October 1969 Vol. Two No. Four

FLUORIDE

QUARTERLY REPORTS

OFFICIAL JOURNAL OF

INTERNATIONAL

SOCIETY for

FLUORIDE

RESEARCH

Vol. Two No. Four October, 1969 Pages 192 - 249

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Issued by

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Editor
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Detroit, Michigan

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ABSTRACTS

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Vienna, Austria

March 22 to 25, 1970

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EDITORIAL

FLUORIDE AND THE THYROID GLAND

In 1854 Maumené (1) administered to a dog from 20 to 120 mg sodium fluoride daily for four months. He observed an enlargement of the neck which he interpreted as a goiter. Ever since then conflicting data concerning the action of fluoride on the thyroid gland have appeared in the medical literature. In the early part of this century several authors concurred that fluoride intake may contribute to the production of goiter. This concept led to the administration of sodium fluoride for the control of hyperthyroidism on a large scale, particularly in Germany, Switzerland and Argentina. Gorlitzer (2) even advocated baths in a dilute HF solution as a treatment for toxic goiter. Subsequently numerous papers favoring and denying a significant goitrogenic effect to fluoride have appeared.

The current issue of "Fluoride" contains two articles bearing on this subject. Both originated in countries where fluorosis is endemic. Siddiqui's observations were made in India and those of Fradà et al., in an area north of Rome.

Although Siddiqui's survey was carried out on a small population, it is well controlled and the results are noteworthy. In the group studied between the ages of 14 and 17 he established a direct relationship between the incidence of small, visible goiter and increasing concentration of fluoride in drinking water. Furthermore, his data suggests the possibility of an inverse relationship between the incidence of type b goiter and increasing concentration of iodine.

As pointed out by Baumann and Metzger (3), the affinity of the thyroid for halides leads to competition by the thyroid for fluoride and iodide ions. A level of fluoride which may produce physiological changes in the thyroid gland at one level of iodine intake, will not do so at a higher iodine intake.

Galletti et al. (4) observed that fluorine did not impair the capacity of the thyroid to synthesize the thyroid hormone when there was an abundance of iodine in the blood. However, when the total iodine pool was low, there was inhibition to the thyroidal concentration capacity. One of his patients was promptly cured of hyperthyroidism, with return of laboratory data to normal, following four months of fluoride therapy; but during this interval he developed a nodular goiter. Treatment with 100 to 120 µg of iodide resulted in a spectacular relapse with disappearance of the goiter. When the hyperthyroidism was subsequently controlled with fluorides, the goiter reappeared.

In Siddiqui's study, goiter occurred exclusively during the mid-teen years when growth and metabolic activity are high; furthermore, females were affected much more frequently than males.

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The calcium content of the water supply in this study was randomly distributed except for the one high value of 7.99 mg/100 ml in one of the wells of Kamaguda. In any study attempting to correlate the fluoride level of water supplies with physiological changes the calcium level in water may be important because increasing calcium concentrations may decrease the effectiveness of fluorides (5).

Siddiqui observed that water containing approximately 5 mg of fluoride per liter will produce small goiters, a gross abnormality. The question arises as to how much more frequently such levels of fluoride produce subtle physiological changes. Such disturbances cannot be ruled out by the absence of macroscopic and microscopic changes in the thyroid in persons exposed to minute amounts of fluorides.

Fradà and co-workers studied the effect of fluoride ingestion on thyroid physiology in 52 patients chosen from 400 with signs of dental, skeletal or visceral fluorosis. The authors, employing radioisotope techniques, observed no significant differences in the experimental and control group. These findings contradict many reports and agree with others. Galetti's series in which he revealed a suppression of the initial and maximum radioactive iodine thyroid uptakes with 5 mg of fluoride, was composed wholly of patients with hyperthyroidism. Other parameters have been operative but not controlled in the studies.

The following points explain negative findings in the presence of possible subtle changes:

1. Whereas the radioactive uptake test is effective in differentiating between the hyperthyroid and the euthyroid patient, it is not reliable in distinguishing between hypothyroidism and euthyroidism. Since fluoride is expected to depress thyroid uptake, the radioactive iodine uptake test is not very discriminating. The wide range of normals (15-40%) for this test illustrates the difficulty encountered in its application for the detection of minor abnormalities. Obviously large control and test groups would be necessary to demonstrate any statistically significant depressing effect of fluorides unless such a defect were marked.

2. Most laboratories have abandoned the 24 hour conversion ratio in evaluating thyroid function. Instead, the serum radioactivity is determined 72 hours after ingestion of radioactive iodine. This time lapse permits the unbound ^{131}Iodine to be excreted by the kidneys.

3. Thyroid scans correlated with careful palpation of the thyroid gland provide some impression as to the gland size. Yet this method is not very accurate. Some workers have injected air into the fascial planes in order to delineate the gland upon AP and lateral X-ray views of the neck. When a lateral scan of the thyroid is added to the anterior scan, the estimation of size can be made more accurately.

4. The average value of the 24 hour thyroid uptake appeared to be unusually high in the paper by Fradà et al. For comparison, I reviewed the 24 hour uptake in the last 50 cases performed in our laboratory. The possibility of bias for low values was eliminated by omitting all studies on patients with a suspected diagnosis of hypothyroidism and of cancer which is frequently associated with low values. Cases with suspected hyperthyroidism were not eliminated. The average uptake in the remaining 40 patients was 25.5%, compared with 39% for 48 subjects reported in the Italian study (four high readings in an obviously toxic range were eliminated). It is apparent that either our group of patients has a lower iodine uptake than Fradà's or the techniques of the tests in the two laboratories must be significantly different. Possibly the Italian technique includes a wider area of the neck and therefore more radioactivity is counted within the blood pool. This may also account for the differences in the short interval readings. All of their 8 hour readings were above 20% whereas the upper limits of normal for our 6 hour uptake is 10%. The differences in these two papers exemplify the conflicting literature during the past century. Although many agree with Hein et al. (6) who concluded on the basis of radioactive fluoride in rats that the thyroid selectivity concentrates fluoride from dilute solutions. Yet it is doubtful that such concentrations cause microscopic, macroscopic or physiological changes.

Nonetheless, the literature does permit some generalizations: 1) Fluoride enters the thyroid gland and under certain conditions suppresses the production of thyroxine. 2) The conflicting data suggest the operation of additional parameters which have not been controlled. 3) The more active the thyroid gland, the more sensitive it is to the effects of fluoride.

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THYROID FUNCTION IN ENDEMIC HYDROFLUOROSIS IN SICILY

by

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As a follow-up of previous observations on hydrofluorosis, a disease endemic in several areas of Sicily, and supplementing research started in 1953, we investigated the function of the thyroid gland in subjects with hydrofluorosis.

The existence of a goiter in dogs, sheep and cattle, affected by fluorosis of industrial origin and of thyroid hypertrophy often encountered in laboratory animals which are treated with fluoride compounds, has given rise to the goitrogenic theory of fluoride and to the conclusion that iodine and fluoride are antagonistic.

The literature on this question is contradictory: Goldemberg (1) had given to rats daily doses of 2-3 mg of sodium fluoride. In all animals he encountered hyperplasia and hypertrophy of the gland, degenerative lesions of blood vessels, emaciation, developmental and mental retardation, which he defined as "Fluoride Cretinism."

Christiani (2) treated calves with sodium fluoride in the same manner as Goldemberg. The thyroid gland exhibited more extensive anatomical lesions than those encountered by Goldemberg who treated only a small number of animals.

Slaviero (3) encountered an increase of colloidal substance of the thyroid in dogs to which sodium fluoride had been administered.

Goldemberg (4) also demonstrated a consistent decrease in basal metabolism in rats treated with a single, large but non-fatal, dose of sodium fluoride. These results were much less evident in chronic intoxication of animals. On the basis of additional experiments on mice, Goldemberg produced evidence that fluorotic cachexia should be considered a manifestation of induced fluoride cretinism and that the changes in thyroid tissues represent experimental goiter.

Goldemberg considered the possibility that in goiter areas, fluoride in water, in soil and in certain foods constitutes the true cause of goiter and that iodine deficiency only accentuates the effect of fluoride, without being its sole cause.

From the Institutes of Industrial Medicine and of Applied Nuclear Physics, University of Palermo, Italy.

Phillips (5) noted an increase in metabolic rate in rats treated with sodium fluoride. This increase is more pronounced when dry thyroid substance is added to the animal ration. Goldemberg and May (6) reported satisfactory results upon administering sodium fluoride in hyperthyroidism.

On the assumption of a fluoride-iodine antagonism and considering the possible therapeutic use of fluoride in thyroid toxicosis, some have prepared fluorinated derivatives of tyrosine analogs of the same amino-acid (mono and di-iodotyrosine). May (6) obtained good therapeutic results in hyperthyroid subjects with 3-fluorotyrosine, and with the 3-fluorohydrobenzoic acid.

Roche (7) confirmed that substitution of one or two atoms of iodine by one or two atoms of fluorine (3-fluoro-3, 5, 5-tyrosine and 3 fluoro-3, 5, 5-tri-iodotyrosine and 3, 5-di-iodo-3', 5'-difluorotyrosine) attained a thyroxin effect whereas 3, 5-fluorotyrosine is completely ineffective.

Anbar (8) demonstrated that the accumulation of fluoroborate in the thyroid gland is superimposed upon iodide ions. Fluoroborate ions do not become organically fixed in the thyroid, and their presence in the gland establishes a specific index of the phase of thyroid uptake. The same author, utilizing fluoroborate ions labeled $^{18}F^-$, demonstrated that thyroid stimulating hormone (TSH) inhibits the accumulation of fluoroborate during the first hour after its administration whereas after 24 hours it increases fluoroborate accumulation.

In the U. S. A. and England endemic goiter, due to lack of iodine, occurs independently of fluoride content of water. Demole (9) asserts that in Switzerland administration of iodine alone eliminated goiter where the fluoride level in water ranged between 1 and 1. 4 ppm and where the population was exposed to industrial pollution by F^-. Daily administration of 1 mg per day of sodium fluoride to students in an endemic goiter area failed to induce thyroid hypertrophy (10).

Present Study

Our investigation was conducted on the population of Acquaviva Platani, in the province of Caltanisetta, a town about 625 meters above sea-level with a rather humid climate. The area is somewhat depressed economically. The 2, 500 inhabitants make their living from agriculture and from mining rock salt which abounds in the area. The nutrition is poor. Because of the scarcity of protein, especially animal protein, a vegetarian and cereal diet prevails. Drinking water is obtained from a public spring which contains 5. 2 ppm fluoride.

We examined 400 subjects. They were free of diseases which could interfere with the results of our investigation. All adult subjects presented typical evidence of dental and skeletal fluorosis of various degrees.

Signs of early aging, features of hypoadrenalism and, not infrequently, evidence of precocious arteriosclerosis were noted. In children evidence of somatic underdevelopment with signs of rachitis was usual, presumably attributable to a deficiency in protein and vitamins. Infantile morbidity was not different from that of other communities of central Sicily. Most adults exhibited degenerative diseases of the joints and of digestive apparatus, namely dyspepsias due largely to chronic catarrhal gastritis and gastroduodenitis accompanied by spastic colitis and by signs of hepatic insufficiency. These conditions must in part be attributed to the action of fluoride. In women data concerning the menstrual cycle, fertility, the course of pregnancy were unremarkable. The incidence of intercurrent diseases was also insignificant. We frequently encountered hypochromic microcytic anemia with normal blood corpuscle-resistance and a deficiency of erythrocyte-forming activity of the bone marrow. Histoplasmocytosis was of some importance. Frequently an increase of the alkaline phosphatase and of inorganic phosphates in the serum, hypocalcemia and hypercalcinuria were found.

In the subjects whom we examined the incidence of goiter was rare, namely about 2%. This percentage is close to the average in any nearby non-fluoride area. A former survey of another center of hydrofluorosis in Sicily disclosed an incidence of goiter between 2% and 2.7%.

Among the 400 subjects, natives of Acquaviva Platani, a group of 52 (Table 1) was selected, ranging in ages from 20 to 60 (39 males and 13 females) for investigation of the thyroid function with radioactive iodine. Two of the 52 were afflicted with a diffuse parenchymatous goiter of moderate size with clinical signs of a slight hyperfunction. In another case a clinically irrelevant increase of the thyroid function but no appreciable morphological changes of the gland were noted.

Our studies were carried out in our mobile unit. The apparatus used was manufactured by the Chicago Nuclear Corporation. It consisted of a group of scintillation probes, one scanner and a deep well counter with analyzer.

All subjects had been fasting since the preceding night. They were given a single oral dose of 50 microcuries of ^{131}I in the form of sodium iodide. The ^{131}I thyroid area was counted at 1, 2, 8 and 24 hours after administration of ^{131}I. Blood samples were taken from the brachial vein after 24 hours for determination of the conversion index (PBI). This was another approach in order to differentiate the high values of fixation due to actual hyperfunction of the thyroid from those that could be indictive of increased uptake without simultaneous hormone hypersecretion.

The results of our investigation as illustrated in the accompanying table can be summarized as follows:

TABLE 1

^{131}I Data on 52 Selected Subjects

Number	Age	Sex	Occupation	^{131}I Uptake in % After				Conversion (PBI) in 24 hrs. in %
				1 hr.	2 hrs.	8 hrs.	24 hrs.	
1	54	M	Miner	12	16	24	39	28
2	27	M	Farmer	8	13	22	32	25
3	27	F	Housewife	13	18	29	50	33
4	30	F	Housewife	35	42	78	92	84
5	60	M	Miner	10	12	20	31	26
6	45	M	Farmer	15	35	50	58	32
7	37	M	Farmer	15	40	55	60	40
8	38	M	Farmer	12	16	38	50	38
9	29	M	Farmer	12	15	22	39	40
10	23	M	Farmer	14	18	28	40	28
11	50	M	Miner	10	13	24	32	32
12	57	M	Sailor	13	25	50	60	39
13	41	M	Miner	16	30	55	60	43
14	53	M	Miner	20	30	60	70	38
15	46	M	Miner	13	18	22	39	25
16	60	M	Miner	12	14	22	29	25
17	60	F	Housewife	12	15	26	40	35
18	46	M	Farmer	19	22	25	30	32
19	31	M	Farmer	11	12	20	31	28
20	48	M	Farmer	14	18	28	48	40
21	57	M	Farmer	15	18	26	42	42
22	21	M	Farmer	12	15	28	38	33
23	48	M	Farmer	16	18	31	41	34
24	47	M	Farmer	14	18	31	36	33
25	44	M	Farmer	30	41	75	89	78
26	44	F	Housewife	18	21	36	41	39
27	51	F	Housewife	10	13	24	30	33
28	40	F	Housewife	13	16	30	37	31
29	27	M	Miner	16	20	36	46	39
30	27	F	Housewife	15	20	36	43	39
31	32	F	Housewife	11	18	26	31	35
32	55	F	Housewife	13	25	34	40	42
33	59	F	Farmer	10	14	28	40	32
34	58	M	Miner	19	22	39	43	38
35	26	F	Housewife	12	13	22	32	38
36	37	F	Housewife	12	16	31	45	40
37	36	M	Farmer	10	12	21	26	31
38	30	F	Farmer	15	16	25	36	40
39	60	M	Miner	15	18	29	40	39
40	28	M	Miner	16	20	31	46	40
41	52	M	Farmer	14	17	25	38	31
42	60	M	Miner	10	13	21	31	36
43	24	M	Farmer	14	18	25	39	36
44	28	M	Miner	14	20	30	39	30
45	40	M	Farmer	18	23	33	41	40
46	20	M	Farmer	10	12	18	30	36
47	52	M	Farmer	15	21	33	45	39
48	60	M	Miner	13	18	29	39	42
49	45	M	Farmer	13	19	33	40	34
50	40	M	Farmer	16	19	28	38	36
51	58	M	Miner	35	48	90	90	80
52	49	M	Miner	18	22	31	43	36

1. In 44 individuals or 84.6% of the cases examined, the percentage of uptake and the value of reported conversion (PBI) in 24 hours was maintained within normal limits from 15 to 50%.

2. In five cases or 10.4% of our cases (6, 7, 12, 13 and 14), the uptake was slightly above normal limits. In one case there was an uptake up to 70%. The reported conversion was within normal limits, varying between 32 and 43%. In these persons there was no clinical evidence of impairment of thyroid function and their PBI levels were within normal limits. In spite of the increased uptake, they could be considered euthyroid.

3. In only 3 cases (4, 25 and 51) or 4.99%, which included two patients with a goitre and one with signs of hyperthyroidism without appreciable morphological alteration of the gland, the percentage of external uptake was above normal. In one case an increase up to 92% was noted. This value was found to correspond with the reported index of conversion (PBI) which amounted to about 80% in 24 hours.

The clinical-statistical and laboratory observations reported here, revealed that the incidence of goiter in the hydrofluorotic area which we investigated was not higher than that encountered in a neighboring nonfluorotic zone. No evidence of hyperfunction was observed in patients with goiter.

The high percentage of euthyroid cases in our hydrofluorotic subjects (up to 95%) indicates that fluoride has no apparent effect on the thyroid function or on the size of the thyroid gland.

Summary

In pursuit of former observations on hydrofluorosis the authors conducted an investigation concerning the incidence of goiter and the function of the thyroid gland in subjects with hydrofluorosis from Aquaviva Platani, a large endemic center of Sicily. To evaluate thyroid function, radio-iodine uptake and the conversion index (PBI) with ^{131}I were adopted. The incidence of goiter was not higher in endemic centers of hydrofluorosis than in nonfluorotic areas. Endemic goiter in hydrofluorotic areas is not attributable to the action of fluoride

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INCIDENCE OF SIMPLE GOITER IN AREAS OF ENDEMIC FLUOROSIS IN NALGONDA DISTRICT, ANDHRA PRADESH, INDIA

by

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For many years, considerable interest has centered upon the metabolic interrelationship of two halogens, fluorine and iodine, and their effect on the thyroid gland. Several studies have been reported concerning the influence of fluorine compounds on the size of the thyroid gland. The reports are conflicting (1-9). It was therefore considered desirable to examine in detail the possible relationship between simple goiter and fluorosis as found in an endemic fluorosis belt in India.

Investigation

The villages of Kamaguda, Yedvelli and Yellareddyguda in Nalgonda District, Andhra Pradesh were selected for the investigation. These villages lie close to each other and are known areas of endemic fluorosis, as indicated in a detailed account published earlier (10). The climate is hot and the temperature in the shade reaches 115° F (46.1°C) in summer. The inhabitants are manual laborers working on tobacco plantations.

From the Osmania Medical College and Osmania General Hospital.

TABLE 1

Thyroid Enlargement Related to Halogen and Mineral Content of Water Supply

Village (Population)	Water Quality								Age (Years)					Incidence of Visible Goiter				
	Fluorine		Iodine															
	(ppm)	Mean	µg/l	Mean	F⁻/I⁻ Ratio	Total Hardness (mg $CaCO_3$/100 ml)	Total Calcium (mg/100 ml)	Total Magnesium (mg/100 ml)	Group 1 (0-13)	Group 2 (14-17) M	Group 2 (14-17) F	Group 2 (14-17) Total	Group 3 (18 and over)	Group 1	Group 2 M	Group 2 F	Group 2 Total (%)	Group 3
Kamaguda (95)	9.6	10.7	18.4	14.4	1.3	19.95	7.99	Negligible	53	4	6	10	32	-	1	3	4(40)	-
	11.8		10.4			8.21	2.40	0.54										
Yedvelli (813)	5.5	6.1	37.4	44.0	7.5	14.68	4.08	1.09	435	82	113	195	183	-	4	20	24(12)	-
	5.8		39.2			22.61	3.07	3.63										
	6.0		41.4			6.07	2.43	Negligible										
	6.2		42.5			6.97	2.79	Negligible										
	6.6		50.1			13.38	2.72	1.60										
	6.8		53.7			23.27	4.72	0.36										
Yellareddy-guda (1100)	2.5	5.4	222.6	175.3	32.4	5.97	2.39	Negligible	573	135	94	229	298	-	4	17	21(9)	
	3.8		198.2			28.60	5.85	3.40										
	4.2		118.4			11.24	4.50	Negligible										
	5.5		248.5			7.17	2.87	Negligible										
	6.0		174.8			2.67	1.07	Negligible										
	6.5		160.4			5.89	2.36	Negligible										
	7.5		104.2			2.75	1.10	Negligible										

The populations of Kamaguda, Yedvelli and Yellareddyguda are 95, 813 and 1100, respectively. The three communities have two, thirteen and eighty-four wells, respectively. Fifteen samples of drinking water consumed by the inhabitants were analyzed for their content of fluorine, iodine, total hardness, total calcium and total magnesium contents - two from Kamaguda, six from Yedvelli and seven from Yellareddyguda (Table 1). Determination of fluorine was made by the thorium nitrate titration method and of iodine by Harvey's method (12). Total hardness of water was calculated from the figures of total calcium and magnesium and expressed in mg of $CaCO_3$/100 ml water.

A survey of the diet was carried out by careful questioning of representative samples of the population in the three villages. Investigations by Pasricha (13) have shown that the oral questionnaire technique, when carried out with proper care, can yield as reliable an estimate of food intake in poor Indian communities as the more conventional, weighing methods.

The nutritional state of the people in the district studied is poor. The diet is deficient in animal proteins, fats, calcium and vitamins A and C (Table 2). The villages are situated far from the sea. Thus no marine foods such as fish and shell fish are being consumed. An average of about 11 g of sea-salt is being consumed daily. The salt is derived from two sources, and a mixture containing roughly equal parts of each is available in the local market. The iodine content of the salt derived from the two sources is 9. 6 and 6. 0 µg/g, respectively.

TABLE 2

Composition of Daily Diet Consumed in the Three Villages

Protein (g)			Calcium (g)		0.48
Animal	5.2		Phosphorus (g)		1.78
Vegetable	73.1	78.3	Iron (mg)		39.1
Fats (g)			Vitamins		
Animal	8.9		Carotene (i. u.)	1048	
Vegetable	19.9	28.8	Vitamin A (i. u.)	176	
Carbohydrates (g)	494.9		Vitamin B_1(mg)	2	
Total Calories	2618		Vitamin C (mg)	23	

The fluorine and iodine content of the solid part of the diet was not examined because the solid food consumed by the local inhabitants is obtained from various neighboring villages.

Observations

Incidence of goiter: This report is not concerned with goiters accompanied by thyrotoxicosis, but with those which are customarily described as simple or endemic. We tried to determine whether or not there is a geographical distribution of goiter. The thyroid gland of every inhabitant of the three villages under review, altogether 2008 cases, was examined. The clinical method of grading and recording the size and possible changes in the thyroid gland, as outlined in a M. R. C. Memorandum was employed. The types of gland were grouped as follows: (a) invisible at rest; (b) visible to the trained observer, but soft, smooth and symmetrical; (c) conspicuously enlarged (clearly visible ++, but showing no palpable asymmetry, firmness or nodular change); (d) showing a degree of firmness, asymmetry or nodularity which can be regarded as definitely pathological. Throughout the whole survey special attention was paid to the patient's age at the time of appearance and disappearance of the thyroid enlargement.

Visible thyroid glands of type (b) were encountered only in the ages 14-17 (Table 1). These cases were examined every 6 months until they were 17 years of age. The visible glands became less noticeable or invisible after puberty and were, therefore, regarded as within the limits of normal. Thyroid enlargement belonging to types (c) and (d) were not encountered in any of the age groups.

No cases of cretinism or of deaf-mutism were seen in the villages under review.

Discussion

An adequate amount of iodine is essential for thyroid function. A number of estimates of minimal and optimal requirements for iodine in man are available. With quantities between 100 and 1000 µg/day the thyroid gland seems to function efficiently (14). Low iodine intake is an established cause of goiter. Among many factors which have from time to time been suggested as favoring the development of goiter or increasing the need for iodine, the following may be mentioned: polluted water, excess calcium, hardness of water, excess fluorine, arsenic, cobalt, bromine and consumption of goiterogenic substances. Heredity and environmental factors also influence the requirements for iodine.

As long ago as 1854, Maumené suggested that fluorine might be the cause of endemic goiter. This possibility was put forward again by Marine (2) and by Wilson (7). Wilson reported a high degree of dental fluorosis in goiterous areas of the Punjab, India, although no mention is made about the iodine intake. In Somerset, England, Wilson also found a parallelism between the incidence of goiter and dental fluorosis. Ornek reported cases of dental fluorosis and endemic goiter occurring together in Isparta, Turkey. On the other hand, Roholm (3) states that, in human cryolite intoxication no change was observed in the size of the thyroid gland. With the exception

of Maumené's insufficiently described case, no clinical observations are known from spontaneous or experimental intoxication to indicate any effect on this gland. The hypothesis of struma-producing effect of fluorine cannot be generally applicable. In two autopsies of cryolite workers, Roholm reported thyroid glands which were normal both macroscopically and microscopically. In Iceland, where fluorosis is present but where goiter is not endemic, the consumption of seafood is very high (6). Murray et al. (15), in a report to the Medical Research Council, were unable to show any correlation between the two variables but, as they point out, the range of fluorine levels which they encountered was not wide.

In the present investigation no instance of marked and permanent enlargement belonging to types (c) and (d) was found. Intake of 11 g sea-salt/day containing an average of 7.8 µg iodine/g will supply 85.8 µg iodine/day. The high level of iodine both in salt and in water presumably insures a large enough intake of iodine to outweigh any possible effect of excess fluorine. A profitable study of the relation of fluorine to endemic goiter could be made in areas such as Isparta in Turkey and the Punjab in India where fluorosis and high goiter incidence have been reported to coexist.

With regard to the slight and temporary enlargement of the thyroid encountered in the age group 14-17 (type b), detailed scrutiny of the data in Table 2 reveals that with a fall in mean fluorine content of the water from 10.7 mg/l in Kamaguda to 5.4 mg/l in Yellareddyguda, there was a corresponding progressive fall in the incidence of pubertal goiters from 40% in Kamaguda to 9% in Yellareddyguda. However, associated with the fall in fluorine content there was also a rise in mean iodine of the water. The figures can be interpreted to indicate that, so far as type b goiters are concerned, (1) fluorine may be actually goiterogenic, and (2) high concentrations of iodine may have a goiter-preventing effect. Investigations in other areas, where the variations in fluorine content are not associated with variations in iodine content of the type encountered here, may throw light on this particular problem.

Summary

The results of analyses of the mineral content of drinking water from three Indian villages with endemic fluorosis surveyed for the incidence of thyroid enlargement are reported.

With the possible exception of temporary thyroid enlargement encountered in pubertal subjects (type b), no relation was found between the incidence of endemic goiter and the fluorine in the water supply.

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"NEIGHBORHOOD" FLUOROSIS

by

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Fluoride fumes, mainly HF and SiF_4, and particulate fluoride compounds, especially NaF, escape from chimneys, storage bins and effluent ponds of factories which employ fluoride compounds as a flux or where fluoride constitutes a by-product of refining and smelting processes. The principal industries involved are those concerned with the production of aluminum, steel, enamel, zirconium, uranium, magnesium, triple phosphate fertilizer, glass, tile and bricks. Fluoride is also liberated into the air wherever wood and coal are burned. Gaseous HF and SiF_4 hydrolyze with the moisture of the air and settle on the ground, on plants, fruit, vegetables and on forage for cattle.

In humans, fluoride-contaminated food and water constitute the bulk of fluoride intake in polluted areas. In the vicinity of an Ontario fertilizer plant, dust contained between 9,000 and 11,000 ppm of fluoride, near a Michigan iron foundry up to 2,000 ppm. The dust from the fertilizer plant covered practically everything in the area and settled on topsoil. Fluoride fumes etched windows and damaged the finish of automobiles.

The data presented here concern 32 patients. Twenty-eight of them resided near the above-mentioned fertilizer factory, 3 near Iowa fertilizer factories, 1 near a Michigan iron foundry. Four of the 32 were hospitalized for diagnostic studies. Fifteen were examined. In the remaining 13, data were obtained from the medical history given to us by the patients. In all cases, characteristic fluoride damage in plants, livestock and materials was established.

The two conspicuous and readily recognizable manifestations of chronic fluoride poisoning, dental and skeletal fluorosis, are not obligatory features of the disease. Dental fluorosis (or mottling of teeth) occurs only in individuals who have consumed, or have been exposed to, fluoride during childhood. The skeletal changes of the disease develop only after many years of persistent fluoride intake. The latter condition need not be considered here, since the air polluting factories have been in operation less than seven years.

History

Table 1 presents the ages and sexes of the 32 cases, the distance of their residence from the factory, and the symptoms which they presented.

TABLE 1

Case Number	Name	Sex	Age	Distance from Factory (miles)	Neuromuscular arthritis	Gastro-Intestinal	Respiratory	Headaches	Visual Deterioration	Epistaxis	Chizzola maculae	Muscular Fibrillation	Additional Data
1	V. F.	M	54	1/4	X	X	X	X	X				Dysuria
2	M. F.	F	13	1/4	X			X	X			X	Spasticity of toes
3	W. R.	F	58	1/4	X			X					Conjunctivitis
4	E. R.	M	56	1/4	X	X	X			X	X	X	AAE*; stomatitis
5	W. W.	F	56	1/3	X	X	X	X	X				Ca of Prostate, osteoarthritis, calcarious tendinitis
6	I. W.	M	53	1/3	X				X	X	X		Stomatitis; purpura
7	D. W.	F	13	1/3		X			X				AAE*
8	W. S.	F	70	1/2	X	X	X						Stomatitis, bursitis; AAE*
9	L. S.	M	61	1/2	X					X			
10	A. Z.	M	63	1/2	X	X	X	X					AAE*
11	G. M.	M	62	1/2	X	X		X	X				
12	M. N.	F	50	1/2	X	X						X	
13	S. N.	M	45	1/2	X			X					Chalazions; pyelocystitis
14	P. D.	F	44	3/4	X	X		X	X				
15	E. P.	M	57	1 1/2	X		X	X	X				
16	M. D.	F	65	2	X	X	X	X	X				
17	J. D.	M	52	2	X	X	X	X	X		X	X	AAE*; syncope while walking, generalized edema
18	A. K.	F	65	2	X	X	X		X	X	X		Repeated renal colics
19	J. V. B.	F	68	2 1/2	X			X	X				Thrombophlebitis
20	D. McK.	F	51	2 1/2	X		X		X				AAE*
21	J. McK.	M	45	2 1/2	X			X					Coronary thrombosis
22	R. McK.	M	12	2 1/2		X	X				X		
23	G. V. L.	F	44	2 1/2	X			X	X				8 cups of tea daily (1.3 mgF)
24	T. B.	F	35	2 1/2	X	X	X		X	X		X	
25	J. C.	F	55	3	X	X	X		X	X	X	X	AAE*; polyuria (3460 cc)
26	A. L. F.	F	48	5	X				X	X	X		Dermatitis
27	D. F.	M	51	5	X		X						
28	J. B. M.	F	46	2 1/2	X	X	X	X	X		X		Coronary thrombosis
29	D. L.	F	56	1/3	X	X	X	X	X	X	X	X	AAE*; pyelocystitis; exostoses
30	L. R. S.	M	51	1/3	X		X	X	X	X	X		Exostoses on fingers and toes
31	M. M. S.	F	52	1/3	X	X				X	X	X	
32	O. S.	M	61	1/3	X			X				X	Periodontal disease

*AAE: Acute abdominal episodes

Ten of the 32 patients manifested the skin lesions described by Steinegger (2) and by Colombini et al. (3) among residents near fluoride-emitting aluminum plants in the vicinity of Chizzola and of Bolzano, Italy. They are bluish-brown, macular areas, round or oval in shape, about the size of a dime (Fig. 1, 2). They are painless, fleeting in character with a duration of 5 to 10 days. They simulate purpuric lesions or traumatic suffusions but do not exhibit histological evidence of capillary permeability. Microscopically, they show lymphocytic perivascular infiltration and edema in the corium with occasional melanin deposits in the epidermis.

Fig. 1

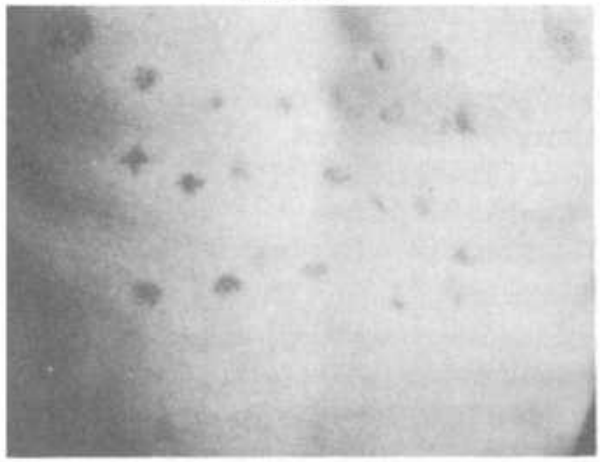
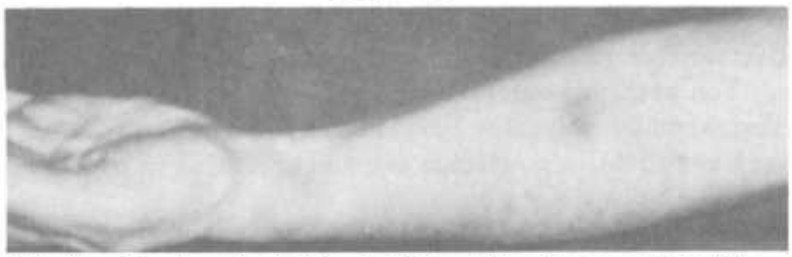


Fig. 2



Similar Lesions in Patient 29 Residing Near a Fluoride-Emitting Foundry.

Gradually increasing general malaise and exhaustion leading to complete disability characterizes the disease. All individuals except two boys, ages 12 and 13, reported pain and stiffness in the lumbar and cervical spine with restriction of spinal movements and arthritis in other joints as well. They complained of myalgia and paresthesias in arms, legs and shoulders with impaired muscular power of hands and legs. Consequently, they were no longer able to grip objects securely and their legs tended to

collapse when walking. Eight patients had muscular fibrillation, 3 intermittent muscle spasms especially in the big toes suggestive of hypocalcemic episodes, a common feature in acute fluoride intoxication (Table 2).

TABLE 2

Symptomatology in 32 Cases of "Neighborhood" Fluorosis

	No. of Cases
1. Neuromuscular-Skeletal	
Arthritis, especially in cervical and lumbar spine	30
Myalgia; myasthenia; paresthesias	30
Spasticity in extremities	3
Muscular fibrillation	8
Migraine-like headaches	20
Visual disturbances (scotomata, blurred vision)	8
2. Gastro-Intestinal	
Gastric (nausea, vomiting, epigastric pain)	18
Intestinal (distention; spastic constipation, diarrhea)	16
Acute abdominal episodes	9
3. Respiratory	
Nasal and conjunctival	28
Emphysema; asthma	4
Epistaxis	7
4. Chizzola Maculae	10

Bilateral migraine-like headaches were encountered in 20 of the 32 cases. Ten patients complained of visual disturbances (scotomata, blurred vision) which were not corrected by glasses. Two had evidence of incipient retinitis, a condition encountered also in hydrofluorosis (1).

Eighteen of the 32 individuals had gastrointestinal disturbances, the second group of symptoms described in fluorosis. They had frequent nausea, occasional vomiting, pains in the epigastrium and in the intestinal tract, bloating and distention of the abdomen. Sixteen of the 32 had spastic constipation, alternating with diarrhea. Nine had repeated acute abdominal episodes simulating, and diagnosed by their physicians as, volvulus, acute gallbladder disease, "intestinal flu" and the like. Several patients identified these episodes with temporary intake of excessive amounts of fluoride-contaminated food grown near the factory (cabbage, lettuce, beans) (Table 3).

In the third group of symptoms concerning the respiratory tract, nasal and conjunctival irritation was common, especially on windy days; but chronic bronchitis and emphysema was noted in only 4 of the 32 individuals.

More significant, however, is the history of frequent epistaxis in six patients which they related to sudden excessive exposure to dust.

TABLE 3

Fluoride Assays of Food

Consumed by Cases #22 and #25

	ppm	"Normal" Values
* Wheat (grain)	2.6	0.7 to 2.0*
Apples	6.6	0.8
Carrots	7.0	2.0
Beets	7.0	0.7 to 2.8
Squash	10.4	
Corn	1.3	0.7
Sauerkraut	10.7	
Currants	8.0	0.7
Cabbage leaves	9.6	
** "Chicken vegetable soup"	4.6	
"Hamburger with onions"	2.4	
Potatoes (boiled)	7.7	0.4
Beans (cooked)	17.3	1.7
Strawberries (frozen)	4.6	
Oatmeal (cooked)	5.1	0.2
*** Lettuce	44.0	0.1 to 0.3

Analysis by

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*** Ontario Water Resources Commission

Examination Findings

Examination and laboratory data were unremarkable as is usually the case in the early stage of chronic fluorosis (4). Two patients manifested exaggerated tendon reflexes, especially in the lower extremities. In others, pain was elicited on palpation of the abdomen. Abdominal distention, joint swelling and restriction of joint movements, especially in the lower spine, were noted. In the hospitalized cases, the spinal X-rays

showed changes indicative of osteoarthritis. Hypercalcemia, hyperuricemia, slight impairment of liver function were noted but were not sufficiently consistent to be attributed to chronic fluoride intoxication.

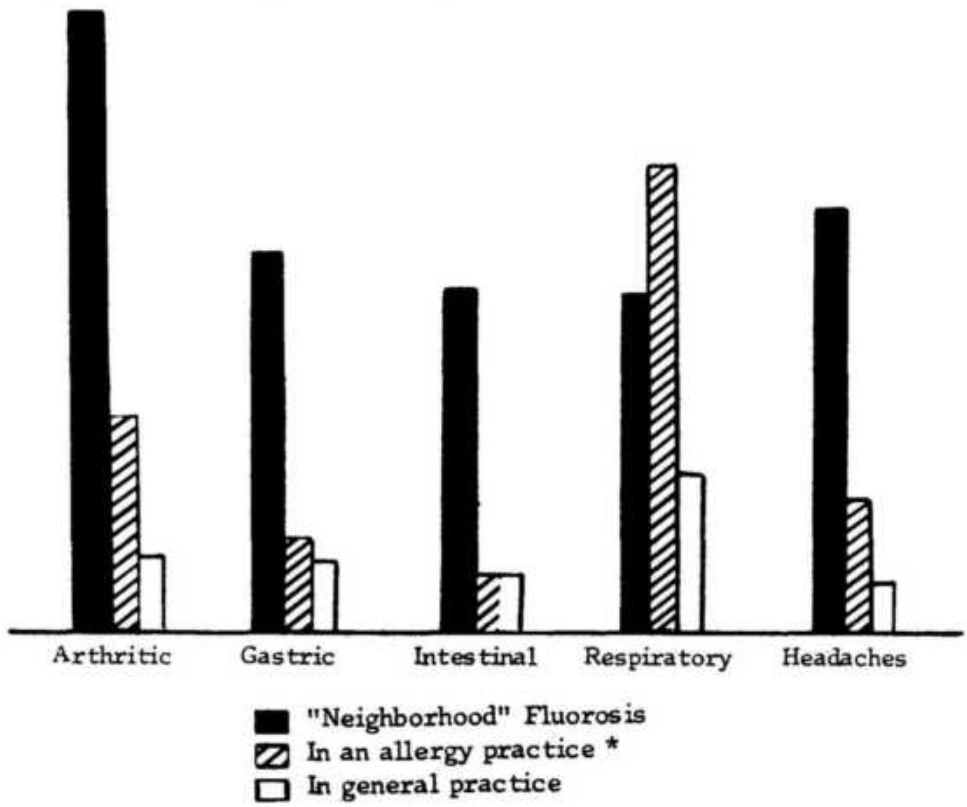
Diagnosis

The diagnosis of chronic fluoride intoxication was based on the following criteria:

1. Excessive exposure to fluoride in food, air and water was established by visible damage to crops, livestock and materials. The vegetation showed the typical fluoride damage of margins and tips of leaves and high fluoride levels. The cattle exhibited the characteristic features of fluorosis i. e. exostoses, joint swellings. Fluoride assays of food consumed by cases 20 to 22 and by case 25 yielded values up to 20 times those grown in non-polluted areas (Table 3)

Fig. 3

Incidence of Symptomatology in 32 Cases of "Neighborhood" Fluorosis



*In an allergy practice respiratory disease constitutes the bulk of admissions.

2. The clinical picture is identical in the 32 individuals, and agrees with the non-skeletal phase of fluorosis in industry (5), of hydrofluorosis (6), of "neighborhood" fluorosis described by Murray and Wilson (7) and by Capps and Hunter (8) and with the recently described symptomatology of chronic poisoning due to fluoride intake for therapeutic purposes (9). Fig. 3 presents the incidence of the symptomatology encountered near the Ontario factory compared with that in 28 consecutive cases seen in an allergy practice (G. L. W.) and 28 in general practice (V. A. C.). The difference in the incidence of arthritis in the 3 groups of cases is particularly noteworthy.

3. In four cases, 24 hour urinary fluoride values were obtained; in three cases, biopsy tissue. Table 4 shows a high fluoride content of bone and soft tissue and an unusually high urinary fluoride excretion, namely 7.04 to 12.63 mg. Since in most instances only a fraction of ingested fluoride is excreted in the urine, the daily fluoride intake must have been considerable.

TABLE 4

Fluoride Assays

	ppm	"Normal" Values ppm
Water *	<1: in 3 persons 1 to 9.9: in 15 persons 10 to 14.9: in 8 persons 15 : in 4 persons 37.8: in 1 person	
Soil *	85 to 520	51 to 361 (G)
Dust *	8,850 to 11,450	
Hay *	3 to 191	1 to 6 (G)
Egg Plant (Leaves)*	52.0	
Wheat (Heads) *	9.1	2.3 to 6.4 (G)
Human Tissue:		
Bone (case 25)	641.3 and 864.1 (O)	<300 (Roholm 5)
Muscle (case 5)	116.4 (O)	<1 ppm (JAMA 204:970, 1968)
Prostate (case 5)	92.2 (O)	
Blood ** (case 5, 6)	0.2 to 0.4	0.013 (Nature 211:192, 1966)
24-hour Urine	7.04 mg to 12.63 mg	

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** The Ontario Dept. of Health
(G) Dr. K. Garber, State's Institute of Botany, Hamburg, Germany
(O) Dr. W. Oelschlager, Agricultural University, Hohenheim, Germany

4. In 10 individuals, the recently described "Chizzola Maculae" were recorded, a condition which we have also encountered near a fluoride emitting aluminum factory and in fluorosis from drinking water.

The above data clearly point to the fact that fluoride emission from the four factories was the source of the illness. Studies in support of our observations are in progress.

Summary

In 32 individuals residing near fluoride-emitting fertilizer factories and an iron foundry where fluoride damage to vegetation, livestock and to materials was established, evidence of the non-skeletal phase of fluorosis is presented.

The symptomatology of the disease is identical with that of the non-skeletal phase of fluorosis recorded by others in industrial fluorosis, in "neighborhood" fluorosis, in hydrofluorosis and in fluoride intoxication from long-term administration of fluoride tablets. Principally involved are musculoskeletal, gastro-intestinal and respiratory systems. Fluoride assays of hay, flydust, food, human tissue and urine are presented. Ten individuals exhibited the skin lesions designated as "Chizzola Maculae" which have been described recently in populations exposed to fluoride emanations near aluminum factories in Italy.

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EXPERIMENTAL STUDIES IN NEWBORN RATS AND MICE ON THE SUPPOSED CAPILLARY-DAMAGING EFFECTS OF FLUORINE AND FLUORINE-CONTAINING INDUSTRIAL POLLUTANTS

by

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This paper refers to the macular lesions described by Cristofolini, et al. (1), Colombini et al. (2) and Monteriolo et al. (3) near an aluminum factory in Northern Italy. Biopsy specimens of the affected skin areas showed foci of pericapillary infiltration and hemorrhages in the subdermal layers (4).

On the basis of their histological pattern we might conclude that the maculae were due to a toxic or allergic mechanism with capillary damage by an agent of unknown origin. Fluorine or other pollutants from the nearby aluminum factory have been suggested as the cause (1-3). This hypothesis must be questioned because no symptoms of fluorosis such as mottled enamel or osteosclerosis have been noted, nor is the urinary fluorine output of the victims higher than that of subjects living in areas free of industrial F^- pollution. Moreover, Balazova and Ripple (5) failed to encounter the maculae in children with mottled teeth residing near a Czechoslovakian aluminum factory where F^- levels were more than twice as high as those recorded in non-polluted areas.

We attempted to clarify the phenomenon under discussion by setting up experiments to establish whether or not fluoride and fluoride-containing pollutants can damage capillaries and induce hemorrhages in both skin and mesentery.

1. Fragility of Skin-Capillaries in Newborn Rats

a. Treatment of Mother Rats During Pregnancy: The following studies were made on newborn Sprague Dowley Rats. Their mothers were treated during the entire pregnancy period as follows: In group 1 six mothers were injected subcutaneously in the dorsal area with an aqueous extract of dust (A. D. E.) collected from the stacks of the above-mentioned aluminum factory. Table 1 presents the composition of the dust, analyzed by spectrogrophy. The dust was extracted with distilled water

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TABLE 1

Composition of Dust Emitted From Aluminum Factory
(Spectographic)

Substance		Weight%	Fluorides
F		7.0	
Coal		26.0	
Al		13.2	Al_2O_3, AlF_3, 3NaF, AlF_3
Na		11.1	Na_2SO_4
S		3.4	Sulphates
Fe		2.7	Fe_2O_3
K		2.1	K_2SO_4
Ca		0.9	CaF_2
Si		0.6	SiO_2
P		0.6	P_2O_5
Ag, As, B, Ba, Bi, Cu, Co, Cr, Ge, Ga, Mg, Ma, Mo, Ni, Pb, Sa, Sr, Ti, V, Zn.	In total	1.0	
Substances extractable in benzene or acetone		12.0	
Oxygen as present in various oxides	Up to	100	

in a Soxhlet apparatus and buffered to pH6. One ml containing 2 mg of F^- was injected daily for 6 days a week throughout the pregnancy up to the day of parturition. The total doses of fluoride ranged from 40 to 52 mg (mean 44 mg). In group 2 six mother rats were treated in the same manner with an NaF solution buffered to pH 6 which provided a daily dose of 2 mg F^-. The total dose of F^- was 40 to 48 mg (mean of 42 mg). Group 3, representing six untreated animals, served as controls. Table 2 gives the number of parturitions and the average weight of the newborn rats by the suction cup test of Wintrobe (6). With a cup 1 cm in diameter applied to the skin of the abdomen on the day of birth, a depression of 100 mm Hg for 20 seconds was found in the control animals to be the maximum degree of suction which failed to induce petechiae on the skin. This depression failed to elicit petechiae in rats born of the mothers which had been injected with A. D. E. and the NaF solution.

The total body fluoride was then determined in 10 newborn rats of each group, after ashing, by the microdiffusion method of Farrah (7), as shown in Table 2.

TABLE 2

Suction Cup Tests and Total Body Fluoride

Treatment (6 Mothers)	Mean Weight grams	Average Total* Body F^- (Wet Wt.) ppm	Positive Suction Cup Test** number of animals
		Newborn Rats	
Controls	5.7 (4.7-7.1)	0.72 (0.3-1.0)	0/67***
NaF 2 mg F daily	6.9 (6.5-7.1)	3.53 (3.12-3.93)	0/62
Dust Extract 2 mg F daily	6.2 (5.7-6.8)	4.16 (3.70-4.59)	0/64

*Determination made on 10 rats per group.
**Maximum depression (100 mg for 20 seconds) which failed to produce petechiae in controls.
***0 in 67 animals.

Mother rats received 2 mg F^- daily throughout pregnancy (total 40-52 mg).

The mothers subjected to the experiment were sacrificed on the day of parturition. Autopsies revealed no macro-or microscopic changes, particularly in the skin as compared with the control animals.

b. Treatment of Newborn Rats: The fragility of skin-capillaries was determined in another group of newborn rats averaging 5.7 g in weight. They were treated during the first 10 days of life as follows: The 10 animals of group 1 received daily doses of 0.1 ml A.D.E. subcutaneously in the dorsal area containing 0.2 mg F^-. In group 2 ten animals were injected daily as above with 0.1 ml NaF solution containing 0.2 mg F. Group 3 consisted of 10 control animals which were not treated.

At the end of the treatment, before the animals' fur had grown, capillary fragility was measured on the skin of the abdomen with the suction cup test. Here too, a depression of 100 mm Hg for 20 seconds was the maximum pressure which failed to cause petechiae in the control animals. The test was negative in the animals treated with A.D.E. and NaF. All rats were sacrificed and the total body F^- content was determined as shown in Table 3.

2. Mesenteric Capillaries

Further studies were carried out on the mesentery of female mice.

Its thin transparent layer of tissue permits a better visualization of the capillary system than that of the skin.

Female Swiss albino mice of an average weight of 14.7 g were divided into 3 groups of 10 animals each, and treated for 6 days a week for 4 weeks: The animals of group 1 were injected subcutaneously with 0.1 ml of A. D. E. containing 0.2 mg F^-; those of group 2 with an 0.1 ml NaF solution containing 0.2 mg F^-. Group 3, the untreated animals, served as controls.

TABLE 3

Suction Cup Tests and Total Body Fluoride

Treatment	Total Body* Fluorine ppm (Wet Weight)	Suction Cup Test Positive Reactions 100 mm Hg for 20 Secs. **
Controls	0.81 (0.38-1.2)	0/10
NaF	8.5 (5.6-10.4)	0/10
Dust Extract	7.6 (5.9-9.2)	0/10

*Average and range.
**Maximum depression which does not produce petechiae in controls.

Newborn rats received 0.2 mg F^- daily for 10 days.

During the treatment no differences between the control and treated animals were observed with regard to food consumption (about 4.5 g per animal per day) and body weight. After 4 weeks at the termination of treatment, the animals averaged 34.6 g. None showed any unusual changes in the skin or on the fur. At the conclusion of the experiment the animals were sacrificed. Pooled skull and long leg bones were ashed and their F^- content was determined as well as that of shaved skin covering one half of the abdomen. The other half was examined histologically with hematoxyline-eosin stain. The mesentery was spread gently over a number of cover slips, fixed in methyl alcohol, stained with the Pickworth method for hemoglobin and with hematoxylin-eosin.

The F^- values in bones and skin are reported in Table 4. The treated animals showed no histological changes as compared with the controls.

TABLE 4

Fluoride in Bones and Skin (Wet)

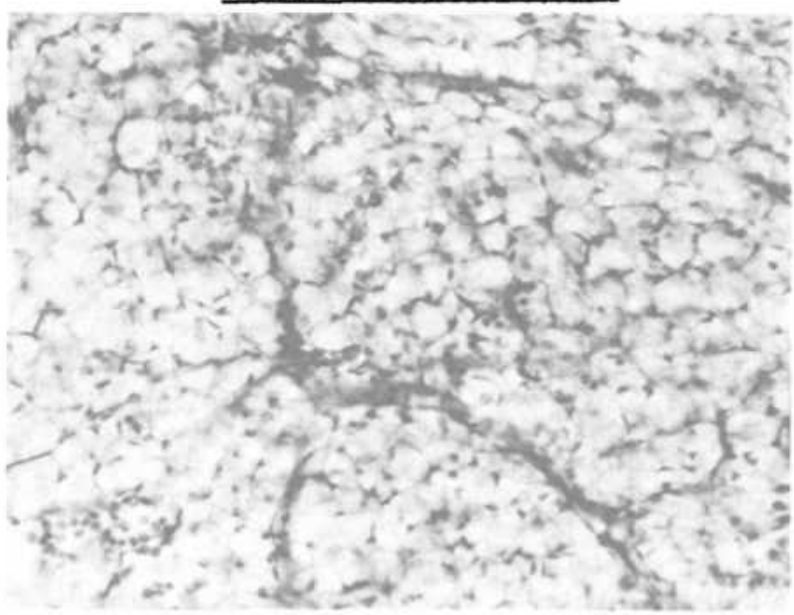
Treatment	Bones * ppm	Skin ppm
Controls	34.3 ±5.5	3.11 ±0.71
NaF **	229.5 ±58.7	8.39 ±1.92
Dust Extract**	224.6 ±74.6	7.02 ±3.4

*Pooled skull and leg bones.
**0.2mgF/day for 4 weeks, 6 days a week.

Typical specimens, illustrated in Fig. 1 and 2, showed no perivascular infiltration or hemorrhages.

Fig. 1

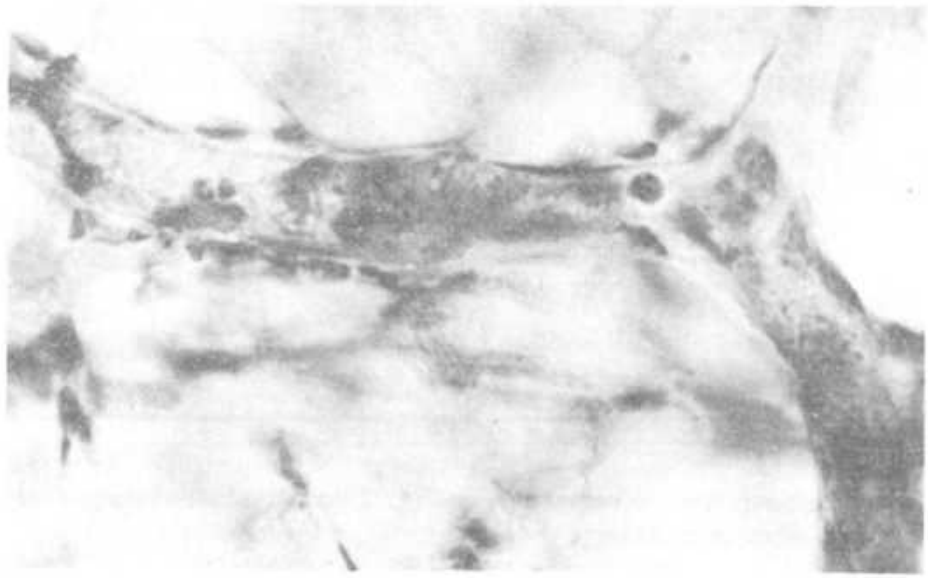
Mesentery of a Female Mouse s.c. Injected with NaF: 0.2 mg F/day, 6 Days a Week, for 4 Weeks.



Pickworth stain X 70. Ramification of capillaries in the serous membrane layer appears normal.

Fig. 2

Mesenteric Capillaries, Female Mouse s.c. Injected with Aqueous Dust Extract Collected from the Stacks of an Aluminum Factory: 0.2 mg F/day, 6 Days a Week, for 4 Weeks



Hematoxylin-eosin. X 500. Normal appearing capillary system.

Discussion

We employed pregnant mother rats and newborn animals for our experiments because the skin lesions recorded by others suggestive of capillary damage by fluoride were encountered exclusively in women and children. The doses of F^- in experimental animals, i.e., 6 mg/kg/day for 20 to 26 days in pregnant rats, 35 mg/kg/day for 10 days in newborn rats, and 14 mg/kg/day for 4 weeks in female mice were calculated to be below those causing pathological changes in bones, teeth and other organs. Hodge and Smith (8) maintain that doses up to 30 to 40 mg/kg/day given for 1 month are not harmful to kidneys.

There was a five-fold increase in total body F^- in the rats born of the mothers treated during pregnancy and a ten-fold increase in the rats injected directly with F^-. No significant differences ($p=0.05$) were observed between the groups receiving NaF and those receiving A. D. E. Despite the increase in total body F^-, the capillary fragility of the skin as tested with the suction cup test was not affected.

In female mice, the experimental treatment produced an eight-fold increase in the F^- content of bones. According to Chang et al. (9) such an increase does not give rise to osteosclerotic changes. Bone changes do occur, however, when the F^- content in the bones increases more than 10 to 15 times. In the skin the F^- content was about twice

that of the controls. No significant differences (p=0.05) were observed between the two groups treated with NaF and with A.D.E. respectively. We did not observe any abnormalities in the skin nor any capillary changes with hemorrhages. Regarding the histology of the mesentery, even in a structure as simple as a serous membrane, fluoride in doses which do not produce skeletal changes induced no changes in the capillary walls.

The extract of dust emitted from the aluminum factory contains many substances other than F^-. However, these substances are present in much smaller amounts. We have demonstrated that these chemicals in association with F^- likewise fail to damage capillary permeability, at least when absorbed in the small quantities employed in our experiments.

Summary

Bluish skin-spots occurring in women and children residing in the vicinity of a F^- emitting aluminum factory have been attributed to a poorly understood F^- capillary-damaging effect. To clarify this phenomenon, a study was carried out on rats born of mothers which were injected subcutaneously for the whole pregnancy period with NaF and an extract of dust emitted from the aluminum factory respectively at a dose of 2 mg fluorine per day: total dose of fluorine given 40-52 mg. No cutaneous lesions nor even an increased capillary fragility of the skin as proved with the suction cup test were observed.

In a second experiment, newborn rats were injected subcutaneously with NaF and the dust extract at a dose of 0.2 mg fluorine per day respectively in the first 10 days of life. Again no cutaneous lesions nor an increased capillary fragility of the skin were observed.

Studies on subacute toxicity were carried out in female mice injected subcutaneously with NaF and with the dust extract respectively, in a dose of 0.2 mg fluorine per day for 24 days. No cutaneous lesions nor capillary alterations, as studied in histologic preparations of the mesentery, were observed.

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Editor's Note:

In order to abide by the principle of presenting divergent viewpoints, this paper is being published essentially as submitted although several significant points are at issue:

Has vascular permeability been established as a histological feature of Chizzola maculae?

Can chronic fluoride poisoning be ruled out on the basis of absence of dental and skeletal changes or of low urinary F^- excretion as postulated by the authors?

Do current data from endemic areas bear out the concept that fluorosis does not occur at less than eight times the "usual" F^- values in bones?

FLUORIDE ACCUMULATION IN LEAVES DUE TO BORON-CONTAINING FERTILIZERS

by

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Introduction

Fluoride damage to vegetation has been observed for a long time near chemical and metallurgical factories. Especially aluminum, glass and superphosphate fertilizer works emit pollutants which contain F^- compounds.

The tolerance of plants to the caustic action of F^- compounds varies greatly with each species. Apricot and peach trees, grape vines, gladiolus and iris plants are most susceptible to damage.

In the Rhone Valley (canton Valais, Switzerland) damage to grape vines and apricot trees has been reported as distant as 23 kilometers from the emission centers in the direction of the prevailing winds. The symptoms observed are typical of F^- damage, namely marginal and apical centripetal necroses in dicotyledons, apical progressive necroses in monocotyledons, twig defoliation in apricot and peach trees.

Fluoride assays of foliage of the damaged plants revealed very high F^- levels with values up to 600 ppm in dry matter (1 ppm = 1 part per million = 1 mg/kg).

Extensive necrosis of leaves as well as high F^- levels occurred several kilometers from the emission points. The damage was limited strictly to particular plots, belonging to the same proprietors, whereas neighboring plots did not show the above-mentioned symptoms.

Neither the composition of the soil, its F^- content, antiparasitic treatments nor irrigation by sprinkling, which is in common use in the Rhone Valley, could be implicated as the cause of the above-mentioned damage. Therefore, our attention was directed to fertilizers as its likely source.

Field Experiments

At first, investigations of the effect of a special form of potassium fertilizer, namely chlorinated and sulphated potassium fertilizer, indicated that the chlorinated type was responsible for the necroses.

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However, investigations conducted in 1968 in open fields as well as in pot-cultures proved that certain special types of fertilizers were exclusively instrumental in causing the high F^- accumulation in plant tissues and the characteristic features of F^- intoxication (Table 1).

TABLE 1

Typical Fluoride-Induced Necroses on Apricot Trees and Grape Vines (Rhone Valley)

Place	Type of Fertilizer Used	Plant Species	Foliage: Necroses*	Foliage: Fluoride ppm **	Soils: pH	Soils: $CaCO_3$ %	Soils: P_2O_5 Test	Soils: K_2O mg%	Soils: Total F^- ppm
Charrat	PKB	Apricot	3,1	630,0	8,0	50,0	250	29,0	760
	PKB	Vine	3,8	620,0					
	NPKMg	Apricot	0	26,4	7,9	48,0	132	19,3	790
	NPKMg	Vine	0	31,6					
	PKB	Apricot	2,0	362,0	7,9	51,0	145	20,0	800
	NPKMg	Apricot	0,1	12,8	7,8	28,5	102	12,7	660
Saxon	PKB	Apricot	2,5	420,0	7,8	12,0	43	3,8	610
	PKMg	Apricot	0	16,8	7,6	14,0	110	5,6	450
Bieudron	PKB	Apricot	3,0	524,0	7,6	3,5	62	17,0	650
	PKB	Vine	2,5	213,0					
	Manure	Apricot	0	38,4	7,7	3,7	200	42,0	620
	Manure	Vine	0	54,4					
Fey	PKB	Apricot	2,6	249,0	7,6	6,8	47	46,5	730
	NPK	Apricot	0,7	11,0	7,3	4,0	580	15,5	810
Coor	PKB	Apricot	2,6	127,2	7,2	----	230	100,0	640
	NPK	Apricot	0,6	12,0	7,1	0,2	200	4,6	720

*Estimated degree of damage

0 = without necroses

5 = all foliage necrotic

**Dry matter

Boron-containing compounds are underlined.

Tests were conducted on a white grape vine in Luins on the shore of Lake Geneva, an area without any atmospheric pollution, and on apricot trees in Fey (Valais) in the Rhone Valley (Table 2). Additional experiments were carried out on forage cultures in Möhlin (Rhein Valley) in the North of Switzerland (Table 3). The special fertilizers were obtained by direct attack of strong acids, i.e. sulfuric or nitric acid, on the mixture of rough phosphates, potassium chloride and borax or boric acid. Only

TABLE 2

F⁻ Content of Grape Vines and Apricot Leaves from Experimental Plots

Type of Fertilizer	Grape Vine (Luins-Lake of Geneva)			Apricots (Fey-Rhone Valley)	
	5.6	26.7 1968	7.10	21.7	19.9 1968
1. Without fertilizer	3,0	11,8	16,5	13,7	18,5
2. Superphosphate + KCl	3,7	8,7	15,5	18,5	18,5
3. Super + KCl + Borax	3,3	8,0	18,5	----	----
4. Superphosphate + K_2SO_4	3,3	7,2	19,5	13,8	16,2
5. Super + K_2SO_4 + Borax	4,3	7,7	16,5	----	----
6. Combined fertilizer PK	3,3	9,0	13,0	9,7	12,5
7. Combined fertilizer PKB	9,7	53,4	45,5	200,1	161,0
8. Mixed fertilizer PKMg	5,3	10,8	16,0	14,5	12,0
9. Mixed fertilizer PKMgB	3,0	9,2	12,0	17,0	17,0

F⁻ concentrations in ppm, dry matter.

TABLE 3

Fluorine, Boron and Chlorine in Forage Plants
Experiment in Möhlin (Rhein Valley)

Type of Fertilizer	Experiment #1 Soil: pH 7,3 Fluorine:540 ppm Crops			Experiment #2 Soil: pH 6,1 Fluorine:260 ppm Crops		
	F⁻ ppm	Boron ppm	Cl. %	F⁻ ppm	Boron ppm	Cl. %
First Cutting (May 14, 1968)						
1. Without fertilizer	7,0	14,0	0,32	11,0	13,5	0,78
2. Superphosphate + KCl	21,5	13,8	1,87	26,5	14,0	1,29
3. Super + KCl + Borax	31,0	23,0	1,96	26,5	20,0	1,30
4. Superphosphate + K_2SO_4	25,5	11,0	0.77	37,5	18,0	0,89
5. Super + K_2SO_4 + Borax	29,0	18,0	0,89	32,5	17,5	0,27
6. Combined PK (chlorinated)	11,5	10,0	1,67	32,5	17,0	1,38
7. Combined PKB (chlorinated)	183,5	36,0	1,69	185,7	44,0	1,12
8. Mixed PKMg (sulphated)	12,5	14,0	0,66	17,0	16,0	0,87
9. Mixed PKMgB (sulphated)	13,0	13,8	0,71	21,5	17,5	0,66

the boron-containing combined fertilizers proved to be responsible for the high F^- content of the plants whereas fertilizers of the same formula, which were obtained by the same process but without addition of boron, failed to cause an increase in F^- uptake by plants. As Table 2 indicates, F^- levels in the leaves of apricot trees and grapevines do not exceed 20 ppm in the dry matter except in parcel No. 7 which received the PKB boron-containing fertilizer.

The same combined PKB fertilizer also caused a very high F^- accumulation in forage (Table 3). In the parcels where the PKB fertilizer was not used, the F^- content did not exceed 40 ppm although the experiments were located in a F^- polluted area near an aluminum factory.

TABLE 4

Pot-Cultures of Winter Rye (1968)
Relation of Type of Fertilizer to F^- and B Accumulation in Leaves

Tested Fertilizers	Fluoride ppm (dry)	Boron ppm (dry)
1. Without fertilizer	6, 0	10, 0
2. Calcium phosphate + KCl	9, 0	13, 7
3. Combined PK 13-26	7, 0	10, 0
4. Combined PKB 13-26-0, 35	34, 5	20, 0
5. Combined NPK 5-8-15	6, 0	13, 7
6. Combined NPKB 5-8-15-0, 35	51, 0	28, 7
7. Combined NPK 6-13-11	5, 0	10, 0
8. Combined NPKB 6-13-10-0, 34	55, 5	40, 0
9. Fertilizer NPK 13-13-21	6, 0	10, 0
10. Fertilizer NPKB 13-13-21-0, 2	7, 0	20, 0
11. Fertilizer NPK 13-13-27	7, 0	12, 5
12. Fertilizer NPKB 13-9-20-0, 12	9, 0	15, 0
13. Fertilizer NPK 9-9-18	10, 0	13, 7
14. Fertilizer NPKB 10-10-18-0, 3	8, 0	20, 0
15. Fertilizer NPKB 12-12-17-0, 1	10, 0	13, 7

"Combined" boron-containing compounds are underlined.

Greenhouse and Laboratory Experiments

Since in the field tests only the special combined boron-containing PKB fertilizer accounted for a marked F^- accumulation in plant tissues, we investigated 13 combined or mixed commercial fertilizers which are commonly used in Switzerland. These experiments were performed on winter rye in pot cultures.

Only 3 of the 13 fertilizers showed the above-mentioned reaction. They were the combined boron-containing type, namely PKB 13-26-0, 35, NPKB 5- 8-15-0, 35 and 6-13-10-0, 34. Mixed boron-containing fertilizers or combined fertilizers without boron (Table 4) failed to accumulate excess fluoride.

The penetration of F^- into plant tissues is obviously proportional to the amount of combined boron-containing fertilizer used in the cultures. A pot-culture experiment on winter barley proved that increasing proportions (from 0 up to 100%) of the combined NPKB boron-containing fertilizer in a NPK fertilizer without boron caused a progressive and parallel accumulation of F^- in the dry matter of barley leaves (Table 5).

TABLE 5

Pot-Cultures of Winter Barley
Relation of Combined Boron Fertilizers on F^- and B Penetration to Leaves

Type of Fertilizer	Fertilizer Ratio (a) with Boron %	(b) without Boron %	Leaf Content Fluoride ppm	Boron ppm
(a) NPKB 5-8-15-0, 35	0	100	23, 0	10. 0
(b) NPK 5-8-15	10	90	116, 0	18, 7
	25	75	237, 0	25, 0
	50	50	499, 5	60. 0
	75	25	807, 0	80, 0
	100	0	945, 0	80, 0
(a) NPKB 6-13-10-0, 34	0	100	9, 0	12, 2
(b) NPK 6-13-11	10	90	69, 5	18, 7
	25	75	153, 0	21, 2
	50	50	358, 0	45, 0
	75	25	508, 0	45, 0
	100	0	822, 0	45, 0

In order to determine the mode of action of the above-mentioned fertilizers on F^- accumulation in the cultures, several tests were made in a greenhouse with some commercial (technically improved) fluoride salts and two of the above-mentioned combined boron-containing fertilizers. Table 6 illustrates the striking analogy of the influence of the above-mentioned special fertilizers and the complex salt potassium fluoborate (KBF_4). Analysis of the combined boron-containing fertilizers proved that a high proportion of F^- in the form of fluoborate, namely 57% of the total F^- content of fertilizer PKB 13-26-0, 35 is indeed present as fluoborate. The total amount of fluoborate in this fertilizer is 1, 16%.

TABLE 6

Pot-Cultures of Goose-foot (Chenopodium)
F^- Penetration into Tissues Under the Influence of F^- Containing Salts Applied to the Soil

Treatment		Fluoride Content ppm (dry) Roots	Stems	Leaves
1. Without Treatment	(a)	76,0	8,0	24,0
2. Sodium Fluoride (NaF)	(a)	207,0	16,0	27,0
3. Sodium Fluoride + Boric Acid ($NaF + H_3BO_3$)	(a)	171,0	14,0	10,0
	(b)	87,0	11,0	14,0
4. Ammonium Fluosilicate ($(NH4)_2SiF_6$)	(a)	215,0	18,0	28,0
	(b)	163,0	26,0	14.0
5. Ammonium Fluosilicate + Boric Acid ($(NH_4)2SiF_6 + H_3BO_3$)	(a)	103,0	19,0	19,0
	(b)	110,0	18,0	28,0
6. Potassium Fluoborate (KBF_4)	(a)	67,0	66,0	475,0
	(b)	155,0	241,0	2365,0
7. Combined Fertilizer without Boron PK 13-26	(a)	70,0	16,0	33,0
	(b)	69,0	19,0	40,0
8. Combined Boron-containing Fertilizer PKB 13-26-0,35	(a)	62,0	129,0	726,0
	(b)	124,0	166,0	2196,0

(a) Temperature, humidity and lighting normal.
(b) Strong insulation, high temperatures and low relative humidity.

TABLE 7

Penetration of F and B into Stems, Grains and Leaves of Beans (Pot-Cultures)
Effect of Washing Leaves on their F and B Content

Type of Fertilizer	Fluoride ppm (dry) Stems	Grains	Leaves Not Washed	Leaves Washed	Boron ppm (dry) Stems	Grains	Leaves Not Washed	Leaves Washed
1. Without fertilizer	7,0	6,0	9,0	8,0	15,0	16,2	21,2	21,2
2. Superphosphate + KCl	6,0	6,0	9,0	6,0	22,5	30,0	22,5	30,0
3. Super + KCl + Borax	6,5	6,0	7,0	6,0	18,7	25,0	140,0	180,0
4. Superphosphate + K_2SO_4	7,0	6,0	10,0	5,5	12,5	13,7	13,7	13,7
5. Super + K_2SO_4 + Borax	5,0	5,0	6,0	5,5	35,0	15,0	223,5	200,0
6. Combined fertilizer PK	7,0	6,0	11,0	7,0	12,5	12,5	16,2	16,2
7, Combined fertilizer PKB	6,0	5,5	55,0	43,5	17,5	17,5	110,0	110,0
8. Mixed fertilizer PKMg	6,0	6,0	5,0	6,0	11,2	10,0	15,0	16,2
9. Mixed fertilizer PKMgB	4,0	4,0	10,0	6,0	11,2	15,0	200,0	190,0

Experiments on beans conducted in a greenhouse with several fertilizers including the special combined boron-containing fertilizer PKB 13-26-0, 35 proved that F^- could be partially eliminated by washing the leaves with water, whereas the boron content of the same tissues remained unchanged by washing (Table 7). Therefore, F^- must have penetrated the plant through the roots. Fluoride in plant tissues must be fairly water-soluble. This experiment further indicates that a part of F^- present inside the plant tissue might be eliminated when the leaves are being washed with water in order to carry away dust deposits. Our observations therefore agree with those of Venkateswarlu et al., who found that F^- is mainly localized in the extracellular spaces.

Summary

In Switzerland's Rhône Valley, apricot orchards and vineyards exposed to F^- emissions from aluminum and phosphate fertilizer factories displayed typical fluoride-induced necroses of the foliage. Up to 600 ppm F^- in dry matter was found in the plant tissue. The damage was limited to a few strictly circumscribed, individually owned parcels of land.

Experiments upon the land as well as in pot-cultures showed that the high F^- content in the plants resulted from certain fertilizers - the end product of a special factory process. These boron-containing combined fertilizers were obtained by direct reaction of sulphuric acid or nitric acid upon the raw products. A particular chemical combination containing fluorine and boron is formed in the fertilizer during the manufacturing process. The action of this special combination is similar to that of potassium fluorborate (KBF_4).

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BIOLOGICAL EFFECT OF FLUORIDE ON PLANTS

by

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In pursuance of investigations by Bovay et al. (1-4) pertaining to fluoride damage to orchards and vineyards in Switzerland, we studied the action of several F^- compounds by means of hydroponics.

We supplemented the nutrient solution with 1 milli-equivalent gram of fluoride/liter (meqg/l) of NaF, $(NH_4)_2SiF_6$, K_2SiF_6, KBF_4. Boron was added to these treatments in doses of 0.01 meqg/l.

Our plant material was seedlings of Prunus armeniaca, Prunus cerasifera, Vitis vinifera and Chenopodium album, with approximately twenty leaves on each plant. The seedlings were maintained 4 to 8 days in the nutrient solution (5). We also employed a 24 hour test with leaves of P. armeniaca, collected from an adult tree and placed in an Erlenmeyer flask containing different F^- compounds in buffer solution.

After drying the plant material for 16 hours at 60°C and then for 1 hour at 110°C, the sample was calcinated with $Ca(OH)_2$. Fluorine was then distilled off as H_2SiF_6 and titrated with $Th(NO_3)_4$ (6, 7).

Damage to Leaves: Table 1 shows the F^- content in leaves of Prunus armeniaca, following 24 hours' growth at 30°C and exposure to 10,000 lux. The nutrient solution contained 1 meqg/l F^-. A good correlation between the extent of necrosis and the leaf's total F^- content (ppm) was noted. Indeed, damage was already in evidence after a few hours of treatment. The proportion of F^-/ molecule of the 4 compounds is not sufficient to explain the greater accumulation of the total F^- present in leaves in solutions containing fluosilicate. Boron plays a spectacular role as it further enhances the expected F^- accumulation, a fact which is not understood.

The boron content remains constant and equal to that in the leaves of the control plant. Greater uptake of boron by leaves takes place only when the boron ion is a constituent of the F^- containing molecule in seedlings of Prunus armeniaca (Table 2).

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TABLE 1

Contents of Fluorine (ppm F^-) in Leaves in Relation to Biological Toxicity

		Control	NaF	NaF + H_3BO_3	KBF_4	$(NH_4)_2SiF_6$	$(NH_4)_2SiF_6$ + H_3BO_3
Prunus * (after 1 day)	ppm F^-	8	19	35	31	77	88
	%N	0	2	5	7	28	62
Prunus ** (9days)	ppm F^-	25	149	161	148	174	181
	%N	1	45	72	32	80	78
Vitis ** (7 days)	ppm F^-	15	84	86	129	319	274
	%N	0	8	6	7	12	51
ChenopodiumII (9days)	ppm F^-	17	194	353	537	1013	562
	%N	0	13	45	8	88	72

*Leaf Test **Hydroponic Experiment

%N = Damage to leaf expressed as % of normal (N) leaf surface.

TABLE 2

Fluorine (ppm F^-) and Boron (ppmB) in Leaves of Prunus Armeniaca

Control	8	41,4	0	24	41,3	1
NaF	19	38,6	2	168	40,2	35
$(NH_4)_2SiF_6$	77	38,1	28	197	41,6	60
NaF + H_3BO_3	35	40,7	5	161	39,4	67
$(NH_4)_2SiF_6$	88	39,9	62	245		64
+ H_3BO_3	88	39,9	62	245	43,4	64
KBF_4	31	45,9	7	185	79,9	6

Initial concentration in nutrient solution.
Fluorine: 10^{-3}N, Boron: 10^{-5}N

The changes which are typical of fluorosis as reported by Bovay (1) on trees are illustrated in Fig. 1. This observation may be of practical importance when leaf samples are chosen from orchards. Both in leaf tests and in hydroponics, boron's action is the same: It raises the content of F^- (Table 1).

Fig. 1

Seedling of Prunus armeniaca growing in soilless culture after 6 days contact with 1 meqg/l of $(NH_4)_2SiF_6$. Lower leaves dry, the upper ones chlorotic. Good examples of necrosis in center.

From the above results we can conclude:

1. Fluosilicates penetrate into plants more readily than the other F^- compounds employed in this study.

2. Boron enhances the accumulation of F^- provided that it is not a part of the fluorine molecule.

3. Fluoborate is less toxic than the other F^- compounds.

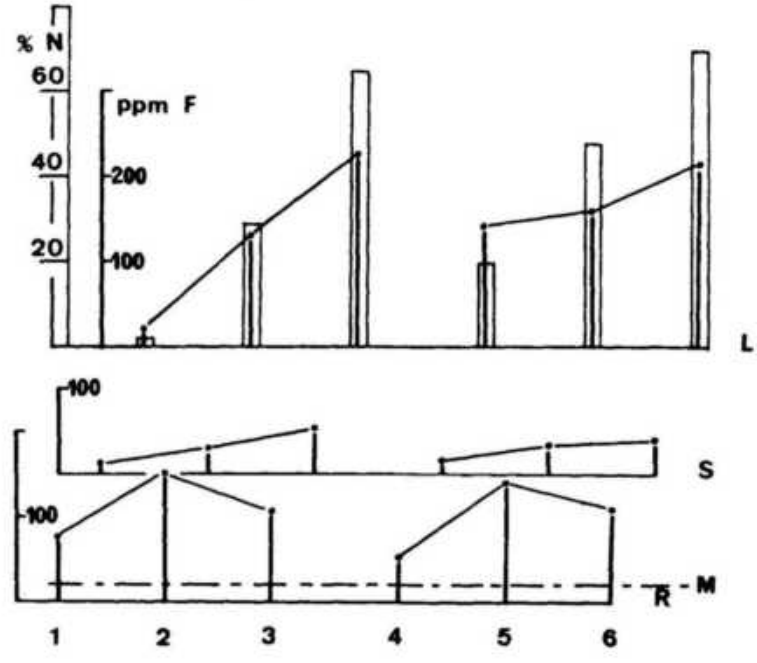
Similar results were obtained with other plant material, i. e. Vitis vinifera and Chenopodium album (Table 1), an observation which suggests that this phenomenon is universal in plant life.

Fluoride Uptake

Fig. 2 demonstrates the effect of the chemical nature of F^- salts upon F^- uptake in plants and upon the translocation and accumulation of F^- in the leaves. The degree of leaf damage by the different F^- compounds is expressed again in % N which indicates the percentage of necrotic leaf surface.

Fig. 2

Average F^- Content (ppm) in Roots (R), Stems (S) and Leaves (L). Soil-less Culture



Necrosis of leaves is expressed in % of the leaf's surface (%N)
M represents the same initial F^- concentration in the nutrient solution, which contains different F^- salts: 1 control; 2 NaF; 3 $(NH_4)_2SiF_6$; 4 KBF_4; 5 $NaF + H_3BO_3$; 6 $(NH_4)_2SiF_6 + H_3BO_3$.

Our results can be summarized as follows:

1. Fluorine accumulation in leaves depends on the kind of F^- salts absorbed by the plant.

2. The magnitude of F^- accumulation in leaves is dependent on the rate of its translocation in the stem. Fluoride is translocated more slowly than the other ions employed in this study.

3. The presence of boron increased F^- accumulation in leaves.

4. The F^- content in leaves correlates with the extent of leaf necrosis except in the case of KBF_4, which appears to be less toxic than other fluoride compounds.

*The buffered medium does not contain boron other than the amount added: 0.01 meq/l.

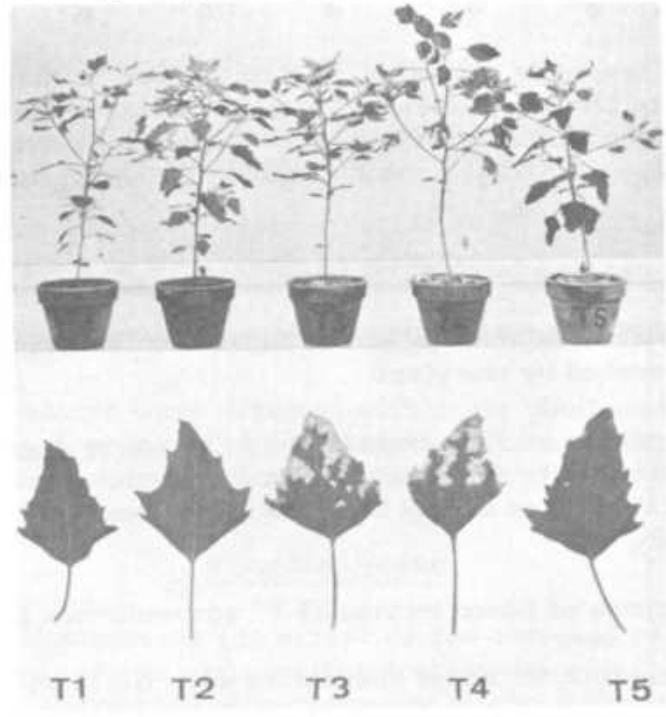
Table 2 shows the same boron content of leaves in the leaf test* and the soilless culture.

Thus F^- accumulation and leaf damage due to F^- depend upon the chemical nature of the F^- compound. If an average content of boron of 40 ppm is applied, the difference after treatment with KBF_4 corresponds quite well to the amount of boron which would be absorbed as BF_4^- complex. By substracting the F and B content of the control plant from the value of BF_4^- in the treated plant, a $\frac{F}{B}$ ratio = 4 is obtained. This correlation proves that F^- moves toward the leaves as BF_4^-. This observation is important because some fertilizers contain KBF_4.

Our recent work about the absorption of F^- was conducted mainly with such salts as NaF and KF (8), but it seems that the fluorine ion is more readily available to the plant in other chemical compounds such as fluoroborate and fluosilicate which are taken up by plants through commercial fertilizers or even through air pollution (8).

Fig. 3

Leaves and Seedlings of Chenopodium album growing in a poor soil (pH 8, 2).



Additions: T1, : control; T2: 600 mg/plant of fertilizer PK 13/26; T3: 600 mg/plant of PKB 13/26/0, 35; T4: 6 ml/plant of KBF_4 10^{-1}N; T5: 6 ml/plant of K_2SiF_6 10^{-1}N.

In the last experiment we employed a poor soil from Valais instead of hydroponics. The test plant was Chenopodium album. In normal cultures we noted again the typical symptoms of fluorosis (Fig. 3) which we had observed previously in Fig. 1.

Here, however, treatment with fluosilicate was found to be less toxic in this experiment than fluoborate in pure form or in fertilizer.

In explanation of this fact, we must consider that the hydrolysis of KBF_4 to BF_3OH^- and HF which occurs in an aqueous solution proceeds more slowly (pK 2, 6) than hydrolysis of SiF_6^{--} which produces H_2SiO_3 and 6 F^- for 3 H^+ (8). The excess F^- derived from SiF_6^{--} or from fluorides by its great solvatation constitutes a larger ion than the small HF (pK 3, 2).

The high pH of the soil or the solution and the presence of other ions such as Ca^{++} are important factors involved in this process. In view of the tendency of F^- to form F^- complexes, the exact chemical nature of the F^- compound which acts upon the site in an organism would be interesting to explore.

According to Venkateswarlu et al. (9) only a small quantity of F^- is localized intracellularly. More recent research by Treshow and Harner (10) indicates the absence of necrosis after fumigation with HF. We must also consider the possibility of paradoxical effect to which Schatz and Martin (11) have drawn attention.

The role of boron is currently under further investigation.

Summary

1. Fluosilicates penetrate into plants more readily than the other fluoride compounds employed in this study.

2. Boron enhances the accumulation of F^-, provided that it is not a part of the fluorine molecule.

3. Fluoborate is less toxic than the other F^- compounds.

Acknowledgements

The author expresses his appreciation to Dr. J. P. Quinche and R. Zuber for dosages respectively of boron and fluoride.

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Correction:

In the article "Interaction of Fluorine with Serum Albumin" in Vol. 2, April 1969, the names of two co-authors were inadvertently omitted. The authors are: C. Mangoni di S. Stefano, F. Gombos, M. Brunese and M. Ruggiero.

MECHANISM OF ACTION OF FLUORIDE IN REDUCING DENTAL CARIES

by

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Several different mechanisms have been suggested for the action of fluoride in caries. Current evidence suggests that all may play a part but their relative importance is uncertain.

Effect of Solubility of Enamel Crystals

Hydroxyapatite, the main constituent of bones and teeth, has a great afinity for F^- which exchanges with the hydroxyl ion to form fluorapatite. The latter has been regarded as a less soluble crystal. It is known that most of the F^- in the body is concentrated by the calcified tissues, especially where they have access to tissue fluid, e.g. the endosteal surface of bone and the outer surface of enamel. When teeth or powdered enamel is shaken with solutions of fluorides in vitro, the introduction of F^- reduces the solubility-rate of the tooth in acids. This simple experiment has such dramatic results that many have assumed that reduction in solubility explains the clinical effect in caries. There have been four attempts to test the theory by comparing the solubilities of teeth from people living with or without F^- in their water (1). All have given general support to the theory because the teeth from the F^- areas were found on the average to be less soluble than the controls, but the effect is small and not always statistically significant. Although it is a reasonable assumption that reductions in enamel solubility do reduce caries, it has never been proved.

The largest effect on solubility has been found in enamel which is already attacked by early caries. In incipient lesions, the enamel becomes permeable and F^- can enter more readily: also, a low pH is known to favor F^- uptake by apatite. The pH in carious areas is lower than that of the mouth as a whole. Enamel already showing carious changes may be 20% less soluble than intact enamel in the same teeth (2).

How Fluoride Reduces Solubility

Recent thinking, while accepting that F^- may reduce the solubility of enamel, has raised doubts as to whether this can be explained on the basis that fluorapatite is less soluble than hydroxyapatite. From recent results it is not certain that this is true (3). Also, it is pointed out that even in outer enamel with the highest recorded F^- level, only one hydroxyl in fifty

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is replaced by F^-. It is doubtful whether this small substitution could affect solubility significantly even if the fluorapatite is eventually proved to be less soluble.

Other mechanisms affecting solubility have been suggested namely, that F^- increases the size of apatite crystals and reduces the number of crystal defects. Both effects are known to occur in bone under the influence of F^- (4). Whether this applies to enamel is less certain (5). Another related suggestion is that F^- competes with carbonate during apatite formation. Some evidence supports the theory that high carbonate concentration in enamel lowers its resistance to caries, but it has been challenged.

Another difficulty in the solubility theory is that ions e.g. zinc, copper, iron and cadmium are known to reduce the solubility of enamel in vitro, but none of these have been shown to influence caries. Fluoride seems unique in its caries-reducing effect but not unique in its effect on solubility. A suggested solution of this difficulty is that F^- reduces solubility in some unique manner. The following reaction may throw light on this possibility. If phosphoric acid is titrated against lime water until a precipitate of calcium phosphate forms, and the mixture is allowed to stand for some days in the presence of F^-, the pH of the supernatant and the composition of the precipitate change (6). One interpretation is that the first precipitate to form is $CaHPO_4$ which, in the presence of F^-, gradually changes into apatite. It is thought that apatite is the most stable and least soluble crystal which calcium phosphate can form. This theory suggests that the action of fluoride in caries is its unique ability to encourage the formation of this substance rather than other less stable crystal forms. This reaction is unlikely to be important during enamel formation because, even in the absence of F^- in the water, the chief crystal of enamel is apatite. Therefore the theory leaves unexplained the reported lower solubility of intact enamel in areas high in water-borne F^-. It could occur, however, in a caries lesion, which is now thought to go through phases of softening and decalcification alternating with rehardening and reprecipitation of some of the apatite dissolved. Fluoride may work on solubility in two ways: (a) modify the enamel during formation so that it is less soluble (due to a higher proportion of fluorapatite or to larger, more perfect crystals?) (b) encourage reformation of crystals in the rehardening phase of caries and increase the tendency for the crystals to be in the most stable and least soluble form (apatite). The final answer is still uncertain.

Morphological Effects

Animal experiments have shown that F^- ingestion during tooth formation leads to smaller teeth with shallower and more rounded fissures (7, 8). Similar observations have been made on human teeth in Hastings, New Zealand (9) although Wallenius (10) found that 1 ppm of F^- was associated with a 1.7% increase in the size of the teeth.

These differences would be expected to lead to less food stagnation, and greater access of saliva which is both alkaline and highly buffered when vigorously stimulated. The effects are very small, however, and it is impossible to decide whether these observed differences would have a significant effect on caries.

Anti-enzymic Effects of Fluoride

The well-known effect of F^- as an enzyme inhibitor raises the question of whether this action is exerted on the oral bacteria responsible for caries. It has been known for years that many oral bacteria are sensitive to F^- but the concentrations needed for marked inhibition (10 ppm) seemed much higher than the concentration present in the mouth. Saliva and plasma were thought to contain about 0.1 ppm F^- but recent analytical refinements suggest that the concentration is as low as 0.02 ppm (11). The situation was transformed when it was discovered that the dental plaque (the layer of modified salivary protein and bacteria on the tooth surface) contained surprisingly high concentrations which were affected by the F^- of drinking water. Dawes, Jenkins, Lardwick and Leach (12) reported an average of 25 ppm in plaques from children in a "low fluoride town" and 47 ppm where the water contained 2 ppm F^- although the range of variation was very large. These concentrations were adequate to inhibit but it was clear that most of this F^- must be bound in some way, otherwise it would not accumulate. The question now arises: Is this F^- bound in a form in which it can inhibit bacterial enzymes? Recent work supports strongly the conclusion that much of the F^- is contained within the bacteria in a form which does reduce acid production. Evidence that plaques from a "high fluoride town" produced less acid when standing with sugar than comparable plaques from a "low fluoride town" (13), has now been confirmed in Newcastle which introduced fluoridation in October 1968: Less acid is now produced here than in plaques from a neighboring area without F^- although samples of both were identical before fluoridation. Oral bacteria have been cultured on media containing varying concentrations of F^-. After thorough washing, the bacteria have been found to contain high concentrations of F^- and to show inhibition of acid production proportional to their F^- concentration.

In vitro experiments have shown that the synthesis by oral bacteria of intracellular polysaccharides from glucose is sensitive to F^- (14, 15). These stores of polysaccharide may be of importance in prolonging acid production in the plaque beyond the time when sugar is available during eating. If this is so, then one action of F^- might be to reduce the storage and therefore the duration of the fall in pH.

The high F^- concentration in plaque raises the question of whether it is wise to remove it by tooth-brushing. The answer seems to be that in spite of its F^- content the overall effect of plaque is to damage the tooth and promote inflammation of the gingivae. Its removal therefore seems desirable.

The probable source of plaque F^- is saliva. The alternative possibilities are the enamel surface (known to be very high in F^-, e.g. 1000 ppm)

or food and drinks. If F^- were constantly diffusing from enamel into plaque it would be expected that the F^- on the outer surface would gradually diminish with age. Most data, however, suggest that it rises. Furthermore, because F^- present in apatite is tightly bound it is most unlikely to be released. The F^- concentration of food is low and, even after chewing and mixing with saliva, unpublished experiments in the author's laboratory have shown that the F^- concentration in the bolus rarely exceeds 0.5 ppm; usually it is much lower. Although certain drinks (tea and beer) contain higher concentrations, their contact with plaque is slight and of short duration. Current evidence suggests that the slow but continuous flow of saliva over the plaque is the most likely source of its F^-

Conclusion

The evidence presented suggests that F^- probably reduces caries because of a unique combination of properties all of which may play some part. The question is not which theory is right, but what is the relative importance of the various effects which F^- seems able to exert? As mentioned previously, F^- must be ingested during enamel formation for its full effect but approximately one third of its total action occurs if F^- is received later. This implies that the greater part of the effect involves tooth structure, presumably depending on solubility or morphological effects. The F^- taken up by early caries and the F^- entering plaque presumably account for the smaller post-eruptive effects although some F^- is incorporated into enamel after eruption. It seems likely that the surprisingly high F^- in plaque in areas without F^- in the water and the far from negligible concentration also in all teeth probably exerts some restraining effect on caries. In other words, the total value of F^- cannot be fully measured by comparing the caries incidence in towns with and without F^- in the water. The small quantities in food and drink which provide the F^- level in teeth without F^- in the water and saliva, probably have an important protective role which cannot be measured as there is no means of knowing how much caries would occur in the complete absence of fluoride. Almost all ideas relating to the mode of action of F^- are based upon the acid theory of caries. Should it be shown that the acid theory is wrong then the mode of action of F^- would have to be reconsidered. The influence of F^- on morphology is probably the only idea which would remain relevant should this occur.

Summary

Three main theories have been suggested to explain the action of F^- in reducing dental caries. (1) Fluoride reduces the solubility of the tooth enamel possibly by increasing the proportion of fluorapatite, by increasing the size of the crystals, or by reducing the concentration of carbonate or in all these ways. (2) Fluoride ingestion during tooth formation makes the tooth more rounded with wider fissures so that it would be expected to be more self-cleansing. (3) Many bacteria are able to concentrate F^- from low concentrations which results in an inhibition of their metabolism and

such an inhibition can occur in the bacteria on the surface of the teeth even with F^- in physiological concentrations. The effectiveness of F^- in caries probably results from its possession of this unique combination of properties.

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Supplement to the Article

REVERSIBLE INHIBITION OF PLATELET FUNCTION BY FLUORIDE COMPOUNDS
(Vol. II No. 3 - Pages 181-182 1969)

by

K. Breddin
Frankfurt, Germany

Fig. 1

Normal Spreading of Human Platelets Magnif. x 400

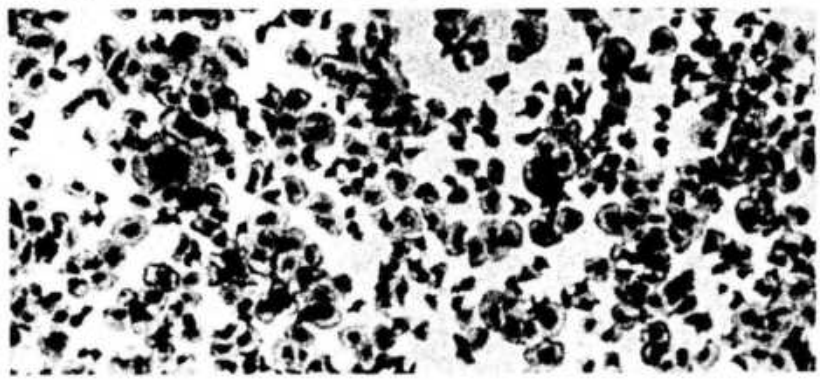
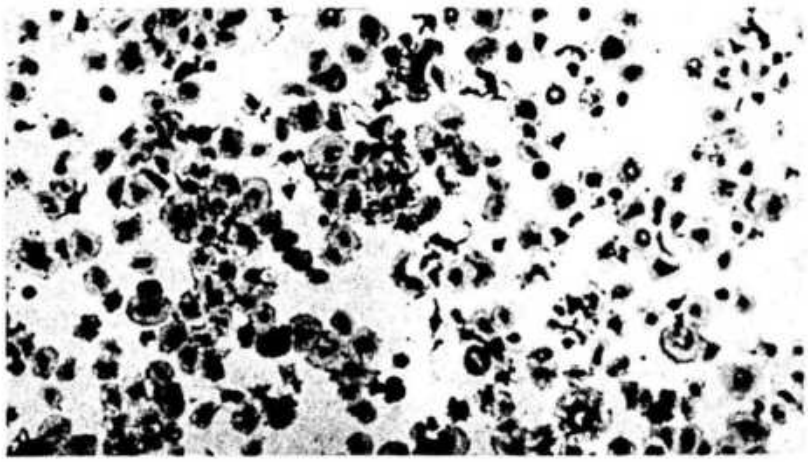


Fig. 2

Nearly Complete Inhibition of Spreading after Exposure to 40 mM NaF

Fig. 3

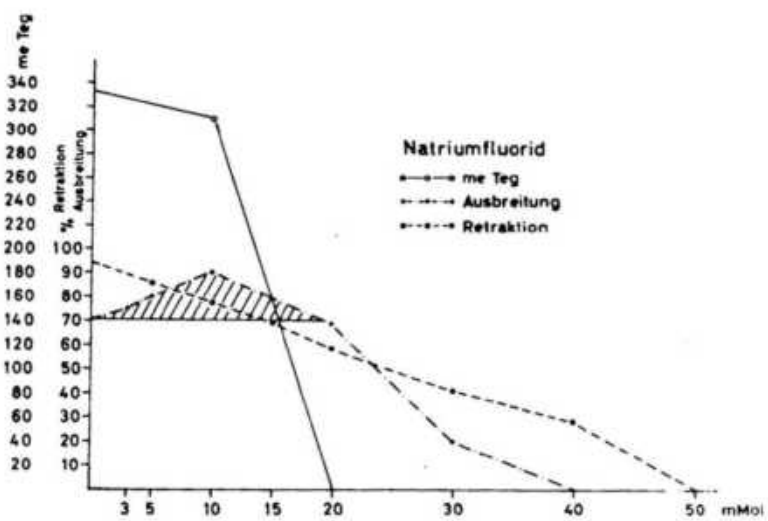
Same Platelets as in Fig. 2 After Removal of NaF by Washing and Resuspension of Platelets in Saline/Citrate Solution.



Spreading almost normal. NaF-effect on platelet function is reversible.

Fig. 4

Effect of NaF on Thrombelastogram (me Teg), on Clot Retraction and on Plateletspreading (Ausbreitung).



Low concentrations of NaF enhance platelet spreading, higher concentrations inhibit all platelet functions.

SIGNIFICANT INCREASE IN INTAKE OF FLUORIDE IN FOOD DUE TO FLUORIDATION

by

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Journal of Food Science 31 (6):941-946, 1966

(Abstract)

An assortment of canned vegetables obtained from various canneries in Southwestern Ontario, Canada, was analyzed for fluoride. Some of these firms use low-fluoride water, while others use municipal water containing 1.0 part per million (ppm) of fluoride. A modified zirconium-SPADNS procedure was employed for the fluoride analyses. They showed a standard deviation not exceeding 0.093 ppm of fluoride (F^-) per test. The authors also analyzed several beverages both before and after fluoridation of the water supply in Ottawa.

Foods and beverages processed with fluoridated water were found to contain 0.5 to 1.0 ppm of F^- with an average of 0.7 ppm. For the same items processed with low-fluoride or essentially fluoride-free water the values were 0.1 to 0.3 ppm (average 0.2 ppm). The increase in fluoride content was thus 3- to 5- fold, with an average fluoride increment of 0.5 ppm on each item for an adult on a typical diet. The average daily intake of F^- from foods processed with fluoridated water was calculated to be between 1.0 and 2.0 milligrams.

Seven male members of the research staff recorded their daily fluid intake from all sources. Some consumed up to 3 times more liquids than their colleagues, even when the results were expressed on a "body-weight" basis. Total intake of liquid was found to be from 1 to 3 liters per day depending on the individual (average 2 liters). Where these beverages were prepared or processed with fluoridated water (1.0 ppm) - they added from 0.9 to 3.0 milligrams of fluoride per day to the 1.0 to 2.0 milligrams ingested with food.

Thus, total intake of fluoride was estimated to be between 2 and 5 milligrams per day. The authors emphasized that this estimate is based on a winter survey of sedentary "indoor" workers, and conclude that fluid intake would be much higher by active "outdoor" workers under summer conditions. The estimated total intake of between 2 and 5 milligrams of fluoride per day, therefore, represents a minimum value.

The authors point to the sparcity of data on current levels of fluoride in food. They suggest that "the total fluoride intake by individuals (not groups as a whole) in a fluoridated community should, therefore, be monitored."

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FLUORIDE CONTENT IN FEED COMPONENTS AND IN ORGANS OF ROOSTERS IN ACUTE FLUOROSIS

by

L. Slesinger and J. Tusl

(Abstract)

Fifty young roosters were fed 200 mg/kg of sodium fluoride twice a day for 24 hours. They developed gastro-enteritis with marked edema of the mucosa of the stomach and the upper bowels, subcutaneous edema, hepatomegaly and atrophy of the pancreas. Twenty young roosters served as controls.

The fluoride content of certain food constituents was unusually high, namely 424.5 ppm in bone meal, precipitated bone substance 5.2 ppm, 120.4 ppm in fish meal and 11,500 ppm in superphosphate. Other constituents of the feed in which fluoride ranged between 16 and 25 ppm were beet sugar and a commercially mixed feed product.

At autopsy the organs contained the following F^- levels:

Organ	Control	Experiment
Bile	0.24 ± 0.07	1.16 ± 0.6
Erythrocytes	0.16 ± 0.08	3.37 ± 3.37
Liver	0.4 ± 0.11	9.9 ± 1.6
Heart	1.28 ± 0.1	10.06 ± 2.1
Bones	289.0 ±36.2	501.5 ±78.6
Beak	34.25 ± 0.7	36.94 ± 1.8
Feathers	6.5 ± 0.8	50.5 ±22.6
Blood Plasma	0.33 ± 0.05	2.9 ± 0.22

The greatest amount of F^- accumulates in the liver, in the heart and in the feathers. Fluoride levels of organs of the control animals were unusually high e.g. 289.0 ppm in bones, 6.5 in feathers, 1.28 in the heart. This is attributed to the high F^- content of the rations which all animals received.

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