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FLUORIDE

Quarterly Reports

Issued by

THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH

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FLUORIDE AND CANCER

Some of the most effective anti-cancer drugs are fluoride containing compounds, such as fluorouracil; others, especially certain inorganic salts, tend to stimulate the growth of a neoplasm.

This paradoxical action of fluoride is well established in other phases of fluoride research. It is illustrated by Berman on page 153 of this issue with respect to fluoride's inotropic effect on the heart muscle.

When the fluoride ion is incorporated into the molecule of dimethylaminoazobenzene, a powerful carcinogen, the cancer-producing ability of this compound is enhanced seven times as much as by substitution with other halogens(1). This occurs even in the 4-position in which all other substituents reduce or eliminate the carcinogenicity.

Among inorganic fluoride compounds, beryllium fluoride and cerium fluoride have produced experimental tumors. In these compounds the role of the fluoride ion is not clear.

Beryllium fluoride is one of the most potent neoplastogenic of all beryllium compounds when inhaled in long-term experiments in extremely small doses (2). According to Cember (3) radioactive cerium fluoride produces cancer in rats by intratracheal insufflation. The radioactivity of this compound rather than the fluoride component appears to be primarily responsible for this action. Whether or not the fluoride ion acts as a synergist was not explored by the authors.

In a Canadian fluorospar mining community, St. Lawrence, Newfoundland, during a 10 year period, 23 of 51 deaths were due to lung cancer among employees with one or more years of underground mining experience (4). The dust in the mine contained, on an average, 62% fluorspar (CaF_2) and 19% quartz. The investigators de Villers and Windish (4) attributed the cancer to radiation with an average alpha energy between 2.5 and 10 times the previous suggested working level of 1.3×10^5 Mev per liter of air. However, in 9 of 60 locations of the mine, where tests for fluoride were made, the average concentration exceeded the official threshold limit values of 2.5 mg of F^-/m^3 . Here too, the role of the fluoride ion was not investigated.

In the environs of two aluminum factories a Russian team, Litvinov et al. (this issue, p. 189) encountered a higher incidence of cancer mortality than in a control area seven kilometers distant where the air was less contaminated. Compared with the cancer mortality in Moscow, there was a substantially higher incidence in both factory areas. The authors were mainly concerned with studying the action of 3, 4-dimethylbenzanthracene. They

attributed the major part of the carcinogenic activity of the flydust to this compound. They also named HF as one of the contaminants. Since fluoride is generally recognized as one of the major air pollutants near aluminum plants, it could have considerable bearing on the findings of the Russian investigators. This question was not investigated by the authors.

More specifically, fluoride was implicated as a carcinogen in experiments by Taylor at the Clayton Foundation Biochemical Institute, University of Texas. He had been testing various chemicals added to drinking water of cancer-prone mice in order to determine whether they might delay or prevent the onset of cancer. The mice which were given a sodium fluoride solution at a concentration of 1 ppm in their drinking water developed cancer at an earlier age than the control animals maintained on fluoride-free water.

Taylor's preliminary work was challenged on the basis that the bone meal content of the mice's ration contained a relatively high concentration of fluoride. In subsequent experiments, Taylor eliminated this factor altogether. He carried out a total of 12 experiments involving 645 mice (5). The data indicated that drinking water containing as little as 1 ppm of fluoride shortened the life span of cancer-prone mice by an average of 9%, regardless of whether they died of cancer or another disease.

Research by two other investigating teams seems to contradict Taylor's findings:

In 1953, Fleming (6) of the Yale University School of Medicine, transplanted a tumor known as sarcoma 37 into young adult mice and guinea pigs. To one group, he administered NaF in concentrations of 20 ppm in drinking water. He injected 1,000 ppm of NaF intraperitoneally into another group. A control group bearing tumor transplants received no injections of fluoride. The daily dose was approximately 0.05 cc of the fluoride solution for the mice; a larger dose, namely 0.5 cc, for the guinea pigs because the latter are larger in size. The fluoride-treated animals bearing the tumor transplants lived longer and lost less weight than the control animals. Growth of the tumors was inhibited by fluoride.

Fleming's animals were subjected to the drug for a few weeks only. Therefore, his work does not precisely parallel that of Taylor whose mice received minute amounts of fluoride daily for a lifetime. Moreover, it is generally recognized that high doses of NaF tend to inhibit the growth of cancer implants as do many other compounds at a dosage toxic to the animal.

In the other experiments Bittner and Armstrong (7), used 36 mice that were given 5 ppm fluoride in their drinking water; a second group of 34 mice received 10 ppm. Thirty-one mice served as controls. The authors reported no significant differences in the age at which cancer developed. Only an abstract of this work was published and the full details are unavailable. The authors concluded that fluoride has no effect on longevity

of mice. The abstract shows that the experimental animals were young; their initial weights were 18 to 25 grams; the tests were continued for less than 10 months (294 days). In order to test the effect of a compound on longevity, it is obviously necessary to keep the experiment going until the animals die of disease or of old age and to keep records of weekly or monthly deaths for each group. Moreover the number of mice, included in each group as reported in the abstract, was far too small to reveal a significant effect of fluoridated drinking water on the entire life span.

In 1964 Taylor reported studies on the effect of sodium bromide upon cancer tissue (8). He observed that sodium bromide in low concentrations stimulated the growth of cancer tissue which was cultivated in eggs as well as that transplanted into mice. Upon comparing the action of sodium bromide with that of two other halides, sodium iodide and sodium fluoride, he found that fluoride's carcinogenic effect was even more pronounced than that of the other two. Sodium fluoride stimulated cancer growth at even lower concentrations than sodium bromide (9).

These observations (9) were based on 54 experiments with 991 mice bearing transplanted tumors and 58 experiments with 1817 eggs implanted with mouse cancer tissue. The statistical significance of the results would appear to be beyond question. At very low concentrations sodium fluoride accelerated the growth of mouse cancer tissue regardless of whether it was cultivated in mice or in embryonated eggs. Stimulation of cancer growth occurred regardless of whether the mice received sodium fluoride in their drinking water, whether they received it by subdermal injection or whether it was added to a cancer tissue suspension before inoculation. Likewise in eggs, the growth-stimulating effect occurred when sodium fluoride was added to the cancer suspension before inoculation into the yolk sac and when sodium fluoride was introduced over the chick membranes of eggs containing an established growth of cancer tissue.

As an interesting sidelight, Taylor noted that when the mouse cancer tissue was cultivated in egg, the cancer grew as an independent body in the yolk sac but shared the circulatory system of the supporting chick embryo. The introduction of fluoride stimulated the growth, not only of the cancer but, of the associated chick embryo as well. This result was uniform and occurred reproducibly in hundreds of tests. On the other hand, fluoride failed to affect growth of the chick embryo when the eggs were not inoculated with cancer. In other words, the acceleration of the growth of these embryos was mediated through the effect of the fluoride on the cancer tissue.

Some of the contradictory results reported above are undoubtedly due to fluoride's well-known paradoxical biological action: In a highly dilute solution fluoride appears to stimulate tumor growth, when more concentrated to inhibit it. The concentration of fluoride added to a suspension of cancer cells before they are inoculated into eggs or mice is much higher than when present in blood plasma where there is a constant tendency toward homeostasis.

The same is true when cancer cells are cultivated in vitro, addition of fluoride to the suspension has been shown to inhibit the growth of cells. Berry and Trillwood, as reviewed in this issue page 157, observed growth retardation of HeLa cells in a fluoride medium at a concentration of 1/10 ppm (10), DeJong at 4.5 ppm (11), Armstrong and Singer at 10 ppm (12). No research is available on whether or not fluoride in more minute amounts than those mentioned above will stimulate growth of cancer cells in vitro.

Other reasons for divergent results concerning the action of fluoride on cancer cells cultured outside of the body are outlined by Berry in this issue page 157. Some of the individual tumor cells undergo adaptive changes and acquire greater resistance to the drug. Furthermore, there is always the possibility of infection of the cells which inhibits cancer growth. In association with fluoride, infected cancer transplants grow much more slowly than the same transplants in control animals. Likewise, minor modifications of the culture medium as well as the strain of cancer cells may affect the results.

It thus appears that the question of the carcinogenic action of fluoride is far from being solved. The matter of its paradoxical action has not received adequate attention. The subject constitutes a fruitful area for further research.

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LIVER DAMAGE IN CHRONIC FLUORIDE INTOXICATION

Cases of chronic fluoride intoxication due to industrial hazards are rarely reported in the medical literature. The following brief case report of chronic fluoride poisoning is of special interest because of liver involvement, a rarely described feature of the disease. It was presented to the editor in a letter by D. M. Gumprecht, M.D. of Coeur d'Alene, Idaho.

On October 7, 1952, J. R. S. age 32, consulted Dr. Gumprecht because of vague pain and abdominal distress, of a few days' duration, in the region of the liver and gall bladder. It had been preceded for approximately three months by episodes of "indigestion", flatulence, loss of appetite and general malaise.

The patient had previously been in perfect health. On examination he was slightly icteric. Tenderness was elicited in the right upper quadrant, in the liver and gallbladder region. The teeth exhibited a distinctly brownish discoloration. The gallbladder X-ray showed poor concentration of the dye without evidence of stones. The patient was given a diet low in fat, bile salts and a high potency vitamin B preparation.

The patient, a waterworks employee, had been adding sodium fluoride to the water supply of a pipeline during the summer months. He had to sift out the powder before adding it to the machine because of contamination by stones and other gross impurities. His health improved after a new shipment of the chemical arrived and it was no longer necessary to sift it.

However, the vague abdominal pains and general malaise persisted. Additional X-rays of the stomach, taken in April 1953, revealed a slightly enlarged duodenal loop above the head of the pancreas. The gallbladder X-ray showed improvement in the concentration of the dye. Again there was no evidence of stones. The "indigestion" and marked general malaise continued.

On November 10th the patient experienced another episode of severe pain in the liver area with slight fever and night sweats, jaundice, anorexia, "staining of teeth" and marked general fatigue. The white blood count was slightly elevated. The symptoms subsided gradually. A consultant W. Myhre, M.D. of Spokane concurred with the diagnosis of fluoride poisoning. With increasing water demands during the summer months, the patient had to handle more fluoride which aggravated the condition.

On May 22, 1954, upon his physician's advice, the patient was transferred to an outside job and avoided all further exposure to fluoride. This change of occupation finally cleared up the disease, although occasional vague pains in the liver area tended to recur.

A legal settlement was made by the water company for damages incurred as the result of the occupational hazard.

Liver damage in chronic fluoride intoxication was reported by Fradà et al. among persons with endemic skeletal fluorosis in Northern Sicily (Fradà, G., Montesana, G. and Nalbone, G.: *Minerva Medica* 54:45-59, 1963). Another reference to liver damage in chronic fluoride poisoning was made at an air pollution District trial in an Oregon court (Reynolds Metals Company versus Paul Martin et al. in U.S. Court of Appeals, June 1958). Testimony was presented on liver damage in three individuals and on the occurrence of sudden acute abdominal episodes.

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METABOLIC STUDIES ON SKELETAL FLUOROSIS WITH A NEW APPROACH TO ITS TREATMENT (PRELIMINARY REPORT)

by

Drs. S. P. S. Teotia, K. B. Kunwar, and M. Teotia
Lucknow, India

Fluoride is deposited on the surface of the hydroxyapatite crystals. It displaces ions present on the surface (1) and also substitutes isomorphically for hydroxyl ions within the hydroxyapatite lattice. The effect of F^- may be explained by partial replacement of carbonate in bone salt (2), decrease in bone citrate (3) accompanied by a diminished solubility (4) and mobility of bone salt and improvement in crystal texture i. e. crystal size and crystal perfection (5). Conceivably these changes in bone salts might reduce the reactivity of the crystal and thereby stabilize it. Fluoride also stimulates osteoblastic activity. Excessive intake leads to osteosclerosis, periosteal bone formation and calcification of ligaments and tendons (6). The in vitro studies by Taves and Neuman (7) suggest that F^- may govern the rate of calcification by enhancing it when the phosphate concentration is low and by inhibiting it when it is high. The microscopic appearance of depressed or absent osteoclastic activity in bones of moderately affected human beings suggests that relatively low doses of fluoride may somehow depress resorption of bone (7, 8).

Crystallographic evidence indicates that the crystals in fluorotic bone have fewer defects and are of a larger size (3) than in normal bone. They are less reactive in surface exchange reactions, since larger crystals offer less surface area for a given weight of bone. It may also be assumed that changes in bone salts increase the resistance of bone to the action of parathyroid hormone.

In our experience, patients with skeletal fluorosis exhibit gross positive calcium balances. We therefore thought that the toxic effects of F^- were probably linked to calcium intake. In this case, clinical improvement should follow regimes aimed at producing calcium depletion.

In this communication we wish to report the calcium, phosphorus, magnesium and nitrogen balances in patients with skeletal fluorosis with particular reference to their treatment with a low calcium diet.

From the Wellcome Metabolic Laboratory, Department of Medicine,
King George's Medical College, Lucknow, India.

TABLE 1

Symptomatology in Twelve Patients with Skeletal Fluorosis

Case No.	Sex, Age	Duration (Years)	Walking	Skeletal Pain	Spine	Other Joints	Myasthenia	Spastic Con- stipation	Fixation of Thorax	Mottled Teeth
1	M 50	3	No	+	S ; Cervical flexion; Thora- cic K ;	Hips, knees elbows	General	+	-	Black
2	M 13	10	-	-	S ; Cervical flexion	P	Hands*	+	-	No
3	M 11	2	No	+	S ; P ; Cerv. flexion; Thora- cic K ; Genera- lized forward flexion	P ; Hips, knees el- bows	Hands*	+	+	Yellow-br brown
4	M 68	9	No	-	P ; Forward flexion defor- mity	P ; Hips knees	-	+	-	Brown
5	M 15	3	-	-	P ; S	S	Hands*	+	-	Black
6	M 36	6	-	+	Thoracic K ; S	S	Hands*	+	-	Brown
7	M 21	4	-	-	P ; S ; Limited Cerv. Movements	P	-	-	-	Brown
8	F 35	3	-	-	P ; K ; S	-	All Limbs	-	-	Brown
9	F 36	5	No	-	P ; Cervical flex- ion deformity	Hips, knees	-	-	-	No
10	F 13	3	-	-	Cerv. flexion de- formity; S	S ; P generalized	Hands*	-	-	Yellow- brown
11	F 25	6	No	+	Thoracic K ; Cerv. flexion; S	Hips, knees elbows	General	+	-	No
12	F 30	5	No	+	Cerv. flexion de- formity; K ; P	Hips, knees	Hands*	+	+	No

S = Stiffness; P= Pain; K=Kyphosis - *Unable to close fists

Materials and Methods

Twelve cases of skeletal fluorosis were studied, 7 males and 5 females. Two of the female patients also had associated nutritional osteomalacia. All were indoor patients. They belonged to a poor socio-economic strata and had spent their entire life in an endemic fluorosis area, namely the village Madheya Khan Ka Purwa in the District of Railballe, U. P. India Table 1.

Laboratory investigations performed in each case included serum calcium, serum phosphorus, serum magnesium, alkaline phosphatase, blood urea, creatinine clearance, chemical analysis of the bone ash for calcium, phosphorus, magnesium and fluoride. Skeletal and dental roentgenograms and histology of undecalcified sections of the biopsied iliac-crest bone were studied. All patients except the two with associated osteomalacia were studied for six balance periods each lasting for six days during which the patients received 900 mg Ca/day. In three cases the histology and the chemical composition of the bone, obtained from the iliac-crest, was determined again at the end of the low calcium diet therapy. The fluoride content was determined in sixteen samples of drinking water obtained from four wells situated at different locations in the endemic area.

The patients with associated osteomalacia had only three control calcium balance periods. They were not subjected to a low calcium diet which might have further exaggerated the symptoms of osteomalacia. All periods were demarcated by carmine powders. The faecal and urine samples were kept in the deep freeze until analyzed.

In three cases detailed phosphorus, magnesium and nitrogen balances were performed on a control diet. During both the 3 control weeks and the 12-24 therapy weeks, the patients were given double distilled water to drink. The calcium content of the diet was the only difference between treatment and control periods.

The fluoride content of the bone, drinking water and urine samples was determined by the procedure based upon diffusion of hydrogen fluoride as detailed by Singer and Armstrong (10).

Radiological Changes: Diagnostic radiological features were observed in each case. These included osteosclerosis of whole skeleton particularly of the spine and pelvis, irregularly outlined osteophytes, periosteal bone formation, calcification of ligaments, of muscular attachments and tendons (Fig. 1, 2). The capsule of the hip joint was often calcified with irregular lipping at the acetabulum (Fig. 3, 4).

Histopathological Data: Histology of the undecalcified sections of the bone obtained from the iliac crest by open biopsy was studied in each case. The most common findings were very thick and well formed bone

trabeculae which contained an excess of calcium. After treatment with a low calcium diet the bones showed thin trabeculae, areas of osteoid tissue and decreased calcification (Fig. 5, 6).

Dental Fluorosis: Mottled discoloration of the teeth was observed in only 8 of the 12 cases. The skeletal changes were very extensive in the 4 remaining cases, even though the teeth were not mottled. Dental radiographs revealed resorption of the alveolar bone around the roots of the teeth as a common finding. Thinning or decreased lamina dura was observed in 2 patients (Fig. 7).

Fluoride in Water Supply: Sixteen samples of drinking water were analyzed from four wells located at different places in the endemic area. They contained 10.35 - 13.5 ppm of fluoride.

Results

After 12 to 24 weeks on low calcium diets, all patients showed a striking clinical improvement. Their bone pains had lessened, they walked quickly and unsupported, the spinal ankylosis had improved, their joint movements had become almost normal and they were able to write and perform their daily tasks.

Following treatment with low calcium diets, radiographs showed decrease in bone density, increased trabeculation and dissolution of calcifications in ligaments and capsules. Skeletal roentgenograms in cases 11 and 12 with associated Vitamin-D deficient osteomalacia revealed a tri-boliolate pelvis, biconcave vertebrae, calcification of vertebral ligaments, coarse trabeculation and less bone density than in other cases.

Our laboratory findings were similar to those reported by Singh et al. (8) (Tables 2-5). They showed a rise in serum alkaline phosphatase, low urinary excretion of calcium and increased urinary excretion of phosphorus and fluoride. Balance studies revealed retention of phosphorus, magnesium, nitrogen and calcium. Skeletal X-rays were diagnostic of skeletal fluorosis in all cases. Histology of the undecalcified sections of the iliac-crest bone biopsies showed thick, well formed bone trabeculae which appeared to be hypercalcified. Chemical composition of iliac-bone revealed an excess content of calcium, magnesium and fluoride. This indicates a close relationship between bone apatite crystal and the composition of the fluids (blood serum) in which crystal formation takes place (9). The phosphorus content of the bone did not vary significantly from normal.

Discussion

On the low calcium regime, calcium balances became progressively negative in all cases. The observed increase in urinary excretion of calcium and fluoride was probably secondary to the effects of low calcium intake. The fact that in our cases of skeletal fluorosis control calcium

TABLE 2

Serum Values of Ca, P, Mg, Alkaline Phosphatase
Before and During Low Calcium Diet

Case No.	Calcium (mg%)		Phosphorus (mg%)		Alk. Phosphatase (K. A. Units)		Magnesium (mEq/L)
	Before	During	Before	During	Before	During	Before
1	10.6	10.2	4.3	3.8	67.0	47	2.20
2	11.0	10.8	3.8	4.1	98.0	93	1.90
3	12.0	10.3	4.2	4.4	65.5	46	1.78
4	9.5	9.5	3.6	4.0	24.0	22	1.70
5	11.2	11.0	3.0	3.8	18.0	16	1.95
6	9.2	9.0	4.6	4.8	36.0	35	1.37
7	10.5	10.0	4.1	4.8	42.0	34	1.54
8	9.6	9.4	3.8	4.0	17.0	17	1.81
9	10.3	9.3	4.0	4.2	22.0	20	1.60
10	9.5	9.3	3.5	3.8	11.0	13	2.25
Mean:	10.34	9.88	3.89	4.17	40.05	34.3	1.81
11	8.7	-	4.1	-	76.0	-	1.68
12	8.5	-	2.8	-	58.0	-	1.72

TABLE 3

Phosphorus, Magnesium and Nitrogen Balances in Three Patients with
Skeletal Fluorosis Before Treatment with Low Calcium Diet

Case No.	Phosphorus mg/day				Magnesium mEq/day				Nitrogen g/day			
	Intake	Urine	Stool	Balance	Intake	Urine	Stool	Balance	Intake	Urine	Stool	Balance
1	1867	1276	491	+ 100	16.0	6.2	8.2	+ 1.6	13.2	11.2	1.5	+ 0.5
2	1838	1405	257	+ 176	14.0	5.8	7.4	+ 0.8	12.1	10.0	1.2	+ 0.8
3	1837	1387	249	+ 201	16.5	6.2	8.8	+ 1.5	12.4	10.4	1.2	+ 0.8

TABLE 4

Calcium Balances (in Six-Day Period) and Urinary Fluoride Excretion
Before and During Low Calcium Diet

Case No.	Calcium Intake mg/day		Urinary Calcium mg/day		Faecal Calcium mg/day		Calcium Balance mg/day		Urinary Fluoride ppm/day	
	Before	During	Before	During	Before	During	Before	During	Before	During
1	900	169	59	76	581	150	+ 260	- 57	3.65	4.80
2	880	165	38	62	581	140	+ 261	- 37	3.30	4.60
3	890	165	50	87	283	88	+ 557	- 10	2.97	3.75
4	920	159	60	62	561	156	+ 299	- 59	3.50	3.56
5	896	160	54	74	570	142	+ 272	- 56	3.40	3.48
6	895	165	56	62	770	102	+ 69	- 1	2.58	3.20
7	900	98	38	58	501	90	+ 161	- 50	1.97	3.30
8	920	92	43	53	561	99	+ 316	- 60	2.65	2.68
9	890	120	34	45	502	87	+ 354	- 12	3.45	3.40
10	900	84	46	72	517	91	+ 337	- 79	2.30	3.20
Mean:	899.1	137.7	47.88	65.1	562.7	114.5	+ 288.6	- 42.1	2.977	3.597
11	920	-	57	-	446	-	+ 417	-	-	-
12	920	-	37	-	756	-	+ 127	-	-	-

TABLE 5

Bone Chemistry (Ileum) Before and at End of Low Calcium Diet
(Per 100 grams dry fat free bone)

Case No.	Calcium (g)		Phosphorus (g)		Magnesium (mg)		Fluoride (mg)	
	Before	After	Before	After	Before	After	Before	After
1	13.6	11.4	5.4	5.0	111	110	695	406
2	12.8	10.8	5.1	4.5	109	108	573	385
3	13.2	12.1	5.6	4.6	107	105	605	280
4	13.6	-	5.4	-	106	-	583	-
5	11.5	-	4.9	-	-	-	495	-
6	12.7	-	5.1	-	-	-	203	-
7	12.1	-	5.1	-	-	-	95	-
8	11.8	-	4.9	-	-	-	85	-
9	11.5	-	5.2	-	-	-	202	-
10	12.0	-	4.9	-	-	-	112	-
Mean:	12.48	-	5.16	-	108.25	-	365	-
Range:	11.5-13.6	-	4.9-5.6	-	106-111	-	85 - 695	-
11	9.8	-	4.1	-	-	-	48	-
12	8.8	-	3.9	-	-	-	56	-

Fig. 1

Radiograph of Lumbodorsal
Spine (Case 3) Age 11



Marked Osteosclerosis

Fig. 2

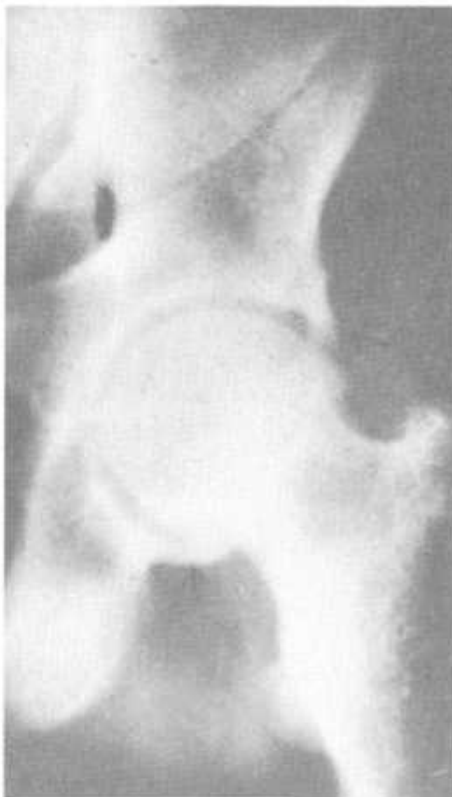
Same Case, After Calcium
Diet 16 Weeks



Decreased Density

Fig. 3

Radiograph of Left Hip (Case 9)



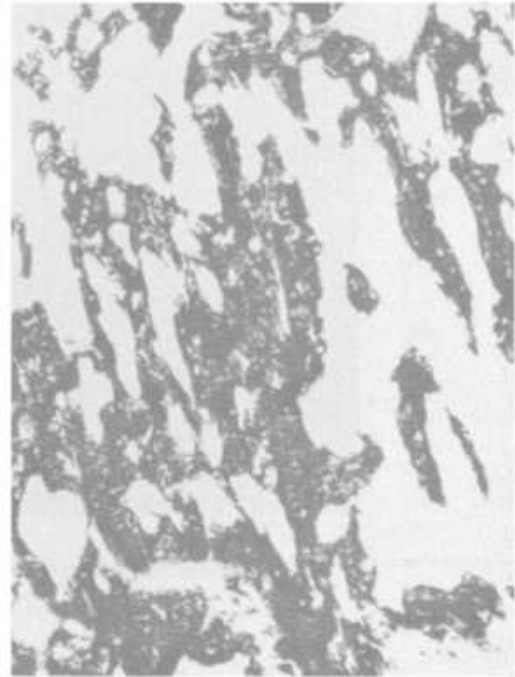
Marked Osteosclerosis; Gross
Calcification of Capsule

Fig. 4

After 16 Weeks on Low Calcium Diet



Decalcification of Capsule

Fig. 5Histology of Biopsied BoneThickened Trabeculae; Excessive
Calcification.Fig. 6After 20 Weeks on Low Calcium
DietThinning of Trabeculae; Normal
CalcificationFig. 7Radiograph of Teeth (Case 1 & 2)

Resorption of Bone; Loss of Lamina Dura

Fig. 8a

Patient with Crippling Fluorosis
(Case 1)



Marked Deformities of the Spine and Joints

Fig. 8b

Same Patient 12 Weeks
After Treatment



Deformity Much Improved

Fig. 9a

Case 3



Ankylotic Spine

Fig. 9b

Same Case After
16 Weeks on Low
Calcium Diet



Marked Clinical
Improvement

balances were positive, suggests that fluoride affects the calcium metabolism. Since most of the calcium and fluoride localizes in the bones, it can be concluded that this is the major site from which these elements are withdrawn during the calcium depleting diet treatment. In three cases following the treatment with a low calcium diet bone histology and chemical composition revealed that much calcium, magnesium and fluoride was removed from the bones. The bone trabeculae became thin and appeared to contain a normal amount of calcium. According to radiographs, these changes were associated with skeletal demineralization, decalcification of calcified ligaments and capsules, decreased bone density and coarse trabeculation. The calcium depleting diet, therefore, is likely to have a direct action on the bone mineral reserves. The effect of the low calcium diets in our cases is probably due to increased osteoclastic activity and mobilization of calcium combined with secondary release of fluoride from the sites where they have been deposited in excess, namely from the bones, ligaments, joint capsules and tendons. Ordinarily the release of these ions is slow or minimal. The lowered intake of calcium hastens their removal from the fluoroapatite crystals.

We are not aware of a calcium depleting regime being previously reported in the treatment of skeletal fluorosis. Dent (11) reported the case of a child with severe congenital osteopetrosis. On a calcium depletion diet, the child became more lively. Less dense bone showed signs of remodelling. Our observations of the increased urinary excretion of fluoride in combination with a low calcium diet and almost fluoride-free drinking water, is consistent with the finding of Siddiqui (12) who noted an average urinary fluoride excretion of 2.75 ppm in fluorotic patients and 3.4 ppm in a patient who had moved from an endemic area to a community with no detectable fluoride in the drinking water.

Two patients (cases 11 and 12) with a vitamin-D deficient osteomalacia, who had lived for their entire life in the same endemic area, had a milder degree of skeletal fluorosis. This fact suggests that dietary deficiency of calcium and vitamin-D may inhibit fluoride deposition in the bones and that fluoride ingestion does not prevent osteomalacia.

Whereas a larger number of patients must be studied for further assessment of calcium depletion therapy, the results of this study are sufficiently striking to recommend the treatment as soon as the diagnosis of skeletal fluorosis has been established.

Clinical improvement was most marked in the latter part of our investigations; it was not observed during short periods of observation. An even more severe calcium depletion regime for a longer follow-up study is recommended.

Summary

Twelve patients of metabolically proven skeletal fluorosis were studied. Two of the five female patients also had an associated vitamin

D deficient osteomalacia. All cases were crippled with severe deformities in spine, hips and knees. All showed positive phosphorus, magnesium and nitrogen balances and excessively positive calcium balances. All patients, except those who had associated osteomalacia, were treated for 12 to 24 weeks with a low calcium diet. Striking clinical improvement was noted in all cases. They became more active, the spinal ankylosis improved, joint movements became almost normal, they were able to write and to resume their daily tasks without much difficulty.

Clinical recovery was accompanied by negative calcium balances and a rise in urinary excretion of calcium and fluoride. The skeletal radiographs showed a significant change. The histology and chemical composition of the bones indicated that calcium, magnesium and fluoride had been removed. The possible mechanisms of action of the calcium depleting diet have been discussed.

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STUDIES ON THE MECHANISM OF THE POSITIVE

INOTROPIC ACTION OF FLUORIDE

by

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Fluoride stimulates the force of contraction of heart muscle (1, 2, 3, 4, 5). This action is paradoxical since stimulation occurs at concentrations of fluoride that inhibit glucose metabolism (6), and markedly reduce the calcium ion concentration (7). According to current knowledge, these actions of fluoride should diminish rather stimulate contraction. It would appear that studies on the mechanism of the positive inotropic action of fluoride will provide new information on the fundamental processes that regulate cardiac contractibility.

Several studies have focussed their attention on the mechanism of the positive inotropic effect of fluoride. Loewi (2) found that fluoride restored the contractile amplitude of the hypodynamic frog heart. He proposed that fluoride and calcium combined with a cellular constituent to form a non-diffusible complex at the cell surface. He attributed the positive inotropic effect to restoration of the membrane to a normal state by the calcium fluoride complex. The mechanism by which this was accomplished is analagous to the action of oleate. That fluoride exerted its effect as a metal complex was confirmed by Berman (8) in mammalian heart muscle. Fluoride had a marked positive inotropic effect when ventricle strips were incubated in a medium containing calcium and fluoride. On the other hand, fluoride was relatively ineffective when the tissue was incubated with fluoride in the absence of calcium. Fluoride also exerted a positive inotropic effect when the tissue was incubated in media in which the calcium was replaced by strontium or magnesium suggesting that fluoride may also exert its effect on contraction as a magnesium or strontium complex. Fluoride action, however, is not confined to the membrane: Rice and Berman (6) demonstrated that fluoride inhibited acetate oxidation by electrically stimulated rat ventricle strips. Inasmuch as the enzymes involved in acetate oxidation are present intracellularly, fluoride must have penetrated the cell to produce this inhibition of metabolism.

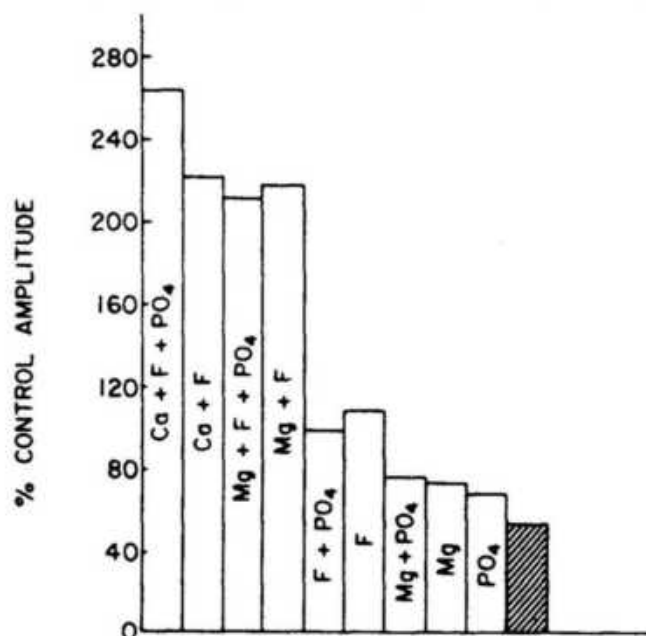
The finding that inhibition of glucose metabolism occurred during the positive inotropic action of fluoride suggested the possibility that the positive inotropic effect of fluoride was related to its action as an enzyme inhibitor. Several enzymes for example enolase, phosphoglucomutase, and succinic dehydrogenase require phosphate for fluoride inhibition. Experiments

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were performed to determine whether phosphate was required in the medium for fluoride action (9). The results are summarized in Fig. 1. In agreement with our previous findings, exposure of quiescent ventricle strips to fluoride produces a positive inotropic effect which persists for a relatively long period of time after removal of the fluoride by washing. The figure

Fig. 1

Effect of Phosphate on the Positive Inotropic Effect of Fluoride on Electrically-Stimulated Rat Ventricle Strips



Tissues were incubated for 30 min. in media containing 154 mM NaCl, 5.6 mM KCl, 5.5 mM glucose and the additions indicated in the bar graph. The concentrations of ions in the incubation media were: Ca (0.8 mM), PO₄ (1.0 mM), F (4.3 mM), Mg. (1.68 mM); when calcium and magnesium were absent from the media, the fluoride concentration was 2.7 mM; when fluoride was absent from the media, the magnesium concentration was 0.88 mM. The concentration of calcium and magnesium were calculated from the solubility products of the fluoride complex using a value of 2.7 mM fluoride. Each value is the mean of 5 to 10 experiments. The details of the experimental procedure are described in reference (8). Cross hatched bar is control (incubation in absence of Ca, Mg, PO₄ and F).

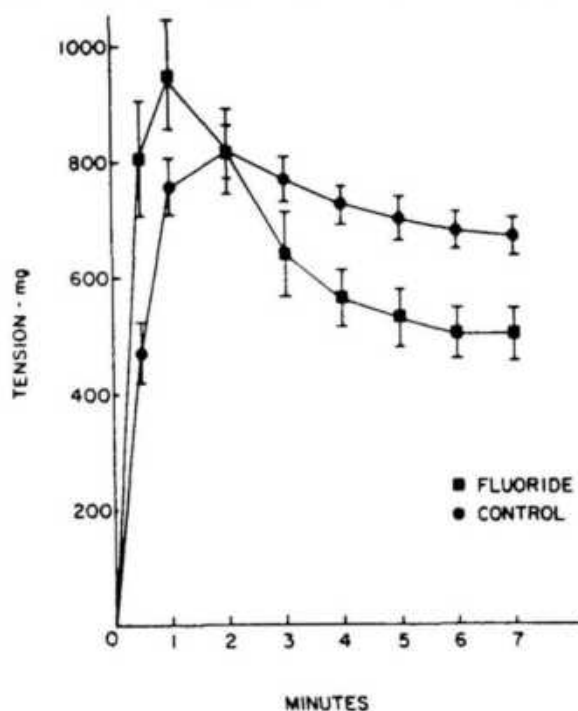
shows that incubation of the ventricle strip with fluoride in the presence of calcium or magnesium produced a greater positive inotropic effect than when these cations were absent from the media. This is in accord with the concept that a metal complex of fluoride is primarily involved in the posi-

tive inotropic action. The relatively slight positive inotropic effect that occurs after exposure of the tissue to fluoride in calcium and magnesium-free media would presumably be due to the action of a complex formed by fluoride with cellular cations. It is also evident that the presence of phosphate is not essential for the positive inotropic action of fluoride. Thus, it would appear that if fluoride exerts its positive inotropic effect by enzyme inhibition, its action would either be upon an enzyme for which there is a sufficient concentration of available intracellular phosphate or upon an enzyme that does not require phosphate.

Reiter (5) proposed that fluoride acted to inhibit the absorption of calcium ions by the calcium pump. He suggested that such a mechanism would increase the amplitude of contraction by delaying the relaxation phase, thus prolonging the rise time of contraction without influencing the initial rate of tension development. Johnson and Berman (10) observed that the effect of fluoride on the contraction cycle characteristics of the electrically-stimulated rat ventricle strip were complex. Fluoride (3 mM) initially increased peak tension, maximum rate of contraction and maximum rate of relaxation; the peak time decreased. Although, slowing of relaxation may

Fig. 2

Effect of Fluoride on K-Contraction of Ventricle Strips
(Tissues Exposed to 150 mM KCl at Zero Time)



The hearts were electrically stimulated at 30/min. in a medium containing 5 mM pyruvate. The curve designated by squares is the mean of 14 experiments in which the hearts have been exposed to 3 mM fluoride for 2 hours. The open circle curve is the mean of 17 experiments of hearts not exposed to fluoride (control). Vertical lines indicate S. E.

be a factor after prolonged exposure to fluoride, the primary mechanism for the positive inotropic effect is more consistent with a facilitation of excitation-contraction coupling rather than inhibition of relaxation (10).

The possibility that the positive inotropic effect of fluoride was mediated by the release of endogenous catecholamines was investigated (10). The stimulation of contraction of heart muscle by fluoride was not significantly modified by the beta-adrenergic blocker, propranolol, or reserpine. Furthermore, the positive inotropic effect was not significantly affected by aminophylline. Fluoride did not significantly alter the positive inotropic effect of epinephrine (11). Thus, neither catecholamine release nor the adenyl cyclase system appear to be major factors in the positive inotropic action of fluoride.

We have recently investigated the effect of fluoride on potassium contractures in heart muscle. It is evident from Figure 2 that fluoride accelerated the rate of development and magnitude of K-contracture. Furthermore, the relaxation rate was significantly increased. These findings are consistent with the hypothesis that fluoride exerts an effect on the excitation-contraction process presumably by facilitating the movement of calcium into the region of the myofibrils. The more rapid relaxation suggests that fluoride also facilitates the removal of calcium from the sarcoplasm. The mechanism of these actions will be the subject of future investigations.

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EFFECTS OF FLUORIDE ON CELLS AND TISSUES IN CULTURE

A REVIEW

by

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The fluoride ion is capable of inhibiting a wide range of enzymes. Metabolically inactive complexes are formed by fluoride, particularly with enzymes dependent upon calcium, magnesium and iron (1). However, the fact that the highly-reactive fluoride ion is an enzyme inhibitor in vitro and in vivo, when administered at dose levels producing acute toxicity, is irrelevant to the study of any potential toxicity of minute amounts of fluoride administered on a long term basis to the intact animal or to humans.

In assessing the risk of toxicity arising from the chronic administration of any pharmacologically-active agent, two factors must be examined separately and independently:

(a) The sites of concentration of the drug within the body and the maximum concentrations and persistence of the drug in those sites after various patterns of administration, and

(b) The sensitivity to the drug of cells of the organs in which the concentration of the drug is maximal.

The former question is beyond the scope of this review; the latter question can best be approached by the use of tissue culture model systems. However, it remains an unanswered question whether the particular tissue culture systems which have been studied so far represent the best models from which to extrapolate potential risks to the intact animal.

Tissue Cultures in Toxicology

Early work in tissue culture used the relatively crude parameter of the survival or failure of histological integrity and gross "growth" of large pieces or organs explanted into culture media to which the drug in question had been added. Organ function was usually difficult to assess in vitro. The "survival" of a tissue explant was often the result of the successful growth of fibroblasts derived from the normally reproductively dormant connective tissues of the organ, rather than from the survival of the parenchymal cells which represented the real interest of the toxicity study. The development of the method of clonal cell culture for mammalian cells by Puck et al. (2) provided a highly sensitive method for deter-

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mining the survival of the reproductive capacity i. e. the ability to divide an unlimited number of times not only for the ubiquitous fibroblasts, but also for epithelial cells derived from various organs, and for cells originating in malignant tumors.

Significance of Cell Growth in Toxicology

A by-product of studies of the effects of ionizing radiation upon mammalian cells was the finding that most irradiated cells which died did so because of failure to complete mitosis. The few exceptions were cell types particularly sensitive to radiation e. g. lymphocytes, oocytes which died a rapid, pyknotic "interphase" death unrelated to their position in the cell division cycle. Other cells, if not called upon to divide after being irradiated, could continue to carry on their many normal biochemical functions unhindered; only their failure to divide revealed the accumulated latent radiation damage. This loss of cell reproductive capacity was far more sensitive as a biological end-point than any other which had been studied. Cells could be rendered reproductively inert by tens of rads*, while depression of the complex synthetic process which replicated deoxyribonucleic acid (DNA) took hundreds of rads (3, 4). Depression of protein synthesis by irradiation required thousands of rads; the inactivation of isolated enzyme systems required even higher doses up to hundreds of thousands of rads. Other recent studies have shown that not only the ability to divide but also the rate at which surviving mammalian cells divide (even several divisions after irradiation) is affected by relatively small radiation doses(5). Thus, the complex phenomenon of cell growth has emerged as the most sensitive biological end-point for the study of radiation damage, a rather non-specific type of damage to mammalian cells. Cell growth has hence been used as criterion for assessing the effects of many other, more specific, kinds of damage, such as the effects of pharmacological agents used in cancer therapy (6, 7).

Sodium Fluoride in Cell and Tissue Cultures

1. Early Studies with Avian Embryo Tissue Cultures: Short-term exposure (one hour) to sodium fluoride added to the balanced salt solution used as temporary incubation medium affected the pulsatile function of the developing hearts of chick embryos only at concentrations above 1125 ppm F^- (2500 ppm NaF); concentrations below 450 ppm of F^- (1000 ppm NaF) did not affect the functional end-point of phenol red concentration by the embryonic chick mesonephros (kidney). Failure of growth of embryonic heart and kidney explants after this short-term exposure to sodium fluoride occurred only above 675-720 ppm F^- (1500-1600 ppm NaF) (8). By contrast, in another study the growth of chick embryo bone was inhibited by a concentration of 94.5 ppm F^- (210 ppm NaF). The highest concentration at which no interference with bone formation was seen was 37.8 ppm F^- (84 ppm NaF) (9).

*A rad is the unit of absorbed radiation dose.

2. Mammalian Organ Cultures: An extremely comprehensive study of effects of added sodium fluoride upon two biochemical parameters in explants of metacarpal bones from young growing rats was reported in a deceptively brief communication by Proffit and Ackerman (10). Their data are shown in Figures 1 and 2. Various concentrations of sodium fluoride were continuously maintained in the culture medium throughout the experiment. Total protein synthesis during the experiment was measured by the incorporation of ^{14}C -labelled proline into collagen, while synthesis of DNA was measured concomitantly by the incorporation of ^3H labelled thymidine. No significant depression of protein synthesis was seen when the concentration of fluoride in the medium was below 10-20 ppm F^- ; no depression in DNA synthesis took place below 20 ppm F^- (44.5 ppm NaF). Concurrent autoradiographic studies showed no structural disturbance in bone cultures grown in medium containing 15 ppm F^- (33.3 ppm NaF). Thus the lack of effect on protein and DNA synthesis could not have been due to a small surviving proportion of bone cells working harder to maintain the total tissue production of DNA and protein.

3. Isolated Mammalian Cells: Carlson and Suttie (11) have shown that the addition of sodium fluoride (30 ppm F^-) to proliferating monolayer cultures of HeLa cells caused a rapid drop in the cellular adenosine triphosphate (ATP) level. This compound contains a high-energy phosphate bond which acts as the immediate energy reserve for many synthetic processes in the cell. There was no specific direct inhibition of glycolysis by this growth-inhibiting concentration of fluoride. Similar results of equivalent inhibition of the growth of HeLa cells on glucose utilization, and lactate and CO_2 production were obtained by appropriate concentrations of fluoride and by the inhibitors ethionine and iodoacetate. However, this does not necessarily imply a common site of action for all three. The specific effect of fluoride on ATP concentration was not shared by the other inhibitors.

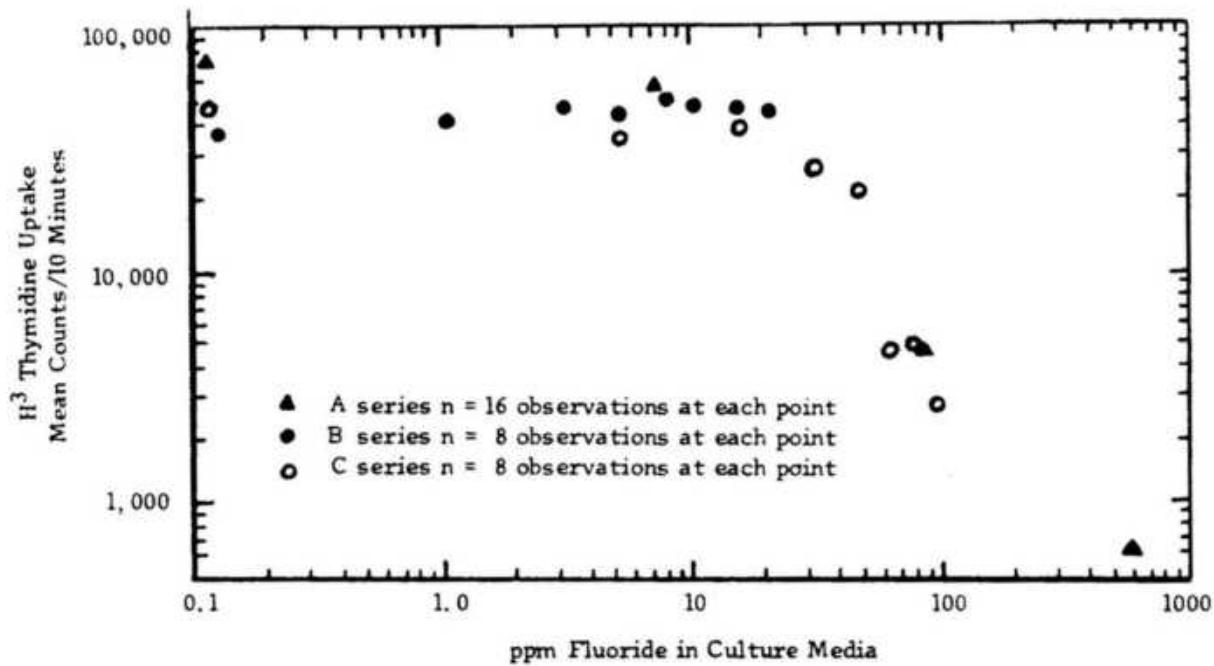
Three types of studies have been reported on effects of sodium fluoride upon the growth of isolated mammalian cells in vitro:

(a) Suspension Culture: Albright (12) used murine leukaemic lymphoblasts which grow in suspension in culture and do not attach to the surface of the culture vessel. He failed to note a significant decrease in the number of cells harvested after 4 days' growth (circa 7 population doublings) unless the growth medium contained fluoride in excess of 5-6 ppm (11.1 - 13.3 ppm NaF).

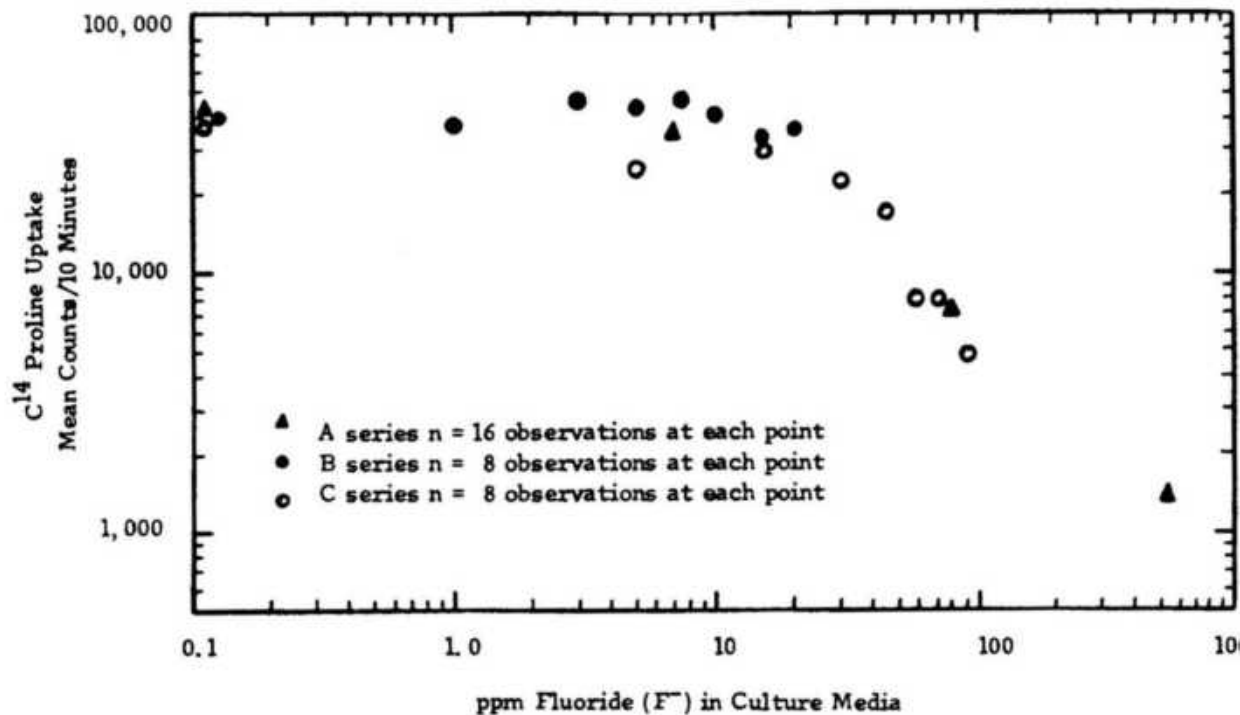
(b) Monolayer growth of surface-attached cells: Berry and Trillwood (13) studied two strains of mammalian cells which grow when attached to the glass or plastic surface of the culture vessel to produce a confluent monolayer of cells. A small but significant decrease in the number of cells harvested after seven days' growth (circa 4-5 population doublings) was seen in glass-attached cultures of human carcinoma-derived HeLa S-3 oxf. cells

Fig. 1

Uptake of thymidine H^3 by Metacarpal Bones in Organ Culture as a Function of Fluoride Concentration from Proffit and Ackerman (10)

Fig. 2

Uptake of Proline $-C^{14}$ by Metacarpal Bones in Organ Culture as a Function of Fluoride Concentration from Proffit and Ackerman (10)



and mouse Strain L_{oxf} dermal fibroblasts to which as little as 0.045 ppm F⁻ (0.1 ppm NaF) had been added. Their data are shown in Table 1.

TABLE 1

Action of Dilute Solutions of NaF on Growth of Mammalian Cells
In Vitro

Percent of Control Growth in 7 Days

<u>Cell Type</u>	<u>Concentration of Sodium Fluoride in Medium</u>		
	0.1 mg/1 (1/10 ppm)	1.0 mg/1 (1 ppm)	10.0 mg/1 (10 ppm)
Human Carcinoma (HeLa S-3 _{oxf})			
Experiment 1	-	72.5	69.8
2	82.3	89.8	73.0
3	92.5	90.2	70.9
4	<u>85.5</u>	<u>85.9</u>	<u>75.5</u>
Average:	86.8	84.6	72.3
Mouse Fibroblast (L, clone 12/ _{oxf} .)			
Experiment 1	-	78.4	72.6
2	68.5	77.0	53.0
3	95.0	81.1	65.7
4	<u>92.1</u>	<u>94.3</u>	<u>65.6</u>
Average:	85.2	82.7	64.2

From Berry and Trillwood (13)

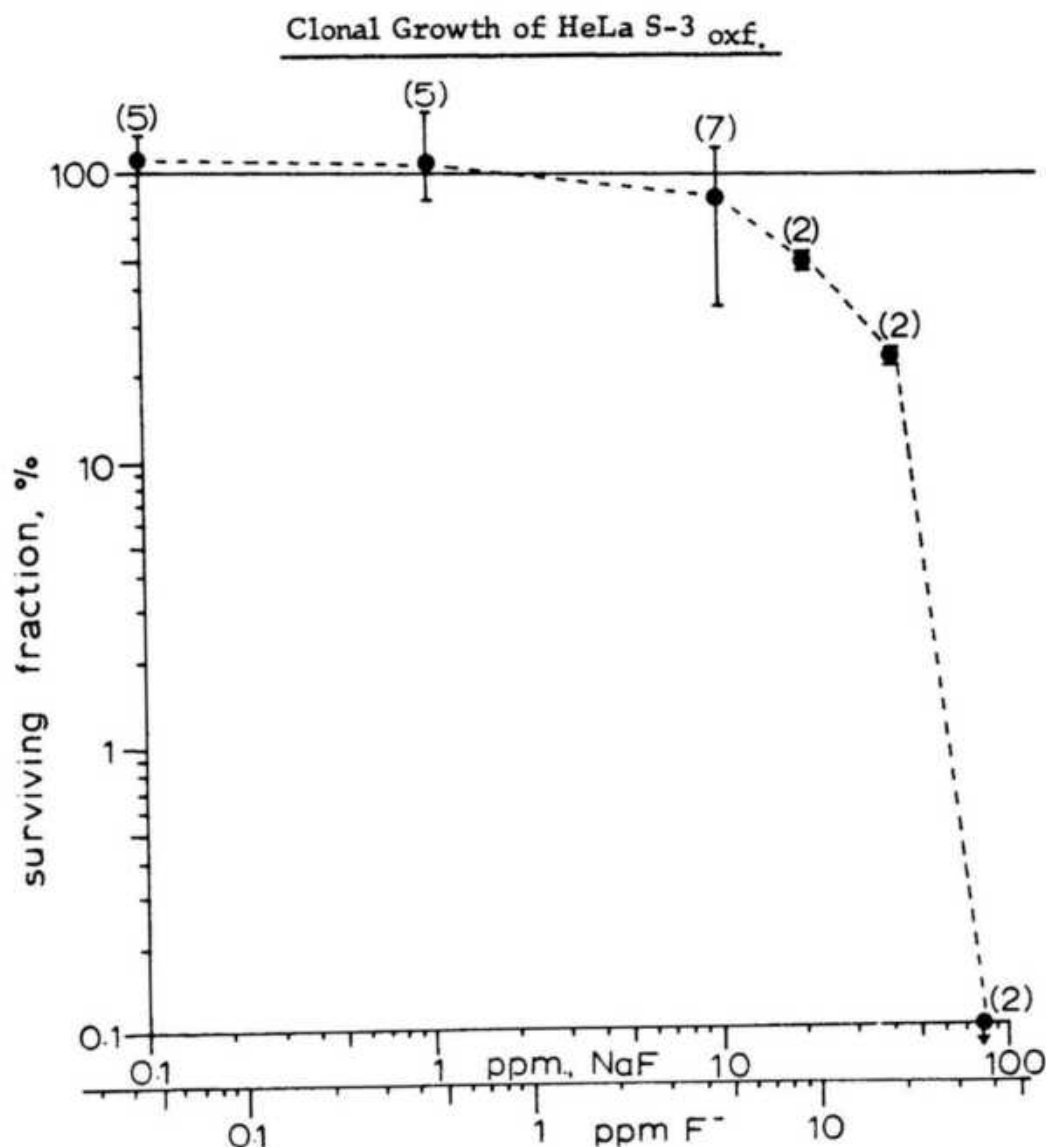
The reduction in cell growth was not due to altered ionic strength of the growth medium: Addition of as much as 10 ppm NaCl to this medium did not affect the number of cells harvested. This fluoride-induced depression of growth rate was also reflected in microcinematographic observations of murine Strain L fibroblasts in a study by the Time-Lapse Research Foundation which was terminated prematurely. For the mouse fibroblasts a decrease in the frequency of cell divisions, and a possible increase in the proportion of abnormal divisions were seen when either 0.018 or 0.038 ppm F⁻ (0.038 or 0.078 ppm NaF) were added to the culture medium. When similar amounts of sodium fluoride were added to cultures of human tooth pulp cells, no such effect was noted. However, these latter cultures were sufficiently crowded so that it was unlikely that the cells would attempt many

further divisions. A similar criticism has been made by Berry and Trillwood (14) of the failure of Armstrong et al. (15) to detect any decrease in growth of glass-attached HeLa or Minnesota EE (human esophageal epithelium-derived) cells in media to which sodium fluoride had been added in concentrations up to 10 ppm F^- (22.2 ppm NaF). However, Nias (16) used glass-attached HeLa cells which had completed over six population doublings in the one-week experimental period. When 0.45 ppm F^- (1.0 ppm NaF) was added to the growth medium, the mean number of cells harvested in five replicate experiments was 4% lower than that in the control cultures. The probability, that this difference was due to chance alone, was 0.20, rather greater than the accepted level of statistical significance, $p \leq 0.05$. Carlson and Suttie (17) also used glass or plastic-attached HeLa cells. Although they did not study the effects of concentrations lower than 10 ppm F^- , they found no difference from control growth at this concentration and only a slight depression of growth rate when 15 ppm F^- was maintained in the growth medium for at least 4 days (circa 4 population doublings). They showed that the decrease in growth was unaffected by alteration in the calcium and magnesium ion concentration in the medium over the range 0.1 to 2 times the normal level. They noted that fluoride-induced growth inhibition was not due to a failure of cells to attach to the surface of the culture vessel but rather to a true decrease in the rate of cell proliferation. Cells grown continuously in high concentrations of sodium fluoride (15-25 ppm F^-) showed adaptation which reduced their sensitivity to an acute challenge with higher concentrations of fluoride; while 75 ppm F^- decimated "naive" HeLa cells, the fluoride-adapted cell line, suffered only a 53% reduction in growth at this concentration. The adapted cell line grew more slowly than the "naive" parent cell line, however, in the absence of added sodium fluoride in the growth medium.

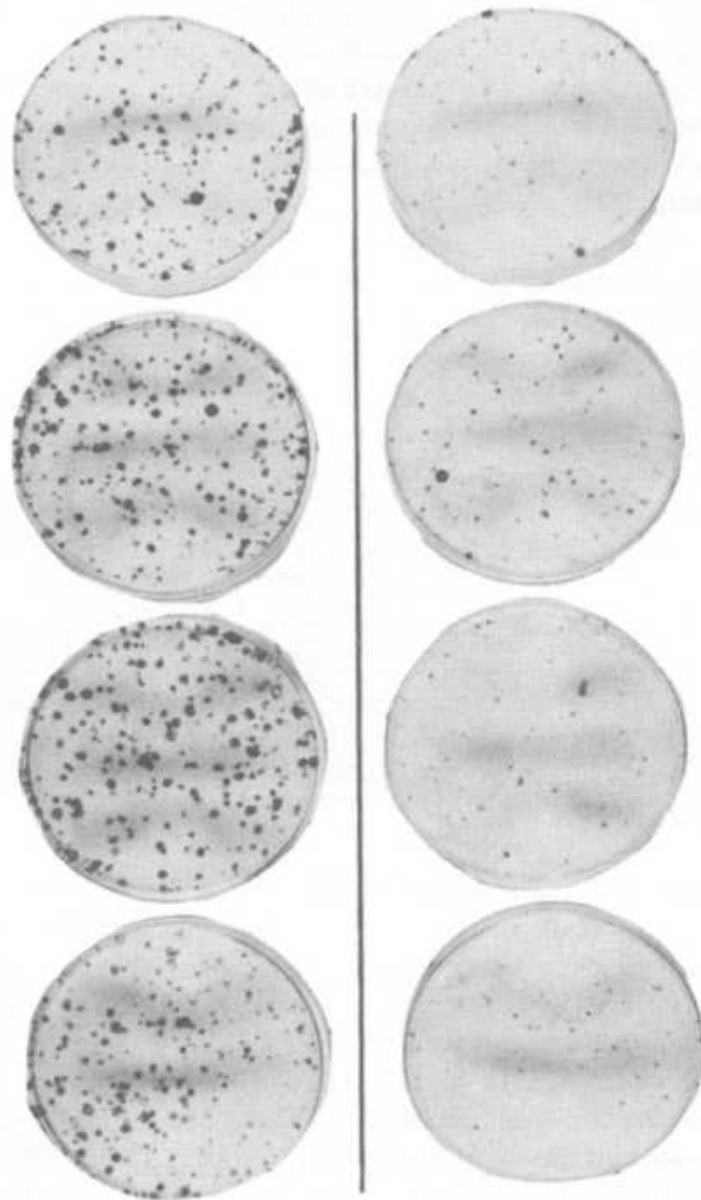
(c) Clonal growth: Armstrong, et al. (18) studied the effect of addition of sodium fluoride (1-10 ppm F^-) to the culture medium, on the numbers of cells in individual clones of HeLa cells growing attached to the polystyrene surface of tissue culture flasks (Falcon TC). Their results indicated that there was no reduction in the rate of growth of the faster-growing clones at a concentration of 5 ppm F^- , although there was a detectable decrease in the growth rate when the concentration of added fluoride was raised to 10 ppm F^- . They rejected slowly-growing clones by abandoning further examination of all clones which failed to reach 4-cell size after 2 days' growth. They also measured survival of cell reproductive capacity by scoring the number of macroscopically-visible clones after 11 days' growth. "The mean number of clones formed in the flasks which contained media of 1 ppm and 5 ppm fluoride equalled or exceeded (my italics) the mean clone count of the control flasks"; at 10 ppm F^- , the mean number of clones was respectively 85% and 79% of the control.

The present author's data for clonal growth of HeLa S-3_{oxf.} cells attached to Falcon TC polystyrene Petri dishes are shown in Figure 3. As in the experiments reported earlier (13), the growth medium was 199 (Glaxo), but it was supplemented with 20% type AB human serum to support clonal

Fig. 3



growth from inocula containing only 125 cells per ml of medium. Both the cells and the appropriate amounts of sodium fluoride were added to the medium in bulk before it was divided into individual aliquots in the Petri dishes. In each experiment, the number of clones was scored on 4-5 dishes to establish one experimental point. Incubation was at 37° C in a humidified 5% CO₂ - 95% Air atmosphere, and the dishes were fixed and stained with Leishman's stain 14 days after plating. The plating efficiency (clones grown/cells initially plated) averaged 43%. When sodium fluoride at a concentration of 0.045 ppm F⁻ or 0.45 ppm F⁻ (0.1 or 1.0 ppm NaF) was added to the growth medium, the plating efficiency regularly exceeded that of three controls; only above 4.5 ppm F⁻ (10 ppm NaF) was there a significant decrease in the number of macroscopic colonies grown. The complete set of culture dishes from one such experiment is shown in Figure 4. Note that at 4.5 ppm F⁻ (10 ppm NaF) not only the number of clones, but also the size of the individual clones is reduced.

Fig. 4Typical Growth of HeLa S3 Cells

CONTROL

4.5 ppm F^-
(10 ppm NaF)

Experiment 98

Discussion

The extreme sensitivity to sodium fluoride of the clonally-selected Oxford HeLa and Strain L cell sub-lines now appears to be somewhat idiosyncratic. Results obtained by several other investigators show similar retardation of cell growth only at concentrations of fluoride ranging upwards from 5 ppm F^- . However, the findings by Carlson and Suttie (17) that cells chronically exposed to fluoride in high concentrations adapt themselves to its presence so that their sensitivity to subsequent growth inhibition by fluoride is reduced, offers the possibility that some cell lines (conceivably even some of the widely-used HeLa lines) may have undergone similar adaptive changes by chance.

An increased plating efficiency of HeLa cells when low concentration of sodium fluoride (0.045-5.0 ppm F^-) were added to the growth medium has been noted both by Armstrong et al. (18) and by the present author. This may, paradoxically, represent a demonstration of a small amount of cell death due to these low concentrations of fluoride. When plating efficiency of a given cell line is less than 100%, the addition of a "feeder layer" of reproductively inert but metabolically active cells has been shown in many cases to increase the proportion of potentially viable cells which grow into macroscopically visible colonies (19). The "dead" cells supply the specific minimal nutritional requirements which the growth medium may lack (despite all the effort and skill which has gone into compounding the particular witch's brews in which mammalian cells thrive in vitro). Only when more marked degrees of cell-killing supervene, in the case of sodium fluoride at levels above 4.5 ppm F^- , would the apparent plating efficiency begin to decrease.

We have previously questioned whether the tissue culture systems which have been used to study effects of fluoride are the best ones from which to extrapolate any potential hazard to the intact animal. From the teleological viewpoint, the types of cells whose response to sodium fluoride is most likely to be relevant are those of bone (where fluoride is concentrated for excretion). Single-cell cultures of true bone cells, as distinct from bone marrow (haematopoietic) cells, are not generally available, but well-documented lines of cells originating from the kidneys of the Rhesus and African Green monkeys, the cow, the pig, the dog, the Syrian hamster and the marsupial Potorous are available from the American Type Culture Collection (20). This author is unaware of published studies in which the response of these cells to fluoride has been reported.

Finally, although the generally accepted levels of plasma fluoride around 0.1 - 0.2 ppm F^- (21) have recently been challenged as being too high (22), it is generally agreed that the concentration of fluoride ion in the urine is of the order of 3 ppm F^- (23). It would not be surprising if cells originating from renal epithelium proved less sensitive to the effects of added fluoride than other cell types which have been studied, due to adaptation to this environmental factor. This would be a reassuring find-

ing, comparable to the decreased sensitivity to high concentrations of fluoride of the HeLa cells grown by Carlson and Suttie (17) after a prolonged period of growth in fluoride-containing medium.

Summary

Various authors have studied the effect of fluoride ion upon mammalian cells using many different biological end-points, of which inhibition of the function of specific cellular enzymes, survival of cell reproductive capacity, production of abnormalities of cell division and alteration in the rate of cell proliferation are a representative sample. In this review the minimum fluoride ion concentration at which biological effects are detectable are compared for each of these end-points. The usefulness of such in vitro studies for evaluation of potential whole-animal toxicity is discussed.

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THE IN VITRO AND IN VIVO EFFECTS OF FLUORIDE ON SUCCINIC DEHYDROGENASE ACTIVITY

by

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Although much work has been done on the inhibitive function of F on succinic oxidase activity, the mechanism of the inhibition is not fully known. Various investigators have suggested a number of different loci of F action. Slater and Bonner (1) confined the reaction site to the primary dehydrogenase. They claimed that F does not act on the respiratory cycle indirectly by affecting some intermediate in the phosphorylating reaction. Examination of the degree of inhibition at different succinate concentrations showed that F is a competitive inhibitor of succinic dehydrogenase. In the absence of F, phosphate was found to be a weak competitive inhibitor. According to Bonner (2) F alone will cause only slight inhibition of the primary dehydrogenase in vitro. In the presence of phosphate, however, this inhibition is increased considerably. The inhibition of the enzyme is believed, therefore, to be competitive with respect to succinate, phosphate and fluoride.

The lack of data concerning the effects of prolonged administration of F on succinic dehydrogenase, that is the primary dehydrogenase in animals, stimulated this present investigation. We studied the effect of F in in vivo metabolism by determining changes in succinic dehydrogenase activity of the kidney and liver tissues of the Golden Hamster, Mesocricetus auratus, following F administration.

Materials and Methods

Twenty-five hamsters of both sexes, between 80 and 100 grams in weight, were maintained on a diet of Purina Chow and distilled water. The animals were sacrificed and their livers and kidneys removed.

Six groups of ten animals each, were also placed on a diet of Purina Chow and distilled water containing fluoride as NaF, in concentrations of 0, 1, 5, 10, and 100 ppm respectively. The animals were of both sexes and weighed between 80 and 100 grams.

All animals were sacrificed after nine months by decapitation or by freezing. Their kidneys and livers were excised, quick-frozen in dry ice,

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and weighed. Each organ was then ground thoroughly with a mortar and pestle in the presence of powdered glass and sufficient 0.15M phosphate buffer (pH 7.6) to yield dilutions of 1:10 of liver homogenates and 1:15 of kidney homogenate. This step was carried out at 0-5°C by pre-freezing the mortar and pestle, powdered glass and buffer solution. The resulting homogenates were then centrifuged at 10,000 rpm for five minutes to remove the powdered glass and tissue debris. The supernatant, a reddish-brown colloidal suspension, was used for the enzyme studies. In those cases when the homogenate was not to be used immediately, 1 ml samples were quick-frozen and stored in the frozen state until further use. Preliminary studies showed that these homogenates suffered no loss of activity for a period of 20 days if stored in the frozen state. Some loss was observed after this period.

Two methods for enzyme determination were used, visual estimation of decoloration of methylene blue (prepared in phosphate buffer pH 7.6 in a 1:15,000 dilution) and spectrophotometric measurement of phenazine metho sulfate (a 1% solution prepared in distilled water and stored at 0°C in the dark for no longer than 48 hrs).

Methylene Blue Method: Into the body of the Thunberg tube was placed 1.0 ml of the homogenate, 1.25 ml of buffer, and 0.5 ml of distilled water with fluoride. The hollow stopper received 0.5 ml of methylene blue and 0.5 ml of sodium succinate (0.2M in phosphate buffer pH 7.6). The stopper was then placed on the tube and the tube was evacuated for 5 minutes at 0.005 mm/Hg by means of a vacuum pump. The tube was then placed in a 38°C glass walled water-bath and allowed to equilibrate for 30 minutes, at the end of which time the contents of the stopper and the tube-body were mixed. The time of complete decoloration of the dye was then measured visually with the aid of a stopwatch while the tube was allowed to remain in the water-bath.

Phenazine Methosulfate Method: Into the body of the Thunberg tube was placed 1 ml of homogenate, 0.5 ml of buffer, 0.7 ml of distilled water, and 0.3 ml HCN (0.01M, pH 8.0, prepared from KCN by the addition of 0.85 equivalents of HCl). 0.3 ml of sodium succinate and 0.2 ml of phenazine methosulfate was delivered into the hollow stopper. The stopper and body were then assembled and evacuated as above followed by a 30 minute equilibration period. After equilibrating, the contents of the tube and stopper were mixed and the tube was placed in a Bausch and Lomb Spectronic "20" and the rate of reduction of the dye measured at 387 μ (3). All the enzyme activity rates in these studies were expressed as reciprocals of time (1/t).

The total protein determinations were made by a modified method of Gornall et al. (4). One-half ml. samples of each homogenate were assayed for protein by means of the biuret reaction calculated against a known standard. The biuret reagent was prepared as follows: 1.5 gms of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 6.0 gms of $\text{NaKC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$, and 300 mls of 10% NaOH made up to a liter with distilled water. The calculations were made on the

basis of optical density readings at 540 μ using a Bausch and Lomb Spectronic "20". Enzyme activity rates were calculated in terms of activity per mg of total protein.

The "t" distribution test (5) was used to determine the significance of the results. A P value of 0.05 or less was chosen as the limit of significance (95% confidence level).

Observations and Results

In-Vitro Studies: The control and experimental enzyme activity rates were established on the basis of the rates of reduction of methylene blue. Since no apparent inhibition occurred when the enzymes were subjected to fluoride in concentrations lower than 25 parts per million, no extensive study was made below this level.

A definite decrease in enzyme activity was noted in both liver and kidney homogenates following the addition of 25 ppm of F. A 27.1% reduction of activity was observed in the case of the liver and 6.8% in kidney homogenates (Table 1).

TABLE 1

In Vitro Studies

LIVER

Number of Animals	ppm in Na F	Mean S. D. Activity (1/t)	Activity Range	Decreased Activity (%)
27	00	0.0583	0.0937 - 0.0457	00.0
23	25	0.0425	0.0499 - 0.0397	27.1
22	50	0.0385	0.0391 - 0.0354	33.8
22	100	0.0356	0.0359 - 0.0333	38.9
24	200	0.0230	0.0261 - 0.0198	60.6
21	300	0.0198	0.0225 - 0.0188	66.0

KIDNEY

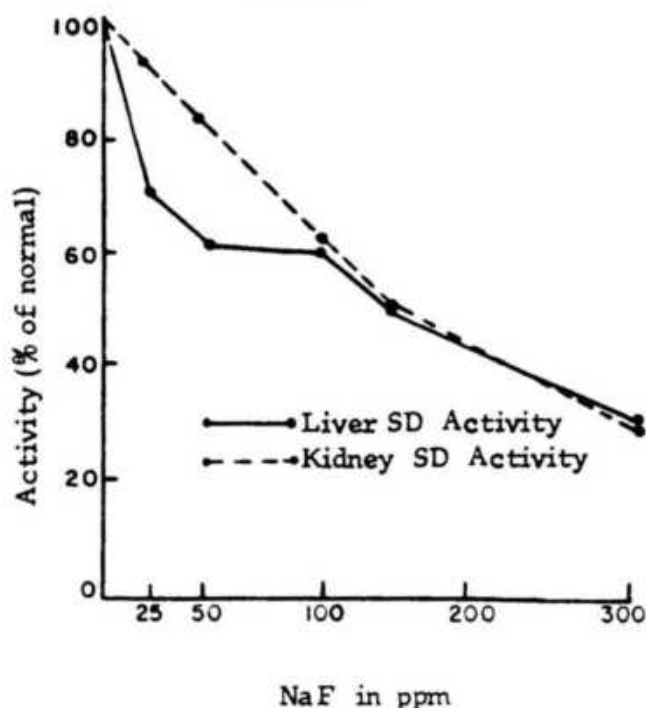
17	00	0.0590	0.0609 - 0.0580	00.0
14	25	0.0551	0.0564 - 0.0546	6.8
16	50	0.0486	0.0499 - 0.0422	17.5
14	100	0.0383	0.0390 - 0.0369	35.0
15	200	0.0247	0.0261 - 0.0232	58.2
15	300	0.0195	0.0204 - 0.0184	67.0

The activity continued to decrease until a maximum decrease was reached upon addition of 300 ppm of F to the homogenates. When the activity rates were plotted as a function of F concentration an asymptomatic curve was obtained beyond 300 ppm (Fig. 1)

At this point the decrease in activity was 66.0% for the liver homogenate and 67.0% for the kidney homogenate (Table 1).

Fig. 1

In Vitro Effect of NaF on Succinic
Dehydrogenase



In-Vivo Studies: Shortly after the animals were subjected to F^- a marked difference in the consumed volume of fluoridated water was noted between the animals receiving pure distilled water and those receiving 100 ppm of F. Due to this self-restraint of the experimental animals they did not receive the full dose intended over the nine-month period.

Animals receiving 100 ppm of F became hypoactive after 6 to 8 weeks of treatment; no further decrease in activity was noted after this period.

A gross examination of the excised organs showed little difference in general appearance of livers in all animals. In the kidneys, however, certain morphological differences between the normal and fluoridated animals were noted. The kidneys of some experimental animals exhibited varying degrees of blanching; in a few the surface was ostensibly rougher than that of normal kidneys and in certain cases a pinkish speckling was noted. These peculiarities were not observed in kidneys of the control animals. No correlation existed between animals showing these variations in morphology and the concentration of fluoride ingested.

Mean liver enzyme activity of control animals was determined at 0.1924 (Table 2).

TABLE 2

In Vivo Studies

<u>LIVER</u>				
Number of Animals	ppm in NaF	Mean S. D. Activity (1/t)	Activity Range	Decreased Activity (%)
7	00	0.1924	0.2280 - 0.1665	00.0
5	1	0.1802	0.2100 - 0.1500	6.4
4	5	0.1459	0.1567 - 0.1363	24.2
6	10	0.1475	0.1970 - 0.0829	23.2
5	100	0.1476	0.1680 - 0.1324	23.2
<u>KIDNEY</u>				
7	00	0.3863	0.5075 - 0.2985	00.0
5	1	0.2016	0.3178 - 0.104	47.8
4	5	0.2075	0.2530 - 0.1702	45.6
6	10	0.2219	0.3330 - 0.1128	42.5
5	100	0.2133	0.2615 - 0.1138	44.8

Compared to this rate, the overall means in the experimental groups were significantly reduced. The liver enzyme activity of the 1 ppm F group was measured at 0.1802, or 6.4% lower than normal. Further decrease in activity was observed in the 5 ppm group, with a mean of 0.1459, or 24.2% lower than the control mean. No appreciable difference in enzyme activity was noted between the 5 ppm group and the 10 and 100 ppm groups.

The mean kidney enzyme activity rate was measured at 0.3863 for the control animals (Table 2). In studies on experimental animals a marked reduction in kidney enzyme activity was noted in the 1 ppm group; it was measured at 0.2016 showing 47.8% decrease over the normal. Animals in the 5, 10, and 100 ppm groups showed no further ostensible inhibition in activity rate.

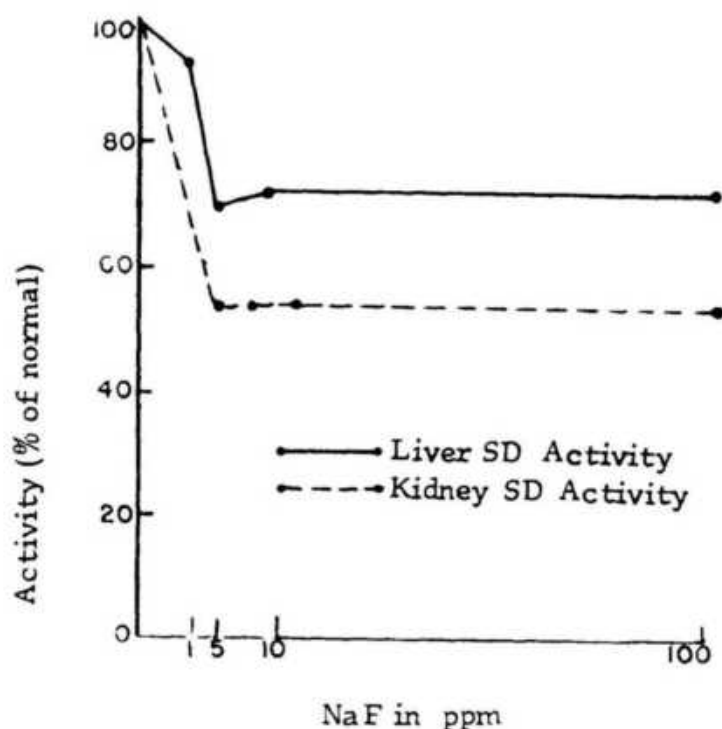
In general, the inhibition of the liver enzyme was less pronounced than that of the kidney enzyme (Fig. 2).

Figure 2 represents liver and kidney succinic dehydrogenase activity rates in vivo as a function of F concentration in drinking water of animals.

The enzyme activity rate of the kidney homogenates was 26.7% lower than the mean inhibition of the liver enzyme of these animals.

Fig. 2

In Vivo Effect of NaF on Succinic Dehydrogenase



Discussion

In Vitro Studies: Measurement of the rate of reduction of methylene blue in vacuo, furnishes a reliable method of determining succinic dehydrogenase activity. The fact that the dehydrogenase is the agent solely responsible for reduction of the dye is shown by the addition of a specific inhibitor of the enzyme, such as malonate. Malonate prevents the reaction of the dehydrogenase with its substrate. It prevents the transfer of hydrogens, leaving the methylene blue in the oxidized state (5).

A definite inhibition of succinic dehydrogenase activity was shown by the decreased rate of reduction of methylene blue after treatment with F. The addition of 25, 50, and 100 ppm of F produced a considerable inhibition of enzyme activity. The addition of 200 ppm of F further decreased the activity of the enzyme; however, this decrease was no longer proportional

to the F concentration. Still higher concentrations of F failed to produce inhibitive effects of any greater significance than those of the 200 ppm concentration. This undoubtedly was a consequence of saturation, which state was attained at the 300 ppm level. At this point maximal inhibition (65%-66%) was observed in the liver enzyme activity as well as that of the kidney enzyme.

In-Vivo Studies: In this phase of the investigation methylene blue was replaced by phenazine methosulfate, the latter being a much more sensitive hydrogen acceptor. Being autoxidizable, the reduction of this compound was also measured in vacuo. In order to measure the rate of reduction of the compound spectrophotometrically the formation of the insoluble leucophenazine methosulfate had to be avoided. In the dilute enzyme preparation the formation of the leuco-compound increases the optical density thus negating any decrease in light absorption. In order to measure this decrease in light absorption, which is a result of the reduction of phenazine methosulfate by dehydrogenase, a more concentrated enzyme suspension had to be employed. The greater concentration of homogenate sufficiently increased the optical density of the solution to prevent the leuco-compound from interfering seriously with increases in light transmittance. Reproducibility within 1% deviation was obtained by measuring the time of complete reduction of the phenazine methosulfate under these conditions.

The above studies demonstrate a significant enzyme inhibition due to prolonged F administration. The succinic dehydrogenase activity rate was suppressed to a much greater degree in the kidney homogenate than in the liver homogenates.

The lack of inhibitive specificity with respect to any one enzyme or enzyme system enables F to affect a number of metabolic processes in a system at the same time. This fact makes it difficult to evaluate the in vivo effect of F on any specific enzyme system, particularly when that system derives its substrate as the end-product of another enzymatic reaction. Any one of these reactions may be inhibited by F and in this way relate the effect to succinic dehydrogenase. Therefore, while the in vitro studies have shown succinic dehydrogenase to be inhibited by F directly, the effect demonstrated by the in vivo studies can be either a direct result of fluoride action on the enzyme, or a combination of this direct result plus the indirect action of other enzyme systems. In any case, this inhibition by fluoride demonstrates the impairment of an important step of cellular metabolism.

Summary

The effect of fluoride on succinic dehydrogenase activity was studied in the liver and kidney tissues of the golden hamster.

In-Vitro: 1. Succinic dehydrogenase is inhibited as a direct result of the addition of fluoride to the enzyme.

2. Maximum inhibition of the enzyme was attained with the addition of 300 ppm of fluoride.

In Vivo: 1. Significant inhibition of succinic dehydrogenase activity rates was demonstrated as a result of fluoride administration.

2. A 25% decrease in activity was registered in liver succinic dehydrogenase of animals receiving 1-100 ppm of fluoride in their drinking water after nine months.

3. A 45% decrease in enzyme activity was registered in kidney succinic dehydrogenase of the same animals.

4. Concentrations of fluoride in the administered drinking water had no ostensible control over the extent of inhibition of enzymatic activity rates.

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ACTION OF FLUORINE AND OTHER TRACE ELEMENTS ON PERIAPICAL OSTEOLYSIS FOLLOWING EXPERIMENTAL PULPAL INFECTION IN RATS

by

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For several years I have been studying the periapical process which arises when the root canal in cats and rats is inoculated with beta-hemolytic streptococcus S 84 (Lancefield's Group A: Griffith's Type B). The operation was carried out as follows: A canal to the pulp was drilled under aseptic conditions by using a rubber dam. An s 84 culture broth was then injected into the pulp lesion. In some infected teeth this procedure gave rise to a periapical process the area of which was measured on the roentgenogram. Thus a quantitative assessment could be made. In order to assess the effect of fluoride on inflammation I employed the following technique.

Method

The size of the periapical process was estimated by determining the area of the periapical space, which was increased by an area of bone destruction when osteolysis occurred. This area was compared with that surrounding the contralateral control tooth. Their ratio was taken as a measure of bone destruction (Fig. 1).

Fig. 1



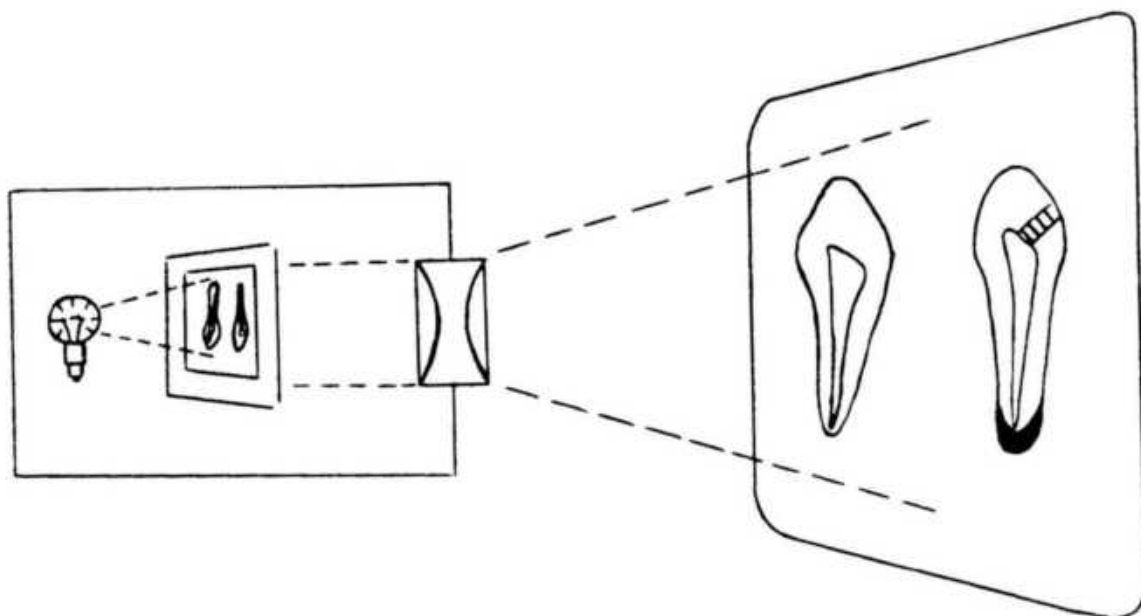
Osteolytic Process Around Tooth
-3 of a Rat and Normal Periodon-
tal Space Around Contralateral
Tooth 3-.

The technique of this procedure was originally described by Hallen. (1). The roentgenograms for each rat were enlarged about 10 times in a projector and traced on ordinary typing paper (Fig. 2). The drawings were cut out and weighed (Fig. 3). This procedure is based on the fact that pieces of paper of uniform quality and size are approximately equal in weight.

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Fig. 2

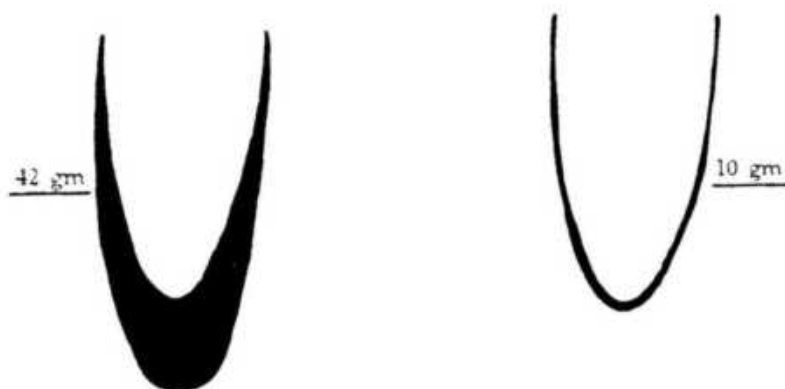
Hallens' Method of Measuring the Affected Area. The Image is Magnified in the Projection and the Outline of the Osteolytic Process is Traced



Roentgenograms Giving a Real Inverted Image with a 10 x Magnification.

Fig. 3

Appearance of Cuttings. Their Weight Ratio Between the Affected and the Corresponding Tooth is Determined.



$$\text{Expansion } \frac{42 - 10}{10} \cdot 100 = 320\%$$

This experimentally induced periapical process was studied in three kinds of experiments.

1. Antistreptolysin O Titer

The relationship to the antistreptolysin O titer was studied in cats. Sera from each cat were assayed at regular intervals after the inoculation. The antistreptolysin O titer was found to be positively correlated to the size of the osteolytic processes as measured on the roentgenograms. These trials enabled me to assess the accuracy of the technique. The extent of the osteolytic process was measured on roentgenograms exposed in such a manner that the central ray was perpendicular to the surface of the alveolar bone (90°). This technique was tested by making further measurements at a projection of 45°. The results were in close agreement, the coefficients of correlation being 0.61 and 0.52 respectively.

2. Inhibition of Inflammation by Fluorine and Other Trace Elements

The effect of the inhibiting action of F^- upon the inflammatory process was studied in 125 rats placed on a low F^- diet (4.8 ppm). Fourteen days prior to the operation the rats were divided into 5 groups whose drinking water contained F^- in concentrations of 0, 10, 20, 30, and 40 ppm. They were then inoculated with bacterial broth. Seven weeks later the presence of periapical osteolytic processes was determined. An inhibiting effect was first noticeable in the group which had been receiving 20 ppm of F^- in the drinking water. A more marked inhibitory effect was apparent in the 30 ppm group. However when the F^- content of the water was raised to 40 ppm no additional inhibition was noted. The values obtained are given in Table 1. The number of cases with widening of the periapical space below and above 100 percent is presented in Table 2.

TABLE 1

Osteolysis After Inoculation of Various Bacteria

<u>Inoculation with</u>				
<u>F in Water</u>	<u>Strept. Viridans</u>	<u>Staph. Aureus</u>	<u>S 84</u>	
		<u>Number of Rats</u>		
0 ppm	Osteolysis	19	11	20
	No Osteolysis	6	14	5
30 ppm	Osteolysis	10	12	13
	No Osteolysis	15	13	12

For comparison, experiments were carried out to determine the inhibitory action on the periapical osteolytic process by manganese, molybdenum, selenium, and vanadium, by themselves and combined with fluorine.

These studies were made on 250 rats which, like those in the preceding trial, had been kept on a low F⁻ diet (4.8 ppm). Fourteen days before the operative intervention, five groups of 25 rats were given drinking water containing, respectively, 30 ppm Mn (manganese nitrate); 30 ppm Mo (sodium molybdate); 10 ppm F + 15 ppm Mn; 10 ppm F + 15 Mo; and, as control, 10 ppm F.

The remaining 125 rats were similarly divided into 5 groups of 25 rats. However, instead of manganese and molybdenum, their drinking water contained selenium (sodium selenite) and vanadium (diammonium vanadotartrate).

When the experimental animals were examined after 7 weeks, selenium and vanadium failed to show any detectable inhibitory action. A probably but not significant effect was obtained with manganese, whereas molybdenum combined with fluoride yielded the most pronounced inhibitory action. Indeed, this effect was greater than that of either substance alone in the same concentrations (Table 2).

TABLE 2

Distribution of Osteolysis in Groups of 25 Rats
Receiving Trace Elements

Elements ppm	Osteolysis	Little or no Widening of Periodontal Space
10 F	19	6
30 Mn	14	11
30 Mo	19	6
10 F + 15 Mn	16	9
10 F + 15 Mo	11	14
10 F	18	7
30 Se	18	7
30 V	19	6
10 F + 15 Se	20	5
10 F + 15 V	19	6

The control groups in these trials deviated from those in the preceding experiment by 15 percent in the degree of the periapical osteolytic process. This discrepancy could probably be ascribed to variations in the virulence of the bacterial strain. Consequently, comparisons must be based on the relative frequencies of the osteolytic process in the control groups and the groups given trace elements.

3. Fluoride Effect on Other Bacteria in Root Canal

As in the above experiment, 6 groups of 15 rats were given a diet low in fluorine. One such group was inoculated with Streptococcus viridans, another with Staphylococcus aureus; for each of these groups a control group received S 84; the fifth group received no bacteria.

The preliminary results so far suggest that the incidence of osseous destruction varies. It was highest in the Streptococcus groups and lowest in the Staphylococcus aureus groups (Table 1).

To elucidate the action of fluorine, 10 ppm of fluorine was added to drinking water of the control groups and 30 ppm to that of the other groups. The preliminary results show that fluorine in a concentration of 30 ppm seemed to inhibit the ability of streptococci to induce osteolysis. Staphylococcus aureus, on the other hand, exhibited the same incidence of osseous destruction when the drinking water contained 10 and 30 ppm of fluorine. In all groups we attempted to recultivate the bacteria. In the staphylococcus aureus groups (Table) we were unable to do so.

With regard to the above experiments concerning the inhibiting action of trace elements and the induction of osteolysis by various bacteria, the following facts should be noted:

The problems presented here have no direct bearing on the production of dental caries. The alveolar bone admittedly exhibits both histological and biochemical similarities to dental enamel tissue, but the two also differ considerably, especially with regard to the effect of extrinsic factors.

The above experiments should serve as models for similar pilot experiments on animals. Conceivably this comparatively simple and precise procedure could make it possible to study various problems in cariology.

SUMMARY

Fluoride added to drinking water of rats inhibited the extent of artificially induced periodontal abscesses. The optimal concentrations of fluoride in water ranged between 10 and 30 ppm. The inhibitory effect was enhanced by the addition of molybdenum to the water. Selenium and vanadium failed to inhibit periodontal infection

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REVERSIBLE INHIBITION OF PLATELET FUNCTION BY FLUORIDE COMPOUNDS

by

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Thrombocytes are cellular fragments lacking a nucleus. They have important functions in hemostasis after vascular damage and they continuously seal microlesions of the capillaries.

Human platelets tend to spread on smooth non-glass surfaces. In clinical conditions this spreading capacity is a valuable and very sensitive test of platelet function. In congenital and acquired thrombocyte defects which cause bleeding disorders, diminished spreading capacity of the platelets occurs invariably. This method of evaluation of platelet function applies even in thrombocytopenia. The spreading of the platelets seems to parallel the so-called "physiological hemostasis", the sealing of endothelial lesions of the capillaries and the precapillaries.

The platelet spreading test (PST) can be easily performed. After sedimentation of erythrocytes in citrate blood, the platelet-rich plasma is diluted 1:10. Plastic slides are covered with the diluted plasma for 30 minutes. Then the preparations are rinsed, fixed and stained. Washed platelets, which have been separated from the plasma, are still able to spread if they are resuspended in an isotonic solution containing magnesium ions and some glucose.

Thrombocyte functional defects may be reversible or irreversible. The reversible inhibition of platelet spreading in paraproteinemia may be caused by adsorption of macroglobulines to the platelet membranes. On the other hand, in thrombasthenia, a primary lack of platelet enzymes leads to an irreversible functional defect. Thus, under experimental conditions, platelet function can be tested easily by the platelet spreading test. Small changes of their metabolic function cause remarkable morphologic changes which can be well demonstrated.

It was of interest to determine whether or not the influence of metabolic inhibitors on platelets could be reversed in vitro. Such investigations might also enable us to check the specificity of some antimetabolites.

One millimole of moniodine acetate inhibits platelet spreading completely. This inhibition is not reversed if moniodine acetate is removed

From the Medizinische Universitätsklinik. Director: Prof. Dr. W. Siede, Frankfurt a.M., Germany.

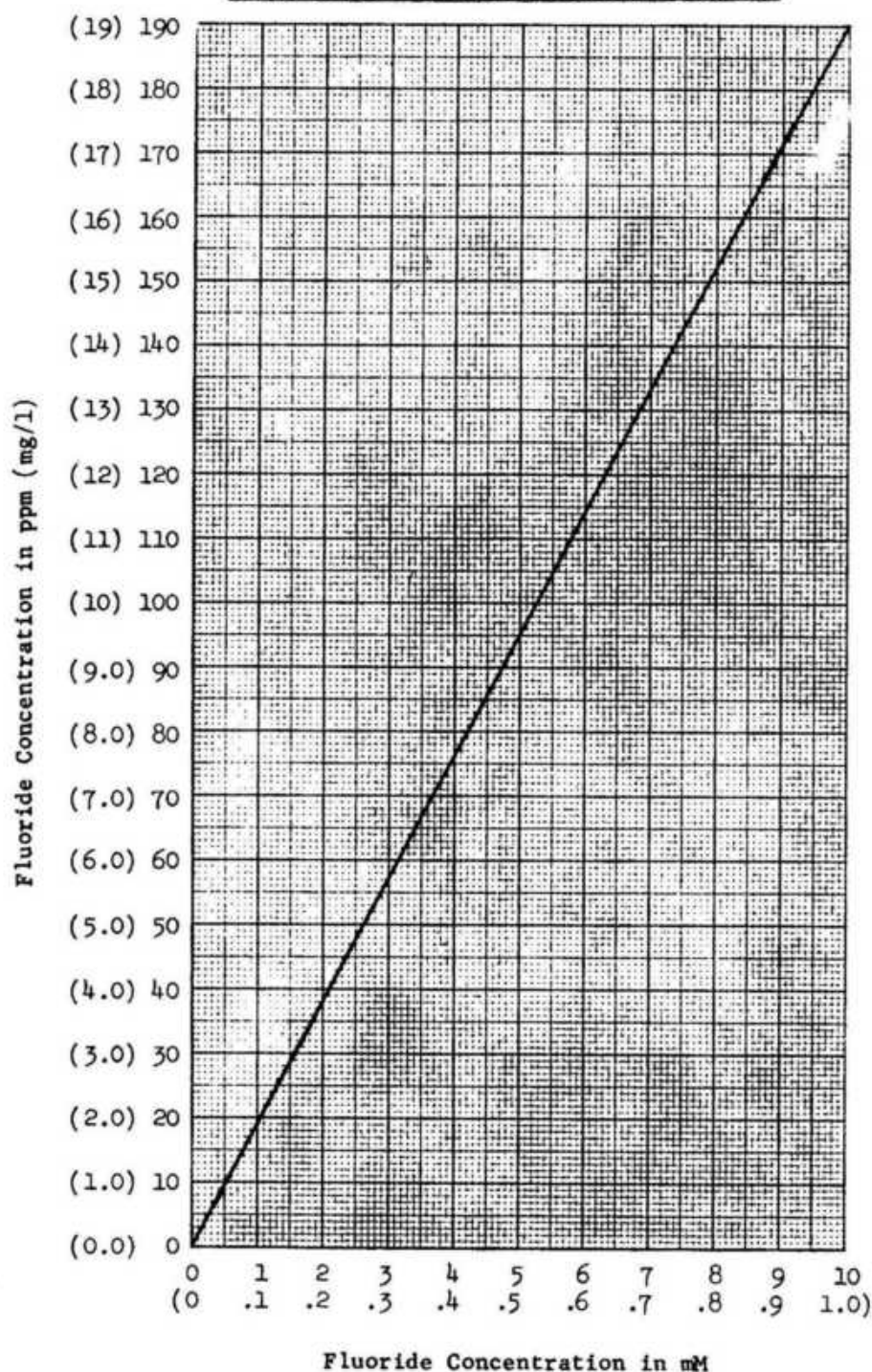
from the suspension medium. 10 millimoles of NaF enhance platelet spreading to some degree if the platelets are suspended in their own plasma. Higher concentrations of NaF inhibit spreading completely. The spreading of washed and resuspended platelets is completely inhibited by as little as 10 millimoles of NaF. If the NaF-poisoned platelets are washed twice by centrifugation, they again spread normally upon resuspension in a NaF-free medium. Thus, NaF has caused reversible inhibition of the spreading function. Even the inhibitory effect of 40 millimoles of NaF can be reversed by further washing of the poisoned platelets. NaF inhibits glycolysis probably on the enolase level. The addition of enolase to a platelet suspension poisoned with NaF does not neutralize the inhibitory effect of NaF. It appears that enolase cannot penetrate the platelet membranes whereas NaF does.

Diisopropylfluorophosphate (DFP) acts primarily as a cholinesterase inhibitor. Addition of 0.1 millimoles of DFP to a platelet-rich plasma caused a profound inhibition of platelet spreading. This effect likewise was neutralized by washing of the platelets. However, the addition of 5 millimoles of cysteine to the DFP-containing plasma neutralized the inhibitory effect of DFP completely. It is difficult to interpret this action of sulfhydryl groups. However, other cholinesterase inhibitors also were partially neutralized by cysteine.

In a similar way the inhibition of 1 millimole of K-cyanide can be reversed. Hydroxycobalamine (Vitamin B₁₂) combines with cyanide to form the non-toxic cyanidecobalamine. Therefore we were able to neutralize in vitro the inhibitory effect of 2 millimoles of KCN on spreading by adding 5 gamma/ml of hydroxycobalamine to the cyanide-poisoned plasma. Platelets are believed to be very fragile. But the close relationship between their functional behavior and morphology makes them very suitable for investigations with metabolic inhibitors and their antagonists.

Our investigations with fluoride compounds were models for research on the action of metabolic inhibitors in general and especially on platelet function. Such investigations can be performed with thrombocytes using simple methods and obtaining reproducible results.

CONVERSION TABLE MOLES TO PPM

Concentration of Aqueous Fluoride Solution

NOTE: In dilute water solution, fluoride concentration in mM (millimoles of F^- per liter of solution) is related to fluoride concentration expressed as mg/l or ppm (milligrams of F^- per liter of water, or parts by weight of F^- per million parts of water) by the equation:

$$\text{mM} = \frac{\text{ppm}}{19.0} \quad \text{or} \quad \text{ppm} = 19.0 \times \text{mM}.$$

Aqueous Fluoride Solution Concentration Conversion Tables

A. Conversion of Molar Concentration to Weight/Weight Concentration:

<u>Molar Concentration</u>			<u>Weight/Weight Concentration</u>
<u>M</u> (Moles/l)	<u>mM</u> (Millimoles/l)	<u>μM</u> (Micromoles/l)	<u>ppm* (mg/l)</u>
1.0	1,000	10 ⁶	19,000
0.1	100	10 ⁵	1,900
0.05	50	5 × 10 ⁴	950
0.01	10	10 ⁴	190
0.005	5.0	5 × 10 ³	95
10 ⁻³	1.0	1,000	19
10 ⁻⁴	0.1	100	1.9
10 ⁻⁵	0.01	10	0.19
10 ⁻⁶	0.001	1.0	0.019
10 ⁻⁷	0.0001	0.1	0.0019

B. Conversion of weight/weight concentration to molar concentration:

<u>Weight/Weight Concentration</u>	<u>Molar Concentration</u>
<u>ppm* (mg/l)</u>	<u>mM</u>
1,000	52.7
500	26.3
250	13.2
100	5.27
75	3.95
50	2.63
25	1.32
20	1.05
15	0.79
10	0.527
5.0	0.263
3.0	0.158
2.0	0.105
1.0	0.0527 (52.7 μM)
0.10	0.00527 (5.27 μM)
0.010	0.000527 (0.527 μM)

*For conversion to ppb (parts per billion, or ug/l), multiply by 1,000.

A.W.B.

THE IMPORTANCE OF DIETARY MAGNESIUM WITH PARTICULAR REFERENCE TO HUMANS

by

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Ottawa, Ontario

Vitalstoffe 13 (4) 144-149, Zivilisationskrankheiten 4/68
National Research Council of Canada Publ. No. 10 173

(Abstract)

In an extensive review of recent data on magnesium metabolism and its role in the human system, Marier discusses the relationship of magnesium to fluoride.

Magnesium is essential for growth and is an activator of many enzyme systems. Approximately half of the body's magnesium is stored in the skeleton (52.4%), slightly less in the intracellular compartments (46.5%), mostly in muscles; only about 1% is present in body fluids. Like potassium, magnesium is very important in intracellular reactions.

Young adults require about 300 mg/day to maintain positive balance, children and expectant mothers approximately 400 mg/day. According to Seelig, about 6 mg/kg/day would be a desirable daily intake.

Sources of dietary magnesium are vegetables, black strap molasses, nuts and whole meal flour. Magnesium is less available in meat and fish. Eggs and dairy products, which are high in calcium, constitute a poor source of dietary magnesium.

Like calcium, magnesium is absorbed from the gut. Deficiency of one ion leads to overabsorption of the other. Citrate facilitates the passage of both calcium and magnesium into the bloodstream and from the blood into the urine. Because both ions are in competition for the same absorption mechanism, large dietary calcium intake reduces the absorption of magnesium. Large dietary intake of phosphates also impedes magnesium absorption whereas a low phosphate intake enhances it.

Magnesium levels in plasma are controlled mainly by the kidneys. The parathyroid hormone does not appear to control magnesium metabolism as it does that of calcium. An inverse relationship between magnesium and aldosterone has been observed. Aldosterone seems to affect intracellular magnesium primarily. Magnesium's concentration in blood plasma is approximately 2.4 mg% compared with 10 mg% for calcium, 3.6 mg% for inorganic phosphorus and 2.3 mg% for citrate. Magnesium is the most vulnerable ionic component of the serum. Unless it is continually replenished a negative balance results with or without hypomagnesaemia. Magnesium prevents clotting of blood, whereas calcium enhances it.

Magnesium tends to delay nucleation and precipitation of apatitic calcium phosphates. Addition of magnesium to urine obtained from persons with urinary calculi alters the urine in such a way that it does not precipitate calcium.

Dietary magnesium deficiency reduces urinary magnesium and increases the output of phosphates. Therefore, addition of magnesium to the diet protects animals against stone formation. In humans, oral magnesium supplements of the order of 420 mg of Mg daily have prevented formation of oxalate and phosphate kidney stones.

Magnesium supplements in the diet also increase urinary citrate excretion. In renal calcinosis, low citrate levels in the urine are found.

Magnesium deficiency or an increase in dietary phosphorus promotes accumulation of calcic deposits in the kidneys. In alcoholics, magnesium depletion occurs due to interference by ethanol with tubular reabsorption of magnesium.

Dystopic calcification of the kidneys and arteries can result from magnesium deficiency. A low incidence of atherosclerosis has been linked with high magnesium and low cholesterol. An inverse correlation between serum magnesium and cholesterol has been suggested. Magnesium might play a vital intracellular role in prevention of soft tissue degeneration.

Epidemiological surveys have established an inverse correlation between total hardness of drinking water and the incidence of cardiovascular disease. Magnesium present in "hard" drinking water had a significant effect in prevention of atherosclerotic lesions in rabbits whose blood serum contained 2% cholesterol. This is in contrast with little protective effect of calcium.

Parathyroid disorders may disturb magnesium balance. Primary hyperparathyroidism causes an increase in urinary output of magnesium. Parathyroidectomy and hypoparathyroidism increase the uptake of magnesium by bone and result in hypomagnesemia. In parathyroid surgery, post-operative magnesium supplements have proved beneficial.

Whereas young rats can lose a large percentage of their skeletal magnesium, adult human bone is probably quite unreactive in supplying plasma requirements of magnesium. The most easily available magnesium reserve in adults is that present in muscle tissue.

The following are the clinical features of magnesium deficiency:

Positive Chvostek sign, without a concomitant Trousseau sign; a low-voltage E. C. G.; epileptiform convulsions and E. E. G. changes, sometimes suggesting a focal cerebral lesion; ataxia, and muscular weakness; irritability, and sometimes depression; tremor, muscular fibrillation; hypertension and muscle rigidity.

TABLE

Symptoms	Fluoride Intoxication	Magnesium Deficiency
Leg cramps, paresthesias	78* Sauerbrunn, et al.	29 Fourman, et al.
Muscular fibrillation	51 Kretchmar, et al.	41 Hanna, et al.
	87 Waldbott	84 Suter, et al.
Tetaniform convulsions (with normal serum Ca)	51 Kretchmar, et al.	29 Fourman, et al.
	87 Waldbott	62 Martindale, et al.
2 to 3-fold increase in serum P at time of convulsion	51 Kretchmar, et al.	62 Martindale, et al.
	87 Waldbott	
Cataracts (optical neuritis)	31 Geall, et al.	29 Fourman, et al.
Bone esostoses and/or soft- tissue calcification	90 Weatherell, et al.	71 O'Dell, et al.

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Magnesium may play a role in Parkinson's disease as well as in certain muscular ailments. Hypomagnesaemia is found in the following conditions: Malabsorption syndrome; prolonged or severe loss of body fluids; chronic alcoholism; diuretic therapy (especially large thiazide doses); renal tubular acidosis or necrosis; portal cirrhosis; primary aldosteronism; primary hyperparathyroidism; hypercalcaemia from any cause; post-operative hypoparathyroidism; epileptiform seizures; malnutrition; hypertension; leukemia. Intravenous, intramuscular or oral magnesium treatment may be indicated in these conditions.

As to the interrelation of magnesium and fluoride, exceedingly high levels of both components fed to growing chicks caused noticeable leg weakness and reduced the mineral content of bone, namely the total ash, calcium, phosphorus and citrate but increased the bone magnesium level. Fluoride may lead to a rachitic-like condition which results from a high-magnes-

ium and low-citrate level in bones. In mangesium-deficient dogs, fluoride supplements prevented soft tissue calcification but failed to improve muscle weakness and convulsions. In rats, fluoride aggravated the hypomagnesaemia which intensified the animals' convulsive seizures.

The symptoms of magnesium deficiency are remarkably similar to those of fluoride intoxication, as indicated in the table above. Marier suggests that this may be due to fluoride-induced increase in the uptake of magnesium by bone and that nonskeletal magnesium depletion may be recognized, someday, as a key feature of fluoride intoxication. However, much additional work has to be done to elucidate the interrelationship of fluoride intake and magnesium deficiency in human beings.

DAMAGE TO VINES BY FLUORIDE EMISSIONS

by

H. H. Hopp

Freiburg, Germany

Wein-Wissenschaft 21, No. 4:141-9, 1966

(Abstract)

In early August, 1965 a sudden outbreak of a serious disease to grape vines occurred in the southern part of Baden. It was attributed to fluoride emanating from a nearby brick factory. The investigation disclosed varying degrees of injury in four zones at increasing distances from the brickworks. In the area 200 to 400 meters from the brickworks, the vines had lost about 1/3 of their leaves. The foliage exhibited typical fluoride damage. Leaves manifested a brownish discoloration and necrosis which progressed intercostally. The stems were not affected. Ripening of grapes was delayed.

Two unexpected findings were reported:

Where there was no visible damage, the leaves contained between 22 and 24 parts per million of fluoride (Table 1). In the area closest to the factory the leaves contained less fluoride than in the more distant areas. The author attributes this unexpected phenomenon to the fact that the leaves were completely dead. Therefore, the usual translocation of fluoride inside of the leaf did not occur. Furthermore, absorption of particulate fluoride hydrolyzed by rain ceases when the leaf becomes necrotic.

The second unusual feature: In another polluted area little damage was encountered. Where this occurred, it was determined that the vines had been sprayed with a mixture containing copper and calcium. The calcium had combined with the fluoride forming the less soluble calcium fluoride and had thus prevented the fluoride ion from penetrating deeper into the leaf tissue.

MORBIDITY AND MORTALITY IN MAN CAUSED BY PULMONARY CANCER AND ITS RELATION TO THE POLLUTION OF THE ATMOSPHERE IN THE AREAS OF ALUMINUM PLANTS

by

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Acta Unionis Internationalis Contra Cancrum 19:742-745, 1963

(Abstract)

The authors studied morbidity and mortality from cancer in the vicinity of two large aluminum factories which utilized the peccoke anodic mass in the electrolytic process of aluminum smelting. During this process harmful substances are emitted, especially 3,4-benzpyrene, resinous substances, silicon dioxide and hydrogen fluoride. Approximately 18 tons a day of a resinous substance and more than 10 kg/day of 3,4-benzpyrene were discharged into the air from the factory. The daily emission of HF and SiO₂ was not recorded.

The plant had been in operation for about 30 years and had maintained a constant level of production. It is the only source of the massive air pollution in the area.

In the flydust which settled on snow, the authors obtained 49.5 gamma of 3,4-benzpyrene per m² per day at a distance of 1 km from the plant. None was detected in snow at a distance of 7 km. The average rate of pollution by 3,4-benzpyrene exceeded that of other areas by 5 to 33 times. The average concentrations of 3,4-benzpyrene in the air exceeded the average figures obtained from Moscow by as much as 17 times.

The authors studied two areas: one at a distance of 4 km; the other 7 to 9 km from the plant. The latter was used for control purpose, although it was not entirely free of the dust.

A six year morbidity survey was made on three groups of the adult population, namely 310, 521 and 377 subjects. There was a definite correlation between the smoke rate of the atmosphere and the susceptibility of the population to bronchitis and focal pneumonia.

The diseases were attributed to 3, 4-benzpyrene which, in conjunction with the other pollutants, were believed to create the soil for the development of cancer.

From the A. N. Sytin Institute of General and Public Hygiene, USSR Academy of Medical Sciences, Moscow, USSR.

With respect to mortality in the two areas, the authors noted an increase in cancer deaths during 1949 to 1960 as compared with the control areas.

In the environs of the two factories the overall cancer rate was 44.1 per 100,000. This was significantly higher than in the control (40.3 per 100,000) area and higher than the national mortality rate from cancer.

This figure refers not only to the incidence of cancer of the respiratory organs but also to that of the liver, biliary tract, rectum and urinary bladder.

EFFECT OF SODIUM FLUORIDE ON CALCIUM ABSORPTION AND BALANCES IN MAN

by

H. Spencer, I. Lewin, J. Fowler and J. Samachson
Hines, Illinois

American Journal of Clinical Nutrition 22:381-390, April 1969

(Abstract)

Calcium and phosphorus balances have been determined in nine patients under strictly controlled dietary conditions during a daily intake of an average of 20.6 mg of sodium fluoride. During sodium fluoride intake, the urinary calcium excretion of three of the nine patients was lower than in the control study. In the majority of patients the plasma levels of ^{47}Ca were lower during the intake of sodium fluoride than in the control studies, indicating decreased absorption of ^{47}Ca . Intestinal absorption of calcium and the calcium balances did not improve during an intake of 20.6 mg of sodium fluoride per day for a period of 22 to 42 days.

From the Veterans Administration, Edward Hines, Jr. Hospital.

ADVERSE EFFECT OF TOPICAL FLUORINATED CORTICOSTEROIDS IN ROSACEA

by

Ian Sneddon, M. B., F. R. C. P.

Sheffield, England

British Medical Journal, 1:671-3, March, 1969

(Abstract)

Since fluoridated steroids were first introduced about two decades ago, their topical application has proven to be most beneficial in skin diseases, especially in allergic dermatoses. They have also been used in the control of certain infectious skin lesions.

Sneddon reported eruptions on 14 patients with rosacea, an acne-like pustular skin eruption, which involves mainly the nose and the face and is often refractory to treatment. In Sneddon's cases fluorinated steroid creams had been used for periods of 2 months to 7 years.

The telangiectasia, a condition associated with rosacea, had become aggravated following the use of fluorinated steroids. Upon cessation of treatment there was usually a severe "rebound" inflammatory reaction with edema and aggravation of pustular eruption. Eventually, about three months after the fluorinated steroid ointment was discontinued, the telangiectasia cleared up.

Hydrocortisone ointment which does not contain the fluorine ion did not produce any untoward effect. In conjunction with oral tetracycline it cleared the condition entirely.

From the Rupert Hallam Dept. of Dermatology, Royal Infirmary.

Correction:

On page 104, Vol. 2, No. 2, third line from bottom "73 g per 40 hectar" should read "73 g per 40 are."

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