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# FLUORIDE

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

Reservations for the Conference should be made at the Coburg Hotel, Bayswater Road, Hyde Park, London W2 4RJ, England.

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**Contributors** will receive copies of the issue of **FLUORIDE** containing their paper, free of charge.

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## EDITORIAL

### FLUORIDE AND THE SKIN

In his classical description of chronic fluoride intoxication, Roholm stated that 8 out of 68 women workers were afflicted with a skin rash, localized mainly on the chest and the back (1). He presented no details concerning the exact nature of this rash but there is reason to believe that it was either a seborrheic dermatitis or an acne-type eruption. This is not surprising since some fluoride is excreted through the skin and since other halogens are known to cause such skin lesions. Iodine, for instance, is often responsible for acne and "Chlor-acne" is recognized as an occupational disease in workers exposed to chlorine gas. The characteristic skin lesions of bromide poisoning are also a form of acne.

The current issue presents several articles which indicate that the skin is indeed susceptible to damage by fluoride. Saunders, a West Virginia dermatologist, described what appears to be a common skin disease among women extending radially from the mouth into the cheeks which improves following substitution of fluoride toothpaste by other dentifrices (see page 40-41).

On the other hand, Epstein, a California dermatologist, gave lozenges containing 2.0 mg of calcium fluoride (approximately 0.65 mg fluoride) to 20 patients with acne daily for 1 to 11 weeks (an average of 6 weeks), and concluded that fluoride neither aggravated nor alleviated the eruptions (2). However, his results cannot be considered conclusive because he administered to his patients other remedies for acne concurrently with this medication.

An entirely different skin disease encountered by several authors (3, 4, 5) during acute fluoride poisoning is urticaria or hives which can involve the whole body surface. In children who receive fluoride applications to the teeth acute episodes of hives confined mainly to the face are occasionally encountered.

Chronic urticaria which is frequently precipitated by drugs and other chemicals has also been described (6, 7) in association with chronic fluoride poisoning and the question arises whether or not fluoride in water, food and air precipitates this ailment much oftener than is currently recognized.

Another allergic manifestation of the skin is atopic dermatitis, which usually affects infants and young children. It is mainly localized

at typical sites of the skin, at the antecubital area of the arm and the retropliteal surface of the legs. That this condition can be induced by fluoride was first recognized by Feltman and Kosel (8) who observed it in 1% of young children and pregnant women who were given fluoride tablets for prevention of tooth decay. Subsequently, Shea and others (9) presented the cases of three infants who developed atopic dermatitis in conjunction with gastrointestinal symptoms following ingestion of vitamin drops containing fluoride. Zanfagna (10) reported this condition in a 48-year-old female due to drinking fluoridated water.

A somewhat similar allergic disease is contact dermatitis which is confined mainly to areas of the skin in contact with the causative agent. For instance, Abelson, a dentist, contracted such lesions on his fingers after he applied a 2% solution of sodium fluoride to his patient's teeth (11). A 4+ positive patchtest reaction established the relation of the dermatitis to sodium fluoride. Reports of similar contact lesions were presented by Fregart and Moller (12) in and about the eyes following the use of eye drops containing a fluoride compound. Two Russian clinicians (13) described contact dermatitis due to hydrogen fluoride in a woman working in a glass factory. In a Czechoslovakian aluminum factory, of 138 women workers Horky (14) recorded a 20.7% incidence of dermatitis on their external genitalia which he attributed to fluoride.

Direct contact is undoubtedly the mechanism responsible for ulcerations in the mucous membranes of the mouth following the use of dentifrices containing fluoride. Douglas (15) described this condition in detail in 133 cases due to fluoride-containing toothpastes. The lesions cleared up promptly upon substituting non-fluoride toothpastes. In 32 patients, the stomatitis was reproduced by reapplying the dentifrice, in several cases as often as six times. In one of my patients, the application of sodium fluoride to the teeth produced an immediate outbreak of urticaria with extensive facial edema followed several hours later by small ulcers in the mouth.

On page 29 Waldbott and Zacks presented data on a skin disease of an entirely different nature. It was first observed near a fluoride-emitting factory in northern Italy and has been subsequently encountered in the USA (16). Colombini and collaborators described the histology of these lesions as follows: "The histological examination of the biopsied skin revealed an edematous-fibrinous leucocytic exudate in the interstice of the adipose lobules. It is most pronounced at the dividing line between dermis and hypodermis. It is associated with degeneration of the adipose cells, partial reabsorption of the fat and formation of frothy, basophilic cells. The regressive phase of the lesions is characterized by mild perivascular

infiltration. These histological changes resemble those of erythema nodosum but cannot be identified as such "(17).

It appears that these lesions occur only in the initial stage of fluoride poisoning, especially in young children, and are not associated with advanced skeletal fluorosis. Roholm made the same observation with respect to the gastrointestinal manifestations in cryolite workers which subside as the disease progresses.

Clinicians consider the skin a mirror on the body's surface which reflects what goes on elsewhere in the system. With this in mind it might be well to search for additional signs of fluoride intolerance after all other causes of the above named skin lesions have been ruled out.

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# THE EFFECT OF FLUORIDE ON THE COMPOSITION OF VOLATILE MONOCARBON ACIDS IN THE RUMEN OF CATTLE

by

W. Leeman, O. Stahel and J. Keeman  
Zürich, Switzerland

**SUMMARY:** Three cows received, after a fluoride-free control period, a supplement to their feed of 1.5 mg fluoride/kg for 11 periods of 3-weeks. By means of gas-chromatography the changes in percentage of acetic acid, propionic acid, butyric acid, n-valerianic and iso-valerianic acid were determined three times daily; the pH values in the rumen content and the urinary fluoride excretion were measured. A mean for fluoride-induced variations in acetic acid, as well as propionic acid, during the eleven periods and the times of day were 70.16-71.24% and 17.35-18.20% respectively. For butyric acid the percentage for the eleven periods showed considerable deviation (10.02-11.16%) but no short-term effect. The same holds true for the n-valerianic acid (0.55-0.72%) which was found only in traces. In all three animals the percentages varied during the eleven periods but, during the hours of the day, variations occurred in two animals only. For the iso-valerianic acid, lesser differences occurred during the eleven periods than at the hourly intervals. The pH values did not differ in two animals. The urinary excretion of fluoride was always below 4 ppm during the control period but rose during the experimental period to a maximum of 51 ppm, with a mean of 30 ppm.

In a former paper we reported on the effect of 2 and 4 mg fluoride/kg body weight upon the composition of monocarbon acids in the rumen of a cow. In that experiment, the time had to be limited to eleven weeks since the animal developed acute fluorosis with such high doses. It refused uptake of food and became emaciated rapidly. Since there is a correlation between

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From the Veterinary Medical Clinic, University of Zurich, Switzerland.

Presented at the Seventh Conference of the International Society for Fluoride Research, Zandvoort, Holland, February 8-10, 1976.

the concentration of the respective fatty acids and their quantitative composition we questioned whether or not with prolonged administration of less than 2 mg/kg body weight the percentual composition of the acids would change. An exact quantitative assay of the fatty acid production, however, is difficult to carry out; therefore, we had to determine the percental composition.

### Materials and Experiments

Experimental Animals: Six cows weighing 490 to 570 kg, aged 5 to 7 years, were studied over a 5 week period. For various reasons, three animals had to be eliminated and the statistical evaluation of the long-term experiments was limited to three cows (numbers 34, 53 and 66) over 30 weeks (=10 periods).

The animals were fed ad libitum. To a feed supplement of about 1 kg, which included 18% raw protein and 120 gm mineral-mixture, 1.5 mg/kg sodium fluoride was added after a 3-week control period. The experimental animals were weighed weekly and the supplementary food was periodically checked for its fluoride content.

Rumen Juice Collections: All experimental animals received a Hart-PVC fistula prosthesis of the rumen fitted according to Snyder. Samples were selected at 10, 13 and 15 o'clock i. e., 3, 6 and 8 hours following the administration of fluoride. The pH value of the rumen content was determined immediately after taking the samples and the gaschromatographic determination of the monocarbonic acid was made within 48 hours after storing the samples at 18°C (1).

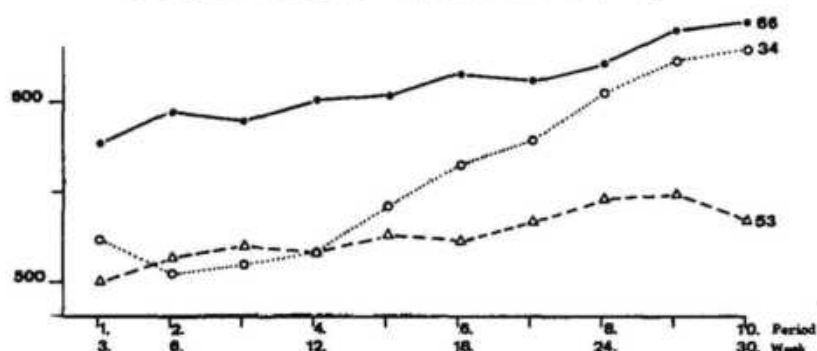
Sampling of the Urine: The spontaneously produced urine was collected between 8 and 12 o'clock and assayed for its fluoride content by means of the fluoride specific electrode, except in animal 53, which was catheterized periodically.

Gaschromatographic Determination of the Short Chain Monocarbonic Acids was made according to the previously described method (1). In addition to the three most important volatile fatty acids, acetic, propionic, and butyric acid (abbreviated C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub>) we also determined the percental portion of the n-valerianic acid (n-C<sub>5</sub>) as well as the iso-valerianic acid (iso-C<sub>5</sub>).

### Results and Discussion

Body Weight of the Experimental Animals: Figure 1 presents the

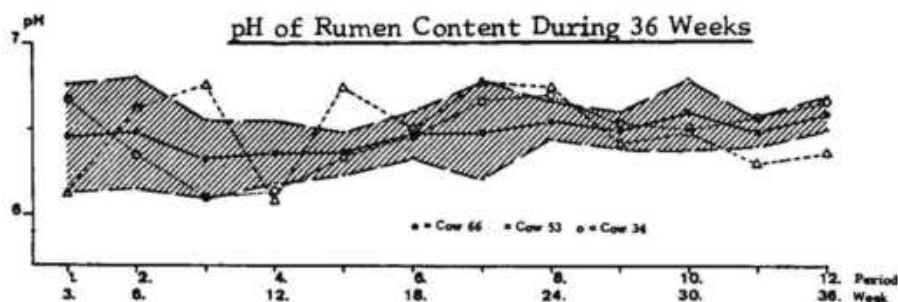
Figure 1

Body Weight of Cows #34, #53 and #66

body weights of the three experimental animals 34, 53 and 66 which increased markedly at varying degrees. At the onset of the experiment they weighed 525, 500 and 575 kg, respectively, but at its termination 630, 530 and 640 kg which corresponds to an average weekly increment of 3.5, 1.0 and 2.2 kg.

Fluoride Content of the Urine: During the first period (1st to 3rd week) the experimental animal 53 eliminated  $2.64 \pm 0.39$  ppm fluoride in the urine (median and standard deviations of 9 determinations within the 3 week period). After the beginning of the fluoride administration during the 5th period (12th to 15th week) we observed a maximum at  $34.71 \pm 11.31$  ppm. The urinary fluoride level declined during the 6th period (15th to 18th week) to  $25.96 \pm 3.65$  ppm and rose to  $36.96 \pm 6.94$  ppm at the termination of the experiment. We noted marked variations during a single period. In spite of a minor rise during the first 6 hours we were unable to determine a statistically significant difference between the three periods of collection. For the purpose of orientation about an increase in fluoride uptake it is, therefore, permissible to determine the fluoride values of the spontaneously produced urine.

pH Value of the Rumen Content: We could again confirm the formerly (1) recognized linear correlation between pH and total concentration of fatty acids, namely a decrease of the pH values by 0.0743 units with an increment of 1 millimol monocarbonic acid per 100 ml rumen content. Figure 2 presents the mean pH values of the three animals as well as the standard deviation of animal 66. The fluoride assays revealed no significant deviation ( $P=0.001$ ) for the three animals and the different times of the day; but a highly significant difference occurred over the 10 periods. These differences of the interactions between the animals and the 3-weekly periods point to the fact that not all animals reacted equally in the same manner to the administration of fluoride. Particularly conspicuous were the deviations in animal 53. How much this change depended upon the amount

Figure 2

of food consumed will have to be examined in a future experiment.

If we compare the first fluoride-free period with the fifth and the tenth period (pH 6.41, 6.51 and 6.50, respectively) no significant differences occurred during the three periods either with respect to the animals, the time of day, or the periods. The total concentrations of the 5 monocarbonic acids, therefore, did not appear to be altered materially.

Molar Concentrations of the Volatile Fatty Acids: It is generally accepted that the molar production rate of fatty acid fractions depends largely upon the kind and the amount of food consumed. The fatty acids which are formed by microorganisms, primarily in the rumen, undergo considerable changes before they reach the intermediate metabolism. They are, therefore, difficult to determine in absolute quantities and are furthermore subject to marked individual variations. Moreover, it is assumed that the action of fatty acid absorption upon the wall of the stomach is dependent on the molar composition and the state of the animals (hungry, growing, lactating). The study of the concentrations of volatile monocarbonic acids nevertheless permits conclusions about certain time-dependent changes in the intensity of fermentation with respect to administration of fluoride (2).

In order to obtain a better insight into this matter the millimol amounts determined by gaschromatography of the individual 5 fatty acids were calculated for their percental portion.

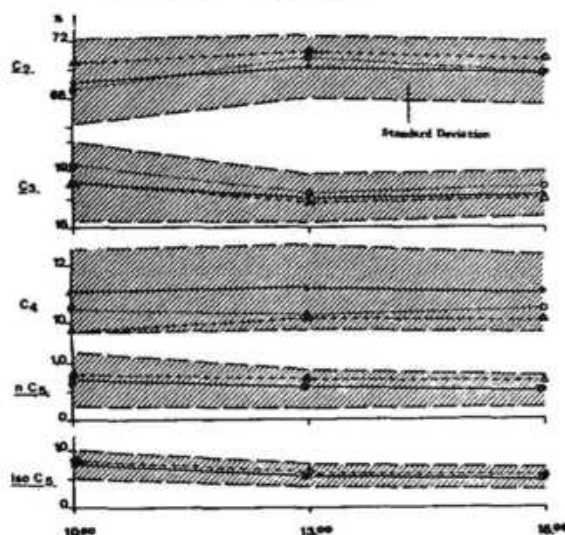
Time Related Effect: If we look at the limits of significance summarized in Figure 1, we recognize highly significant differences ( $P=0.01$ ) of the percentages for the first two monocarbonic acids  $C_2$  and  $C_3$  in all three animals both for the 3-week periods and for the three samplings during the day. The butyric acid, however, showed differences with a 99.9% significance only for the periods. The n-valerianic and iso-valerianic acids showed highly divergent conditions from one animal

to another.

With regard to the behavior of the individual fatty acids during the first fluoride-free control period by means of the t-test, there was at no time any systematic deviation at the three daily samplings. Therefore, the varying intensity of fermentation seemed to be at the basis of the observed differences which occurred over all 10 periods due to the administration of fluoride.

The data concerned with the daily sampling is presented in Figure 3. It shows that the portion of the acetic acid increased between 10 and

Figure 3  
Percental Changes of the 5 Monocarbon Acids at the  
3 Daily Samplings



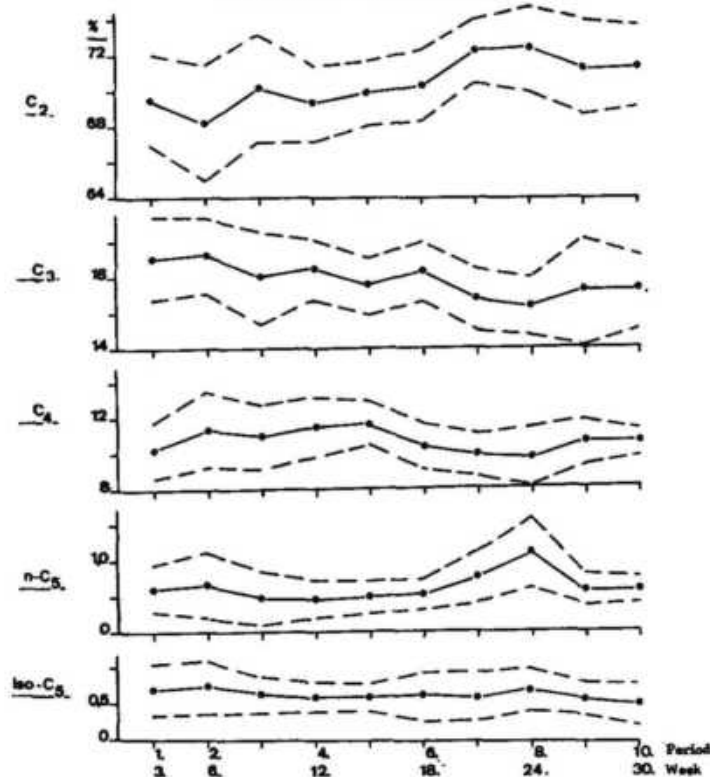
13 o'clock from 69.88 to 70.97% and declined subsequently to 70.32% at 15 o'clock. The limit of significance amounted to 99.9%. The values for the propionic acid, however, showed a different behavior: Three hours after administration of fluoride we found 18.27%, but at 13 o'clock only 17.11%, and at 15 o'clock, 17.95%. Here too, all three differences are highly significant at 99.9%. The butyric acid rose from 10.40% at 10 o'clock to 10.78% at 13 o'clock and subsequently decreased slightly to 10.6% at 15 o'clock. These differences are also significant at 95%. The n-valerianic acid decreased highly significantly from 0.72% to 0.58% to 0.56%, respectively. Similar results were obtained with the iso-valerianic acid, namely: From 10 to 13 o'clock its portion declined at a 99% significance from 0.74 to 0.57% and remained stable up to 15 o'clock at 0.58%. In summary, the

effect in each animal was markedly less with respect to the interaction of the various acids. Only for the first two fatty acids could we determine significant differences ( $P=0.01$ ).

Changes in the Molar Concentration During the 10 Periods: Upon considering the molar conditions of the individual fatty acids during all 10 periods we established highly significant changes ( $P=0.001$ ) in all except the iso-valerianic acid. Also, the interaction of the various acids showed highly significant variations and each cow reacted differently. In Figure 4, the means and standard deviations for the 5 monocarbonic acids for all animals and times are presented.

Figure 4

Mean Percentages for the 5 Monocarbon Acids for the 11 Week  
Experimental Periods



With respect to the acetic acid the mean value for all animals was 69.43% during the first period. Following the beginning of fluoride administration its percentage decreased to 68.13% but rose again distinctly during the 3rd period to 70.08%, then more slowly to the 8th period up to 72.26% and declined again slowly to the 10th period to 71.40%.



Table 1

Molar Concentrations of the Volatile Monocarbon Acids With Respect to Significance Limits of the Individual Animals, Periods and Times

Acids	Cow 34			Cow 53			Cow 66		
	Means and Standard Deviations	Significance Periods	Times of Day	$\bar{x}$ , $s_{\bar{x}}$	Periods p	Times p	$\bar{x}$ , $s_{\bar{x}}$	Periods p	Times p
Acetic	70,22 $\pm$ 3,08	0,001	0,001	71,24 $\pm$ 2,02	0,001	0,001	70,16 $\pm$ 2,56	0,001	0,001
Propi- onic	18,20 $\pm$ 2,42	0,001	0,001	17,35 $\pm$ 1,90	0,001	0,001	17,52 $\pm$ 2,11	0,001	n.s.
Butyric	10,43 $\pm$ 1,67	0,001	n.s.	10,02 $\pm$ 1,40	0,001	n.s.	11,16 $\pm$ 1,42	0,001	n.s.
n-valer- ianic	0,55 $\pm$ 0,41	0,001	n.s.	0,72 $\pm$ 0,28	0,001	0,01	0,58 $\pm$ 0,37	0,001	0,05
iso-val- erianic	0,60 $\pm$ 0,30	n.s.	0,001	0,65 $\pm$ 0,29	0,01	0,001	0,59 $\pm$ 0,25	n.s.	0,001

The authors express their gratitude to E. Lehmann and H. M. Halter, Institut für Tierernährung, Eidgenössische Technische Hochschule, Zürich for the statistical evaluation and to F. Bürge, R. Wernli, R. Dürler and J. Wyss for the analyses.

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#### Discussion

Dr. Waldbott: Why did the weight gain take place soon after the experiments started?

Dr. Leeman: This was a question of proper management, since the animals received better care in the clinic than previously by the farmers. The protein intake during the experiment was 18 percent, which in actual practice is rarely supplied by farmers in my country.

Dr. Burk: Why in your treatment was your highest dose 1.5 mg fluoride/kg

- body weight? The human dose is several mg per 100 kg.
- Dr. Leeman: Because in fluorotic regions the hay contains high concentrations of fluoride.
- Dr. Roelofs: In which form did you add fluoride and is there a difference in sensitivity to fluoride between animals?
- Dr. Leeman: We gave fluoride as NaF. Sheep and cattle are more sensitive than horses. I have had no experience with cats and dogs.
- Dr. Cook: Would this sensitivity be concerned with the Ca content of the diet?
- Dr. Leeman: Calcium exerts some influence. If the mineral supplement is low, fluorosis develops much more easily.

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## AUTOMATIC ANALYZER OF FLUORINE IN AIR

by

B. Boeuf  
Les Milles, France

The following is the description of an automatic analyzer for atmospheric fluorides, which is designed to measure HF near aluminum smelters, brick factories, iron smelting industries, fertilizers and super-phosphates factories, and other exposed areas.

Measuring Principle: The instrument is based on the principle that the air to be analyzed bubbles continuously in an electrolyte which scrubs total fluorine as fluoride. The increase in the concentration of fluoride in the solution is measured with an ion electrode which is specific for fluorides. The potential of such an electrode is based on Nernst law. At constant temperature and ionic strength this potential is a function of the concentration  $CF^-$  of fluorides trapped in the solution. The Nernst law is usable for concentrations between  $10^{-6}N$  and  $10^{-1}N$ .

To avoid interferences by  $OH^-$ ,  $Al^{3+}$ ,  $Fe^{3+}$ ,  $Si^{4+}$ ,  $PO_4^{3-}$ ...it is necessary to use a complexing agent, namely TISAB, made by mixing acetic acid, CDTA and sodium chloride. We add fluorides also to obtain a solution  $5 \times 10^{-6}N$ .

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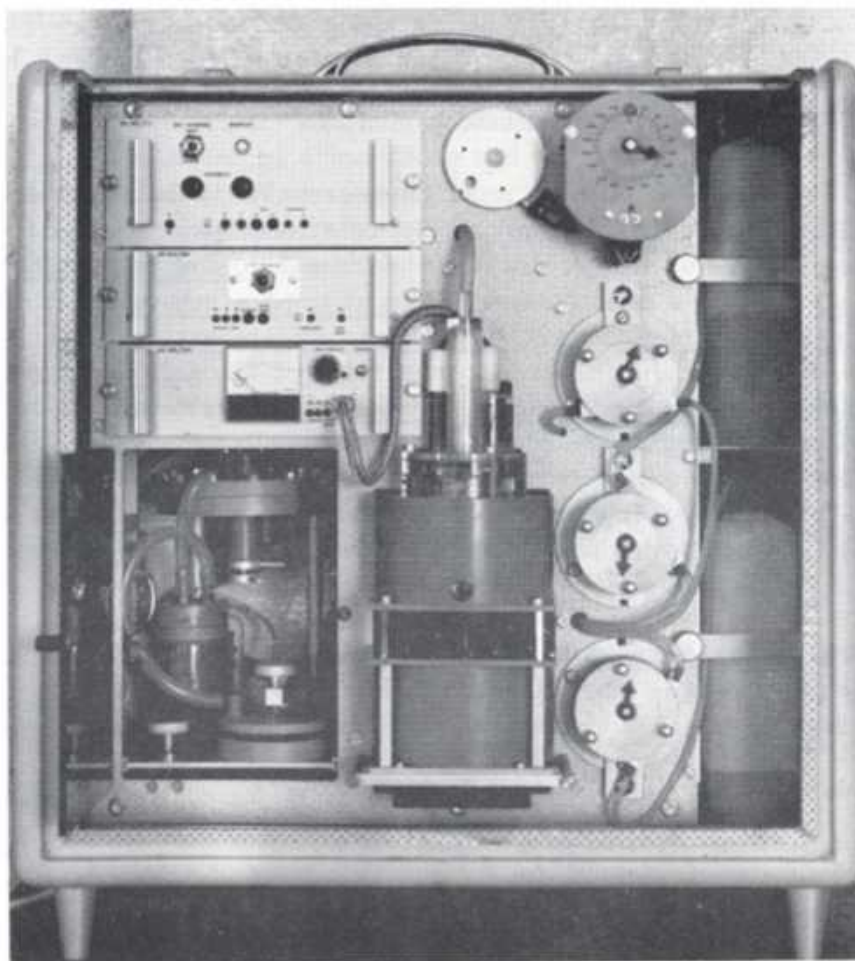
From the Société d'Étude et de Réalisation d'Équipements Spéciaux, Rue Albert Einstein, 13290 Les Milles, France.



Description: The instrument consists of two cabinets, one for analysis, the other for recording and for its power supply (Fig. 1). The air to be analyzed is drawn through the lower cell where it is mixed with the reagent. It then passes by the upper cell and the air pump before it exits. At the start, the upper cell is filled with the reagent; a peristaltic pump feeds the lower cell slowly and continuously. The reagent is conducted to the upper cell by the air flow after it passes a reaction coil where it mixes with the air. The increase of the fluoride concentration is measured with an ion specific electrode in the upper cell. A mixer assures a homogeneous solution; the temperature is regulated with "Peltier" elements.

Each cycle lasts three hours. During the first half hour the air pump is stopped in order to allow a good stabilization of the electrodes. During the following 150 minutes the air pump operates and the values are recorded every 30 minutes. At the termination of the cycles a pump empties the cells and a new cycle is begun. The portable unit requires a power supply of 220/110 V. It is sensitive to  $1 \mu\text{g}/\text{m}^3$ .

Figure 1



### Discussion

Dr. Cook: Is the system portable?

Dr. Boeuf: Yes, but it requires a generator.

Dr. Waldbott: Could you give an idea of the cost?

Dr. Boeuf: We have just started production, but it will cost about 25,000 francs.

Dr. Johnson: Do you filter and remove the particulate?

Dr. Boeuf: Yes, only the gaseous fluoride in the air is measured. The concentration range for measurement in the solution is  $10^{-6}N$  to  $10^{-1}N$ . The temperature is regulated within 0.1 C.

Dr. Franke: Did you measure levels of fluoride in factories? Do you have human industrial fluorosis in France?

Dr. Boeuf: Yes, we have industrial fluorosis in France, but I am not sure of the levels found in the factories.

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### THE RELATIONSHIP OF AN INSECT INFESTATION ON LODGEPOLE PINE TO FLUORIDES EMITTED FROM A NEARBY ALUMINUM PLANT IN MONTANA

by

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**SUMMARY:** Stepwise, multiple regression techniques were used to statistically analyze the relationships between damage caused by the pine needle sheath miner, Zellaria haimbachii (Busck); a needle miner, Ocnerostoma strobivorum (Zeller); sugar pine tortrix, Choristoneura lambertiana (Busck); and ambient and foliar concentrations of fluoride in lodgepole pine (Pinus contorta v. latifolia Engelm.) near the Anaconda Aluminum Company at Columbia Falls, Montana. Foliar fluoride concentration was significantly related (0.01 level) to needle miner and pine needle sheath miner damage. The data strongly indicate that fluoride is a contributing factor in predisposing pines to damage by these insects.

The Environmental Protection Agency (1) in 1970 studied fluoride emissions from an aluminum plant near Columbia Falls, Montana. They showed that fluorides were carried in the air from the aluminum plant

northeastward toward Glacier National Park. Injury to vegetation was observed throughout the area in which airborne fluorides were found. Gordon (2) showed that forest vegetation over a wide area from Columbia Falls, Montana, to Logan Pass in Glacier National Park accumulated abnormally high amounts of fluoride and showed varying amounts of injury. In addition, he showed that animals feeding on contaminated vegetation accumulated high quantities of fluoride in skeletal tissue. Carlson and Dewey (3) showed that excessively high amounts of fluoride were found in vegetation over 200,000 acres in the area, and that visible fluoride injury could be found over 69,000 acres. The most severe injury was found on lodgepole pine (Pinus contorta var. latifolia Engelm.), ponderosa pine (Pinus ponderosa Laws.), and western white pine (Pinus monticola Dougl. ex D.). Within 30,000 acres of the 30 isopol(2) east of Teakettle Mountain, a severe insect infestation was detected on the lodgepole pine. It was estimated in 1970 that about 50 percent of the visible injury on lodgepole in this area was caused by fluoride and about 50 percent was caused by insects. The defoliating insects were identified as pine needle sheath miner, Zellaria haimbachi (Busck); a needle miner, Ocnerostoma strobivorum (Zeller); pine needle scale, Phenacaspis pinifoliae (Fitch); and the sugar pine tortrix, Choristoneura lambertiana (Busck) (3).

The association between the insects and fluoride was quite striking. The infestation expanded to nearly 150,000 acres in 1973 and was contained primarily within the area in which vegetation accumulated fluoride emitted by the aluminum plant. The most serious insect damage appeared to be within the area moderately influenced by fluorides. Interestingly, little insect damage was found close to the aluminum plant where high amounts of fluoride prevailed in the atmosphere and vegetation. The objective of this study was to determine if significant correlations existed between atmospheric fluoride, pine needle tissue fluoride concentration, fluoride injury on needles, and damage caused by four insect species.

#### Methods

Thirty-six plots were randomly distributed in lodgepole pine stands throughout the area moderately and lightly influenced by fluorides between the 100 and 10 isopols as shown by Carlson and Dewey (3). No plots were put within the area included by the 100 or greater isopols because of a lack of lodgepole pine stands. Four control plots were placed in areas not influenced by airborne fluorides: two, 30 miles south of Columbia Falls in the Swan River Valley and two, 20 miles west of Kalispell, Montana, near Rogers Lake. At each plot location, five lodgepole pine between 20 and 40 feet in height (dominant and codominant trees on the site) were selected as sample trees. The nearest neighbor technique (4) was used in tree selection to reduce personal bias. In all, 200 trees were sampled. Sample trees at each plot were then classified, based on condition as affected by both insects and fluoride:

<u>Tree class</u>	<u>Vigor description</u>
0	No apparent insect feeding or fluoride damage. Needle retention normal in appearance.
1	Some damage evident; tree crowns becoming thin or light, insect activity visible. Needle retention below normal.
2	Damage by insect feeding quite evident or crown thin. Needle retention may be poor.
3	Heavy insect damage evident. Excessive insect feeding visible. Crowns thin and needle retention poor.

From each of the sample trees on each plot, four branches 18-20 inches in length were removed from midcrown and transferred to our laboratory. Here, estimates of the populations of tortrix, needle miner, and needle sheath miner were made. Total insect counts were made on each sample tree for each insect species following procedures described by McGregor (5) and Fischer (6). The sugar pine tortrix and sheath miner had completed their feeding and some were in the pupal stage, whereas the needle miners had pupated and started to emerge as moths. The pine scale was not counted because of a very low population.

Next, the relative amount of foliar damage caused by each insect and by fluoride was estimated. Needles were stripped from each sample branch and kept separate by year of origin: 1973, 1972, or 1971. Then, for each tree, 100 needles from each growth period were randomly selected and examined individually. From these 100 needles, the number sustaining damage caused by the pine needle sheath miner, needle miner, and fluoride was determined based on the characteristic damage of each. Current tortrix and sheath miner damage was confined to the 1973 growth because of their feeding habits. Needles mined by the sheath miner were discolored and hollow and the tortrix confined its feeding to elongating needles of the candles. However, because of difficulty in separating sheath miner damage from that caused by tortrix, these variables were lumped. The needle miner fed on 2- and 3-year-old needles and damage was easily recognized by the hollow needles and exit holes of mature larvae. Fluoride damage was identified by necrotic needle tips and dark brown band appearing at the junction of healthy and necrotic tissue.

Approximately 10 grams (fresh weight) of needles from each growth

period on each sample tree were prepared and analyzed in our laboratory for fluoride content by the specific ion method described by Gordon (2). The 10 grams included healthy needles and needles damaged by various agents. Fluoride concentrations were given in parts per million (p p m ) based on the dry weight of the tissue. Two static sodium formate monitors to estimate the amount of gaseous airborne fluoride were placed in each plot. Ambient concentrations of fluoride were computed in micrograms of fluoride per square centimeter per day.

### Results and Discussion

Stepwise multiple regression was used to analyze the data. Means and standard errors for each variable measured are given in Table 1. The average concentration of fluoride in the 1971 and 1972 foliage was computed and used for regression purposes. Fluoride concentration in 1973 foliage was

Table 1

#### Means and Standard Errors for Independent and Dependent Variables

<u>Variable</u>	<u>Code<sup>a</sup></u>	<u>Mean</u>	<u>Standard error</u>	<u>Standard error as percent of mean</u>
Tortrix population	X <sub>1</sub>	0.9330 <sup>b</sup>	0.1770	0.19
Ambient Fluoride	X <sub>2</sub>	.0027 <sup>c</sup>	.0002	.07
Average F concentration	X <sub>3</sub>	10.2000 <sup>d</sup>	.9300	.09
Needle miner population	X <sub>4</sub>	39.3000 <sup>b</sup>	7.0400	.18
Sheath miner population	X <sub>5</sub>	2.8100 <sup>b</sup>	.3600	.13
Tree rating	Y <sub>1</sub>	1.1200	.0500	.04
1971 needle miner damage	Y <sub>2</sub>	11.0400 <sup>e</sup>	.8100	.07
1971 fluoride damage	Y <sub>3</sub>	1.3600 <sup>e</sup>	.3000	.22
1972 needle miner damage	Y <sub>4</sub>	9.3600 <sup>e</sup>	.6700	.07
1972 fluoride damage	Y <sub>5</sub>	.6200 <sup>e</sup>	.1500	.24
Sheath miner and tortrix damage	Y <sub>6</sub>	11.7300 <sup>e</sup>	.9000	.08

<sup>a</sup> X = independent; Y = dependent.

<sup>b</sup> Tortrix, needle miner, and needle sheath miner populations are expressed as total insects per four-branch sample per tree.

<sup>c</sup>  $\mu\text{gF}^-/\text{CM}^2/\text{day}$ .

<sup>d</sup> Parts per million fluoride, dry weight basis.

<sup>e</sup> Damaged needles per 100 observed.

not used because that foliage had been exposed to ambient air for only 1 month prior to collection and would have accumulated little fluoride. Data from one plot were discarded because no information on ambient fluoride



concentrations was obtained. Therefore, the analysis was done on data from 195 trees instead of 200. All standard errors were less than 25 percent of their respective means; thus we considered the sampling adequate.

Stepwise multiple regression selectively computes regressions based on a predetermined level of significance. Different independent variables are entered or deleted in a stepwise fashion based on a selected "F" ratio level. We selected  $F = 2.0$  as the minimum acceptable level for any independent variable entering the regression ( $t^2 = F$ ,  $t = 1.41$ ). Values of  $t$  at the 0.05 and 0.01 levels for 195 degrees of freedom are 1.97 and 2.35, respectively. We selected the slightly lower value of 1.41 so as to include as many independent variables as reasonably possible in the regression. Results are given in table 2. All of the independent variables entered in each

Table 2

Regression Coefficients, t ratios, and Coefficients of Multiple Determination and Correlation for Six Dependent Variables

Dependent variable	Independent variable	B <sup>a</sup> /	t ratio	R <sup>2</sup>	R
Y (tree rating)	X0 <sup>b</sup> /	0.540		0.51	0.71**
	X2 ambient F <sup>-</sup>	119.500	5.41** <sup>c</sup>		
	X3 av. F <sup>-</sup> content	.023	5.96**		
	X5 sheath miner population	.003	4.01**		
Y <sup>2</sup> (1971 needle miner damage)	X0	6.600		.45	.69**
	X3 av. F <sup>-</sup> content	.150	3.28**		
	X4 needle miner population	.070	11.71**		
Y <sup>3</sup> (1971 fluoride damage)	X0	-.530		.15	.39**
	X2 ambient F <sup>-</sup>	692.200	5.92**		
Y <sup>4</sup> (1972 needle miner damage)	X0	6.110		.14	.38**
	X3 av. F <sup>-</sup> content	.170	2.89**		
	X4 needle miner population	.037	4.70**		
Y <sup>5</sup> (1972 fluoride damage)	X0	.250		.04	.20**
	X3 av. F <sup>-</sup> content	.036	3.09**		
Y <sup>6</sup> (sheath miner tortrix damage)	X0	6.250		.23	.47**
	X1 tortrix population	.830	2.55**		
	X3 av. F <sup>-</sup> content	.310	4.97**		
	X4 needle miner population	-.020	-2.18**		
	X5 sheath miner population	.800	4.90**		

<sup>a</sup>

B = partial regression coefficient.

<sup>b</sup> X0 = Y intercept.

<sup>c</sup> \*\* = significance at 0.01 level.

of the regressions were statistically significant at the 0.01 probability level.

Populations of insects are usually quite mobile and difficult to measure. However, the feeding injury caused by an insect remains for a period of time and the amount can be estimated with a reasonable degree of accuracy. This is supported by the data in table 1. Standard errors for 1971 and 1972 needle miner damage, sheath miner and tortrix damage, and percent damaged candles are much less than standard errors for population estimates. For this reason, damage caused by the different insects at Columbia Falls was used dependently in regression analyses even though populations were measured. Insect populations, along with ambient and foliar concentrations of fluoride, were used as independent variables.

Visual rating of pines was significantly affected by ambient fluoride, foliar fluoride, needle miner populations, and sheath miner populations, although this effect was primarily caused by the insects. However, presence of ambient and foliar fluoride in the regression at substantially higher "t" values than computed for insect populations indicates a strong relationship between the insects and fluoride, i. e., the insects preferred trees having moderate fluoride loads. This relationship is supported in that for each of the cases in which insect feeding damage on needles was a dependent variable, foliar fluoride concentration contributed in a positive highly significant way to the regression as indicated by the "t" ratio for partial regression coefficients. For the cases in which fluoride injury was the dependent variable, only atmospheric fluoride and needle concentration and not insect populations contributed significantly to the regression, indicating the tests were definitive.

Consistency of the 1971 and 1972 needle miner regressions is striking. Foliar fluoride concentration and needle miner populations were highly significant and had roughly the same coefficients in both regressions. This consistency was not found in relation to fluoride damage. Fluoride injury to 1971 needles was related to ambient fluoride, but for 1970 needles was related to foliar fluoride. The simple correlation coefficient between ambient and foliar fluoride was 0.67, indicating a high association. Standard errors of fluoride damage estimates, however, were relatively large (table 1), and this variability probably affected the independent variable entering the regressions. Nevertheless, fluoride damage was related to either ambient or foliar fluoride and not to some other variable.

Pine needle sheath miner damage was negatively related to needle miner populations, as indicated by the regression equations. Populations of pine needle sheath miner or sugar pine tortrix could reduce subsequent nee-

dle miner populations, because both of the former destroy current year's needles. During heavy populations of either or both insects, the complete needle complement can be destroyed for several consecutive years, leaving no oviposition or feeding sites for the needle miner. Also, needle miner populations could indirectly affect pine needle sheath miner damage if parasites of the needle miner were also parasitizing the pine needle sheath miner. Sheath miner was about three times as numerous as tortrix, and little feeding damage by tortrix was found during laboratory work. Thus, even though we lumped tortrix and sheath miner damage, most of it must be attributed to sheath miner.

It is recognized that many so-called "indicator" organisms show that traces of toxic substances can cause harmful effects in the environment. Entomological literature, both published and unpublished, during the last half century contains numerous references to suspected or documented associations of airborne toxicants and outbreaks of forest insects (7). In a majority of these outbreaks, toxicants either preconditioned coniferous host trees making them more susceptible to attack of insect pests, or they induced the buildup of epidemic populations of arthropods by killing important invertebrate parasites, predators, or competing species that normally cause these populations to be endemic.

Several outbreaks of forest insects similar in nature to the problem we report have been tied to airborne pollutants in the Western United States and Canada (8, 9, 10, 11) and many species of insects were shown to be secondary in nature, confining their attacks to unhealthy and weakened trees. In 1929, Keen and Evenden (9) studied the effects of either smelter fumes or a combination of smelter fumes and other destructive agents on rather large volumes of timber near Northport, Washington. After examining 1,000 trees, they concluded that it was nearly impossible to separate the importance of insects from other factors that might have contributed to death of the trees. They also found that the two or three previous dry years might have weakened ponderosa pine within the study area making them more susceptible to western pine beetle attacks. This same condition might have precipitated population buildup of various defoliators in their respective hosts. They also found that many Douglas-fir infested with bark beetles were dying principally from *Armillaria mellea*. Keen and Evenden (9) concluded that in lodgepole pine, insects (defoliators, bark beetles, and wood borers) appeared to be entirely secondary although contributing to mortality of many trees which were severely defoliated by fume damage in the vicinity of a smelter at Northport, Washington.

We have demonstrated a strong statistical relationship between industrially emitted fluorides and damage caused by a complex infestation of insects



on lodgepole pine. Mechanisms for this relationship were not studied but research on the effect of different foliar and/or ambient fluoride concentrations on insect feeding habits, fecundity, parasites and predators, or other factors affecting population dynamics is warranted.

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## FLUORIDE AND BONE DISEASE IN UREMIA

by

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**SUMMARY:** Fluoride stimulates osteoblastic activity with formation of poorly mineralized osteoids, inhibits pyrophosphatase activity causing a decrease in bone mineralization; inhibits many bone resorptive pathways; causes parathyroid hyperplasia and excess secretion of parathyroid hormone (PTH) resulting in bone demineralization. The effect of fluoride on bone is influenced by the availability of calcium and Vitamin D. The action of fluoride in uremia and the use of fluoridated water in hemodialysis are reviewed.

Ever since Roholm's (1) description of bony changes due to fluoride, the role of fluoride in bone disease and in healthy subjects has been an area of considerable research and controversy. Fluoride exerts profound effects on bone in various ways. Bony changes of fluorosis, such as increased bone density, increased thickening of cortical bone and periosteum, and exostosis, are found only in about 15% of people who ingest fluoridated water for prolonged periods. Even in people who ingest water the fluoride content of which is high (8 ppm), it has been reported (2) that no hypertrophic bone changes, no unusual incidence of fractures, arthritis, or interference with bone healing were seen. In the presence of renal failure, complex pathophysiological processes such as phosphate retention, depressed calcium concentration, impaired formation of active form of Vitamin D ( $1.25(\text{OH})_2\text{D}_3$ ), excess parathyroid hormone concentration, impaired collagen synthesis, acidosis and other unknown factors all compromise the integrity of bone. Bone lesions of renal osteodystrophy consist of varying degrees of osteoporosis, osteomalacia, osteitis fibrosa cystica and osteosclerosis. These lesions are by no means universally found and several renal failure patients, even on prolonged hemodialysis, exhibit little or no bony changes. Any added significance of fluoride accumulation consequent to renal failure or hemodialysis with fluoridated water, in the genesis or worsening of renal osteodystrophy, is difficult to evaluate.

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This paper will review normal fluoride metabolism and summarize the current views regarding the role of fluoride in uremic bone disease.

Normally, fluoride enters the body through ingestion in food and water although miniscule amounts may be inhaled or absorbed through the skin (3). Over 90% of ingested fluoride is absorbed mainly in the upper gastrointestinal tract but, when it is consumed with large amounts of calcium, phosphate, or other ions with which it forms insoluble salts, absorption can be greatly reduced (4). Total fluoride ingestion is estimated to be 3-5 mgs/day for healthy indoor workers ingesting fluoridated water (5). Absorbed fluoride rapidly leaves the blood stream, and plasma concentration of fluoride is regulated by a combination of skeletal deposition and urinary excretion. In bone, fluoride is deposited in a non-uniform fashion in the apatite mineral. Accumulation of fluoride in this exchangeable compartment is progressive but whether saturation has been reached is difficult to estimate. In the kidney, fluoride is filtered through the glomerulus and approximately 51-63% is reabsorbed in the tubule (6). Normal fluoride clearance is 58 ml/min/1.73M<sup>2</sup> or 51% of creatinine clearance (7). Excretion of the normal dietary load of fluoride becomes increasingly unlikely when the creatinine clearance is below 16 ml/min (8). Sweat losses of fluoride are negligible.

Plasma fluoride concentration is low, approximately  $\mu$  mole/liter (0.02 $\mu$ g/ml) in normal adults (8, 9, 10), rising significantly in uremic patients.

Siddiqui et al. (10) reported serum fluoride concentration of  $0.054 \pm 0.025 \mu\text{g/ml}$  in patients whose serum creatinine concentration was less than 3 mg/100 ml, and  $0.094 \pm 0.01 \mu\text{g/ml}$  in patients whose serum creatinine was above 3 mg/100 ml. Similar findings have been reported by other workers (7, 8) and fluoride clearance in uremics has been reported to be 3.1 ml/min (7). Further elevation of serum fluoride concentration is found in patients with renal failure who undergo hemodialysis with fluoridated water (9, 11, 12), because of net transfer of fluoride from the dialysate to the blood.

The effects of fluoride on bone are numerous, few of which have been proven in man, but the following results are available from animal experiments:

I. Stimulation of osteoblastic activity resulting in formation of poorly mineralized osteoid, simulating osteomalacia (13). Availability of Vitamin D and large doses of calcium result in mineralization of osteoid (14).

II. Altered enzymatic activity such as a) inhibition of pyrophosphatase activity in the presence of excess magnesium resulting in decrease in bone mineralization (15, 16); b) inhibition of citrate metabolism (17) and turnover of bone lipids (18), synthesis of citric acid cycle inhibitors (19), causing alterations in bone resorption.

III. Hormonal effects, primarily induction of secondary hyperparathyroidism due to failure of bone resorption and decrease in ionic calcium (20). This will enhance bone demineralization.

IV. Direct physical effects due to substitution of hydroxyl radicals within hydroxyapatite to form fluorapatite (21).

In presence of normal renal function, these diverse effects of fluoride produce unpredictable bone changes, consisting predominantly of increased bone density, increased thickening of cortical bone and periosteum (2).

#### Fluoride, Renal Failure and Hemodialysis

As mentioned, serum fluoride levels are elevated in the presence of renal insufficiency. Patients in the end stage renal failure, who undergo hemodialysis, are exposed to large amounts of fluoride. Fluoridated water usually contains 1 ppm (1mg/l) of fluoride. Taves et al. (22) were first to note that during regular hemodialysis using fluoridated water, there is "reverse dialysis" of fluoride, resulting in uptake of fluoride by patients from the dialysate. By serial fluoride measurements in arterial and venous blood, they calculated the net transfer of fluoride from the dialysate to the blood to be 3.4  $\mu$ moles/min (23).

Fluoride uptake during the course of hemodialysis was estimated to be 14-20 mg per dialysis by Prosser et al. (24), 30 mgs per dialysis (50  $\mu$ g/min) by Siddiqui et al. (25) and 10+3  $\mu$ moles per dialysis by Nielsen et al. (26). As a result of this influx, the blood fluoride level increases post-dialysis, but returns to pre-dialysis levels before the next dialysis. By administering  $^{18}\text{F}$  intravenously and in the dialysate, and by total body scans, Nielsen et al. have demonstrated that fluoride, retained during hemodialysis is stored almost exclusively in bones (26). Kaye et al. (27) described 3 uremic patients with osteosclerosis in whom the bone fluoride concentration was 2 to 5000 ppm, but the distribution of sclerotic lesions differed from that of classic fluorosis. Posen et al. (28) found a fluoride level in bone as high as 22,700 ppm in dialysis patients. Similar bone fluoride levels are reported by Kin et al. (29), Taves et al. (22), Parsons et al. (30). The question arises does fluoride thus accumulated aggravate renal osteodystrophy or is the prior bone disease responsible for the fluoride accumulation. It is claimed

also that fluorides might be beneficial by stimulating osteoblastic activity and inhibiting bone resorption.

#### Fluoride and Bone Lesions in Uremia

Siddiqui et al. (25) found a significant correlation between elevated serum fluoride levels and severity of bone disease in dialysis patients. The highest fluoride concentration was found in patients with evidence of osteomalacia and osteitis fibrosa with increase in total bone mass and osteoid and decrease in mineralized bone. In a study by Kin et al. (28) bone fluoride content was similar in dialysis patients whether the bone lesion was osteoporosis, osteomalacia, or osteitis fibrosa. Jowsey et al. (31) in their studies with low and high fluoride content of the dialysate, noted disabling bone pain, muscle weakness, and spontaneous fracture in the latter group, which they ascribed to fluorides. Cordy et al. (32) who found an increased incidence of osteomalacia in patients dialyzed with fluoridated water suggested use of non-fluoridated water for the dialysate. In contrast to these studies, Siddiqui (11) and Nielsen (12) were unable to substantiate a role for fluoride in renal osteodystrophy. Oreopoulos et al. (33) in a double blind study concluded that, at least over a two year period, fluoride did not increase the incidence or progression of osteomalacia in dialysis patients. The findings of Posen et al. (34) are consistent with the thesis that fluoride-caused bone disease is preventable. In dialysis patients, they were able to prevent the development and promote healing of prior osteomalacia by employing deionized water. Deionization removes fluorides and possibly other "toxic" factors. From these conflicting reports, the role of fluoride in metabolic bone disease must be judged unsettled.

In summary, during hemodialysis with fluoridated water, there is evidence of uptake of fluoride from water to blood which is deposited in bones. Bone fluoride levels progressively increase with duration of hemodialysis but they cannot be correlated with the nature of bone lesions. At present, no studies confirm the harmful or beneficial effects of fluoride in bone disease of uremia.

Because of known toxic effects of fluoride in animals, and the potential harmful effects of large amounts of fluoride retained during hemodialysis, it is prudent at present to provide dialyzed patients with approximately normal internal milieu. The use of water for hemodialysis which has been treated to remove fluoride and other contaminants, is a sensible precaution at the present time.

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Discussion

Dr. Waldbott: Is there any information to indicate the percentage of fluorosis among kidney patients? Do you consider the bone disease often encountered in advanced kidney disease identical to fluoride osteosclerosis?

Dr. Rao: No, in most cases of renal failure as such, the predominant bone lesions are of the osteotic fibrosis osteomalacia type. Only a minority of patients have osteosclerosis. The main reason for this may be that in renal failure they develop vitamin D deficiency and cannot transport calcium. We are not dealing with normal cases and when calcium is deficient there is an excess of fluoride.

Prof. Jolly: I have one or two comments: First, the kidney is a very important organ in the handling of fluoride which explains why, when all people of a group are exposed to a particular level of fluoride, some develop fluorosis and some do not. Thus, the handling of fluoride clearance varies between individuals. Second, it is true that fluoride in combination with calcium deficiency will not produce osteosclerosis but it will produce diminished bone as has been demonstrated repeatedly. It is difficult to obtain autopsies in India but, of five cases of mine with fluorosis, two had renal disease which may have been responsible for aggravated fluoride toxicity.

Dr. Waldbott: Have you run across calcification of arteries in your work with dialysis?

Dr. Rao: Cases have been reported with fluoride in the arteries up to 8000 ppm. Almost always we find calcification of arteries. The incidence of calcification is very high and fluoride probably plays an important role in this calcification.

Dr. Franke: I believe your explanation on how fluoride affects bone is too simple. You indicated that fluoride causes osteomalacia. In studying industrial fluorosis we found no evidence of osteomalacia but we did find signs of osteomalacia in patients with renal disease. Also, we found signs of osteomalacia in osteoporotic patients treated with high doses (80-100 mg/day) of sodium fluoride. We feel that three factors influence the bone changes namely, the dose of fluoride, kidney function and calcium intake.

Dr. Rao: This is the point I was trying to make to explain why we don't find classical fluorosis in kidney patients. The two critical factors are vitamin D and calcium.

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## BLOOD CLOTTING IN PATIENTS WITH "CHIZZOLA"MACULAE

by

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In previous publications (1, 2) a condition termed "Chizzola Maculae" was described, characterized by round or oval-shaped, brownish, bruise-like skin lesions. First encountered in the village of Chizzola in Northern Italy in proximity to a fluoride-emitting aluminum smelter, they occurred predominantly in children and women. Histologically, the lesions represent a toxic pericapillary infiltration with blood cells which fade spontaneously within about a week but tend to recur in other parts of the body surface, especially the extremities. This dermatosis was also observed in fluoridated communities (3) and was prevented by placing the patient on distilled water for drinking and cooking and avoiding food high in fluoride. Other symptoms of intolerance to fluoride associated with the skin disease also subsided on this management.

Because the above-described lesions resemble suffusions of the kind seen in certain blood dyscrasias, it was of interest to determine whether or not this condition was associated with abnormalities in the blood-clotting mechanism. In eighteen subjects who manifested the maculae the blood studies outlined in Table 1 were carried out. The patients ranged in age from 3 to 38; ten were females; eight, males. All coagulation tests were performed by the Department of Physiology, Wayne State University, Detroit, Michigan, (Professor E. Mammen). As the study progressed several additional tests became available which accounts for the fact that not all patients were given the same number of tests. The two syringe technique was used; one part of a 3.2% sodium citrate was added to nine parts of blood.

According to Table 1, of the 18 persons 7 had no abnormalities in the hemostatic parameters measured; 9 manifested abnormalities in the coagulation parameters and 2 had Von Willebrand disease, an inherited coagulation defect which is characterized by an abnormality of the Factor VIII molecule which also causes a defect in the adhesive properties of the platelets and, clinically, by a tendency to epistaxis and mucosal and cutaneous bleeding. The seven remaining patients had signs of an activation of the fibrinogen levels and prolonged thrombin times. Compared with patients not showing the skin lesions this finding was considered too high to be merely coincidental. However, this evidence by itself does not constitute proof of a direct relationship to fluoride intoxication.

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Presented at the 7th ISFR Conference, Zandvoort, Holland, Feb. 8-10, 1976.

Table 1  
Lab. Findings in 18 Cases with Maculae

	Range	Normal Range	Abnormal Tests and Total Patients Tested
Whole Blood Clotting Time	5/30 to 12 min/sec	6 to 12 min	0 (16)
Bleeding Time	1/30 to 4/10 min/sec	2 to 4 min	2 (11)
Prothrombin Consumption	7 to 38 Units/ml	< 25	2 (14)
Partial Thromboplastin Times	37.4 to 94.5 Sec	50 to 70	5 (18)
Prothrombin Time	9.6 to 16.0 Sec	11.8 to 15.8	6 (18)
Snake Venom Times	9.9 to 18.8 Sec	11.5 to 17.5	1 (18)
Factor V	8 to 36 Units/ml	10 to 42	1 (18)
Factor VIII	46% to 260%	60% to 200%	3 (18)
Factor IX	55% to 125	80% to 120%	3 (18)
Factor VII & X	77% to 175%	70% to 135%	0 (18)
Fibrinogen(clotting)	140 to 331 mg%	200 to 300 mg%	6 (18)
Euglobulin lysis times	32 to 180 min	>180 min.	7 (18)
Thrombin Times	10.5 to 17.9 Sec	11.1 to 15.9	4 (18)
Platelet Count	160,000 to 412,500	200,000 to 350,000	
Platelet Aggregation in Response to ADP	Normal in all but one patient		1 (15)
Clot Retraction	Complete in 60 min. at 37° in all but 4		0 (14)
Prothrombin	178 to 339 Units/ml	250 ± 50	0 (13)
Antithrombin	47% to 62%	40 to 60	1 (8)

Table 2  
Symptomatology in 11 Cases with Abnormal Coagulation

Name	Age	Sex	Asthma	Allergic Nasal Disease	Atopic Dermatitis	Arthritis	Headaches	Gastro-Intestinal Symptoms	Abnormal Coagulation
CC	25	F		X		X	X	X	↓ Fibrinogen ↑ Antithrombin ↓ Euglobulin lysis ↑ Thrombin times
DT*	4	M	X	X					↑ Bleeding time ↓ Factor VIII & IX ↑ P. T. T. ↓ Euglobulin lysis
KW	7	F	X	X	X		X		↑ Prothrombin Time ↓ Euglobulin lysis
PT*	20	M	X						↑ Prothrombin Consumption ↓ Factor VIII Abnormal Platelet Aggregation
JC	5	F	X	X					↑ P. T. T. ↓ Factor IX
KB	8	M		X			X	X	↓ Factor IX
TR	7	M		X		X	X	X	↑ P. T. T. ↓ Euglobulin lysis ↓ Factor VIII & IX ↑ Thrombin Time
SK	28	F		X			X	X	↓ Factor V ↓ Fibrinogen ↓ Euglobulin lysis
RS	3	M	X	X	X				↓ Euglobulin lysis
LK	22	F		X		X	X	X	↑ Thrombin Times ↓ Fibrinogen ↓ Euglobulin lysis
RG	7	F				X	X	X	↓ Fibrinogen

\*Von Willebrand Disease

Since these subjects had additional manifestations of the preskeletal phase of fluoride intoxication it was of interest to relate the severity of their symptoms to the above findings. Table 2 shows the symptomatology of the 9 cases with abnormal coagulation. Whereas no readily apparent relationship between any specific symptom and a particular coagulation defect was observed, 3 out of the 4 patients with multisystem complaints (K.B., T.R., and S.K.) had evidence of activation of the fibrinolytic system with decreased fibrinogen levels of the blood and prolonged euglobulin lysis times.

#### Comment

Only little information is available in the literature on the effect of fluoride on blood clotting. During the 1930's Stuber, in several articles, brought forth evidence that fluoride prolonged coagulation time. He also found a high serum fluoride content in hemophiliacs (0.94%) (4, 5). In acute fluoride intoxication with  $\text{Na}_2\text{SiF}_6$  Heydrich (6) likewise observed considerable delay in coagulation but Shortt et al. (7) in a series of ten cases of chronic skeletal fluorosis reported no change in bleeding and coagulation time.

In his classical study on fluorosis in cryolite workers Roholm (8) recorded an average bleeding time of 1 to 7 minutes; the coagulation time averaged 3.23 minutes. The number of platelets varied from 343,000 to 561,000 with an average of 461,000 platelets. In several cases of preskeletal fluorosis, Waldbott noted high platelet counts which, in one instance, amounted to 1,200,000; however, this observation was not consistent. In 7 of the current cases, the platelet count was above 300,000 with a high of 411,000 which is considered the upper limit of normal.

In connection with this study it should also be noted that Breddin (9) reported reversible inhibitions of platelet function by fluoride. Ten millimoles (190 ppm) of NaF enhanced spreading of platelets suspended in their own plasma whereas higher concentrations inhibited spreading completely, a process which is reversible by washing the poisoned platelets.

The above data suggest that, in the incipient stage of chronic fluoride poisoning, certain abnormalities in blood coagulation occur. Whether or not these changes account for or contribute to the formation of the lesions described as "Chizzola maculae" requires further investigation.

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#### Discussion

Dr. Cook: You spoke of "Chizzola" maculae at previous meetings. Are they bluish or purplish?

Dr. Waldbott: No, they are brown not blue and their color does not change or fade as is the case with traumatic suffusions which have many different shapes and forms.

Prof. Sinclair: May I ask you more about these lesions? You mentioned they are the size of a gold piece. This is a unit with which most of us are unfamiliar. What produces their brown color?

Dr. Waldbott: They are rarely larger than a U.S. quarter, always round or oval. They contain very little pigment. Histologically they show pericapillary extravasation of lymphocytes.

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# THE EFFICACY OF ACETAMIDE IN THE PREVENTION AND TREATMENT OF FLUOROACETAMIDE POISONING IN CHICKENS

by

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**SUMMARY:** The antidotal effect of acetamide (AA) was studied in chickens. For this purpose the acute  $LD_{50}$  of orally administered fluoroacetamide (FAA) was determined in 2 to 3 month old Leghorn chickens and was found to be 4.25 mg/kg. AA was administered orally at dose rates of 0.5g and 2.5 g/kg. Only at the higher dosage rate, did AA prevent the lethal action of FAA (10 mg/kg) when given one hour before, at the same time or 10 or 20 minutes after the administration of FAA. The same dose of AA failed to prevent the death of chickens when given 30 to 60 minutes after the administration of FAA.

FAA ( $F \cdot CH_2CO \cdot NH_2$ ) is an extremely effective chemical for rodent control. It is approximately 1/4 to 1/5 as toxic as sodium fluoroacetate (FAC) (1), yet FAA is one of the most common sources of accidental poisoning in domestic livestock (2, 3, 4) and wild birds (5) under special environmental conditions. The metabolism and mechanism of the toxicity of FAC has been studied thoroughly (6) and repeated efforts have been made to find efficient antidotes for treatment of humans and animals poisoned by it (7, 8, 9, 10). FAC bears a pronounced structural resemblance to acetic acid (acetate) and the two can compete with each other with regard to incorporation in the Krebs cycle.

Acetate donors (ethanol, acetamide, monoacetine), the recommended antidotes in FAC and FAA poisoning, are regarded specific, since they block metabolic formation of poison from the less toxic precursor (11). Unfortunately, the efficacy of these therapeutic agents is limited to treatment of clinical poisoning in humans (11) and experimental animals (1, 10).

An outbreak of FAA poisoning in greyleg geese and teal and possible mass poisoning in other wild birds (4) necessitated the determination of the toxicity of FAA in chicken and attempts were also made to establish the preventive and therapeutic value of one antidote, namely AA in experimental FAC poisoning in the same species.

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Material and Methods1) Determination of acute oral LD<sub>50</sub> of FAA in chickens:

Sixteen 2 to 3 months old male Leghorn chickens were divided into four groups. A single dose of FAA, dissolved in distilled water, was given in each group. The dose rates were 1.5, 3.0, 6.0, and 12.0 mg/kg body weight. The toxicity data were plotted on a dose-response curve (Table 1).

2) Antidote experiments with AA in chickens poisoned with FAA:

(2-3 months old male Leghorn chickens were used)

Twenty-two chickens of the same age, sex, and breed were used and divided in eleven groups. In one control group, FAA was given orally alone in a dose of 10 mg/kg body weight. In a second control group, AA dissolved in H<sub>2</sub>O was administered orally alone in a dose of 2.5 g/kg. In the actual antidote experiments, FAA was given in the same dose as in the control group, whereas AA was administered in two dosage rates: 0.5 and 2.5 g/kg (Table 2).

Table 1

Acute Oral Toxicity of FAA in Chickens (4 in each group)

<u>Dose</u>	<u>Survived</u>
1.5 mg/kg	4
3.0 "	4
6.0 "	1
12.0 "	0

Table 2

Schedule of administration of FAA and AA and results of experiments (2 chickens in each group)

	<u>Dose of FAA</u> <u>(oral)</u>	<u>Dose of AA</u> <u>(oral)</u>	<u>Design of Application</u>	<u>No. of</u> <u>Survival</u>	<u>Remarks</u>
1.	Control	2.5 g/kg		2	No symptoms
2.	10 mg/kg	-		0	Death 7-18 hrs.
3.	"	0.5 g/kg	AA 1 hour before FAA	0	Death after 31 hrs.
4.	"	"	AA and FAA at same time	0	Death after 25-31 hrs.
5.	"	"	AA 1 hour after FAA	0	Death after 5-7 hrs.
6.	"	2.5 g/kg	AA 1 hour before FAA	2	Slight depression
7.	"	"	AA and FAA at same time	2	No symptoms
8.	"	"	AA 10 minutes after FAA	1	One died after 25 hrs.
9.	"	"	AA 20 minutes after FAA	2	No symptoms
10.	"	"	AA 30 minutes after FAA	0	Died after 9-25 hrs.
11.	"	"	AA 1 hour after FAA	0	Died after 5-25 hrs.



### Results

- 1) Determination of acute oral LD<sub>50</sub> of FAA was measured in chickens by increasing the dose by a constant multiple (2 in our case). We had an all or none response (quantal effects) since the two lower doses did not cause any harm whereas the highest dose was lethal for all chickens in the group (Table 1). The acute oral LD<sub>50</sub> was estimated 4.25 mg/kg.
- 2) Antidote experiments: 10 mg/kg of FAA administered orally was found to be lethal in every instance. The animals became slightly restless, their respiration and heart rate increased. Congestion and later cyanosis of the comb and wattles became apparent. The birds became progressively weak and died 7 to 18 hours after the administration of FAA (Exp. 2). No toxic effect resulted from 2.5 g/kg AA administered orally (Exp. 1).

AA in a dose of 0.5 g/kg failed to prevent the lethal action of FAA in the combinations seen in experiments 3, 4, and 5. A dose of 2.5 g/kg AA administered orally prevented the lethal action of FAA when given one hour before FAC (Exp. 6), or simultaneously with FAC (Exp. 7) and not later than 20 minutes after the administration of FAA (Exp. 9). The same dose of AA failed to prevent the death of chickens when given 30 to 60 minutes after the administration of FAA (Exp. 10 & 11). One chicken died and another survived when AA was given 10 minutes after administration of FAA (Exp. 8). These findings indicate that the efficacy of AA in FAA poisoning is dose and time dependent.

### Discussion

FAA is hydrolyzed to FAC in the body. This compound enters the tricarboxylic acid cycle in competition with acetate and is then metabolized to fluorocitrate (FC). FC acts by blocking aconitase, the enzyme responsible for the conversion of citrate to isocitrate.

Acetate itself or acetate donors (ethanol, acetamide, monoacetine) might serve as antidotes by competing with FAC. It was assumed that AA is capable of penetrating into the mitochondria and interfering with the formation, or accumulation, of FC. Since AA is unable to split the FC molecule, its antidotal efficacy in FAA poisoning is indeed dose and time dependent: 0.5 g/kg of AA had no apparent beneficial effect on the course of FAC poisoning, whereas AA in a dose of 2.5 g/kg given one hour before FAC, or together with it, but no later than 30 minutes after the administration of FAA, was able to prevent its lethal action, obviously before the lethal formation or accumulation of FC took place. In the light of our experimental results, it



can be stated that always the dose and time factor should be taken into consideration in prevention and treatment of accidental or experimental FAC or FAA poisoning.

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## ACUTE FLUORIDE POISONING

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(Abstracted from Pediatrics, 58:90-93, 1976)

A 2 1/2 year old colored girl was brought to the emergency room with progressive vomiting and lethargy of about six hours' duration. The respiratory rate was only 6 to 8 breaths per minute. The child had a disconjugate gaze with coarse horizontal nystagmus and muscular fasciculation throughout the body. A soft systolic ejection murmur was audible at the left sternal border.

The child had been playing with a laundry powder called "Rayline Brand Laundry Sour" (manufactured by BASF, Wyandotte Corporation in Michigan) which contained sodium silicofluoride  $\text{Na}_2\text{SiF}_6$  as its major ingredient. Laboratory data showed a BUN of 31 mg/100 ml, 2+ protein and 40 red blood cells/high power field. The plasma sodium was 138 mEq/liter, potassium 6.7, bicarbonate 13 and chloride 107. Serum calcium was 3.4 mg/100 ml, the lowest ever reported in fluoride poisoning. The EKG showed peaked t-waves which were inverted in the chest leads.

After admission the patient developed acute respiratory failure which required assisted ventilation for 48 hours. Repeated episodes of ventricular tachycardia and fibrillation were treated with lidocaine and eight separate courses of direct current cardioversion. The hypocalcemia was treated with three intravenous infusions of 10% calcium chloride (0.3 gms) followed by calcium gluconate and 0.1% calcium hydroxide (lime water) and aluminum hydroxide by nasogastric tube which brought the serum calcium up to 13 mg/100ml. Aspiration pneumonia required penicillin, kanamycin, and dexamethasone. Peritoneal dialysis was instituted with a calcium concentration of 10 mg/100 ml and continued for 48 hours.

The patient became responsive 18 hours after admission and returned to full consciousness two days later. There were no mucosal burns or ulcerations and the upper gastrointestinal examination was normal.

The serum fluoride levels were extremely high (14 mg/liter) but dropped to 1.8 mg/liter after 11 hours and to 0.1 mg/liter 21 hours after the ingestion of the poison. Urinary fluoride excretion amounted to 24.8 mg over the first three days. The average fluoride clearance was 98 ml/min/1.73 sq m. In view of the fact serum levels above 3 mg/liter had been fatal in other cases, the authors attributed the improvement mainly to

the gastric lavage with calcium salts and to the maintenance of a urinary output. Peritoneal dialysis resulted in no significant removal of fluoride. The fluoride level of the effluent was less than the fluoride level of the bottled dialysate, which had evidently been prepared from fluoridated water.

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#### FLUORIDE TOOTHPASTE: A CAUSE OF PERIORAL DERMATITIS

by

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(Abstracted from Arch. of Dermatol. 112:730-731, 1976)

The authors reported two cases of perioral dermatitis which failed to respond to therapy until the use of fluoridated toothpaste was discontinued.

In the first patient, a 63 year old woman, the skin lesions which appeared in 1971 had persisted for 24 months and had been refractory to treatment with cortisone, coal tar, diiodohydroxyquin and tetracycline. When it was ascertained that the onset of the lesions coincided with the use of a fluorinated toothpaste she was instructed to substitute a nonfluorinated dentifrice and to continue the cortisone, coal tar and diiodohydroxyquin cream. The dermatitis cleared up within 2 weeks. Three months later, resumption of use of the fluoride dentifrice induced a prompt recurrence of the lesion. Upon changing to a nonfluoride toothpaste the condition again subsided and for 16 months she has remained free of the lesions without treatment merely by avoiding fluoride toothpaste.

The authors observed a similar case in a 20 year old woman in July 1974 who had been using a fluoridated toothpaste for "several years". Here too, the lesions cleared promptly after substituting a nonfluoride toothpaste and recurred in April 1975 when she resumed use of a fluoridated toothpaste.

The authors quoted Stone and Willis (Toxicol. Appl. Pharmacol. 13:322-338, 1968) who reported positive reactions when they patchtested traumatized skin with sodium fluoride and stannous fluoride in concentrations equal to those in dentifrices; patchtesting over non-traumatized skin gave no reaction. The authors concluded: "In this setting, we feel fluoride dentifrices may act topically as a pro-inflammatory agent potentiating and perpetuating a chronic perioral inflammatory dermatosis."

The intensity of the glycolysis among the cancerous animals increased considerably but fluoride in doses of 1.5 and 5 ppm inhibited completely the increase of glycolysis. These doses, however, normalized the activity of phosphofructokinase which increased as the cancer progressed. Also, hexokinase activity increased in the animals receiving 1.5 and 5 mg fluoride. There was no change in the activity of pyruvokinase as the cancer advanced and no effect of fluoride was observed.

In the liver of the rats which received no fluoride the activity of enolase increased with the development of cancer. Also, in the non-cancerous control animals, increasing fluoride supplements in food led to a rise in enolase activity. With respect to phosphoglucomutase, the level of activity in cancerous rats did not change but the large doses of fluoride inhibited the activity of this enzyme. Higher fluoride levels in the food favored enhancement of glucosophosphatisomerase activity in the cancerous animals.

The authors concluded that there was a negative correlation between glycolysis and phosphofructokinase in the development of cancer in the "non-fluoride" rats; with the addition of fluoride this correlation disappeared.

The long-term administration of 1.5 mg F/kg induced less distinct, macroscopically visible tumors in the liver than smaller doses or complete absence of fluoride. The rate of glycolysis in the liver of rats increases with the development of cancer. A correlation between the cancer retarding action of sodium fluoride upon glycolysis and the alteration of the phosphofructokinase activity was observed.

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J. F.

## FLUORIDE TOOTHPASTE: A CAUSE OF ACNE-LIKE ERUPTIONS

by

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Virginia Beach, Virginia

(Abstracted from Arch. of Dermatology 111:793, 1975)

The author encountered a skin disease in approximately 65 women ranging in age from the early 20's to the early 40's, characterized by comedonal or papular acne. The lesions extended in a slightly fan-like distribution from the corner of the mouth to the chin and the proximal area of the cheek.

He had instituted the standard acne therapy such as special washing agents, dietary control, tetracycline at varying dosages, and a variety of lotions of different strengths. He had also instructed his patients to avoid lipsticks and cosmetics for long periods of time to no avail.

Aware that fluoridated steroids applied to the face of women resulted in erythema-type eruptions resembling acne, also that industrial halogen fumes cause acne-like eruptions, he requested the patients to switch, on a trial basis, from fluoride toothpaste to one without fluoride. Within two to four weeks, in approximately one-half of the patients the previously persistent lesions cleared up. The other 50% tended to persist without change. When the remaining patients switched from their present dentifrice which contained "brightening and flavoring agents and other unknown chemicals", to baking soda and a commercially available mouthwash (Scope) after brushing, nearly all improved and an almost complete clearing of the eruptions ensued.

When several patients at their own request resumed the use of the fluoride toothpaste everyone, without exception, experienced a recurrence of the former lesions with the same distribution around the mouth.

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#### EVIDENCE FOR A LACK OF AN EFFECT OF DIETARY FLUORIDE LEVEL ON REPRODUCTION IN MICE

by

S. Tao and J.W. Suttie  
Madison, Wisconsin

(Abstracted from J. Nutr., 106:1115-1122, 1976)

In response to reports by Schwartz and Milne and Messer et al. that fluoride is an essential nutrient, these authors undertook a set of experiments to determine the essentiality of fluoride, especially as it relates to reproduction.

Webster strain mice were fed the same basal diet as that used by Messer et al., low in fluoride (0.2-0.5 ppm) for controls, +2 ppm fluoride and +100 ppm for experimental animals.

No significant difference in growth rate, reproductive response, or litter size was observed. The ash content of femurs was not influenced by fluoride treatment. The plasma fluoride concentration showed

FLUORIDE

a significant increase in mice fed the 100 ppm supplemental diet.

Except for the finding that the 100 ppm supplemental group had slightly lower copper values in liver and kidneys, no significant differences between the three groups involving copper and iron concentrations or hematocrit could be observed.

In conclusion the authors state: "Although fluoride may yet be shown to be essential for some physiological process, sound evidence for a claim of essentiality of fluoride for reproduction is still lacking."

\*\*\*\*\*

J. Y.

#### THE EFFECT OF LONG-TERM ADMINISTRATION OF FLUORINE WITH FOOD ON THE CARCINOGENESIS AND BIOCHEMICAL CHANGES IN THE LIVER OF RATS

by

Gorban', G. P., M. B. Pliss, E. D. Karpilovakaja, A. S. Petrun',  
V. I. Svatkov and B. L. Rubencik  
Moskva, USSR

(Abstracted from Voprosy pitaniya, Moskva, 6:21-24, 1975)

The authors carried out two kinds of experiments on 370 mixed breed male rats which received a special fluoride-free nutrient mixture and distilled water.

In the first experimental series, 170 animals were studied for the effect of varying doses of sodium fluoride upon the appearance of macroscopically visible liver tumors induced by p-dimethylaminoazobenzol (DAB). The animals were divided into three groups: Group I received no fluoride in the diet, Group II, 0.1 mg/kg body weight and Group III, 1.5. After six weeks half of the animals in each group received 0.06% DAB daily per ration. Normally, without fluoride in the diet, within 4 to 5 months cancer in the liver was visible macroscopically.

The second series of 200 rats was divided into 4 groups which received 0, 0.1, 0.5 and 5 mg/kg of fluoride with their daily ration. The animals were sacrificed after consuming the DAB for three months and the biochemical changes in the liver preceding the tumor formation were studied.



The intensity of the glycolysis was evaluated by the increase in lactic acid according to the method of Hohorst. In addition, the activity of the following enzymes was determined: phosphofructokinase, hexokinase, pyruvatokinase, glucosophosphatisomerase, phosphoglukomutase and enolase.

Results: Eighty-five percent of the rats which received no fluoride developed liver tumors (Group I); in the second group (0.1 mg F<sup>-</sup>) the percentage was 77 and in the third group (1.5 mg F<sup>-</sup>), 51. The difference between the first and third groups was significant ( $P < 0.01$ ).

\*\*\*\*\*

J. F.

#### PROPHYLACTIC FLUORIDE TREATMENT AND AGED BONES

by

J. Inkovaara, R. Heikinheimo, K. Jarvinen, U. Kasurinen, H. Hanhijarvi and E. Iisalo  
Tampere, Finland

(Abstracted from the Brit. Med. J. 3:73-74, 1975)

In view of the fact that osteoporosis has been shown to be less frequent in areas with a high fluoride content in water, the authors investigated whether fluoride prophylaxis slows down the development of osteoporosis in the aged.

They selected two groups of patients, over 65 years of age in the Koukkuniemi municipal home for the aged in Tampere, Finland for double-blind tests: One group received a capsule of sodium monofluorophosphate, corresponding to 25 mg fluorine per day, the other 30 mg sodium bicarbonate as a placebo. The capsules were distinguished only by color and the contents were known solely to the manufacturer. The fluoride group comprised 237 patients including 41 men, aged 65-95, with an average age of 78.5; the controls numbered 223 (58 men), aged 65-94 with an average of 78.4. Patients with known osteoporosis were included and their former treatment continued. Random samples were taken from each group, comprising 10 normally mobile, 10 poorly mobile, and 10 moving only with assistance. X-ray films were taken of the thoracic and lumbar spine at the start of treatment and again after six months. When bone fractures occurred, the dose of fluoride was lowered to 25 mg twice a week. The free fluoride content of the plasma was measured in 21 patients on fluo-

ride and in 25 controls. The blood samples were taken while fasting, 24 hours after the last dose of fluoride. The data were analyzed statistically by means of the Student's t test and  $\chi^2$ -test.

**Results:** In the fluoride group treatment had to be discontinued more frequently than in the control because of "abdominal discomfort". The height and weight of the patients showed no essential differences between the two groups but the "fluoride patients" lost 0.9 kg, whereas the control group gained 0.7 kg ( $P < 0.01$ ). The rate of admission to the hospital was larger in the fluoride group. In six cases of this group and in one of the controls arthrosis became aggravated. Fractures occurred in 11 "fluoride cases" compared to 6 of the controls. After the treatment was terminated, three additional "fluoride cases" developed fractures but none did in the control group. X-rays of the spine showed no differences in changes in the bone calcification between the two groups, nor was osteosclerosis encountered in any case.

In five of the fluoride-treated patients the dose of 25 mg per day led to concentration of ionized fluoride in plasma which averaged  $9.8 \pm 1.6$  mol/l. When this dose was lowered to twice weekly the fluoride levels diminished in two months to  $3.3 \pm 0.04$  mol/l in 21 patients. Even two months after the termination of the treatment the plasma ionized fluoride content was  $1.8 \pm 0.31$  mol/l and had not yet reached the level of the controls, namely  $0.80 \pm 0.02$  mol/l. Spinal fractures occurred when patients showed 2.1 to 5.0 mol/l fluoride. A cross-over comparison of absorption and excretion of sodium fluoride and sodium monofluorophosphate with respect to plasma and urinary fluoride revealed no difference between the two products in four patients.

The authors felt that the fluoride treatment was, "probably partly responsible for the fractures in our cases". They did not consider adding calcium and vitamin D to the treatment as suggested by Jowsey et al, because patients were drinking an average of one liter of milk per day and, therefore, were receiving more calcium than the recommended 600 mg per day.

The authors considered the 25 mg fluoride ion per day used in their study too high. The occurrence of numerous fractures in the group receiving fluoride contraindicates prophylactic administration of fluoride in geriatric patients, unless it is possible to observe the plasma fluoride content and to ascertain that it remains within the desirable level of 3 mol/l at the most.

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**CORRECTION:** The article by J.A. Cooke, 9:204-212, 1976 contains several printing errors which are herewith corrected.

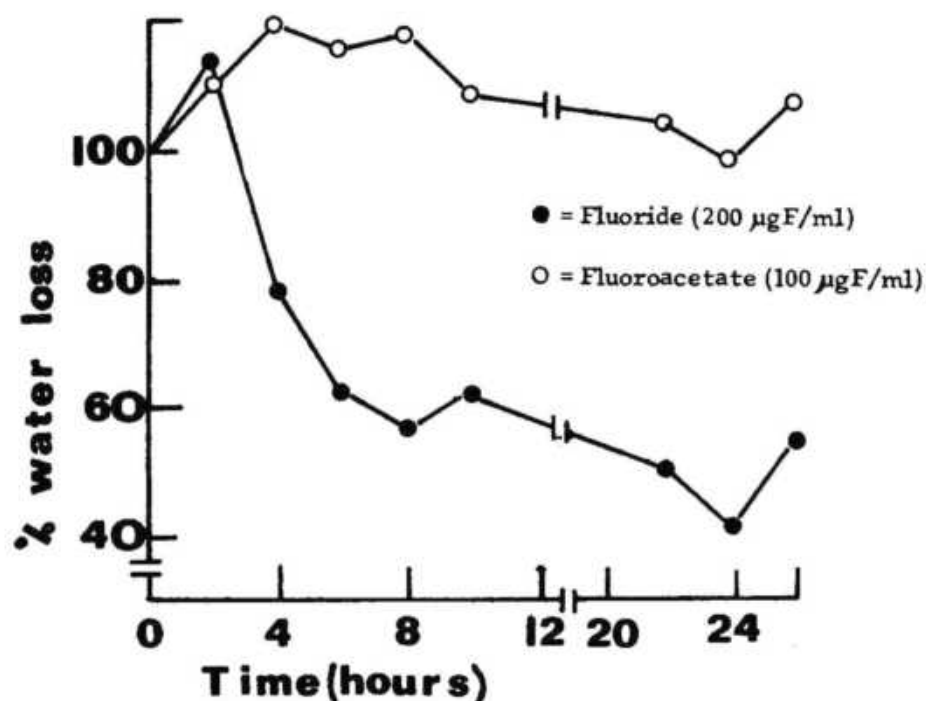
On Page 207, after line 4, the following sentence should be added, "As well as these marked effects on growth, particularly root growth, fluoroacetate reduced the fresh weight/dry weight ratio (Figure 5)".

On the last line of the text on the same page (207) Figure 5 should read Figure 6.

On page 208, line 13, Figure 6 refers to a Figure which was omitted. It is herewith reproduced and designated 6a.

Figure 6a

Water loss ( $\text{g}/\text{m}^2/\text{hr}$ ) expressed as a % of the control of Glycine max treated with fluoride and fluoroacetate



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