10 minutes. The serum was stored on ice and frozen until used.

2) Enzyme Assay: Spectrophotometric and colorimetric methods for

the determination of the various enzymes were carried out, as described in Bergmeyer (12), on the same day as the blood was taken.

Isocitrate dehydrogenase (ICDH) levels were determined by the oxidation of isocitrate with triphosphopyridine nucleotide (TPN) and the increase in reduced triphosphopyridine nucleotide (TPNH) formation was measured spectrophotometrically. 0.8 cc of a mixture of 0.5 M Tris buffer, 4.6 mM d-1-isocitrate and 0.52 M NaCl, at pH 7.5 was added to 0.15 cc of fresh unhemolyzed serum. After incubation at 25°C for 5 minutes, 0.04 cc of a mixture of 9.1mM TPN and 0.12 M $MnSO_4$ was added and the contents mixed well in a 1 cc cuvette. The rate of reaction was recorded at 340 amp on the spectrophotometer, at 25°C. The ICDH levels were measured as milliunits/minute (mμ/min.), the amount of ICDH which, under the conditions described above, causes a change in optical density of 0.001 in one minute.

The lactic dehydrogenase (LDH), ICDH, sorbitol dehydrogenase (SDH) and alkaline phosphatase levels were measured on a Unicam recording spectrophotometer model SP 800 A. The levels of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were measured on a Bausch and Lomb Spectronic 20 colorimeter.

3) Calcium Magnesium and Phosphorus: Serum calcium and magnesium were measured by atomic absorption spectrophotometry using a Unicam SP 90A spectrophotometer.

Serum inorganic phosphorus was measured by the method of Fiske and Subba Row (12), on a Klett-Summerson colorimeter with a red filter (number 66) on the same day as the blood was taken.

Results

Effect of FAA Dose on Serum Enzymes of Guinea Pigs: Increasing doses of FAA resulted in higher levels of ICDH, LDH and SDH (Table 1, Fig. 1).

Liver specimens taken from poisoned animals showed various stages of cloudy swelling in the cells.

Effect of Time After FAA Injection on Serum Enzymes, Calcium, Magnesium and Phosphorus Levels: Increases in enzyme levels following the injection of lethal doses (20 mg/kg) of FAA were seen with LDH, ICDH, SDH, SGOT, and SGPT (Table 2, Figs. 2 and 3). No definite changes were observed in the levels of alkaline phosphatase, calcium and magnesium. The concentrations of inorganic phosphorus also rose in relation to the interval following injection (Table 2, Fig. 3).

Guinea pigs killed when moribund showed parenchymal degeneration and vacuolization of nuclei and cytoplasm in the liver cells.

TABLE 1

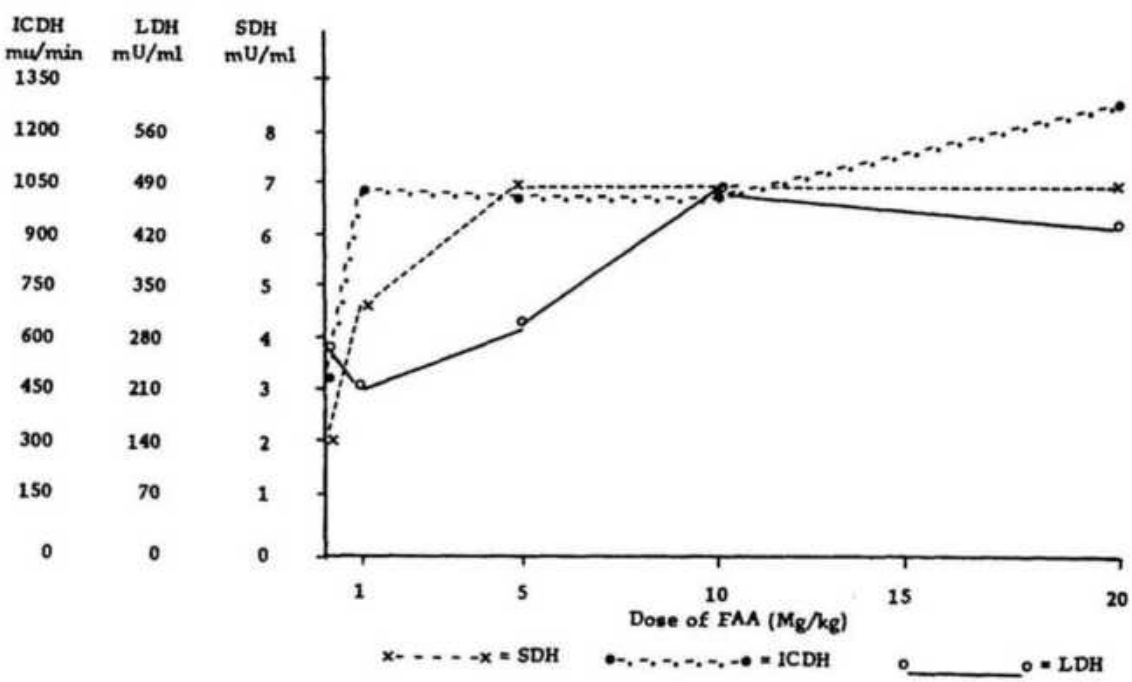
The Effect of Various Doses of Fluoroacetamide on Certain Serum Enzymes of Guinea-Pigs

Dose of FAA Injected (mg/kg)	ICDH mμ/minutes	LDH mμ/ml	SDH mμ/ml
0	510	275	2.0
1	1070	235	4.8
5	1050	315	7.2
10	1070	505	
20	1310	450	7.2

The conditions for LDH and SDH are as described in Bergmeyer (12), for ISDH as described in "Materials and Methods".

Fig. 1

Enzyme Activity Related to Dose Following Injection



The conditions for LDH and SDH are as described in Bergmeyer (12), for ICDH as described in "Materials and Methods".

TABLE 2

Lethal FAA Poisoning in Guinea Pigs
(Serum Enzymes and Inorganic Phosphorus, Calcium and Magnesium Related to Time after Injections)

Time (hours)	(mμ/min.)	(mU/ml)	(mU/ml)	Alkaline phosphatase (mU/ml)	SGOT (mU/ml)	SGPT (mU/ml)	Phos-phorous (mg%)	Cal-cium (mg%)	Magne-sium (mg%)
0 (control)	310	250	2.4	91	45	12	3.2	9.8	3.2
1	430	290	2.4	88	-	17	3.5	-	-
2	730	420	3.1	94	52	15	3.4	9.4	3.1
3	750	505	4.2	105	55	22	4.8	+	-
4	-	-	4.8	89	120	-	6.8	-	-
5	1230	780	8.4	110	-	-	6.4	9.7	3.5
Died at 4 to 5 hours	-	-	30.6	-	175	26	8.1	-	-

TABLE 3

The Effect of Tissue Extracts from FAA Poisoned Sheep on Guinea-Pig Serum Enzyme

	ICDH (mμ/min)	SDH (mU/ml)	LDH (mU/ml)
Control extract	710	2.0	290
Kidney extract	950	3.0	260
Muscle extract	1850	6.4	310

The conditions were as described in "Materials and Methods".

Fig. 2a

Lethal FAA Intoxication on Guinea Pigs
(Effect of Time Following Injection on Enzyme Activity)

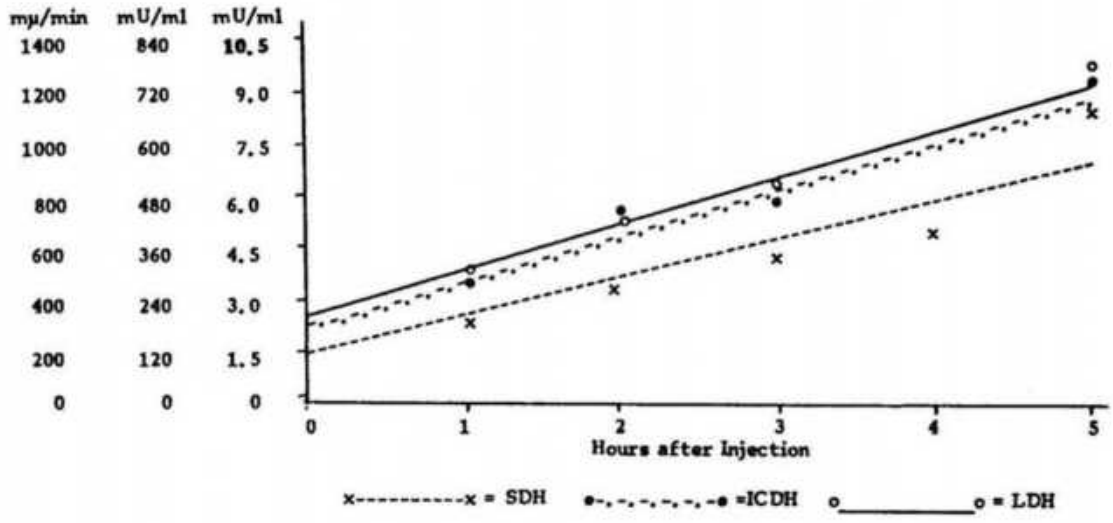
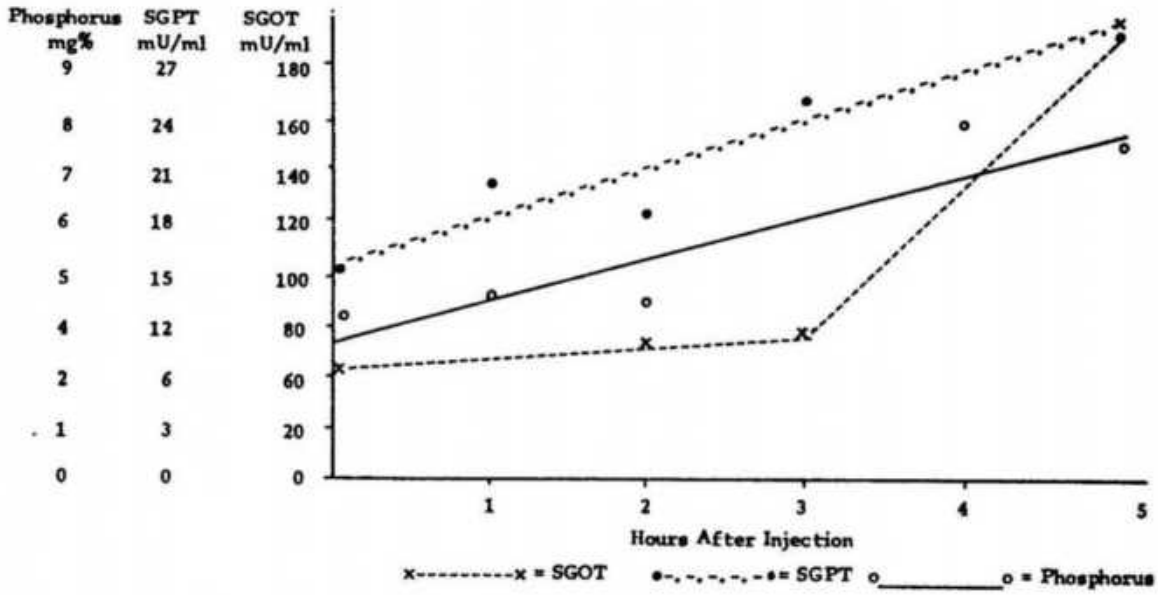


Fig. 2b



Effect of Tissue Extracts from FAA Poisoned Sheep Injected into Guinea Pigs, on Guinea-Pig Serum Enzyme Levels: The levels of ICDH and SDH were higher than in control guinea-pigs. LDH levels were similar in control and experimental guinea-pigs (Table 3). Extracts from muscle had the greatest effect on both SDH and ICDH levels. There were also increases in SDH and ICDH levels, but to a lesser degree, with kidney extract.

Discussion

An increase in enzyme levels in the sera of animals is usually associated with cellular malfunction, damage or death and can be induced by the presence of toxins in the serum (14).

Past evidence has indicated that the dose of a poison administered to an animal is proportional to the consequent rise in serum enzyme levels (15, 16, 17). This trend was seen in our dose study experiments with SDH and ICDH, and to a lesser degree with LDH (Table 1, Fig. I). It is apparent that the more poison administered, the more cellular malfunction and the more damage or death was caused and thus the more enzyme appeared in the serum.

However, the pattern of enzyme rise differed in that LDH levels showed a rise of 50 to 100% above normal only at a dose of 10 mg/kg and above of FAA, whereas SDH and ICDH showed the same rise (50 to 100% above normal) at a dose of only 1 mg/kg FAA (Table 1, Fig. 1). Thus it would appear that low doses of SDH and ICDH are more sensitive indicators of FAA poisoning than is LDH

At lethal doses (20 mg/kg) in the time study, the 3 enzymes SDH, ICDH, LDH and in addition SGPT, showed similar rises in serum levels with increasing time (Table 2, Fig. 2). The levels of SGOT were not elevated until 3 hours after administration of the poison. Thus the serum enzymes SDH, ICDH, LDH and SGPT showed changes earlier than did SGOT. After 3 hours, the levels of SGOT rose at a higher rate than the levels of SDH, ICDH, LDH and SGPT had risen.

The steady rise of serum enzyme as seen with SDH, ICDH, LDH and SGPT might be explained by the metabolic effects of the poison and by the presumed progressive leakage of these cytoplasmic enzymes into the serum, as the membranes of the cells lost the power of retaining these enzymes. This loss of efficiency of the cell membrane in maintaining a normal equilibrium with its surroundings might be associated with the blockage of the tricarboxylic acid (TCA) cycle in some of the cells of the body, due to the fluorocitrate inhibition of the enzyme aconitase (7, 8. 9, 10).

A sufficient energy source is needed to maintain the efficiency of the cell membrane. The reduction in available energy at the cell membrane by a partial shutdown of the TCA cycle may have been sufficient to allow leakage of enzymes in abnormal amounts (11).

In isolated perfused rat liver an induced hypoxia leads to large rises in extracellular enzyme levels (18). This feature resembles the leakage in our experiment. Both experiments ultimately resulted in less energy available at the cell membrane.

The difference in the pattern of enzyme level rise with SGOT compared with SGPT, LDH, ICDH and SDH (Fig. 2 and 3), may be explained by the fact that a portion of the SGOT is situated inside the cell mitochondria. In rat liver cells, all the SGPT, LDH, ICDH and SDH and 30% of the SGOT is in the cytoplasm whereas 70% of the SGOT is inside the mitochondria (18). In human liver 40% of the SGOT is inside the mitochondria (19). Thus perhaps in guinea-pigs some, probably most, of the SGOT is situated inside the mitochondria. Therefore, if these changes occur at the cell membrane as described above, only the SGOT in the cytoplasm is able to leak out of the cell which may account for the slow rise up to 3 hours after administration of the poison. Probably only in cases of poisoning which lead to cell death when the cell and mitochondria are broken large quantities of SGOT enter the blood. Thus in less severe poisoning namely some chronic poisoning, or in the early stages of acute poisoning (as in our time study experiments) the SGOT levels are low. Probably they only rise to high levels when the poisoning leads to cell death.

In the tissue extract experiment, the rises seen in enzyme levels above the control indicate that the toxic principle (presumably fluorocitrate) was also present in the kidney and muscle (Table 3). It is probable that fluorocitrate is synthesized in these tissues, and to a greater degree in muscle than in kidney, and that when the levels in the tissues could be expected to be similar, it is not just passively brought to these tissues in the blood. This observation agrees with findings related to citric acid elevations found in guinea pigs following the injection of kidney and muscle extracts taken from FAA poisoned sheep (20).

A steady rise in the level of serum inorganic phosphorus was seen in the time study experiment. This can be explained by the action of fluorocitrate i.e. the blocking of the TCA cycle by inhibition of the enzyme aconitase. The TCA cycle is an energy forming process whereby inorganic phosphorus is esterified to form adenosine triphosphate (ATP). In normal cell metabolism ATP is broken down, supplying the required energy and yielding ADP and phosphorus, a process which continues unchanged in the poisoned guinea pigs. However, the inorganic phosphorus which has been formed cannot be reincorporated to produce more ATP. Thus the levels of inorganic phosphorus build up, and this accumulation is seen as an increase in serum levels.

Acknowledgement: We would like to thank Dr. U. Klopfer, Dept. of Pathology, for carrying out the histological examinations.

Discussion

Dr. H.M. Sinclair: Why does hydrogenase activity increase in these cases?

Dr. Egyed: The exact mechanism is not known; we know that citrate causes a marked increase in dehydrogenase activity. Citric acid is elevated in the poisoned tissues.

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SPECIAL ARTICLE

EFFECT OF FLUORIDE AIR POLLUTION ON FLORIDA CITRUS

by

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Introduction

Fluorine is found in green plants throughout the world, but it has never been shown to be an essential nutrient element. On the other hand, varying levels of fluorine in the leaves and other plant parts have been found by many workers to be injurious to different species and varieties of plants. In most agricultural areas, fluorine occurs in plant tissues at levels too low to cause injury. Many soils contain several hundred parts per million (ppm) of fluorine and the widely used superphosphate fertilizers may contain from 1.5% to more than 2% fluorine. However, little of this fluorine is in available form and much of that which is absorbed by the roots tends to remain in the root system.

During the past 15 years, gaseous fluorides have become serious air pollutants in agricultural areas adjacent to many phosphate manufacturing plants, aluminum plants, steel plants, and ceramic plants in various parts of the U.S. These plants employ processes that release gaseous fluorides into the air. The amounts of fluorides released depend on the materials used in manufacture and the extent to which waste gases are washed or "scrubbed" to remove fluorides. Different species of plants vary widely in their sensitivity to gaseous fluorides. Many species are injured by prolonged exposure to less than 1 part per billion (ppb) fluoride in the air.

Plants exposed to elevated levels of gaseous fluorides often build up leaf concentrations of fluorine many thousands of times higher than the fluoride concentrations in the air surrounding them. Levels of fluorides in the air usually are reported in parts per billion while leaf concentrations normally are expressed as parts per million, a 1,000-fold increase in concentration. Mature leaves of citrus are relatively resistant to injury from moderate concentrations of airborne fluorides. In fact, they may accumulate several hundred parts per million of fluorine without showing chlorosis or other evidence of injury. However, young citrus leaves often show fluorine chlorosis when they contain as little as 20 to 30 ppm fluorine.

With the rapid expansion of phosphate manufacturing in central Florida, and particularly in Polk County, the problem of fluoride air pollution became a

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From the University of Florida Citrus Experiment Station, Lake Alfred, Florida.

matter of serious concern to citrus growers and other agricultural producers in the 1950's and continues to the present time. Fifteen phosphate manufacturing plants are now operating in southwestern Polk County within the area of a circle with a radius of 8 miles centered 4 or 5 miles southeast of Mulberry, Florida. Three additional plants are operating in adjacent Hillsborough County and another started operating in Manatee County in 1966. Among various phosphate products which these plants produce, triple superphosphate appears to elicit the largest amounts of gaseous fluorides.

The work reported here was carried out to study the effect of different concentrations of fluorides in the leaves on yield and quality of 'Valencia' and 'Hamlin' oranges and 'Marsh' seedless grapefruit.

Review of Literature

In 1955, Wander and McBride (1) reported a new type of chlorosis found on the leaves of citrus trees near Bartow in Polk County, which was caused by airborne gaseous fluorides from a nearby phosphate manufacturing plant. The investigators produced the same chlorotic pattern on the leaves of grapefruit trees 19 miles from the nearest phosphate plant by spraying either hydrofluoric acid (HF) or fluosilicic acid (H_2SiF_6) at 0.1 N on the trees 7 times during a period of 2 months. They found no clear correlation between degree of chlorosis and fluorine content of the leaves.

In 1960, Brewer et al. (2) planted 4 trees each of 7 different citrus varieties in a greenhouse fumigation chamber in which HF gas was added to the air. A similar group of trees was grown in a duplicate control chamber that received clean, filtered air. After 4 months of exposure to HF, the fumigated trees were less vigorous, their leaves were noticeably smaller, lighter green in color and more chlorotic than those of the controls. 'Valencia' orange leaves absorbed most fluoride. The apparent order of decreasing sensitivity to airborne fluoride was : Navel orange, 'Lisbon' lemon, 'Valencia' orange, 'Eureka' lemon, red grapefruit, 'Marsh' grapefruit and 'Temple' orange.

Brewer, et al (3) grew navel orange trees in deep soil beds in a greenhouse and exposed them to 1 to 5 ppb fluorine as HF gas for about 26 months. After 13 months, the fumigated trees were much smaller than controls grown in clean air. Significant reductions in trunk diameter, height of tree, crown volume, and average leaf size in the fumigated trees as compared with trees grown in clean air, as well as a detrimental effect on yield and quality of fruit were recorded. Fluorine chlorosis and necrosis were found in leaves containing 75 or more ppm fluorine.

Under California grove conditions, Brewer et al. (4) found that more fluorine was absorbed by leaves of navel orange trees sprayed with NaF and HF than by those sprayed with NH_4F and H_2SiF_6 at concentrations of 0.10, 0.01, 0.001, and 0.0004 N solutions. They suggest that larger ionic and molecular

sizes in NH_4F and H_2SiF_6 may account for lower absorption of fluoride from these materials than from NaF and HF. At the lowest concentration of spray, HF gave highest absorption rate of fluorine.

In 1967, Brewer et al. (5) sprayed bearing navel orange trees periodically from about March 15 to September 15 over a 6 year period with .0025 and .00125 normal NaF solutions. These sprays resulted in accumulation of about 75 ppm fluoride in spring flush leaves from the lower NaF concentration and about 150 ppm from the higher concentration of NaF. In order to eliminate Na as a factor in the experiment, the control trees were sprayed with .0025 N NaCl. Fluoride toxicity symptoms from these treatments could not be distinguished from those produced by exposure of citrus trees to HF gas in the greenhouse. These symptoms were (1) the characteristic interveinal chlorosis pattern, (2) premature leaf drop, and (3) reduced leaf size. Also significant reductions in fruit yield beginning with the third year of treatment were encountered. No effects of the fluoride treatments on fruit quality criteria (taste, vitamin C, citric acid, soluble solids, size, juice content and rind thickness) were observed in this experiment.

Important information of interest to citrus growers has been obtained with other types of plants. Allmendinger, et al.(6) applied to gladiolus plants exposed to airborne fluoride, periodic sprays and dusts of hydrated lime and other materials. Lime sprays applied weekly gave the lowest leaf fluorine values in washed leaves; it also showed the lowest injury index, the latter being based on length of the scorched area on leaves. Longer intervals between sprays resulted in increased injury. Lime dusts reduced damage considerably but were less effective than the lime sprays.

Adams et al. (7) exposed about 40 different varieties of plants to HF fumigation at concentrations of 1.5, 5 and 10 ppb of HF in both daylight and darkness. The varieties fumigated averaged 91.3% as responsive to HF in darkness as in daylight. The varieties fumigated were somewhat more responsive to a daily low fumigation concentration than to twice weekly higher concentrations of approximately equivalent exposure factor (time x concentration of HF). Varieties fumigated included vegetables, ornamentals, field crops, fruit and shade trees.

McNulty and Newman (8) studied the rate of change of the chlorophylls with respect to the rate of change of suspected chlorophyll precursors and related compounds to investigate the mechanism of fluoride-induced chlorosis. In leaf tissue cultures, the addition of sodium fluoride prevented accumulation of chlorophyll a and chlorophyll b and protochlorophyll in bean leaves that were etiolated at the start of the fluoride treatment. These authors concluded that fluorides may affect the early stages of pigment synthesis or induce the degradation of chloroplast structure.

Fluorine Survey

From May 1, 1961 to May 1, 1962, a survey of 16 'Valencia' orange groves

in Polk County and 16 similar groves in Hillsborough County, Florida, was conducted to determine the extent to which airborne fluorides were being absorbed by citrus leaves at various distances and directions from phosphate manufacturing plants (9,10).

One hundred or more 1961 spring flush twigs on each of 8 trees per grove were marked with paint during April, 1961, to identify them for sampling later in the year. The 1961 spring flush leaves were sampled once a month for 12 months from each of the survey groves. A control grove was selected in northern Polk County about 30 miles from the nearest phosphate plant. Each leaf sample was thoroughly washed in a solution of Dreft detergent and rinsed several times in deionized water. The leaves were dried at 70° C. and ground in a Wiley mill. The ground samples were analyzed for fluorine in the Winter Haven Laboratory of the Florida State Board of Health under the direction of K. K. Huffstutler, Sanitary Engineer in Charge, by a modification of the Willard and Winter (11) method. The highest fluorine values were found in leaves taken from grove No. 14 southwest of Bartow, where the oldest leaves contained 365 ppm fluoride in May 1961 (Table 1) and 1961 spring flush leaves accumulated 346 ppm fluoride by February 1962 (Table 2). Young leaves showed much more severe fluorine-chlorotic symptoms than did older leaves. Fluorine chlorosis patterns were found in a few samples of young leaves containing only 20 ppm fluoride. Leaves that apparently had matured before exposure to high levels of airborne fluoride were found to accumulate large amounts of fluoride without showing appreciable chlorosis.

Groves nearest triple superphosphate manufacturing plants showed the highest levels of fluoride in the leaves. At a given distance from the fluoride source, however, the prevailing wind direction and time of exposure to airborne fluorides largely determined the absorption of fluoride by the leaves. Due evidently to the prevailing easterly to southeasterly winds during much of the year, groves north, northwest and west of the phosphate plants showed higher leaf fluoride than groves south or east of these plants.

The old leaves and the 1961 spring flush leaves sampled May 1, 1971, were analyzed for Mn, Zn, Fe, B, N, P, K, Ca, and Mg. These assays indicated that fluorine level had no significant effect on levels of the above nutrient elements.

Fruit samples were taken in May 1961 and April 1962 from fluorine survey groves. No significant effect of elevated fluorine levels on the interior quality of citrus fruit was found. Analysis of whole fruit showed little absorption of fluorine by the fruit. Most of the fluorine found in the fruit was in the peel, with very little in the pulp and juice.

Survey groves which showed moderate to high fluoride levels during the survey and some additional groves, have been sampled periodically between 1962 and 1968 and analyzed for fluorine in the Citrus Experiment Station laboratory by a modification of the volumetric method of Willard and Winter (11). Instal-

TABLE 1

Fluoride Content of Old Leaves from Orange Groves Sampled from 1961 to 1968 in Polk and Hillsborough Counties, Florida

Grove No.	Date Sampled: May 1961	Dec. 1962	May 1965*	Jan. 1966	June 1967	Feb. 1968
2	87	172	139	155	86	66
4	156	484	245	335	165	142
7	59	---	40	44	---	---
10	75	164	107	115	104	97
10-A	---	---	---	---	140	308
11	69	144	150	181	120	108
12	80	188	152	183	118	119
13	241	333	284	---	---	---
14	365	894	353	580	---	---
16 (check)	10	---	1	9	---	---
21	67	77	17	---	---	---
27	151	226	174	235	81	127
30	249	342	151	232	192	168
31	146	223	75	133	---	88
32	252	279	56	106	142	126
36	---	---	301	510	---	316
37	---	---	---	740	295	250
43	---	---	---	---	---	219
47	---	---	---	---	340	355
48	---	---	---	---	184	188
50	---	---	---	---	193	226
55	---	---	---	---	216	226
56	---	---	---	---	224	280

*Since virtually all survey trees were defoliated by the severe December 1962 freeze, these "old" leaves are no older than 1963 spring flush.

lation of scrubbers in recent years to remove fluorides from the gaseous effluents of these plants has lowered the total amount of fluoride released through the stacks into the surrounding air. This has resulted in lower levels of fluoride in many citrus groves in the area in the past few years than those found prior to 1965.

TABLE 2

Fluoride Content of Spring Flush Leaves from Orange Groves Sampled from 1961 to 1968 in Polk and Hillsborough Counties, Florida

Grove No.	1961 spring flush Sept. 1961	1961 spring flush Feb. 1962	Dec. 1962 flush Feb. 1964	1963 spring flush Sept. 1963	1964 spring flush Sept. 1964	1965 spring flush Aug. 1965	1965 spring flush Jan. 1966	1965 spring flush Sept. 1966	1966 spring flush Sept. 1966	1967 spring flush Sept. 1967	1967 spring flush Feb. 1968
2	73	73	203	44	57	18	39	119	107	31	40
4	100	129	136	94	76	97	135	325	100	57	75
7	22	36	58	30	--	7	19	46	18	12	---
10	17	44	120	44	31	12	37	154	40	40	47
10-A	---	---	---	--	--	--	---	---	---	90	118
11	11	26	102	13	17	25	67	145	28	24	60
12	15	48	132	17	23	18	71	118	19	17	50
13	67	110	178	49	48	61	---*	---	---	--	---
14	101	346	426	86	84	66	141	---*	---	---	---
16(ck)	7	8	5	2	--	1	1	5	4	1	---
21	33	33	23	10	---	5	5	---	---	--	---
27	63	132	123	54	36	33	69	167	26	34	48
30	54	203	286	43	39	13	68	168	29	41	77
31	59	66	---	31	38	29	44	---	---	16	33
32	48	117	180	31	15	15	42	131	24	22	41
36	---	---	---	--	--	--	388	433	119	128	218
37	---	---	---	--	--	--	260	393	96	64	102
43	---	---	---	--	--	--	---	---	---	51	112
47	---	---	---	--	--	--	---	---	246	163	326
48	---	---	---	--	--	--	---	---	---	81	123
50	---	---	---	--	--	--	---	---	---	115	163
55	---	---	---	--	--	--	---	---	---	61	147
56	---	---	---	--	--	--	---	---	---	112	198
57	---	---	---	---	--	--	---	---	---	51	175

*Grove sold to phosphate company, sampling discontinued.

The amount of fluoride air pollution near central Florida phosphate plants and its effect on citrus groves have been matters of controversy between phosphate companies and neighboring citrus growers for about 15 years, and remain so today. The director of the new Florida Air and Water Pollution Control Commission recently reported that fluoride evolution in Polk and Hillsborough Counties, Florida, from May to October 1967 was the lowest in the his-

tory of the Florida phosphate industry (12). According to this report, while sampling the phosphate industry for the purpose of issuing permits to operate, the Winter Haven Laboratory of the Florida State Board of Health found a total of 3,016 pounds per day of gaseous and/or water-soluble fluoride emission. Due to a reduction in phosphate fertilizer production during that period, that laboratory estimated in October 1967 that fluoride emissions amounted to only 1,500 to 2,000 pounds per day. This compares with a maximum of 5,537 pounds of fluoride emissions per day allowed under the permit system adopted by the former Florida Air Pollution Control Commission.

Fluorine analyses of citrus leaves sampled during 1967 and 1968 from fluorine survey groves indicate that substantial amounts of airborne fluorides are still present in certain areas of Central Florida. Some of these high leaf fluoride levels (Tables 1 and 2) are now of serious concern to the owners of citrus groves in these areas. Relatively low scrubber efficiency in some phosphate plants, periodic breakdown of scrubbing equipment, building of new phosphate manufacturing plants, expanding phosphate production facilities at various older plants, and/or release of some gaseous fluorides from phosphate plant waste ponds are possible causes of high leaf fluoride concentrations shown for certain groves.

Alleviation of Fluorine Chlorosis

Late in 1960 an effort was made to alleviate fluorine chlorosis symptoms by application of various liming materials to the soil and by spraying mixtures containing 20 or more pounds of hydrated lime per 100 gallons (12). None of the soil treatments regreened fluorine-chlorotic leaves or reduced fluorine absorption by the leaves. However, a mixture containing 25 pounds of hydrated lime plus zinc and manganese sulfates, copper, boron, molybdenum, magnesium, and urea nitrogen applied as a spray in October 1960 caused at least 90% greening of the fluorine-chlorotic leaves by January 1961 (Treatment 9, Table 3). Treatment 17, with much less lime, produced about 70% greening of similar leaves. A few of the other sprays showed some reduction of leaf fluorine, but did not regreen chlorotic leaves. The leaves were prepared for analysis by vigorously scrubbing them in a Dreft solution and thoroughly rinsing them in 5% HCL before drying.

Separate trials made later with each of the above nutrient elements showed that hydrated lime, manganese sulfate, and urea applied together were the effective materials causing regreening of the leaves. Sprays of this mixture greatly reduced fluoride absorption by the leaves and regreened fluorine-chlorotic leaves when at least 2 months of relatively dry weather followed application of the sprays. Effects of these sprays on leaf content of various nutrient elements are shown in Table 4. Leaf Mn was quite low except for those trees receiving sprays that included Mn. In this connection, it should be noted that the first symptom of fluoride toxicity on young citrus leaves in Florida is often identical with Mn-deficiency chlorosis. Where this occurs, the apparent Mn-deficiency pattern changes to the typical fluoride-toxicity pattern within a few weeks. This fact, plus the fact that the leaves of only those trees re-

TABLE 3

Effect of Lime Sprays with Various Nutrient Elements on Fluoride Content and Color of 'Valencia' Orange Leaves Showing Fluoride Chlorosis

Treatment No.	Materials applied per 100 gallons							F ppm	Greening of fluoride chlorotic leaves
	$Ca(OH)_2$	$ZnSO_4$	$MnSO_4$	Urea*	$CaCl_2$	Neutral copper	Other		
3	50	10	--	--	--	--	--	44	None
5	25	5	--	--	10	--	--	53	None
9	25	5	15	15	--	5	**	19	Est. 90%
12	30	5	5	--	--	5	--	26	None
17	2.5	5	10	15	--	2.5	**	38	Est. 70%
26	55	5	--	--	--	--	--	34	None
31	15	5	5	--	10	--	***	45	None
34	55	--	--	--	--	--	--	38	None
Check	No treatment							56	None

*Low biuret urea.
**Also, 1 pound borax, 5 ounces sodium molybdate, and 10 pounds magnesium nitrate per 100 gallons.
***Also, 25 pounds light soda ash (Na_2CO_3) per 100 gallons.

TABLE 4

Content of Fluoride and of Various Nutrient Elements in Fluoride-Chlorotic 'Valencia' Orange Leaves Sprayed in October 1960 with Mixtures Shown in Table 3 and Samples in January 1961*

Treatment No.	F ppm	N %	Ca %	K %	Mg %	Mn ppm	Zn ppm	Fe ppm
3	44	2.52	2.41	1.96	.38	13	66	70
5	53	2.64	2.96	1.96	.34	13	78	65
9	19	3.08	2.96	2.04	.31	103	58	75
12	26	2.55	2.72	2.06	.36	65	38	95
17	38	3.37	2.81	1.94	.33	206	57	70
26	34	2.35	2.72	1.94	.33	30	36	52
31	45	2.76	2.72	1.85	.39	130	165	58
34	38	2.23	3.04	1.75	.33	21	23	58
Check	56	2.29	2.56	2.38	.36	15	18	80

*Leaves scrubbed with Dreft solution, then rinsed in 5% HCl solution to remove spray residue.

ceiving 10 or 15 pounds of $MnSO_4$ per 100 gallons in the lime spray regreened suggest that leaf fluorides may interfere with absorption of Mn by the leaves. The addition of urea to high Mn sprays appears to increase the absorption of Mn by the leaves over similar high Mn sprays without urea. Regreening of fluoride-chlorotic leaves did not occur with high lime, high Mn sprays without urea. Lime sprays with a high Mn and urea have not been effective in regreening fluoride-chlorotic leaves during the rainy season in Florida since much of the spray residue is washed from the leaf surfaces within 2 to 3 weeks after application.

Field Greenhouse Experiment

In 1963, a field experiment was started in which 6 medium-sized bearing 'Valencia' orange trees were enclosed in individual plastic greenhouses and 3 trees were not enclosed for use as outdoor checks. This experiment was located in fluorine survey grove No. 14 southwest of Bartow, Florida. This grove showed the highest levels of leaf fluoride during the 1961-62 fluorine survey. The 6 greenhouses were 15 ft square, 12 ft high at the eaves, and 15 ft high at the ridge. A change of air in each greenhouse was made about every 35 seconds with electrically operated blowers and louvered air outlets. The fluorides were removed from the air entering 3 of the 6 greenhouses by drawing ambient air through dry calcium carbonate filters. Unfiltered ambient air was moved through the other 3 greenhouses. The 3 trees not enclosed were used as outdoor controls.

All trees received the same fertilizer and spray programs and were irrigated as required. The blowers were operated continuously for a 3 year period except for brief shutdowns for minor greenhouse repairs, spraying and heating when necessary. All of the trees to be used in the experiment were defoliated by the severe December 1962 freeze. Late in December new leaves developed on all trees. When the greenhouses were closed and the blowers started on May 6, 1963, the December flush of leaves on all trees contained more than 200 ppm fluoride. The fluoride content of these leaves on trees receiving filtered air decreased to less than 100 ppm fluoride within a few months. The fluoride increased to more than 250 ppm in leaves of the same flush on outdoor check trees during the same period.

The limestone filters did not remove all of the fluoride from the air entering the filtered greenhouses. They did, however, reduce fluorides to a relatively low level. The leaves of trees receiving unfiltered air in greenhouses absorbed much less fluoride than those of the outdoor check trees. Air samples taken by the Winter Haven Laboratory of the Florida State Board of Health showed slightly less fluoride in the unfiltered greenhouses than was found outdoors. These differences may have been due to interference with air circulation by the trees in greenhouses. The lower fluoride content of the leaves of trees in unfiltered greenhouses as compared with those on the outdoor check trees possibly was due to the fact that the leaves in the greenhouses remained dry whereas those outdoors were moist much of the time from rain or dew. Wet citrus leaves apparently absorbed more gaseous fluoride than is absorbed by dry leaves.

Chlorophyll, Photosynthesis and Respiration

In February 1964, leaf samples were taken from the greenhouse trees and outdoor checks. Conventional Warburg manometry was used to measure apparent photosynthesis by oxygen evolution and respiration by oxygen consumption (13). Each determination was replicated 2 or 3 times in addition to the replication of the experimental treatments. These determinations showed that greenhouse effects and filtering effects combined to lower the fluoride content of the leaves, to increase the net photosynthetic efficiency, and to increase the chlorophyll content of the leaves of both the 1963 fall and spring flushes (Table 5).

TABLE 5

Net Photosynthesis and Chlorophyll Content of 'Valencia' Orange Leaf Disks from Grove No. 14 Sampled February 28, 1964

Tree location	Fluoride content ppm dry wt.	Leaf area sampled: Whole PS*	Whole Ch**	Base PS	Base Ch	Tip PS	Tip Ch
		Fall flush					
Filtered greenhouse	3	113	2.9	79	2.8	61	2.1
Unfiltered greenhouse	24	83	1.6	115	2.2	4	1.2
Outdoors	65	59	1.3	83	2.0	16	0.8
		Spring flush					
Filtered greenhouse	12	110	3.4	83	3.4	71	3.3
Unfiltered greenhouse	113	96	2.9	118	3.3	44	2.5
Outdoors	234	98	1.7	77	2.0	41	1.3

*PS: Net photosynthesis expressed in ml x 10^{-1} oxygen evolved per square centimenter of leaf area for one day. Calculated for 12 hours light at 1, 250 foot candle intensity and 12 hours dark, both at 30° C.

**Ch: Chlorophyll content of same eight disks used in Warburg apparatus, mg per gm fresh weight.

The fall flush leaves apparently were damaged more by fluoride than the spring flush leaves, even though the spring flush leaves contained more fluorine than the fall flush leaves. As pointed out above, young citrus leaves are much more susceptible to chlorosis by airborne fluorides than old leaves. Separation of leaf disks into categories corresponding to tip of leaf and base of leaf showed that the leaf tip was the more severely affected by airborne fluorides with respect to both net photosynthesis and chlorophyll content. This is in agreement with the pattern of fluorine chlorosis on citrus leaves since the leaf tips and

margins are characteristically more chlorotic than other leaf areas. Fluorine-chlorotic leaves were sampled from a grove in December 1964, and used to determine the fluorine content of the yellow (chlorotic) parts of the leaves, the green parts, and that of the whole leaves. The fluoride content of the whole leaves was 113 ppm, of the yellow parts 138 ppm, and of the green parts 63 ppm.

For 1964 spring and summer flush leaves, sampled on June 24, 1964, from trees in the greenhouse experiment, the chlorophyll contents and rates of photosynthesis of leaf disks were lowest for outdoor check trees, intermediate for unfiltered greenhouse trees, and highest for filtered greenhouse trees (Table 6). The spring flush was more severely affected than the relatively young summer flush. There was a trend toward increased respiration rate associated with increased fluoride content in the spring flush. Net photosynthesis and increased respiration apparently caused by airborne fluorides severely limit the gain in useable photosynthate or food supply produced by the leaves.

TABLE 6

Photosynthesis, Respiration, and Chlorophyll Content of 'Valencia' Orange Leaf Disks. Leaves Sampled June 24, 1964

Tree location	Fluoride content	Fresh wt. mg per cm^2	Mg chlorophyll x 10^{-3} per cm^2	Respiration*	Photosynthesis** per cm^2 area	mg fr. wt.	mg Ch	NPE***
			Spring flush					
Filtered greenhouse	5	21	77	16	60	2.9	7.9	44
Unfiltered greenhouse	35	21	62	21	46	2.2	7.4	25
Outdoors	64	24	49	26	38	1.6	7.8	12
LSD 5% level								12
			Summer flush					
Filtered greenhouse	26	22	23	26	59	2.7	26.0	33
Unfiltered greenhouse	21	22	22	24	50	2.3	22.7	26
Outdoors	45	24	21	29	47	2.0	22.4	18
LSD 5% level								NSD

*Respiration expressed as microliters oxygen consumed per square cm leaf per hour at 32°C.

**Photosynthesis expressed as microliters oxygen evolved per hour at 32°C, 1,250 foot candles of light, in terms of leaf area, disk weight and chlorophyll (Ch) content of disks.

***NPE: Net photosynthesis efficiency, the calculated net oxygen evolution per square cm during one hour photosynthesis and one hour respiration from data in the table.

Fluorine Content of Fruit: The highest levels of fluorine in the fruit were found in the peel. There was slightly more fluorine in the juice than in the pulp (Table 7).

TABLE 7

Effect of Airborne Fluorides on Fluorine Content of Peel, Pulp and Juice of 'Valencia' Orange Fruit, Fruit Sampled March 11, 1965.

	Fluorine content			
Treatment	Juice ppm	Pulp ppm	Peel ppm	Whole fruit ppm*
Filtered air	.63	.40	.87	.52
Unfiltered air	.52	.39	2.31	.85
Outdoor checks	.69	.54	6.22	1.78

*Weighted mean of average ppm F in pulp and peel, based on relative weights of pulp and peel.

Leaf Size: In May 1964 and in July 1965, width and length of 200 current spring flush leaves were measured on each tree. Average leaf areas are shown in Table 8. There was an average 20% reduction in leaf size on the greenhouse trees receiving unfiltered air and a reduction of about 50% on the outdoor check trees as compared to the leaves of trees receiving filtered air.

TABLE 8

Effect of Airborne Fluorides on Size of Spring Flush Leaves of 'Valencia' Orange Trees

	Mean leaf area			Relative area
	cm^2	cm^2	Average	2 years
Treatment	1964	1965	cm^2	%
Filtered air	42.70	32.00	37.35	100.0
Unfiltered air	33.49	26.18	29.84	79.9
Outdoor checks	17.18	19.89	18.54	49.6

Fruit Quality: During 1965 and 1966, three separate samples of mature fruit were analyzed for internal quality. Data for fruit sampled March 28, 1966, are shown in Table 9. These data show a trend toward higher acid content and resultant lower Brix/acid ratio of the juice with increasing leaf fluorides. However, these results are not statistically significant.

TABLE 9

Effect of Airborne Fluorides on Internal Quality of 'Valencia' Oranges

Treatment	Juice by weight %	Brix %	Acid %	Ratio Brix/acid
Filtered air	61.0	11.75	0.83	14.26
Unfiltered air	58.5	12.17	0.91	13.59
Outdoor checks	59.2	11.52	0.97	12.10

Yield: Defoliation of the trees by the December 1962 freeze, before the trees were enclosed in the greenhouses, prevented production of a crop for the 1963-64 season. Yields for both 1964-65 and 1965-66 are shown in Table 10. A rapidly developing, severe mealybug infestation occurred in the spring of 1964 on 4 of the greenhouse trees and caused excessive dropping of small fruit. Two of these trees received filtered air and 2 received unfiltered air. The infestation was light with little or no effect on yields on the remaining 2 greenhouse trees. There was no mealybug infestation whatever on the outdoor check trees.

TABLE 10

Effect of Airborne Fluorides on Yield of 'Valencia' Orange Trees

Treatment	Yield of fruit pounds A	B	C	Mean*	Yield %	Mean leaf fluorine, ppm 1965 spring flush 8-20-65	1-5-66	Old leaves 7-2-65
1964-65 Crop Picked March 11, 1965								
Filtered air	413**	301***	30*+	248	100.0			
Unfiltered air	427**	102*+	170***	233	94.0			
Outdoor checks	29++	80++	166++	92	37.1			
1965-66 Crop Picked March 28, 1966								
Filtered air	397	372	363	377[c]	100.0	5	10	34
Unfiltered air	302	273	316	297[b]	78.7	43	71	196
Outdoor checks	140	59	116	105[a]	27.8	86	197	307

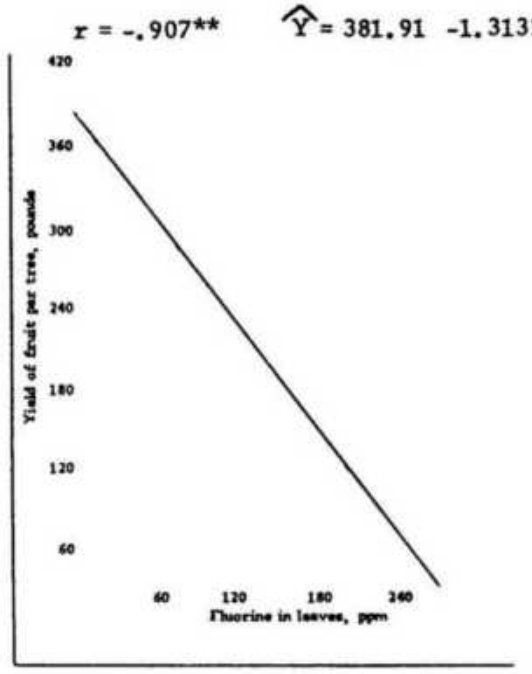
*Mean yields followed by different letters are significantly different at P = 0.05.
**Slight damage by mealybugs.
***Moderate damage by mealybugs
*+Severe damage by mealybugs
++No mealybug damage

No statistical analysis of the 1964-65 yield is reported here because of the mealybug infestation. It should be noted, however, that the mean fruit production of greenhouse trees, both filtered and unfiltered, was about 2 1/2 times greater than the yield of the outdoor check trees. This is particularly noteworthy since there was no mealybug infestation on the outdoor check trees.

The mealybug infestation on the greenhouse trees was eliminated by several sprays of parathion and guthion during 1964. The outdoor check trees were given the same sprays even though they had no mealybug infestation. A heavy spray program throughout 1965 prevented any damage to the 1965-66 crop. For the 1965-66 crop, the trees receiving filtered air produced significantly more fruit than the trees receiving unfiltered air. Both of these treatments produced significantly more fruit than the outdoor check trees. There was a highly significant negative correlation, $r = -.907$, between yield of fruit and fluorine content of 10 month old 1965 spring flush leaves sampled in January 1966. This linear regression formula was $\hat{Y} = 381.91 - 1.3132X$, where X = ppm F in the leaves and the regression coefficient $\hat{Y}$ is expressed as pounds of fruit per tree (Fig. 1). There was also a highly significant negative correlation, $r = -.869$, between yield of fruit and fluorine content of old leaves sampled in July 1965. This linear regression formula was $\hat{Y} = 417.25 - .8797X$. There was a significant negative correlation, $r = -.685$, between yield of fruit and fluorine content of 5 1/2 month-old 1965 spring flush leaves sampled in August 1965. The linear regression formula was $\hat{Y} = 345.61 - 1.912X$.

Fig. 1

Linear Regression of 'Valencia' Orange Yields and Fluorine in Ten-Month Old Spring Flush Leaves, 1965-66 Crop



Fluoride Spray Experiments

In December 1963, 2 fluoride spray experiments were started in the Citrus Experiment Station grove at Lake Alfred, Florida. Varieties used were 'Marsh' grapefruit and 'Hamlin' orange, both on rough lemon rootstock. Each experiment consisted of 4 tree plots in randomized blocks with 4 replications. The 4 treatments included 3 different concentrations of hydrofluoric acid (HF) with a wetting agent and a check treatment sprayed each time with water plus the same wetting agent. The trees had been damaged by the December 1962 freeze, so the first year's yield taken for the 1963-64 crop was considerably lower than the yield in subsequent years.

The concentrations of HF used for the first 4 applications were .015 N, .03 N, and .05 N. One of these applications was made in December 1963, 2 in January 1964, and the fourth in late April 1964. One spray of half those concentrations was applied during bloom in March, on half the trees in each treatment. Sprays of .01 N and .02 N, and .03 N HF were used on and after June 5, 1964, except that the concentrations were reduced to .01, .015, and .02 N HF when sprays were applied during the development of a major new flush of growth. During the fourth year of these experiments, only 3 sprays of HF were applied to the experimental trees. The first 2 sprays were applied on March 10 and March 14, 1967, at concentrations of .005 N, .010 N, and .015 N HF. The third spray was applied on March 22, 1967, using concentrations of .005, .0075, and .010 N HF. All of these sprays were applied during the spring bloom period.

TABLE 11

Three-Year Average Yields of 'Marsh' Grapefruit Trees Sprayed with Different Concentrations of Hydrofluoric Acid

HF Treatment	Yield, boxes*	F in leaves ppm
None	7.46[a]	16
Low	6.27[b]	59
Medium	5.50[bc]	116
High	4.62[c]	165

*Mean yields not followed by the same letter are significantly different at P = 0.05.

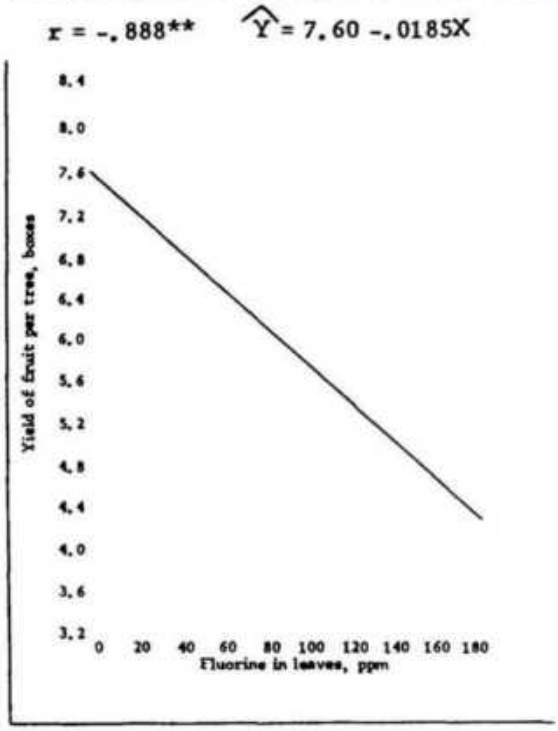
Results

'Marsh' Grapefruit: The average yields of 'Marsh' grapefruit for the first 3 years and the average fluoride content of spring flush leaves sampled in September are shown in Table 11. The difference in yield between the check and all 3 of the fluoride spray treatments was significant.

The high HF spray treatment produced a yield significantly lower than the low fluoride spray treatment. The yield difference between the low and medium spray treatments was not significant. The correlation coefficient relating yield of fruit to fluorine content of the leaves for the 3 year average was highly significant at r = -.888. The linear regression curve for the first 3 years of this experiment is shown in Fig. 2. The linear regression formula was $\hat{Y} = 7.60 - .0185X$.

Fig. 2

Linear Regression of 'Marsh' Grapefruit Yields and Fluorine in Six-Month Old Spring Flush Leaves. Three Year Averages



No significant differences in internal quality of the fruit were found during the first 3 years of the experiment. Yield data for the fourth year (1967-1968 crop) are not yet available.

'Hamlin' Oranges : Three year average yields of fruit and fluorine concentration in spring flush leaves sampled in September each year are shown in Table 12. During this period, the check treatment produced significantly more fruit than the medium and high rates of fluoride sprays. Although the yield of the trees receiving the low fluoride rate was somewhat less than the yield of the check, the yield difference between these 2 treatments was not significant. There was a highly significant negative correlation between yield and fluorine content of the leaves, with the correlation coefficient r = -.841. The linear regression formula was $\hat{Y} = 5.19 - .0097X$.

As pointed out above, the concentrations of HF sprays and the time of application were changed from the first 3 years for the fourth year of the experiment covering the 1967-68 crop. Since the only sprays applied for the fourth year were applied during the spring bloom period, the spring flush leaves were sampled for fluorine analysis on May 8, 1967. The fluoride contents of the leaves were, of course, much lower for the fourth year than for the first 3 years of the experiment. The mean differences between yield from the check and yield from the low, medium, and high HF spray treatments were all highly significant (Table 12). There were no significant differences between yields from the low, medium and high HF spray treatments. The negative correlation between yield of fruit and fluoride content of the leaves was highly significant at $r = -.758$. The linear regression formula was $\hat{Y} = 8.74 - .0319X$.

TABLE 12

Yields of 'Hamlin' Orange Trees Sprayed with Different Concentrations of Hydrofluoric Acid

	3 year mean		1967-68 crop		4 year mean	
HF Treatment	Yield boxes*	F in leaves ppm	Yield boxes**	F in leaves ppm*+	Yield boxes**	F in leaves ppm
None	5.25[a]	12	8.83[A]	5	6.00[A]	10
Low	4.63[ab]	66	7.38[B]	42	5.32[AB]	60
Medium	3.99[b]	138	6.55[B]	52	4.63[BC]	117
High	3.13[c]	188	6.79[B]	70	4.05[C]	159

*Mean yields not followed by the same letter are significantly different at P = 0.05.

**Mean yields not followed by the same letter are significantly different at P = 0.01.

*+Trees sprayed during bloom, 3 times only.

Yields and fluorine contents of leaves for the first 4 years of the experiment are also shown in Table 12. There was a highly significant difference in yield between the check treatment and the medium and high levels of fluoride spray. The difference in yield between the check and the low fluoride spray was not significant. In spite of much lower leaf fluorine values for the fourth year, due to application of only 3 sprays during bloom, the correlation between yield of fruit and fluorine content of the leaves for the 4 year period was highly significant at $r = -.850$. The linear regression formula was $Y = 6.04 - .01206X$.

No significant differences in internal quality of the fruit were found during the first 4 years of the experiment.

Discussion

The mechanisms by which airborne fluorides may produce leaf chlorosis and leaf burn (necrosis), reduce leaf size, retard tree growth and reduce yield of fruit (3) are not fully understood. Under Florida conditions, exposure of citrus trees to relatively high levels of airborne fluorides during spring bloom period is believed to cause the greatest losses in fruit production. This is indicated by the significant reduction in yield of 'Hamlin' oranges by each of 3 different concentrations of HF sprays applied only 3 different times during the 1967 bloom period, as reported above. Where the concentration of airborne fluorides is high enough during the bloom period to cause extensive leaf chlorosis, leaf burn, dropping of young leaves and excessive dropping of bloom and small fruit, the mechanism causing yield reduction is obvious.

Heavy drop of young leaves, bloom and small fruit as a result of exposure to high levels of airborne fluorides, however, has occurred in relatively few Florida citrus groves. These symptoms of acute or severe fluoride toxicity did not occur on the trees in the greenhouse experiment reported above. In the spray experiments, leaf burn and dropping of young leaves occurred only moderately when the highest concentration of HF sprays was applied while the trees had an extensive new flush of immature leaves. No dropping of young leaves nor excess dropping of bloom and small fruit occurred during the bloom period in the first 3 years of the spray experiments. A little of this occurred in the fourth year of the 'Hamlin' orange experiment with the highest HF spray concentration, when all 3 of the HF sprays were applied during bloom. For these reasons, other mechanisms less obvious than heavy dropping of bloom and fruit would appear to be largely responsible for the yield reductions found in both the greenhouse experiment and the HF spray experiments.

Fluoride has been reported to inhibit the activity of certain enzymes that occur in plants (14). Development of fluorine chlorosis on previously green leaves indicates some destruction of chlorophyll. In addition, fluorides may inhibit the synthesis of chlorophyll (8). In either case, the amount of photosynthesis per unit of leaf area is reduced since chlorophyll is required for photosynthesis. Airborne fluorides also reduce total photosynthesis by decreasing the average size of the leaves. This directly reduces the area of photosynthetic activity. The resulting decrease in production of food by the trees is followed by decreased growth and lower fruit production. This probably accounts for much of the decrease in yield found in the experiments reported here.

Acknowledgments: We thank Dr. R. F. Brewer, Department of Soils and Plant Nutrition, University of California, Riverside, for his many helpful suggestions in the planning and carrying out of the work reported here. We also thank Mr. K. K. Huffstutler, Director of the Regional Environmental Office of the Florida State Board of Health in Winter Haven and members of his staff for making fluoride determinations on leaf samples taken for the 1961-62 fluoride survey and for collecting and analyzing air samples representing the 3 treatments in the greenhouse experiment.

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IRREVERSIBLE ACUTE OLIGURIC RENAL FAILURE, A COMPLICATION OF METHOXYFLURANE ANESTHESIA

by

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Boston, Mass.

(Abstracted from the New England Journal of Medicine, 286:877-879, 1972)

The authors describe three cases of extremely prolonged renal failure following anesthesia with methoxyflurane (Penthrane). These patients were on hemodialysis for several months to more than a year due to persistent renal failure in the absence of cortical necrosis.

Methoxyflurane anesthesia had been used on these three patients for 2.2 hours, 3 hours, and 7.5 hours respectively. In the first patient, the renal failure became apparent on the 9th postoperative day, in the second on the 13th and in the third - who had been under anesthesia longest - on the 4th postoperative day. The pattern of the disease was that of acute renal failure. The daily urinary output increased up to 2 to 3 liters during the first few days. The blood urea nitrogen had risen to 130, 108 and 105 mg% respectively.

The features of the biopsy, autopsy and nephrectomy specimens were quite uniform in the three cases. The kidneys had a bluish tint. The glomeruli showed an expansion of the mesangial area in the matrix and hypercellularity. There was edema in the interstitium, occasional fibrosis and focal round cell infiltration.

Two prominent features were thickening of the small arteries with subintimal and medial fibrosis and hyalinization as well as abundant crystalline birefringent material resembling oxalate crystals present in the tubules, in the interstitium and in the adventitia of the blood vessels. One of the patients expired, in spite of continued hemodialysis, on the 111th post operative day. The other two were maintained on dialysis for 98 days and 25 months respectively following surgery.

Although the oxalate deposition was the most outstanding morphological feature, the authors did not consider the tubular obstruction by oxalates the cause of the renal failure. They pointed to the biodegradation of methoyxflurane to fluoride ion, a number of halogenated hydrocarbons and oxalic acid, all of which are excreted by the kidney. Serum fluoride levels are markedly elevated in patients with renal failure following methoxyflurane anesthesia. The authors concluded that the fluoride ion was the nephrotoxic moiety.

METHOXYFLURANE NEPHROPATHY

by

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Bethesda, Maryland

(Abstracted from the U.S. Navy Medicine 59:30-34, 1972)

The author reported two non-fatal cases of nephrogenic diabetes insipidus following the use of methoxyflurane anesthesia.

One of the patients, a 69 year-old retired naval captain, had surgery for removal of a carcinoma of the rectum, the other for duodenal ulcer. The duration of the anesthesia was approximately 4 to 6 hours. Two to five days postoperatively large volumes of dilute urine were excreted with a rise in blood urea nitrogen and serum sodium. In the first patient, this development was transient but the second case continued to manifest azotemia and creatininemia. The urine of both individuals failed to concentrate under stimulation of an infusion of hypertonic saline solution and following administration of Pitressin. The urine-to-plasma osmolality was less than 1. The first patient responded to Pitressin within 2 weeks; for the second, response was delayed one month following exposure to the anesthetic.

The author comments on the incidence of nephrogenic diabetes insipidus with or without azotemia following the use of methoxyflurane which occurs in 4 to 24% of the cases receiving this anesthetic. With regard to the mechanism, it has been pointed out that methoxyflurane leads to deposition of oxalate crystals in the renal tubules and in the medullary interstitium. Another theory is based on the finding of elevated levels of both inorganic fluoride and nonvolatile organic fluoride in the blood as reported by Taves et al. Some clinicians have postulated a synergism of the anesthetic with tetracycline given prior to surgery. According to the author, a wide spectrum of nephropathies are associated with methoxyflurane anesthesia. They range from a mild to moderate concentrating defect with a good prognosis to a fulminant uremic state which may be fatal.

From the Naval Hospital, National Naval and Medical Center, Bethesda, Md.

STUDIES OF PATIENTS WITH OSTEOGENESIS IMPERFECTA

by

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(Abstracted from the Journal of Bone and Joint Surgery 53A:1415-1424, 1971)

In their attempt to learn more about osteogenesis imperfecta and the effects of fluoride therapy upon it, the authors studied 25 patients. Thirteen of these were treated with sodium fluoride.

Patients

The diagnosis of osteogenesis imperfecta was made clinically and roentgenographically in each case. All patients had a history of multiple fractures and growth was severely retarded. Most individuals were below the 3rd percentile for both height and weight.

The patients received fluoride in doses ranging from 0.25 to 0.90 mg/kg of body weight per day. Repeat metabolic studies were done approximately one year after starting treatment.

Methods:

Calcium, magnesium, phosphorus, nitrogen and potassium balance studies were performed. Diets were given to duplicate patients' normal intake. Analysis were performed on urine, stool, rejected food and duplicate diets. Distilled water was used instead of tap water, mouthwash instead of toothpaste and any medications the patient had been taking were included in the duplicate diet analyses.

Calcium and magnesium analyses were performed by atomic absorption spectrophotometry, potassium by flame photometry, phosphorus by the method of Fiske and Subbarow, and total nitrogen by Kjeldahl digestion and direct Nesslerization. An evaluation of calcium kinetics was performed with ^{48}Ca as described by McPherson. Bone Density was evaluated by visual inspection of roentgenograms. Routine serum chemistries were done in the hospital clinical chemistry laboratory. Genetic screening studies for amino acids and sugars, reducing substances and mucopolysaccharides, keto acids, nitroprusside and alkaptones were performed on urine specimens.

To evaluate the affect of fluoride, the fracture rate during the first 12 to 24 month period of treatment was compared with the rate for an equal period immediately prior to treatment.

From the Yale University School of Medicine, Dept. of Orthopaedic Surgery and Pediatrics. The Newington Children's Hospital, Newington, Connecticut, and the Yale-New Haven Hospital, New Haven, Connecticut.

Results

Using each patient as his own control, the average incidence of fracture decreased from 2.44 to 0.96 fractures per year after fluoride treatment. The clinical status paralleled the change in fracture rate, but no patient showed any dramatic improvement.

Serum sodium, potassium, chloride, bicarbonate, calcium, phosphate and BUN were normal throughout the control and treatment periods. Free hydroxyproline was not elevated. Calcium and magnesium balance showed no significant variation from normal, either before or after fluoride treatment.

No significant change in bone density was found on roentgenograms following fluoride treatment. Genetic screening studies were normal in all patients under observation.

The authors conclude that the over-all results do not warrant the general use of fluoride for the treatment of osteogenesis imperfecta.

FLUORIDES IN COMMUNITY PROGRAMS: RESULTS AFTER TWO YEARS FROM A FLUORIDE GEL APPLIED TOPICALLY

by

L. F. Szwejda
Lansing, Michigan

(Abstracted from Journal of Public Health Dentistry 31:241-242, 1971)

Children from second grades of selected schools were given two annual prophylaxes with a rubber cup and mixture of pumice and water and two single annual applications of acidulated phosphate gel in wax trays. The gel was permitted to remain in contact with the teeth for approximately three minutes. The gel contained 1.23% of the fluoride ion.

Annual dental examinations were made and compared to a control group which received prophylaxes with a bland non-abrasive paste and a mild saline solution applied topically.

The two year incidence of carious lesions indicate little difference between the experimental and control groups. The findings from this study do not confirm the successful results found in other studies of the gel.

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A CASE OF SKIN SCALDING WITH HF-HYDROFLUORIC ACID

by

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(Abstract)

A 61 year old man spilled carelessly 67% hydrofluoric acid on his leg and remained in the contaminated atmosphere. Death occurred suddenly within about two hours after contact with the acid.

The body was exhumed for an autopsy. The following fluoride concentrations in tissues were found:

	Fluoride Content ppm
Small intestine with contents	9.8
Liver	8.0
Kidney	9.7

The method which was used for the assays follows:

Ashing of samples with excess $CaOH_2$, separation of fluoride from ashes by distilling in Pietzka-Ehrlich apparatus, and spectrophotometric determination of fluoride content in the distillate with the reagents SPADNS-Zr according to Ballac and Schouboe.

Normal levels in samples assayed by this method in the Institute ranged between 0.5 to 4.5 ppm fluoride. In other cases of fatal poisoning by fluoride compounds the fluoride concentration amounted to 10.0 to 46 ppm in liver and kidneys and to 100 ppm in the small intestines.

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