July, 1981 Vol. Fourteen No. Three

FLUORIDE

OFFICIAL QUARTERLY JOURNAL

OF

INTERNATIONAL

SOCIETY for

FLUORIDE

RESEARCH

Vol. Fourteen No. Three July, 1981 Pages 93-146

FLUORIDE

Quarterly Reports

Issued by

THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH

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FLUORIDE is published quarterly by THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH, INC.,

SUBSCRIPTION RATES 1979 — Price per annum in advance including postage $25.00, Single copies $7.00.

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EDITORIAL

HYDROGEN BONDING BY FLUORIDE ION: MECHANISM OF TOXICITY?

In the Journal of the American Chemical Society, Vol. 103, No. 1, pp. 24-28, January 14, 1981, John Emsley and co-workers at King's College, London, England, and Brock University, St. Catharines, Ontario, Canada, report findings on the hydrogen bonding properties of fluoride ion that shed considerable light on the problem of the mechanism of fluoride toxicity. On the basis of experimental and theoretical data, they demonstrate the existence of an unusually strong hydrogen bond between the fluoride ion and amides (RCONHR') which they suggest may be involved in how fluoride interferes with normal biological functioning.

Hydrogen bonds have the structure X-H··Y, in which X-H is a normal covalent bond and H··Y is the weaker hydrogen bond. Elements X and Y that participate best in hydrogen bonding are strongly electronegative ones like oxygen, nitrogen, and fluorine (as F^-). It is the O-H··O hydrogen bond that keeps water liquid at room temperature and makes it so high boiling for a molecular weight of only 18 atomic mass units. In macrobiomolecules, N-H··O and N-H··N hydrogen bonds between amide groups and between thymine-adenine and cytosine-guanine base pairs bind protein and nucleoprotein chains together, respectively, as in the double helix of deoxyribonucleic acid (DNA).

According to Emsley et al., the hydrogen bond between fluoride ion and the nitrogen-hydrogen linkage of amides, N-H··F^-, has a bond strength of 148 kJ/mol (35 kcal/mol), which makes it "the second strongest type of hydrogen bond and the strongest heteronuclear hydrogen bond."

The strongest hydrogen bond is that of the bifluoride ion, F-H··F^-, estimated to have a bond energy of 214 kJ/mol (51 kcal/mol). By contrast, the strength of intermolecular amide hydrogen bonds, N-H··O, is only about 20-40 kJ/mol (5-10 kcal/mol). It therefore appears likely that in the presence of fluoride the N-H··O hydrogen bond can be replaced by the much stronger N-H··F^- hydrogen bond.

In the view of Emsley et al., such disruption of and interference with normal hydrogen bonding in proteins and nucleoproteins by fluoride offer a sound mechanistic basis for "the profound biological effects that are being linked to the simple fluoride ion such as genetic damage, birth defects, allergy responses, and cancer," as documented in the book Fluoridation: The Great Dilemma, by G.L. Waldbott, A.W. Burgstahler, and H. L. McKinney, with a foreword by A. Ochsner, Coronado Press, Inc., Lawrence, Kansas, 1978.

Whereas the fluoride ion is comparatively stable in aqueous solution and not very reactive in normal covalent bond-forming and bond-breaking reactions, "its strong hydrogen bonding potential toward the NH group of amides and related biomolecules," provides, in the words of Emsley et al., "an explanation of how this reputedly inert ion could disrupt key sites in biological systems."

A.W.B.

EDITORIAL

FLUORIDE AND SOFT TISSUE CALCIFICATIONS

One of the most characteristic features of the biological action of fluoride is its strong affinity to calcium. Calcified tissues (bones, teeth and nails) are the major target organs of fluoride. Not only is the absorption and retention of fluoride affected by dietary calcium (1-2) but calcium metabolism itself is altered by the presence of fluoride(3-6). Acute fluoride intoxication is associated with low serum calcium (7). In chronic intoxication, calcium metabolism is often disturbed: the calcium content of the blood may be either elevated or reduced (8,9).

Whereas much research is available on the relationship of calcium to fluoride in bones and teeth, calcification of soft tissue organs has received relatively little attention. In a survey of 127 autopsies in Utah, Call et al. (10)found as much as 258 ppm fluoride in calcified aortas. In a similar study of 165 autopsies, Geever et al. (11) recorded up to 8400 ppm of fluoride in calcified aortas of two Grand Rapids individuals and 2340 in another subject from New York State, but they observed no correlation between the calcium and fluoride levels. This lack of a direct relationship between calcium and fluoride in the aorta suggests that deposition of fluoride is not dependent on the presence of calcium but can occur independently as outlined by Waldbott (12).

Calcification of the arteries of the Mönckeberg type, reported by several investigators (13-17), has most recently been recorded by Huo in association with the skeletal fluorotic changes due to fluoride in food(18).

Especially noteworthy is the finding by Kour et al. pertaining to soft tissue calcification, namely calcified areas in the liver of rats that had received large doses (1000 ppm) of fluoride in their drinking water (this issue page 119). It is true, calcific areas were found in only 3 out of 10 animals and only after long-term (3 months') fluoride intake at elevated levels (1000 ppm). Nevertheless, the observation of the Indian authors deserves attention because lifelong intake in humans of small amounts of fluoride is likely to produce effects similar to those from large doses administered experimentally to animals for short periods (19).

Connective tissues such as ligament and joint capsules are known to be subject to fluoride-induced calcifications. In calcium-contaning kidney stones, fluoride up to 10,650 ppm (20) has been recorded, whereas kidney stones in which little or no calcium is found are low in, or almost free of, fluoride. Therefore fluoride is likely to be etiologically involved in the production of calcium-containing uroliths.

The presence of calcium deposits in arteries, connective tissue, liver and kidney stones calls for further studies on whether or not fluoride-induced calcifications occur in other soft tissue organs. In pursuance of such studies, the role of other minerals, especially that of magnesium will have to be explored. As shown by Marier (21) magnesium appears to play an important role in the production of tissue calcifications.

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FLUORIDE BRIEFS

Bone biopsies were made on 52 patients with osteoporosis treated daily for two years with 50 mg NaF, 8000 units Vitamin D_2 and 1 gm calcium. The osteoblast population, the volume of osteoid and the volume of the bone trabeculae increased significantly. Bone resorption increased less. In six subjects histological examination revealed osteomalacia.

Briancon, D., Charhon, S., Edouard, C. and Meunier, P.J.: Histological Effects of the Treatment of Osteoporosis with the Combination of Sodium Fluoride, Vitamin D and Calcium. Revue Du Rhumatisme et Des Maladies Osteo-Articulaires, 47:693-8, 1980.

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In Wistar rats that received 20 mg NaF per kg body weight for four weeks, the activity of alkaline phosphatase in granulocytes decreased and the leucocyte count of the peripheral blood became elevated. This finding, which is interpreted as a toxic effect of large doses of fluoride on the red blood cells, should be borne in mind in evaluating health effects on humans in the environs of factories which contaminate the atmosphere by fluorides.

Orzechowska-Juzwenko, K., and Orzechowski, V.: Effect of Contamination of Drinking Water with Sodium Fluoride on the Alkaline Phosphatase. Med. Pr. 31:371-377, 1980.

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FLUORIDE ACCUMULATION IN AQUATIC ORGANISMS IN THE LAGOON OF VENICE

by

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SUMMARY: As some industries are likely to discharge fluoride into the Lagoon of Venice a survey was undertaken of the environmental levels of this element. However, determination of the fluoride level of water alone tells little about the entry of this element into the food chain. We therefore surveyed organisms for their ability to accumulate fluoride in tissues. The biological indicators chosen for this purpose were the crustacean Balanus amphitrite (barnacles) and the mollusk Mytilus galloprovincialis (mussels). Both species were found to accumulate fluoride in their soft tissues to above-ambient levels. Maximum fluoride concentrations found for barnacles and mussels were 81 ± 6 µg/g and 85 ± 20 µg/g (dry weight) respectively. Seasonal variations in soft tissue fluoride levels were recorded and are probably related to the reproductive cycle in both species. Only the barnacles are able to accumulate fluoride in proportion to water levels, even in heavily polluted environments. Thus to monitor the fluoride pollution in the Lagoon of Venice, B. amphitrite seems to be the more efficient and reliable indicator. Before using this organism however in monitoring programs, the biological variables that affect accumulation should be taken into account.

Introduction

Fluoride is generally considered to be a major component of sea water and it is present in ocean water at a concentration of about 1.3 mg/kg with a ratio to chlorinity of 6.7×10^{-5} (1). In coastal waters of the northern Adriatic Sea, a positive linear correlation between fluoride concentration and chlorinity was found (2). The same relationship was observed along chlorinity gradients in the Baltic Sea (3) and in the northern part of the Lagoon of Venice where urban and industrial disturbance is minimal (4). However fluoride pollution may alter this relationship. In fact in the central part of the Lagoon of Venice, especially in the industrial area of Portomarghera where some industries discharge fluoride into the lagoon, no relationship between fluoride concentration in water and chlorinity was found and all fluoride values exceeded the levels considered normal for seawater having the same chlorinity (personal data).

Risk assessment for marine ecosystems and consequently for human health, however, can be based partially upon surveying the pollutant con-

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centration in water, as this gives no information about the entrance of the substance into food chain. Therefore it seemed appropriate to extend the survey by examining organisms for their ability to accumulate pollutants in tissues, the further advantage being that if organisms are able to accumulate in relation to ambient levels, they can be used as biological indicators of marine pollution in environmental monitoring programs. In the Lagoon of Venice two aquatic organisms were found to be potential indicators of pollution: the crustacean Balanus amphitrite-barnacle-(5) and the mollusk Mytilus galloprovincialis - mussel - (6). However the accumulation of pollutants by indicator organisms can be affected by environmental variables and physiological conditions of the organisms themselves (7).

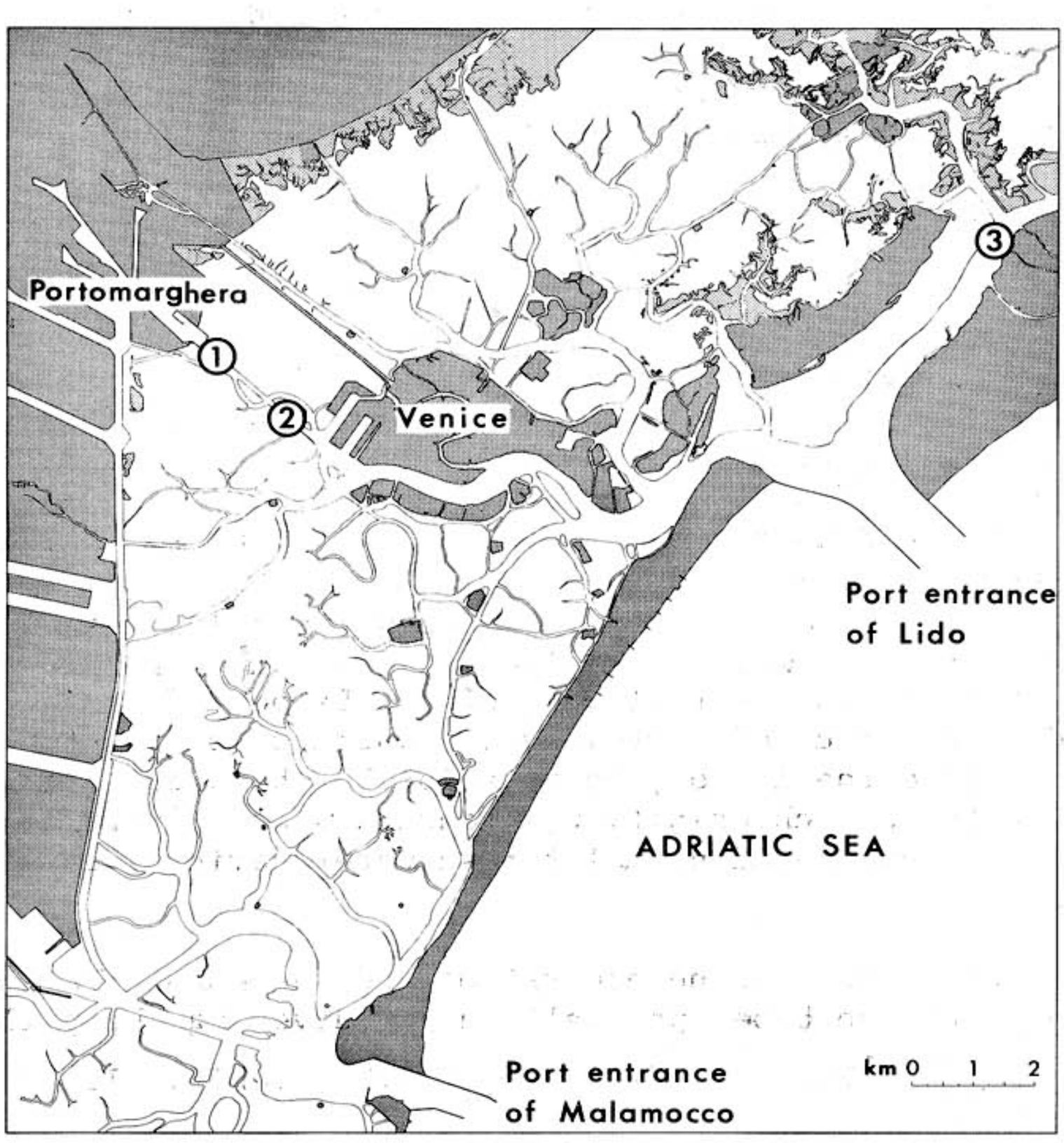
The aim of this work was to determine whether season and extremes in environmental conditions can affect fluoride accumulation in barnacles and mussels.

Materials and Methods

The investigation was carried out at three stations in the central part of the Lagoon of Venice (Figure 1). Two stations were close to the

Figure 1

Sampling Stations in the Lagoon of Venice



industrial area of Portomarghera (station 1 and 2); the last station (Station 3) was located in a part of the lagoon in which industrial and urban disturbance is much lower. The three stations were sampled four times, in March, June, September and December 1977; the water and organisms were collected about 1 meter below the mean water level. Fluoride concentration in unfiltered water was determined colorimetrically with lanthanum alizarin complexone method(8), measurements of optical density being made with a double beam spectrophotometer. Barnacles were collected from stations 1 and 2, and mussels from stations 2 and 3. Tissue analyses were conducted on soft parts of the organisms and fluoride concentration was expressed on dry weight basis. Fluoride extraction was performed by steam distillation according to the modified Willard-Winter method (9, 10); to determine fluoride concentration in the distillate, the same colorimetric method as for water was used.

Results and Discussion

At all stations, the same seasonal fluctuation of water temperature was recorded (Table 1); the highest temperature was measured in July (about 26°C) and the lowest in January and December (about 7°C).The different levels of fluoride concentration in water, decreasing from station 1 to station 3, were observed during the entire year (Table 1); at all stations the water fluoride levels showed no significant ($P < 0.01$) seasonal fluctuation.

Table 1

Temperature and F^- Concentration of Water at Three Stations

	St.	Jan.-Mar.		Apr.-June		July-Sept.		Oct.-Dec.	
t °C	1	11.4	1.7(10)	22.4	2.7(9)	22.3	2.7(9)	10.4	3.1(7)
	2	10.2	1.8(10)	21.8	2.9(9)	21.5	3.3(9)	9.1	3.0(7)
	3	9.5	1.9(10)	20.5	3.2(9)	20.7	2.9(8)	10.4	4.4(5)
F^- mg/l	1	1.43	0.10(10)	1.38	0.06(9)	1.48	0.08(9)	1.42	0.06(7)
	2	1.31	0.07(10)	1.30	0.05(9)	1.35	0.07(9)	1.31	0.08(7)
	3	1.14	0.07(10)	1.13	9.08(9)	1.24	0.08(8)	1.24	0.09(5)

Barnacles and mussels were both found to accumulate fluoride in their soft tissues to above ambient levels (Table 2); the maximum fluoride concentration found for barnacles was 81 ± 6 µg/g at station 1 and for mussels 85 ± 20 µg/g at station 3. The concentration factor calculated as the ratio of mean concentration of fluoride in dry soft tissue of the animal to mean concentration in water, was between twenty and seventy times for both species.

Table 2

F^- Accumulation in Barnacles and Mussels

	St.	March	June	September	December
Barnacles	1	61±3 (8)	81±6 (8)	50±8 (8)	54±4 (8)
F^- µg/g	2	27±8 (8)	50±7 (8)	33±6 (8)	23±9 (8)
Mussels	2	45±8 (8)	31±12 (8)	38±6 (8)	23±9 (8)
F^- µg/g	3	85±20 (8)	27±6 (8)	61±21(8)	83±12(8)

Differences between stations in the fluoride concentration of water were clearly reflected only by the barnacles. At station 1, where the highest fluoride levels in water were recorded, the fluoride concentration in barnacles was always higher than at station 2. Mussels, on the contrary, do not accumulate fluoride in proportion to the concentration of this element in the water; in fact they had higher fluoride levels at the least polluted station (station 3). This could be explained by the fact that at station 2 mussels are under stress, being at the limit of their survival; at station 1, the nearest to the polluted industrial area, no mussels were found.

In the Lagoon of Venice, thus, the barnacle seems to be the more efficient indicator of fluoride pollution since it is able to accumulate this element in proportion to water levels even under stress. Indeed, a necessary requirement for a biological indicator is relative insensitiveness to the adverse effects of pollutants as pointed out also by Phillips (7) and Zaroogian et al. (11).

Observations of seasonal variations in fluoride tissue concentrations showed that maximum levels in barnacles occurred in July at both stations. For mussels, significant seasonal fluctuation in fluoride concentration was found only at station 3, with maximum levels in spring and winter. As no significant seasonal fluctuation was recorded in aqueous fluoride concentration, these variations are probably related to processes within the animals themselves, for example in the reproductive cycle. In the Lagoon of Venice, barnacles spawn during the summer months exclusively; mussels spawn in autumn, winter and spring and are inactive only in summer. Thus in both species the highest fluoride levels coincide with the spawning periods. The influence of the reproductive cycle on pollutant accumulation has been noted in the literature for barnacles (12), mussels (13) and other mollusks (14, 11).

Moreover, barnacles and mussels are included directly or indirectly in the human diet, especially during their spawning periods; mussels

are eaten directly and certain fishes - usually included in a local dish - feed on barnacles. Fluoride can be toxic at a certain concentration, behaving as a general inhibitor of oxidative metabolism and damaging the nervous and skeletal system (15). According to the literature (16) a daily intake of about 3 mg of fluoride from water can induce fluorosis in humans. Daily ingestion of 20 mussels in spawning condition, could provide daily intake of about 1.2 mg fluoride. In consequence, more attention should be paid to fluoride levels in environments like the Lagoon of Venice, in the vicinity of highly industrialized communities in which a large amount of local seafood is included in the diet.

In conclusion, to monitor the fluoride pollution in the Lagoon of Venice, the crustacean Balanus amphitrite seems to be an efficient and reliable indicator because it can reflect differences in the water fluoride concentration even in heavily polluted environments. However, before using this organism in monitoring programs the biological variables (i.e. reproductive cycle) that affect pollutant accumulation should be taken into account.

Acknowledgements

The authors thank Mr. G. F. Antonini and Mr. M. Cardinaletti for technical assistance. The research was supported by the Prógramma Finalizzato "Promozione della Qualita del'Ambiente."

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COPPER AND IRON IN TISSUE FOLLOWING EXPERIMENTAL FLUOROSIS

by

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SUMMARY: We studied the distribution of copper and iron in the liver, kidney and bone of mice subjected for 16 weeks to varied fluoride concentrations in drinking water. Whereas copper registered a significant fall, there was a marked increase in iron levels of the above-mentioned organs following fluoride administration.

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Introduction

Because fluoride is highly reactive, it complexes with many organic and inorganic biological components. Reportedly, fluoride interacts and alters the metabolism of calcium (1-3) and magnesium (4-6). However, data on the effect of fluoride on other essential metallic nutrients are both sparse and inconclusive. According to Ruliffson et al. (7) and Wegner et al. (8), fluoride enhances absorption and utilization of iron. On the other hand, in fluoride-treated animals, Kahl et al. (9) observed a decrease in ^{59}Fe incorporation in the blood with concomitant increase in ^{59}Fe uptake in the bone marrow and the liver. Furthermore, anemia has been reported in fluorosis (10, 11).

The present paper discusses, in detail, alterations in the iron and copper levels of various tissues following fluoride administration. These two essential micronutrients are vitally involved in various anemic manifestations (12). Copper deficiency, according to Prasad (12), not only causes anemia but also produces skeletal abnormalities.

Material and Methods

Forty-eight female albino mice, each weighing 19-25 g, were divided into 6 groups of 8 each. The animals were fed a balanced pellet diet supplied by Hindustan Levers, Bombay, India and were subjected for 16 weeks to fluoride concentrations of 0 ppm (control), 10 ppm (Group I), 25 ppm (Group II), 50 ppm (Group III), 100 ppm (Group IV) and 200 ppm (Group V) in the drinking water.

After the treatment, the animals were anestheized with ether, and their liver, kidneys and bone (femur) removed by means of stainless steel dissecting instruments. These tissue samples were processed for analysis by Atomic Absorption Spectrophotometer employing the technique of Barker et al. (13). The processing, in brief, involved sequential digestion of a known amount of dried tissue in concentrated nitric and perchloric acid (70%) followed by appropriate dilution in deionized water.

Utmost precautions were taken to avoid contamination of the samples during processing. All glassware and sampling bottles were first cleaned in hot nitric acid whereafter they were thoroughly washed with deionized water. A blank, devoid of tissue and treated in exactly the same way as the tissue samples, was also run. Appropriate corrections were made for the amount of copper and iron appearing in the blank.

Results

Depletion of copper in the bone and liver occurred in Groups III, IV and V (50 ppm, 100 ppm and 200 ppm fluoride) whereas depletion in the kidney was recorded only in Groups IV and V. The degree of copper depletion in these tissues increased with ascending fluoride concentrations in the drinking water; the depletion in the liver approximated 15%, 18% and 23% in groups III, IV and V, and in the kidney 26% and 39% in Groups IV and V respectively. In the bone the depletion of copper was most pronounced. It reached 52%, 60% and 64% in Groups III, IV and V respectively(Table 1).

Table 1

Copper Levels[a] Following Fluorosis

	Groups				
	Control	I 10 ppm F	II 25 ppm F	III 50ppm F	IV 100 ppm F	V 200 ppm F
Liver	20.42 ±2.37	19.12 ±1.83	21.07 ±2.12	17.44[b] ±2.05	16.83[c] ±1.84	15.70[c] ±1.93
Kidney	23.07 ±2.67	25.23 ±2.29	22.17 ±2.97	20.09 ±2.16	17.08[c] ±2.04	14.07[d] ±1.87
Bone Femur	9.64 ±1.30	10.09 ±1.27	9.08 ±1.07	4.61[d] ±0.53	3.94[d] ±0.47	3.7[d] ±0.44

a: Values are mean ± S.D. and expressed as ppm dry tissue weight. Significantly different from control, b: $p<0.05$; c: $p<0.01$; d: $p<0.005$

Table 2

Iron Levels[a] Following Fluorosis

	Groups				
	Control	I 10 ppm F	II 25 ppm F	III 50ppm F	IV 100 ppm F	V 200 ppm F
Liver	1058.85 ±192.6	996.7 ±113.6	1188.7 ±142.7	1711.5[d] ±282.5	1816.35[d] ±210.25	2496.1[d] ±293.05
Kidney	928.73 ±141.25	1098.85 ±181.25	1058.23 ±129.2	1268.3[d] ±140.35	1586.3[d] ±227.25	2032.2[d] ±288.55
Bone Femur	285.67 ± 54.72	293.29 ± 43.17	271.63 ± 34.37	294.68 ± 34.30	326.76[b] ± 39.43	340.37[c] ± 38.3

a: Values are mean ± S.D. and expressed as ppm dry tissue weight. Significantly different from control, b: $p<0.05$; c: $p<0.01$; d: $p<0.005$

On the other hand, the iron content of the liver and kidney increased tremendously in the wake of excessive ingestion of fluoride; in liver it was elevated by 61%, 72% and 136% and 37%, 71% and 119% in the kidney in Groups III, IV and V respectively. The elevation in iron content in the bone was marginal following fluoride ingestion, except in Groups IV and V where it was statistically significant (Table 2).

Discussion

The present study indicates a physiological interrelation of fluoride with copper and iron resulting in overall depletion of copper and concomitant increase of iron in the various tissues studied following experimental fluorosis.

Copper depletion is known to adversely affect overall metabolism, especially the iron metabolism (12). According to Prasad the deficiency of this nutrient results in anemia which is frequently reported during fluorosis and attributed to impaired hemoglobin synthesis (10, 11) due to fluoride intake. In fact, in copper deficiency, impairment of iron utilization - vital for hemoglobin synthesis - is well established (14).

In the present study, the storage of iron in the bone and liver following administration of fluoride implies that iron uptake exceeds iron metabolization. This can be explained on the basis of concomitant depletion of copper from these tissues following fluoride administration. Copper depletion not only impairs iron utilization but also affects its release from iron-storing organs such as the liver (15). In fact, a copper-dependent protein, ceruloplasmin - considered necessary for the release of iron from hepatic parenchyma and reticuloendothelial cells - reportedly suffers depletion during copper deficiency (14).

The present study which establishes accumulation of iron in the liver and bone of fluoride-treated animals, is supported by Kahl et al. (9) who, following fluoride administration in experimental animals, reported elevation of ^{59}Fe uptake by liver and bone marrow. In addition, these workers also observed a decreased ^{59}Fe incorporation in blood, implying anemia despite iron abundance.

The suggestion that the hemoglobin synthesis is impaired by fluoride despite abundance of iron is strengthened by Bernard et al. (16), who reported that anemia in fluoride toxicity is due to inhibition of ^{59}Fe incorporation in protoporphyrin, a precursor of hemoglobin - and not for want of iron.

In addition to impairment of iron metabolism, copper depletion is also associated with defects in connective tissue metabolism (12). In copper deficiency, the cross-linking of bone collagen is impaired due to reduced activity of lysyl oxidase - a cuproenzyme which plays a key role in cross-linking of collagen (12). Interestingly, alterations in collagen metabolism have been reported in fluoride toxicity (17, 18).

Acknowledgement

The authors are grateful to Dr. I.P. Abrol, Director, Central Soil Salinity Research Institute, Karnal (Haryana), for allowing the use of Atomic Absorption Spectrophotometer.

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CONTENTS OF FLUORIDES IN VEGETABLES FROM AREAS CONTAMINATED BY INDUSTRIAL EMISSIONS: A PRELIMINARY REPORT

by

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SUMMARY: In vegetables grown in 1978 and 1979 near a chemical plant where phosphorites and apatites are processed, according to preliminary data, fluoride was significantly elevated in roots of carrots and parsley and in the leaves of parsley.

Introduction

Chemical and metallurgical industries are known to process most of the world's raw materials containing fluoride. From these facilities fluorides are emitted into the air and contaminate the environment. Therefore control of the fluoride content of food produced in the neighborhood of such factories, is vitally important.

The fluoride content of vegetation is indicative of the degree of atmospheric contamination. It assists in delineation of the border of a contaminated area and in estimating fluoride values attributable to food consumed by people and animals (1).

The purpose of this paper is to determine the fluoride content of the roots of carrots and parsley and of the leaves of parsley grown within the range of emissions from a factory processing fluoride containing raw materials. The facility which emits fluoride into the atmosphere has been in operation for several years. Our investigation of the fluoride content of vegetables grown in that area began in 1978.

Materials and Methods

The vegetables under investigation originated from areas marked A, B and C (Fig. 1), 0.5 to 3 km distant from the chemical plant. The prevailing winds in the region come from the southwesterly direction. Locality C was the only one not in the main pathway of the prevailing winds from the chem-

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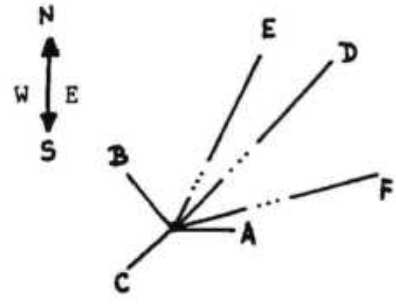
ical plant. In the control group, vegetables were cultivated in an area 100 km southwest of the chemical plant in the opposite direction from the prevailing winds.

Figure 1

Sampling Sites



The vegetables were dried at a temperature of 105°C. Preparation of the samples for fluoride assays proceeded at three stages (2): Alkalization, incineration and distillation. The alkalization consisted of adding to 10 grams aliquots of the vegetables 0.5 g CaO in the form of an aqueous suspension in order to convert fluorine into sparingly volatile CaF_2. The fluoride determination of vegetables grown in 1978 was made by the colorimetric method with the use of Alizarin Complexone (3) and for vegetables grown in 1979 by the Ferric-Sulphosalicylic method (4). Figure 1 shows the direction from the plant where the vegetables were grown. Location A is about 1 km distant from the plant; B - 1.5 km, C - 3 km and D, E, and F - 100 km.

Table 1

F^- Content of Vegetables from Different Areas in mg/kg of dry mass/ppm

		Carrot		Parsley Root		Parsley Leaves	
		1978	1979	1978	1979	1978	1979
Locality:	A	5.46	6.56	5.09	6.57	14.94	18.99
	B	1.98	3.13	5.30	4.67	12.84	17.96
	C	0.92	2.27	2.82	4.47	10.37	8.82
Controls:	D	2.19	2.96	1.75	2.38	10.77	10.12
	E	2.57	2.37	2.21	2.42	11.04	9.64
	F	1.31	1.83	2.40	2.32	11.85	10.06

Results

Vegetables grown in region A that is located closest to the chemical plant (about 1 km distant) contained the highest amount of fluoride compared with the controls. Therefore in the immediate neighborhood of the chemical plant fluoride accumulates in vegetation. (Rippel (5) found that the fluoride content of corn and vegetables grown at distances of 100 m, 500 m and 1 km from an aluminum smelter is directly related to the distance from the emission source, but in his control tests and those of other researchers fluoride levels were lower than ours. However our results are comparable with those found by other investigators (1,6,7) who recorded in vegetables grown in regions of industrial emissions higher fluoride

levels than in controls. The accumulation of fluorides in plants is strikingly variable not only between individuals of a given species, but also between individual parts of the same plant (8,9). This observation has been confirmed by our fluoride assays of roots and leaves of parsley. In areas contaminated by industrial emissions the levels of fluorides in vegetables are likely to continue to increase from year to year (10,11) provided that the production and the technology in such plants remains unchanged.

It should be emphasized that the vegetables used in this study originated from small plots where no chemical fertilizers were used. We believe therefore, that the fluoride content of the plants may serve as an indicator of progressive contamination of cultivated areas by industrial emission. Since vegetables grown in contaminated areas constitute an important source of fluoride uptake into living organisms, humans as well as animals already exposed to an excess intake of atmospheric fluoride through respiration, should be subjected to continuous control.

We intend to follow up our preliminary observations with further studies and additional fluoride assays of food grown in the vicinity of the above-mentioned chemical facility.

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EFFECT OF SODIUM FLUORIDE ON BLOOD AND LIVER ENZYMES IN CHANNA PUNCTATUS (BLOCH.)

by

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SUMMARY: Channa punctatus (Bloch.) was treated with sodium fluoride (LC_{50} at a concentration of 10 ppm) both at room temperature (30.22 ± 0.54°C) and at 15°C. At room temperature the increase in blood cholesterol, glucose and acetylcholinesterase was significant compared to the controls. In the liver, only GOT activity increased. At 15°C the levels of blood glucose and whole animal oxygen consumption decreased significantly whereas blood cholesterol became elevated compared to control. An increase in plasma proteins, transaminases and a decrease in LDH activities was noted. In the liver extracts, a significant decrease in LDH and an increase in GOT levels was observed. The physiological significance of the above-described variations in response to sodium fluoride stress are discussed.

Introduction

The determination of enzyme levels in various tissues of fish, particularly in blood and serum, is helpful in establishing safe concentrations of therapeutic agents, control of water pollution and early and rapid diagnosis of diseases.

The toxicity of chemicals on some aquatic animals has been investigated by Racicot et al. (1) and by Angelovic et al. (2). In Channa punctatus (Bloch.) no such studies have been made. We have observed certain changes in biochemical factors and hematological indices caused by sodium fluoride in Channa punctatus (B.) (3-4). In the present paper we are recording the effect of sodium fluoride (10 ppm) on enzyme activities in Channa punctatus (Bloch) maintained at room temperature (30.22 ± 0.54°C) and at 15°C.

Material and Methods

Healthy fish were obtained from local markets and subjected to laboratory conditions for over a week. The lethal concentration (LC_{50}) of sodium fluoride was determined (10 ppm) for more than 96 hours both at room temperature (30.22 ± 0.54°C) and at 15°C.

The mean weight of the fish both at room temperature and 15°C was 102.43 ± 2.93 gms and 118.80 ± 4.23 gms. Their length was 19.90 ± 0.44 cms and 20.42 ± 0.68 cms respectively. After a week's acclimatization,

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blood samples were collected in the heparinized vials by severing the caudal fin. The samples were spun at 3000 rpm and the plasma was separated. Subsequently red cells were washed three times with saline (0.9% NaCl). The red cells were then lysed with three parts of Saponin (0.01%) and centrifuged to obtain clear hemolysate. The liver homogenate(10%) was prepared with the use of 0.05M sucrose.

Both blood and liver tissue samples were processed to estimate the following parameters: LDH (5), (Lactate Dehydrogenase L-Lactate, Nad-oxidireductase ECl. 1. 27). Transaminases - Glutamate Oxaloacetate transaminases (GOT E C 2. 6. 1. 1.) and Glutamate pyruvate transaminases (GPT E C 2. 6. 1. 2.) (6), Cholinesterase (E C 3. 1. 1. 7.) (7), total plasma protein and tissue protein (8), glucose (9) and cholesterol (10). The whole animal oxygen consumption was measured following Winkler's method as described by Saroja (11). Control samples were also maintained similar to the experimental samples. Hemolysates were used to estimate the enzyme levels whereas whole blood was used for the estimation of glucose and cholesterol content.

Results

Variations caused in the biochemical effects from sodium fluoride treatment at 15°C were more pronounced than at room temperature (Tables 1, 2). Fluoride-treated fish showed a significant increase in blood cholesterol, glucose and acetylcholinesterase levels at room temperature when compared to controls. In liver extracts such an increase was observed only in GOT activity (Table 1).

At 15°C a significant decrease in the mean oxygen consumption and in the blood glucose levels occurred, whereas blood cholesterol increased as compared to controls. Plasma protein increased by 4 gms percent in fluoride-treated fish in conjunction with an increase in transaminases and a reduction in LDH activities. In the liver extracts a significant decrease in the LDH and an increase in GOT levels were observed, whereas acetylcholinesterase activity did not vary much from the control values (Table 2).

In general, sodium fluoride (10 ppm) at low temperature caused an elevation in blood cholesterol and GPT and liver GOT levels as compared to the levels at room temperature (Table 1-2).

Discussion

The above results suggest that variations in the toxicity of fluoride on the blood of Channa punctatus (B.) are likely to be temperature dependent. At low temperature (15°C), fluoride causes anoxia as indicated by the low oxygen consumption. Furthermore it increases protein synthesis as evidenced by increased total protein content and GOT and GPT activities. Metabolic activity seems to be reduced as indicated by low LDH (blood and liver) and blood glucose levels.

Table 1

Effect of NaF on Blood and Liver Enzymes in Channa Punctatus at Room Temperature

Parameter	Control No.	Control Mean	Control S.E.**	Acclimated No.	Acclimated Mean	Acclimated S.E.	t value	Degree of Freedom
O_2 consumption (ml/hr)	6	1.86	0.83	6	0.77	0.08	0.91	10
BLOOD								
LDH IU/100ml	15	1.31	0.15	15	1.34	0.38	0.08	28
GPT IU/100ml	15	1895.00	109.41	15	1634.00	80.04	-1.88	28
GOT IU/100ml	15	2370.00	18.67	15	2082.50	202.97	-1.22	28
AchE IU/100ml	15	0.0007	0.00010	20	0.0019	0.00038	2.65*	33
Glucose (mg%)	36	21.60	7.33	21	34.28	9.68	2.13	55
Cholesterol (gm%)	36	4.03	0.19	21	6.99	1.56	3.19*	55
Plasma protein (gm%)	36	2.78	0.31	21	2.45	0.15	-1.55	55
LIVER++								
LDH IU/hr/mg	10	0.09	0.02	12	0.07	0.01	-1.45	20
GOT IU/hr/mg	10	145.38	23.90	12	399.05	67.44	3.26*	20
GPT IU/hr/mg	10	276.91	36.52	12	346.93	60.77	0.94	20
AchE IU/hr/mg	10	0.0009	0.00004	10	0.0016	0.0009	0.0009	18
Protein (gm%)	10	31.18	6.46	12	35.83	2.49	0.72	20

* = P < 0.01
** = Standard Error

Table 2

Effect of NaF on Blood and Liver Enzymes in Channa Punctatus at 15°C.

Parameter	Control No.	Control Mean	Control S.E.	Acclimated No.	Acclimated Mean	Acclimated S.E.	t value	Degree of Freedom
O_2 consumption (ml/hr)	6	2.72	0.03	6	0.36	0.003	-23.80*	10
BLOOD								
LDH IU/100ml	15	1.72	0.36	15	0.72	0.17	- 2.59*	28
GPT IU/100ml	15	1350.92	80.05	15	1413.33	219.14	4.77*	28
GOT IU/100ml	15	1458.54	73.38	15	2376.00	200.15	4.31*	28
AchE IU/100ml	15	0.00065	0.0001	15	0.0012	0.0004	0.41	28
Glucose (mg%)	20	31.56	5.92	24	21.48	2.83	- 2.21*	42
Cholesterol (gm%)	20	3.42	0.67	24	7.30	1.09	5.79*	42
Plasma protein (gm%)	20	1.77	0.19	24	2.25	0.21 0.21	3.20*	42
LIVER++								
LDH IU/hr/mg	10	0.05	0.01	10	0.01	0.002	- 3.24*	18
GOT IU/hr/mg	10	135.45	10.47	10	500.32	124.09	8.99*	18
GPT IU/hr/mg	10	213.95	23.03	10	208.32	45.85	- 0.11	18
AchE IU/hr/mg	10	0.00055	0.00005	10	0.0022	0.0012	1.90	18
Protein (gm%)	10	50.14	3.95	10	48.87	4.89	- 0.22	18

There appears to be a greater demand for AchE activity at room temperature under stress condition leading to increased glucose levels which provides energy for the AchE formation. Further, the effect of fluoride may be specific on the liver transaminases where only GOT activity is increased.

Further studies on the toxic effect of NaF on biological systems at a wider range of temperature, would help to confirm their synergistic effect as indicated by the present results.

Acknowledgement

The authors wish to thank the University Grants Commission for financial assistance and Prof. P. Ramachander Rao, Head of the Department of Zoology, Osmania University, Hyderabad for providing the necessary facilities.

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HISTOLOGICAL CHANGES IN LIVER FOLLOWING SODIUM FLUORIDE INGESTION

by

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SUMMARY: The present study was undertaken to assess the effects of fluoride ions on liver. Sixty guinea pigs were divided into four groups of 15 each. Group A was given 1000 ppm of sodium fluoride in drinking water, Group B, 500 ppm, Group C, 10 ppm and Group D served as control with plain drinking water without fluoride. An equal number of animals from each of the groups were sacrificed at the end of one, two and three months. The liver from each of these was removed, processed and stained with hematoxylin and eosin stain. Fatty degeneration and necrosis of liver cells were found. Damage was greater with the higher dosages.

Introduction

The adverse skeletal and dental changes due to fluoride intake have been extensively studied, both clinically and epidemiologically, but information regarding the nonskeletal effects of fluoride.especially after prolonged intake,is rather incomplete. The aim of the present study was to assess the effects of chronic fluoride ingestion on liver in experimental animals.

Material and Method

Sixty adult guinea pigs were divided into four groups. Group A was given 1000 ppm of sodium fluoride in drinking water; Group B,500 ppm and Group C,10 ppm. Group D received plain drinking water without fluoride as a control. About five animals from each group were sacrificed at the end of one month and at the end of two months, the remaining animals at the end of three months. The livers were fixed in normal saline, then embedded in paraffin wax, 5-7 μ sections were cut and stained with hematoxylin and eosin. Von Kossa's stain was also used in order to show areas of calcification in the liver.

Results

Gross Pathology: On gross examination, the livers in the experimental groups were pale in color but normal in size, whereas in the controls they were of a reddish brown color.

Microscopic Changes: The histological changes in the liver were in direct proportion to the dosage and the period of time of fluoride admini-

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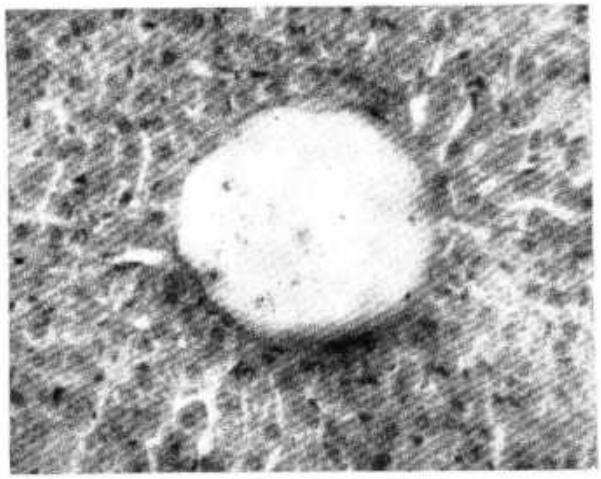
stration. In the group with the highest dose of fluoride (1000 ppm), the central vein was dilated after one month of administration and the liver cells showed focal necrosis (Fig. 1). At the end of the second month, fatty changes in liver cells were the most characteristic feature seen in all three zones (Fig. 2). There were focal areas of reticuloendothelial

Figure 1

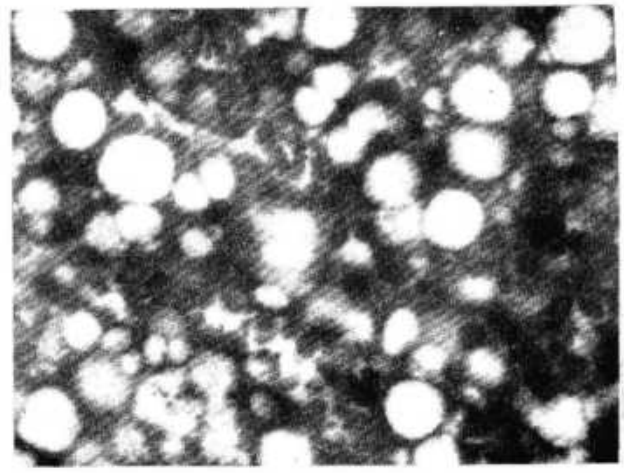
Guinea Pig Liver
(1000 ppm NaF; 1 month)



Dilatation of central vein and sinusoids; focal areas of cell necrosis
(x 200)

Figure 2

Guinea Pig Liver
(1000 ppm NaF; 2 months)



Degeneration of Liver Cells
(x 200)

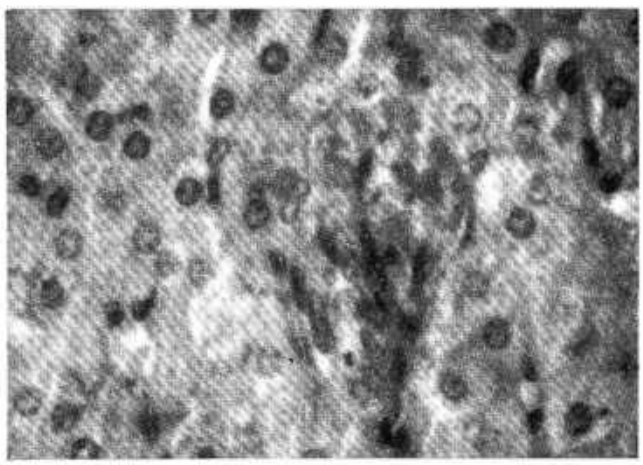
cell hyperplasia accompanied by areas of liver cell necrosis (Figs. 3 and 4). These structural changes were far advanced at the end of three months of fluoride administration, when areas of massive liver cell necrosis were found (Fig. 5). The central veins and sinusoids were markedly congested and dilated. In these animals the liver section revealed marked hypoplasia of reticuloendothelial cells.

In addition to the above findings, the livers of 3 out 10 animals which received 1000 ppm of fluoride for three months, presented calcified areas surrounded by degenerated liver parenchyma. These findings, though evident in hematoxylin and eosin stained sections, were further corroborated by the Von-Kossa's staining technique which is specifically used for staining of calcium in tissues (Fig. 6).

In the liver section of the animals in Group B (500 ppm) we observed structural alterations similar to those mentioned above. However, the changes were degenerative and less pronounced. In these sections, the necrosis of liver cells occurred mainly in the regions surrounding the central vein. In addition to necrosis, dilation and congestion of the central vein, scattered areas of fatty degeneration of liver cells were evident. All these changes were most extensive at the end of three months.

Figure 3

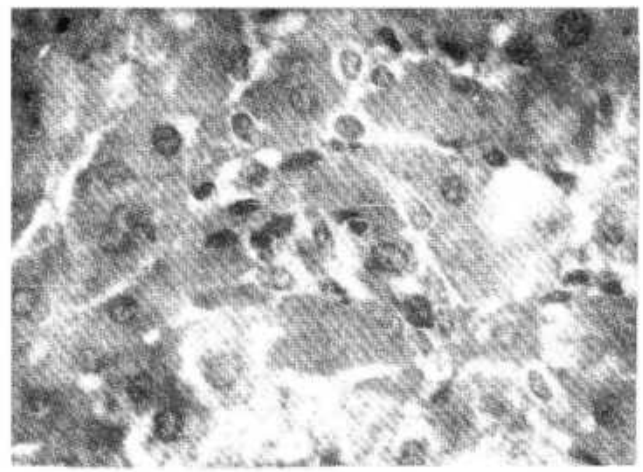
Guinea Pig Liver
(1000 ppm NaF; 2 months)



Extensive cell necrosis; nuclear degeneration (x 700)

Figure 4

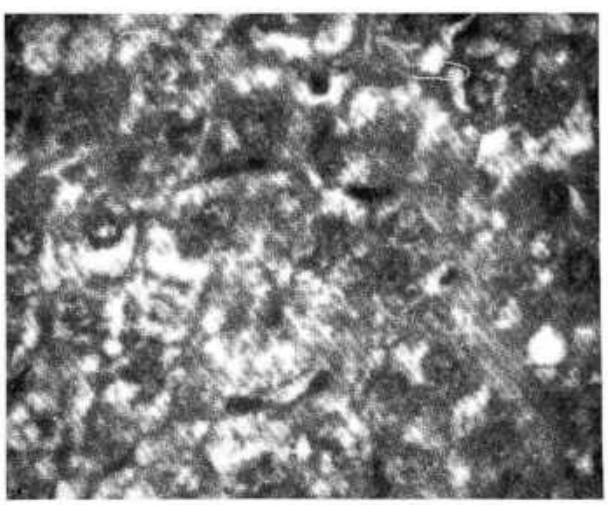
Guinea Pig Liver
(500 ppm NaF; 2 months)



More extensive cell necrosis; areas of reticuloendothelial cell hyperplasia (x 700)

Figure 5

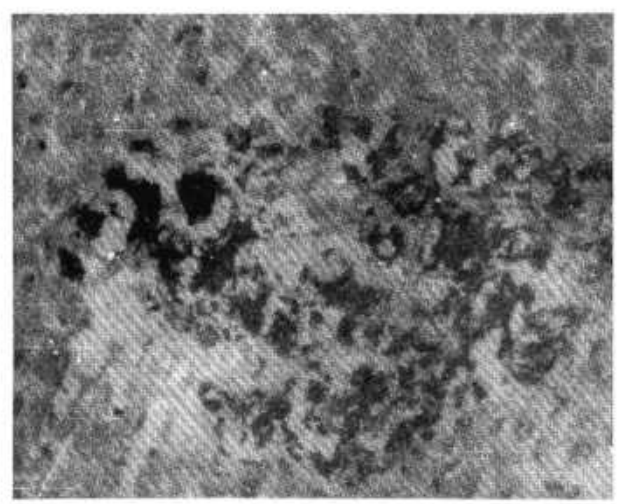
Guinea Pig Liver
(1000 ppm NaF; 3 months)



Massive cell necrosis; cell outline disappeared; nuclei degenerated (X 700)

Figure 6

Guinea Pig Liver
(1000 ppm NaF; 3 months)



Liver cell necrosis; area of calcification stained darkly
(Von-Kossa Stain x 200)

In distinction to the above findings, the animals in Group C (10 ppm) appeared least affected and showed no changes in liver parenchyma. The liver sections of control animals, sacrificed at the end of three months showed normal parenchymal cells and sinusoids and the central veins were normal in size and structure (Fig. 7).

Discussion

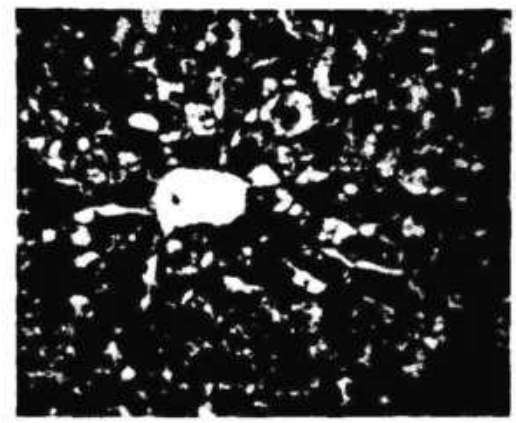
Liver, an organ of vital importance, was severely damaged by fluoride toxicity. Excessive fluoride intake by the experimental animals resulted in extensive degenerative changes in the liver which varied from focal areas of liver cell necrosis to complete fatty degeneration of the liver. Focal areas of hyperplasia of the reticuloendothelial system, congested central veins and liver sinusoids were seen. A similar picture of degenerative changes in the liver of experimental animals fed excessive amounts of fluoride has been reported earlier by Muehlberger (1) and by Phillips (2). However, Ogilvie (3) failed to find any structural alteration in the livers of fluorosed rats.

The discrepancy in the findings of these authors may be due to species variation as these investigators studied different species of animals. The present study has shown that fluoride ions exert toxic effects on liver tissues. Our findings have been further substantiated by Haber (4) who reported mid-zonal liver cell necrosis caused by fluoride ions in patients receiving fluorinated anesthetics.

The presence of calcified areas in liver sections observed in the present study may possibly be due to the interference of fluoride with the calcium metabolism (5). Malmquist and Low (6) observed accumulation of electron dense material in the mitochondria of rats fed large doses of sodium fluoride. This material turned out to be calcium fluorophosphate. In their opinion, after fluoride treatment, calcium is deposited as fluorophosphate which is less soluble than corresponding phosphate. As a consequence, calcium phosphate deposited in the mitochondria might be transformed into calcium fluorophosphate. Under these conditions, this compound gradually accumulates in the form of needle-like crystals. It is likely that, as the result of degeneration of liver cells, calcium fluorophosphate is released in the cell debris. This view is supported by the fact that each of the calcified areas (Fig. 6) in the liver sections were surrounded by necrotis areas.

Figure 7

Guinea Pig Liver (Control)



Normal central vein, liver cords and sinusoids (X 200)

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FLUORIDATED WATER AND TEETH

by

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SUMMARY: The relation between fluoride naturally in drinking water, mottled enamel and dental caries in children is analyzed. The prevalence of mottled enamel (dental fluorosis) in children of 73 communities with drinking water containing 0.4 - 6.6 ppm (parts per million) F^- is log-normally distributed with the concentration of natural fluoride in drinking water. Signs of intoxication may be anticipated at levels below 1 ppm F^-; at 0.4 ppm F^-, approximately 3% of the children, on the average, show dental fluorosis.

The prevalence of dental caries in children aged 12 to 14 from 136 communites with drinking water containing 0.15 - 5.8 ppm F^- shows no relationship with the concentration of fluoride naturally in drinking water.

Presented at the Eleventh I.S.F.R. Conference on April 9, 1981 in Dresden GDR. From the Institut für Umweltforschung, am Forschungszentrum, Graz.

It can be concluded from the above results that at the so-called "caries prophylactic level" of fluoride (1 ppm) some signs of intoxication must be expected but no caries prophylactic effect.

Introduction

It is a widely propagated thesis that fluoridation of drinking water (1 ppm) reduces the incidence of dental caries and causes no harm (1). Dean recorded "mild" dental fluorosis in midwestern cities with as little as 0.4 to 0.5 ppm fluoride in water. He observed in Marion, Ohio, at 0.4 ppm "very mild" and "mild" in 6.1% of the 12 to 14 year olds and in Kewanee, Illinois, with 0.9 ppm, in 12.2% of the children of the same age group (2).

In this article, the relation between the presence of fluoride in water naturally and "mottled enamel" and dental caries in children is evaluated statistically.

Material and Methods

The data for analysis are from the studies on "mottled enamel" and dental caries in North America and Europe as published in all literature available to the author, without any exclusions. The sole criteria for inclusion of data are that they are concerned with the content of fluoride in water naturally and with dental fluorosis and with dental caries. Dental fluorosis is measured as the percentage of children with mottled enamel. The degree of dental caries is evaluated by the percentage of DMF (Decayed, Missing and Filled Permanent) Teeth per child computed on the basis of the complete set of permanent teeth (28 teeth).

Results

Figure 1 presents the relation between the concentration of fluoride naturally in drinking water and the incidence of "mottled enamel" in more than 12,000 children, 9 to 14 years old who had been examined in 67 communities from 12 states of the USA (2-10) and in 6 communities of Denmark (11). The fluoride concentrations of the various water supplies which ranged from 0.4 to 6.6 ppm and the incidence of dental fluorosis are plotted in a coordinate system of a log-normal distribution. Each point shows a mean value of measurements and is representative of a water supply. It is evident that the incidence of "mottled enamel" is positively correlated with the concentration of natural fluoride in drinking water and follows a log-normal distribution function ($R^2 = 0.8496$).

Figure 2 shows the relation between the concentration of fluoride naturally in drinking water and the presence of dental caries in permanent teeth of more than 48,000 examined children, 12 to 14 years old, in 136 communities from 13 states in the USA (2,6,12-22) and from Canada (21), Great Britain(23), Denmark(11), Spain(24), Austria(25), Hungary(26-28)and the German Democratic Republic (GDR) (29-31). Fluoride concentrations in

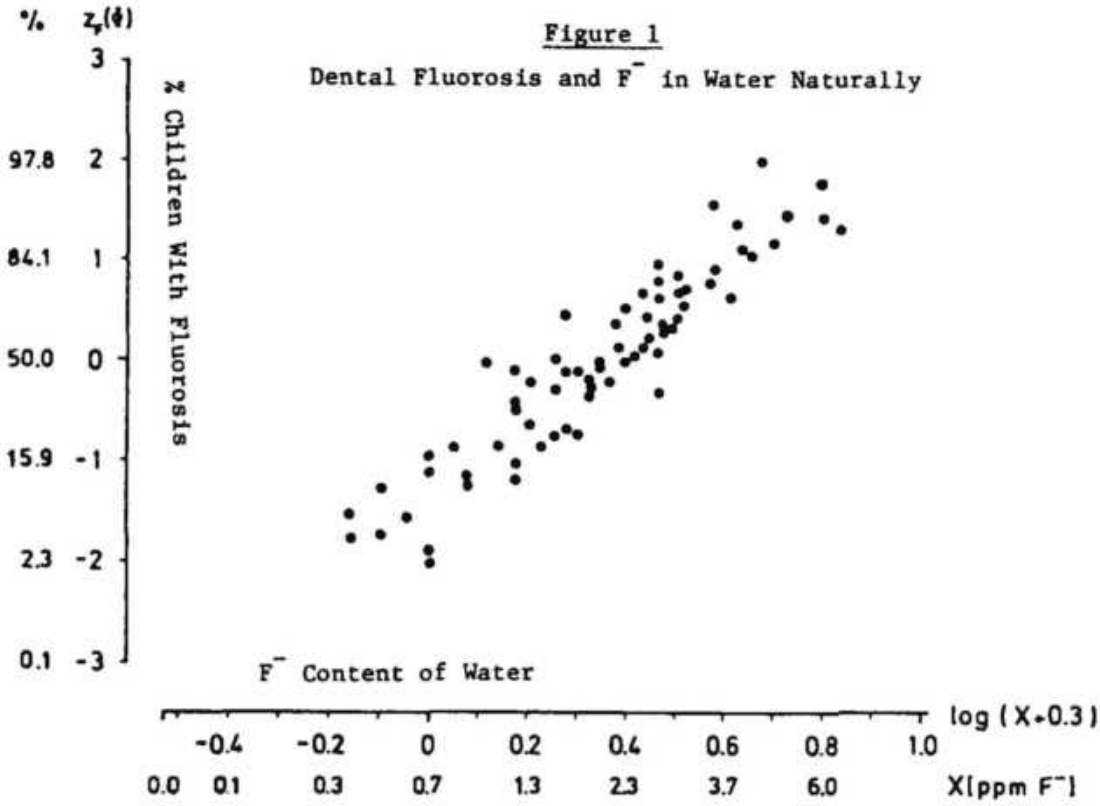


Figure 1

Dental Fluorosis and F^- in Water Naturally

water range from 0.15 to 5.8 ppm F^-. Figure 2 is again plotted in a coordinate system of a log-normal distribution. The communities of the survey of 21 communities by Dean et al. (2) are also included. It is evident that dental caries in children does not correlate with the concentration of fluoride naturally in drinking water (R^2 = 0.0098).

Comment

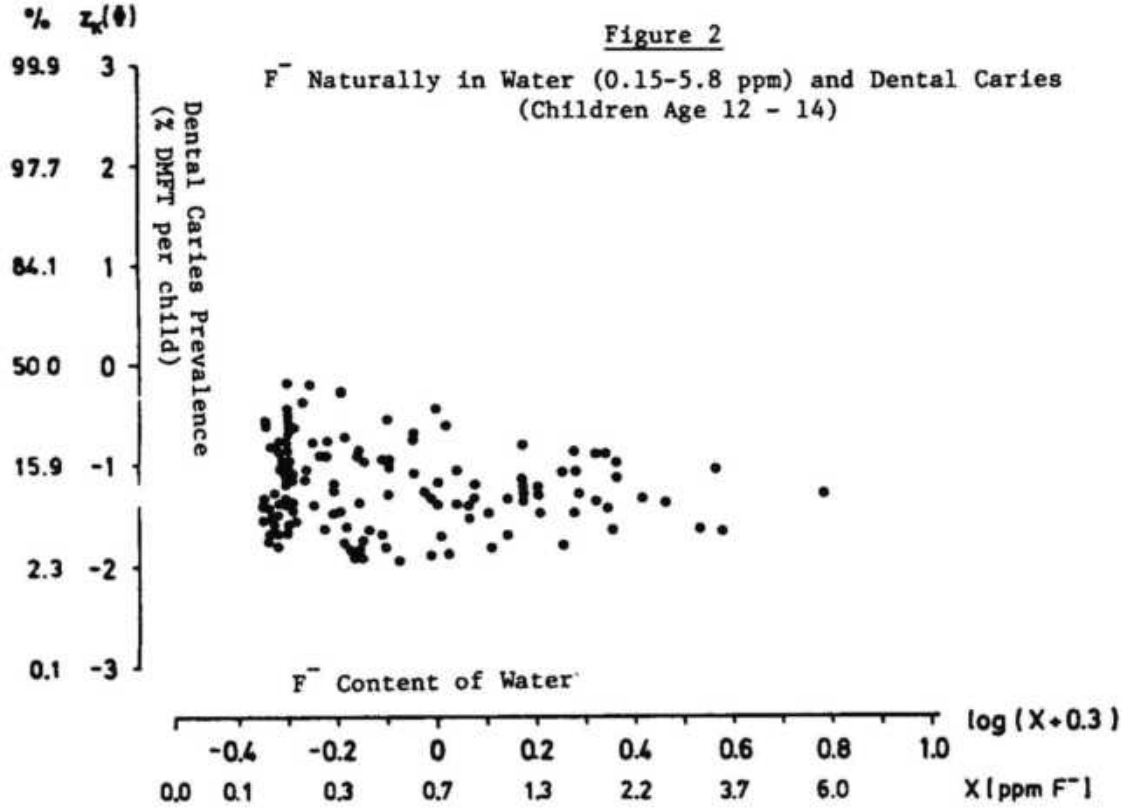
The following is an example for computing a point in Figure 2: The dental caries of children in Colorado Springs, aged 12 to 14 years, was 2.46 DMFT/child, the mean fluoride content in the water supply was 2.6ppm F^- (12). In the abscissa (x-axis), the fluoride concentration is x = 2.6 ppm F^- or log (x + 0.3) = log (2.6 + 0.3) = log 2.9 = 0.46239.

If the DMFT/child = 2.46 then Φ in the ordinate (y-axis) is $\Phi = -\frac{2.46}{28}$ = 0.0879 (Φ % = 8.79%). To Φ = 0.0879 belongs a z (Φ) =-1.353, which may be taken from a table of standardized normal distribution (32-34). The values were weighted when there was more than one value for the water supply of a particular community.

The regression equation for Fig. 1 is $z(\Phi) = -1.29489 + 3.706843, \log (x + 0.3)$, $R^2 = 0.849646$, $0.4 \leq x \leq 6.6$ ppm F^- and for Fig. 2 $z(\Phi) = -1.22108 + 0.16215, \log^* (x + 0.3)$, $R^2 = 0.0098281$, $0.15 \leq x \leq 5.8$ ppm F^-.

Figure 2

F^- Naturally in Water (0.15-5.8 ppm) and Dental Caries (Children Age 12 - 14)



Originally the fluoridation theory was based upon the survey of 21 cities by Dean et al. (2) carried out prior to 1941. In 1955 and 1960 (35), Dean acknowledged that the statistics derived from this survey are invalid, according to his own criteria, because the cities in his survey did not meet the two basic requisites which he had established (3) namely, continuous exposure of the group under observation during childhood and an unchanged water source. The second requisite was lacking in all 21 cities.

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* The number of points decreases as the fluoride concentration rises because, in nature, there is a progressive decrease in the number of high fluoride water supplies.

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FLUORIDE BRIEFS

Corticosteroids applied topically are known to produce a variety of side effects such as acne, premature aging of the skin, hypertrichosis, perioral dermatitis, glaucoma and adrenal suppression. These side effects are markedly enhanced by the use of fluorinated compounds, i.e. Kenalog Aristicort, Synalar, Lidex, Valisone, Diprosone, Topicort, Halog and are much more pronounced and more frequent with a fluorinated derivative than with hydrocortisone.

Morman, M.R.: Possible Side Effects of Topical Steroids. American Family Physician, 23:171-174, 1981.

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TOOTH EROSION BY BEVERAGES: PROTECTION BY FLUORIDE

by

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SUMMARY: The hydrogen (H^+) ion concentration of six commonly marketed soft drinks and fruit juices showed mild, insignificant changes during atmospheric exposure over a period of 40 minutes. The acidic pH of these beverages causes significant erosion ($p > 0.01$) both in normal and fluoridated caries-free human premolar teeth which had been extracted from persons aged 12 to 20 years because of orthodontic problems. In vitro exposure to a 2% solution of sodium fluoride for 15 minutes affords over 50 to 78% protection above the normal degree of erosion.

Introduction

A high incidence of dental disorders in humans by food mastication, acidic beverages (1), glandular secretion (2) has been related by Mannerberg (3) to their H^+ ion concentration. Dental caries has been attributed to citric, hydrochloric and nitric acids (4,5).

In the present study, pH variations were determined of the commonly marketed, widely consumed, clear, colored, carbonated, non-aerated and fresh fruit juices. They were related to the degree of enamel erosion in normal and fluoridated, caries-free human premolar teeth which had been extracted for orthodontic purposes at dental clinics.

Materials and Methods

We chose among clear, aerated drinks, soda (Parle, Fab); among colored carbonated preparations, Limca, Goldspot (Gujarat beverages) and among non-aerated beverages, grape, orange and lemon juices. In five samples of each category, the pH values were determined at 0, 5, 20 and 40 minute intervals after preparation when the beverage bottles were opened to atmospheric air. The H^+ ion concentrations were recorded with the Beckman glass electrode and in some with standard Merck pH papers.

Caries-free human premolars from subjects 12-20 years of age were ground in buccolingual direction with the help of coarse and fine abrasive wheels in wet condition and finished by hand with carborundum stone. Before preservation in water, the teeth were gently rubbed with pumice glycerine paste (6) on a ground glass. Twenty fractions in each group comprising 10 before and 10 after treatment with a 2% solution of sodium fluoride for 15 minutes were exposed to one of the soft drinks for 24 hours. The

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enamel erosion of normal and fluoridated teeth after exposure to each soft drink was calculated with a special micrometer screw gauge and also by volume displacement technique (micrometer syringe method) and expressed as percentage of erosion in mm.

Results

The changes of the H^+ ion (pH) concentration of the six beverages on exposure to air are presented in Table 1. The carbonated beverages showed greater variation of the pH than fruit juices. In all drinks except in soda, the pH remained on the acidic side of 2 to 3, even during the atmospheric exposure of 40 minutes.

The effect of fluoride on dental caries is presented in Table 2. The maximum erosion of 2-4 mm occurred with lemon, Limca and in orange drinks (60 and 75%) compared to the matched controls ($p < 0.01$). Goldspot and grape juice produced only a moderate erosion of 1.5 to 2.2 mm (64 - 76%) following exposure to fluoride. The soda caused only a mild erosion and its prevention by having the teeth fluoridated was only moderate (56.3%).

Table 1

pH Variation of Beverages
40 Minutes After Preparation

Kind of Beverage	Sampling	0 min. M±SD	5 min. M±SD	20 min. M±SD	40 min. M±SD
Pure Lemon Juice	5	1.86±0.11	1.90±0.14	2.04±0.08	2.08±0.08
Limca	5	2.28±0.16	2.48±0.13	2.72±0.08	2.88±0.13
Orange Juice	5	2.18±0.16	2.30±0.15	2.40±0.12	2.52±0.08
Gold Spot	5	2.18±0.16	2.36±0.14	2.58±0.19	2.78±0.16
Grape Juice	5	2.40±0.15	2.46±0.11	2.54±0.11	2.58±0.13
Soda	5	2.96±0.20	3.12±0.13	3.20±0.15	3.42±0.08

Table 2

Tooth Erosion and Protection by Fluoridation
on Exposure 15 minutes to Beverages

Type M±SD	Sampling	Before Fluoridation/A	After Fluoridation/B	Safety Index %-(A-B/A)100
Lemon Juice	10	3.49±1.04	1.04±0.27	70.2
Limca	10	2.32±0.47	0.62±0.32	73.3
Orange Juice	10	2.52±0.35	0.97±0.30	61.5
Gold Spot	10	1.99±0.67	0.72±0.39	63.8
Grape Juice	10	1.57±0.46	0.38±0.19	75.8
Soda	10	0.74±0.24	0.32±0.11	56.7

Discussion

The pH variation of the beverages following exposure to air, showed minimal changes during the span of 0 to 15 minutes which is the usual period of exposure when a person consumes the drink. Our results were consistent and confirm a similar trend to that reported by Shourie et al. in 1965 (7), with a number of beverages. The acidic pH of these drinks (Table 1) correlates clearly with the degree of erosion or decay regardless of whether consumption is immediate or delayed. Smokers, who want to conceal their mouth odor, usually chew the fruits in locally prepared juices or drink them slowly. Habits of sucking through crupiece (a cubicle of sugar) under the incisors with support of the tongue in tea and coffee drinkers of kurds in Iraq, Persia, Turkey and Russia induce enamel erosions of anterior maxillary teeth (4).

In the second phase of our study (Table 2), the degree of tooth erosion both in carbonated and non-carbonated fruit juices, and the effect of exposure of the teeth to fluoride was shown. Exposure to fluoride for 15 minutes of prepared human teeth in vitro prevented erosion by more than 50% ($p < 0.01$). The degree of protection by fluoride differs with the kind of beverage. Such drinks as soda which are not colored and do not have a pleasant taste are consumed immediately. Therefore they rarely cause erosions since the teeth are not exposed for a long time to the non-tasty beverages. On the other hand, colored and aerated drinks or fruit juices, especially lime, cause more severe damage to the tooth's surface because they are consumed more slowly and therefore exposed to aeration for a longer period of time.

Damage by citric acid or acidic pH is delayed by the formation of a fluoride layer around, and at, the sites of erosion (8). Another means of reducing tooth erosion is the use of a waxpaper flap tube which delivers the drink to the lower palate and therefore tends to counter the effect of habitual use of soft drinks.

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EFFECT OF FLUORIDE ON TISSUE ENZYME ACTIVITIES IN RAT: BIOCHEMICAL AND HISTOCHEMICAL STUDIES

by

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SUMMARY: Activities of various enzymes have been determined biochemically and histochemically in the liver and kidney of rats subjected for 10 months to fluoride concentrations of 0 ppm (control), 10 ppm (Group I) and 25 ppm (Group II) in drinking water. The activity of alkaline phosphatase, acid phosphatase and succinic dehydrogenase decreased appreciably. However, adenosine triphosphatase activity increased in liver and kidney of Group II (25 ppm) animals. Lactic dehydrogenase activity also decreased but only in the kidney histochemically. The alterations in enzyme activities were very pronounced in proximal and distal convoluted tubules of the kidney. The biochemically determined activity of glutamic oxaloacetic transaminase increased slightly in the liver in Group II. The observations suggest that fluoride interferes with intracellular metabolism in liver and kidney.

Introduction

It is now well established that teeth and bones are not the only parts of the body affected by fluoride; fluoride ingestion over prolonged periods can adversely affect many other organs as well (1). The nonskeletal phase of fluorosis was recognized by Roholm (2) as early as 1937. Subsequently many studies described the toxic effects of fluoride on soft tissues(1, 3, 4). Since the kidney is the main organ excreting fluoride (5) in fluoride toxicity, it is one of the most severely affected among soft tissue organs. Renal lesions have been widely reported after prolonged ingestion of excessive fluoride (6-10). However, little attention has been paid to metabolic changes caused by fluoride. Furthermore, no serious attempts have been made to locate histochemically the sites of such metabolic lesions. According to Manocha et al. (11), squirrel monkeys given 5 ppm F in drinking water exhibited various cytochemical changes in the kidney. Altered enzyme activities in the kidney tubules and liver parenchyma have also been reported due to fluoride toxicity(12).

The present study has been designed to study the extent and sites of metabolic lesions caused by fluoride in liver and kidney. For this purpose, the activities of various enzymes have been studied biochemically and histochemically in liver and kidney of rats given 0, 10 and 25 ppm F in drinking water for 10 months.

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Materials and Methods

Female albino rats obtained from the Central Research Institute, Kasauli, were used in the present study. Eighteen animals weighing 125-155 g each were divided into three groups of 6 each and subjected for 10 months to fluoride concentrations of 0 ppm (control), 10 ppm (Group I) and 25 ppm (Group II) in drinking water. The animals were fed ad libitum a balanced pellet diet obtained from Hindustan Levers, Bombay. After the fluoride treatment, the animals were anesthetized with ether, sacrificed and their liver and kidneys removed immediately. The tissues were then processed for biochemical and histochemical investigations.

Biochemical Investigations: Each tissue was cut into small pieces and homogenized for two minutes in Potter Elvehjem homogenizer which was maintained at 4-6°C. The activities of alkaline phosphatase - E.C. 3.1.3.1 and acid phosphatase - E.C. 3.1.3.2 (13), adenosine triphosphatase (Mg^{++} activated) - E.C. 3.6.1.3 (14), succinic dehydrogenase - E.C. 1.3.99.1 (15), lactic dehydrogenase - E.C. 1.1.1.27 (16), glutamic oxaloacetic transaminase - E.C. 2.6.1.1. and glutamic pyruvic transaminase - E.C. 2.6.1.2 (17) were determined at 37°C. in the whole homogenate (10% w/v) of liver and kidney. The total protein was determined according to Lowry et al. (18) with the use of bovine serum albumin as standard.

Histochemical Localization of Enzymes: The frozen tissue pieces were sectioned at -25°C by means of a cryostat. The sections (8-10u thick) were then processed for cytochemical localization of acid phosphatase, alkaline phosphatase, adenosine triphosphatase (Mg^{++} - activated), lactic dehydrogenase and succinic dehydrogenase.

The histochemical methods employed during the course of present studies, have been detailed by Pearse (19), namely for dehydrogenases the method of Nachlas et al. (20) using nitroblue tetrazolium, for the demonstration of acid phosphatase, the azo dye method of Barka and Anderson(21) for alkaline phosphatase the naphthol phosphate method of Gomori (22).Adenosine triphosphatase was localized by the lead nitrate method (23).

Sections belonging to all groups of animals under study were incubated in the same incubating medium for the same length of time under similar conditions in order to eliminate any variable due to extraneous factors. The incubating media were freshly prepared each time by mixing various amounts of stock solutions. In addition, all enzyme procedures were always accompanied by requisite controls; the incubating media for the controls contained all necessary ingredients except the substrate/cofactor of the respective enzyme.

Results

Biochemical Studies: Biochemically determined activities of various enzymes in liver and kidney of the three groups of animals are summarized in Table 1. The activity of alkaline phosphatase and acid phosphatase significantly decreased in liver and kidney of Group II (25 ppm F)as compared to the control group; the decrease in alkaline phosphatase activity

was recorded to be 27% and 22% and that of acid phosphatase in liver and kidney 10% and 15% respectively. Similarly, the activity of succinic dehydrogenase (SDH) also decreased significantly in both liver and kidney in Group II (25 ppm F).

On the other hand, the activity of adenosine triphosphatase (ATPase) increased very significantly in both tissues, namely 63% and 27% in liver and kidney respectively. The activity of both glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) decreased in the kidney whereas GOT increased slightly in the liver in Group II.

No significant change was recorded in the activity of any enzyme studied in kidney of Group I (10 ppm F) animals. However, in liver of Group I, ATPase was the only enzyme the activity of which differed significantly (18% higher) from that of the control.

Histochemical Studies: Histochemical preparations of various enzymes, namely lysosomal (acid phosphatase), those concerned with active transport (alkaline phosphatase) and energy production (ATPase) and representative of anerobic pathways (LDH) and of the Krebs cycle (SDH) were studied and sites of enzyme activities precisely determined. The results are shown in Table 2.

Table 1
Enzyme Activities Following F^- Ingestion (Mean±S.D.)

Enzyme	Organ	0 ppm F	10 ppm F	25 ppm F
Alkaline phosphatase (n mole of p-nitrophenol liberated/mg protein/ 10 min)	Liver	103.8± 6.7	95.2± 9.7	75.3± 4.5**
	Kidney	301.0±23.4	285.4±22.0	257.0±21.6**
Acid phosphatase (n mole of p-nitrophenol liberated/ mg/protein/10 min.)	Liver	206.0±20.3	211.0±17.2	185.0±16.2*
	Kidney	104.4± 9.6	96.2± 9.8	81.3±10.2**
Adenosine Triphosphatase (n mole phosphorus release/mg protein/10 min.)	Liver	78.7±10.4	93.8± 9.3*	128.0±10.8**
	Kidney	250.8±41.0	276.0±48.8	317.0±45.0*
Lactic Dehydrogenase (n mole of pyruvate formed /mg protein/10 min.)	Liver	202.5±12.7	220.2±12.2	215.3±11.8
	Kidney	482.0±29.0	478.0±23.4	471.0±33.4
Succinic Dehydrogenase (n mole of tetrazolium reduced/mg protein/10 min)	Liver	73.2± 5.5	68.1± 6.9	48.4± 7.6**
	Kidney	54.3± 6.4	52.1± 9.8	43.1± 4.3**
Glutamic Oxaloacetic transaminase (n mole of pyruvate formed/mg protein/ 10 min.)	Liver	64.2± 5.1	58.8± 7.6	75.8±11.0*
	Kidney	97.6±12.2	82.6±19.2	71.8±12.4**
Glutamic Pyruvic Transaminase (n mole of pyruvate formed/mg protein/10 min.)	Liver	188.7±10.6	193.0±14.5	186.7±20.8
	Kidney	116.5±10.5	105.8± 5.6	91.3± 6.6**

Significance of difference from Control * p 0.05; ** p 0.005

Table 2

Enzyme Activities Following Fluoride Treatment

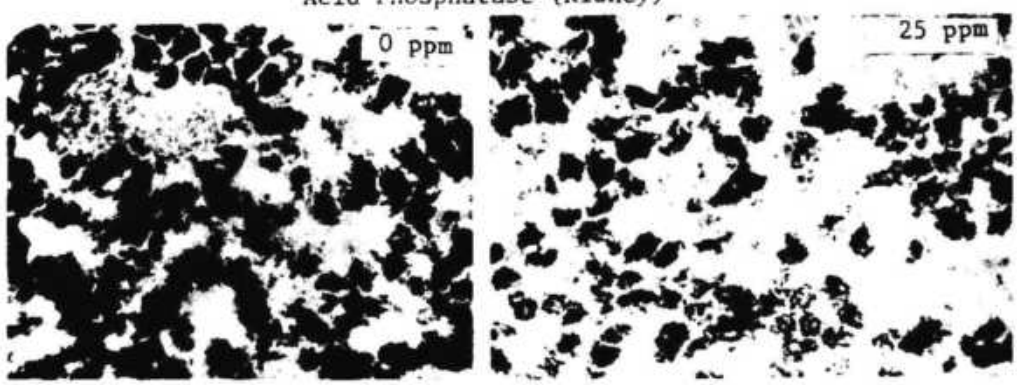
F^- Levels (ppm)	0	10	25	0	10	25	0	10	25	0	10	25	0	10	25
	Alk. phosphatase			Acid phosph.			ATPase			LDH			SDH		
KIDNEY															
Outer cortex	++++	++++	++	+++	+++	++	+	++	++	++++	++++	+++	++++	++++	++
Proximal tubules				to +++			to +++		to +++						to +++
Distal tubules	++++	++++	++	+++	+++	++	+ to ++	++	+++	++++	++++	++ to +++	++++	++++	++ to +++
Glomeruli	±	±	±	+	+	±	+	+	+	+	+	+	±	±	±
Inner cortex	+	+	+	+	+	+	+	+	±	++	++	++	++	++	++
Outer medulla	±	±	±	±	±	±	±	±	±	++	++	++	+ to ++	+ to ++	+ to ++
Inner medulla	±	±	±	-	-	-	-	-	-	±	±	±	±	±	±
LIVER															
Parenchymatous cells	+++	+++	++ to +++	++++	++++	+++	++	++	+++	++++	++++	++++	++++	++++	+++

Activity: ++++ very strong; +++ strong; ++ moderate; + low; ± negligible; - none

The activity of acid phosphatase in cells of proximal and distal convoluted tubules in Group II (25 ppm F) was less than that of the control animals (0 ppm F). The reaction for this enzyme in the glomeruli was weak in both, control and experimental, groups. Similarly, the reaction for alkaline phosphatase in the cells of proximal and distal convoluted tubules in Group II animals appeared weaker compared with control animals. The glomeruli showed negligible activity of alkaline phosphatase in both control and experimental groups. Activities of acid and alkaline phosphatase in the inner cortical and outer medullary regions remained almost unaltered following fluoride treatment. SDH activity was very strong in cortical tubules (proximal and distal) of control animals. However, following fluoride ingestion the SDH activity was moderate in some tubules and moderate to strong in others. In control group, the activity of SDH was negligible in glomeruli and appeared unchanged following fluoride ingestion.

Figures 1 and 2

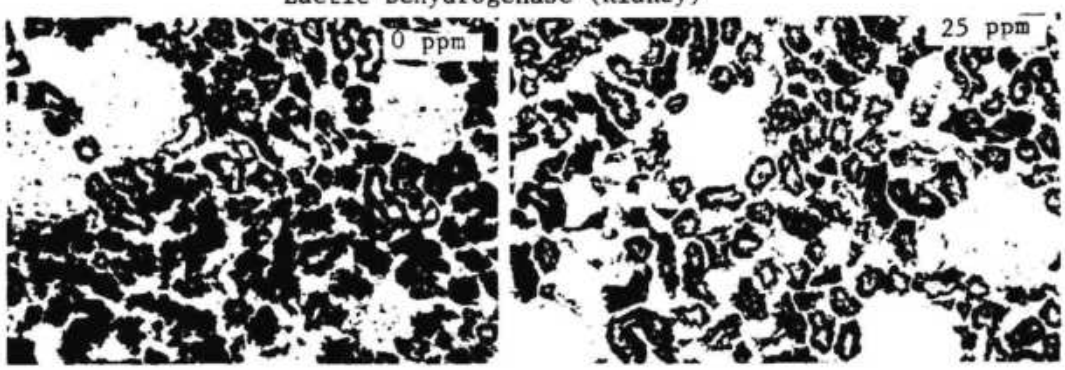
Acid Phosphatase (Kidney)



Reduced activity in convoluted tubules (230X)

Figures 3 and 4

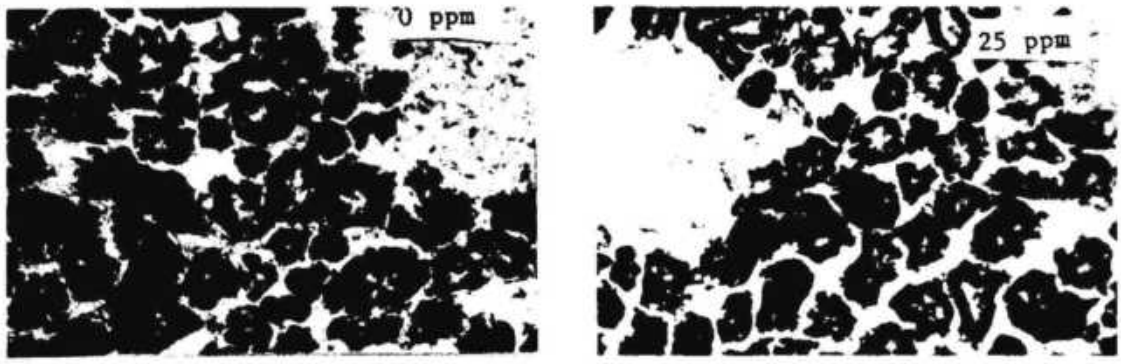
Lactic Dehydrogenase (Kidney)



Activity in convoluted tubules and glomeruli (115X)

Figures 5 and 6

SDH Activity (Kidney)



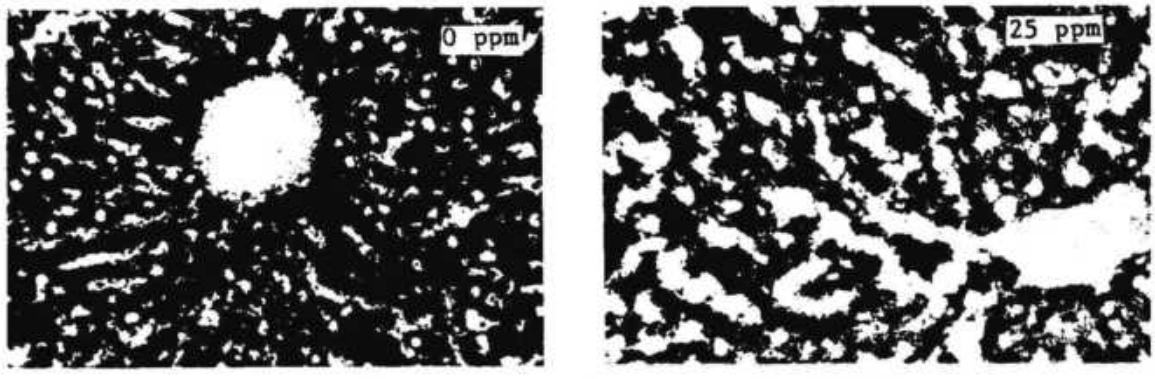
Reduced activity in convoluted tubules (230X)

The distribution pattern of the LDH reaction in kidney of the control animal was almost similar to that of SDH. In general, this enzyme yielded a very strong reaction in cortical tubules though its activity in glomeruli was low in the control group. However, following fluoride ingestion (25 ppm F), the LDH activity decreased appreciably in the proximal and distal convoluted tubules and slightly in the glomeruli.

On the other hand, the activity of ATPase increased appreciably in the kidney in Group II. The ATPase activity in the control animals was low to moderate in tubules of outer cortex and low in glomeruli. However, following ingestion of water contaminated by 25 ppm fluoride, the activity of ATPase was enhanced (moderate to strong) in cells of cortical tubules but remained unchanged in the glomeruli as compared to the control group. In particular, some proximal as well as distal convoluted tubules in Group II showed strong activity of ATPase whereas others gave only a moderate reaction.

Figures 7 and 8

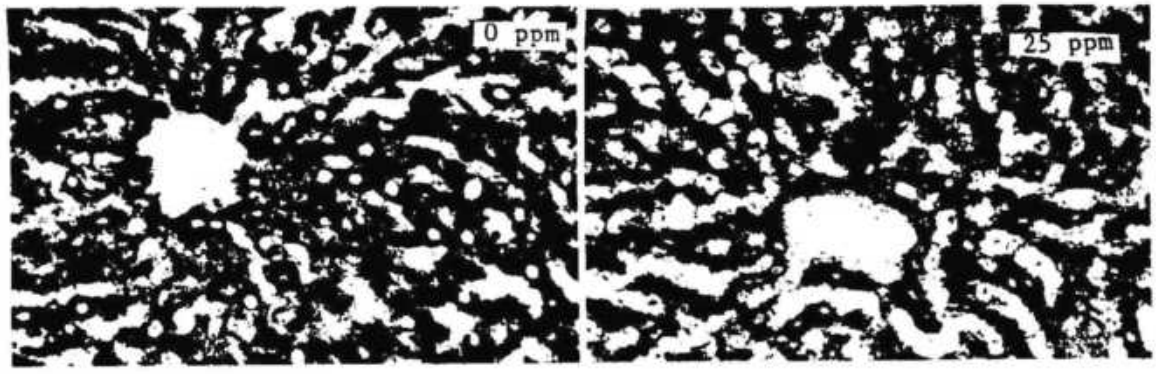
SDH Activity (Liver)



Low activity in parenchymatous cells (230X)

Figures 9 and 10

LDH Activity (Liver)



No change in activity (230X)

Although the activity of some of these enzymes was somewhat affected in the sections of liver in Group II (25 ppm F^-), the change was marginal and not as pronounced as in the kidney. The activity of acid phosphatase, alkaline phosphatase and succinic dehydrogenase decreased in parenchymatous cells in Group II as compared to control. On the other hand, ATPase activity in liver of Group II was somewhat higher than in control animals. In general, the activities of enzymes studied were found to be uniformly distributed in the liver sections of all three groups of animals.

Discussion

The present study on biochemical and histochemical alterations in enzyme activities reveals that both liver and kidney of rat are affected following intake of 25 ppm in water for 10 months. Following fluoride ingestion the activities of acid phosphatase, alkaline phosphatase and SDH decreased and that of ATPase increased both histochemically and biochemically in liver and kidney. In addition, LDH activity also decreased although histochemically only in kidney.

Manocha et al. (11) reported histochemically increased activity of acid phosphatase, glucose-6-phosphate dehydrogenase and SDH and slightly reduced activity of LDH in kidney of squirrel monkeys maintained on 5 ppm fluoride in water for 14 months. Our observations on acid phosphatase and SDH do not agree with those of Manocha et al. This disagreement can be attributed to administration of much higher levels of fluoride in the present study. However, our biochemical and histochemical findings are in agreement with biochemical findings of Sullivan (24) on SDH in kidney and liver and with histochemical findings of Gabovich(12)

who reported reduced activities of acid phosphatase, alkaline phosphatase and SDH in liver and kidney of fluoride-treated animals. Bogin et al. (25) too reported decreased activity of acid phosphatase and SDH in liver of mice given fluoride.

In general, our biochemical findings reveal that activities of acid phosphatase, alkaline phosphatase and SDH are markedly reduced following fluoride treatment. It is interesting to note that these enzymes are also known to be inhibited in vitro even by low fluoride concentrations (26). Furthermore, it is known that the fluoride content of liver and kidney is elevated following fluoride treatment (1). Whether the observed inhibition of these enzymes at the levels of fluoride currently administered orally is due to a direct inhibitory effect of fluoride as seen in vitro, remains to be investigated. Nevertheless, the results indicate that the changes in enzyme activities are considerable and are likely to be associated with pathological conditions.

In the present study, ATPase activity was increased histochemically and biochemically in both liver and kidney following fluoride ingestion. However, this increase does not seem to be a direct effect of fluoride on this enzyme. It is now well known that fluoride impairs carbohydrate metabolism and interacts with the intracellular oxidative system (26), thus impairing energy production. The increased ATPase activity suggests an increased rate of ATP breakdown as a metabolic response to fluoride ingestion in view of depleted energy availability from carbohydrate sources. This view is supported by Handler (27) who reported increased serum phosphate level in fluoride intoxicated rats. He attributed this to increased breakdown of ATP and creatine phosphate when energy from carbohydrate sources is not sufficiently available.

The present histochemical observations reveal that the alterations in the enzyme activities are very pronounced in the proximal and distal convoluted tubules of kidney. These findings, therefore, suggest that fluoride drastically affects intracellular metabolism of the kidney tubules. It is known that kidney handles fluoride by means of glomerular filtration and tubular resorption (28). Tubular degeneration has been widely reported in experimental animals ingesting excessive fluoride (7-10).

In general, we found a correlation between biochemical and histochemical observations following fluoride ingestion indicative of decreased activity of acid phosphatase, alkaline phosphatase and SDH and an increase in ATPase activity both in liver and kidney. It should be mentioned that, in liver of Group II, the histochemically determined activity of alkaline phosphatase and SDH was slightly decreased and that the decrease in activity was uniform throughout the liver section which explains large biochemical changes despite small histochemical alterations in liver. However, in the case of the kidney, the histochemically observed decrease in alkaline phosphatase and SDH activity, although very appreciable, was only confined to the outer cortical region of the kidney which accounts for the fact that the biochemically observed decrease in activity was less pronounced. Furthermore, the same explanation can be extended to LDH, the activity of which decreased in the outer cortex of kidney but remained unchanged biochemically.

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****

Abstract

FLUORIDE METABOLISM IN PATIENTS WITH CHRONIC RENAL FAILURE

by

H. Spencer, L. Kramer, C. Gatza
C. Norris, E. Wiatrowski and V.C. Gandhi
Hines, Illinois

(Abstracted from Arch. Int. Med., 140:1331-1335, 1980)

Fluoride metabolism studies were carried out on 8 men with chronic renal failure on a basal low protein diet containing 1.2 mg fluoride plus approximately 2.4 mg of fluoride in drinking water. The duration of the fluoride balance study ranged from 18 - 40 days.

Urinary fluoride excretion was significantly lower in the nephritic patients than that in subjects with normal renal function, an indication of a significant retention of fluoride. The fecal fluoride excretion was slightly. but significantly, increased but not sufficiently to compensate for the decrease in urinary excretion.

Since patients with chronic renal failure are often given aluminum hydroxide for prolonged periods in order to reduce the elevated serum phosphorus, the authors administered to six of the eight individuals 30ml aluminum hydroxide 3 times daily for an average of 26 days. The aluminum hydroxide solution showed a fluoride content of 0.64 to 1.03 mg/d. Addition of this antacid induced a very marked increase in fecal fluoride excretion in relation to the fluoride intake. The factor of this increase ranged from 6-14 and was related to a markedly significant (46%) decrease in the fluoride balance. These changes in fluoride metabolism are similar to those induced by aluminum hydroxide in subjects with normal renal function.

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OBSERVATIONS AND IMPLICATIONS OF THE [Mg vs F] INTERRELATIONS IN BIOSYSTEMS: A REVIEW and COMMENTS ON MAGNESIUM INTAKE AND FLUORIDE INTAKE IN THE MODERN-DAY WORLD

by

J. R. Marier
Ottawa, Canada

(Abstracted from Proc. Finn. Dent. Soc. 76:82-92 and 76:93-102,1980)

In the first of two articles, Marier reviews his own studies as well as the literature on magnesium, intake of which into the human body increased during the period of 1910-1965 throughout the world. Current magnesium intake, however, is between 6 - 28% lower than required.

The interrelation between dietary intake of fluoride and magnesium can affect soft tissue calcinosis. According to experimental data obtained in short term studies, the "toxic effect of fluoride is a direct function of the severity of magnesium deficiency." In other words, low levels of dietary fluoride may be toxic when dietary levels of magnesium are ultralow. However, large amounts of fluoride are innocuous in the presence of increased magnesium intake. Where intake of fluoride has risen during recent years, magnesium intake has declined. The escalation of the total daily fluoride intake is attributed to its availability in drinking water plus its use in processing food and beverages; currently many concentrated foods and beverages are being diluted with fluoridated water. Daily fluoride intake, which ranged between 0.2 to 0.8 mg in the 1940's and 1950's, has risen during recent years to 2 - 5 mg.

The second paper deals with the chemical interaction of the two ions, magnesium and fluoride. Marier shows that in sea water, 50% of the total fluoride is present as the double ion (MgF^+). In pine needles contaminated by airborne fluoride, the magnesium content is reduced by 50%. On the other hand tomato plants, susceptible to fluoride toxicity, are protected by magnesium supplementation.

With respect to fluoride accumulation in bones, in experimental animals reduction of bone mineral has been associated with an increase in bone magnesium and fluoride, possible as $[MgF]^+$, at intracellular sites during bone mineralization. "Leg weakness" in rapidly growing chicks caused by high dietary levels of magnesium and fluoride is alleviated by administration of calcium. Soft tissue calcification can be induced by magnesium deficiency. High dietary magnesium protects animals against fluoride toxicosis. In fluorotic cattle, the magnesium content of erythrocytes showed a decrease by 47%, but the serum magnesium increased by 12%.

Marier concludes that kidney cells are the most likely sites for the interaction of magnesium with fluoride and that the intracellular concentration of $[MgF]^+$ in the kidneys resembles that of sea water.

****

Abstract

PHARMACOKINETICS OF FLUORIDES IN THYROID DYSFUNCTIONS

by

W. Borner, R. Eichner, D. Henschler, E. Moll and G. Ruppert
Wurzburg, W.Germany

(Abstracted from Fortschr. Med. 98:1083-1096, 1980)

The authors studied the pharmacokinetics of fluoride in 54 patients, 9 of whom were euthyroids, 14 hypothyroids and 31 hyperthyroids. Twenty-four received sodium fluoride in slow absorbing form and 30 in regular form. Heparinized blood was taken prior to, and 20, 40, 60, 120 and 180 minutes after oral application of a 40 mg tablet.

The baseline concentration of the plasma averaged 0.02 ppm. In the hypothyroid cases, the maximal fluoride concentration in plasma was reached after 40 to 60 minutes after which it gradually declined. Both, the increase and the decline appeared to be slower in this group than in the hyperthyroids and the euthyroids. With the slow acting tablet, there appeared to be some delay in absorption and in the decline of serum concentration of fluoride. The findings are explained on the basis of the well-established increase in gastrointestinal absorption and in kidney and circulatory function in hyperthyroidism.

****

FLUORIDE CONTENT OF COMMERCIALLY PREPARED STRAINED FRUIT JUICES

by

S.M. Adair and S.H.Y. Wei
Rochester, N.Y. and Iowa City, Iowa

(Abstracted from Pediatric Dentistry, 1:174-176, 1979)

Analysis of strained fruit juices prepared by the Gerber Co., Fremont, Michigan and Heinz Co., Pittsburgh, Pennsylvania revealed less than 0.33 ppm fluoride in most of the products; but 6 of the 18 products contained more than 0.50 ppm. Two of the Heinz products, apple and apple-grape, averaged over 1.2 ppm. The type of soil in which the food is grown, contamination by fertilizers or by atmospheric sources of fluoride account for wide variations in the fluoride content of strained fruit juices. Particularly the use of fluoridated water in processing the juices and in diluting the concentrates accounts for an increase in their fluoride content. In infants and small children of low body weight "downward revision of older supplementation schedules may be ncessary to prevent fluorosis."

****

THE EFFECT OF DRINKING WATER FLUORIDATION ON THE FLUORIDE CONTENT, STRENGTH AND MINERAL DENSITY OF HUMAN BONE

by

E.M. Alhava, H. Olkkonen, P. Kauranen, and T. Kari
Kuopio, Finland

(Abstracted from Acta Orthop. Scand. 51:413-420, 1980)

The authors analyzed 158 autopsy samples of the anterior iliac crest from the town of Kuopio, fluoridated since 1969, and from a nonfluoridated area. The fluoride content of the Kuopio bones increased significantly with age as compared with the controls. In women in Kuopio, the fluoride in bones ranged from 399 to 4140 with somewhat lower values, 347 to 2360 ppm, in men. This compares to control levels of 144 to 1500 in women and 106 to 769 in men.

The fluoride content of iliac bone increased steadily with age without leveling off at a higher age as suggested by Jackson and Weidmann, 1958. The highest amounts of fluoride had accumulated in bones in women with severe osteoporosis. In general cancellous bone showed higher fluoride values than cortical bone.

The bone strength of cancellous bone, measured by a strain transducer, was significantly higher in women with chronic immobilizing disease from Kuopio compared with the control. But in men no significant difference in bone strength was found between the two areas. In humans with fluorosis, osteoid seems thicker and mineralization is increased resulting in denser bone tissue. However the physical properties of this denser bone tissue may be abnormal.

Bone mineral density as measured by gamma ray attenuation, showed no significant difference in the samples from the fluoridated and nonfluoridated areas. The authors stated that, according to Jackson and Weidmann, fluoride levels above 2000 ppm in fat-free, dried cortical bone are toxic.

Their conclusion: "The physical parameters used in this study, viz. bone mineral density and cancellous bone strength, did not show any significantly beneficial effects of fluoridation, apart from some evidence that fluoridation may preserve mineral density and bone strength better in woman with chronic immobilizing diseases compared with women from the nonfluoridated area. This effect was not seen in men, but the comparison between men of rural and urban populations is difficult because of the difference in bone density between these populations."

****

Abstract

EFFECT OF INORGANIC FLUORIDE SALTS ON URINE AND TISSUE 3'5' CYCLIC-AMP CONCENTRATION IN VIVO

by

D.W. Allmann and M. Benac
Indianapolis, Indiana

(Abstracted from J. Dent. Res. 55 Suppl. b: B192, 1976)

Twenty-four rats, divided into four groups, were fed a diet containing less than 1 ppm fluoride. One group was given distilled water (less than 0.1 ppm fluoride), the other groups NaF at 1 ppm, Na_2PO_3F at 1 ppm and Na_2SiF_6 at 1 ppm in drinking water. Their 24-hour urine was collected each week and tissue and urine cAMP were determined by the cAMP protein binding assay. All three fluoride groups excreted significantly more cAMP than the control. In the fluoride groups, elevation of the concentration of cAMP in liver, heart and submaxillary glands was significant. The rats ingesting fluoride at 1 ppm had an elevation of cAMP in both tissue and urine.

****

INFLUENCE OF SHIFTS OF FLUORIDE INTAKE ON PLASMA FLUORIDE LEVELS

by

R.H. Ophaug and L. Singer
Minneapolis, Minnesota

(Abstracted from J. Dent. Res. 55 Suppl. b: B192, 1976)

Rats on a low fluoride intake were given 25 ppm of fluoride for 28 days, after which they were restored to the low fluoride regime for 28 days. The ionic plasma fluorine level, at the baseline, was 0.005 ppm and the total, 0.03 ppm; both levels rose rapidly to 0.09 and 0.13 ppm respectively after 10 days on the high fluoride intake and remained constant for the following 18 days. The increase of bound fluorine started within one day after the dietary transfer to high fluoride intake and persisted throughout the entire transfer period. Following the return to the low fluoride intake, both the ionic and total fluorine levels decreased promptly. After 28 days these levels had fallen to 0.013(ionic) and 0.05 (total) which are significantly higher than those prior to the high fluoride intake.

****

ACUTE FLUORIDE POISONING LEADING TO FATAL HYPERKALEMIA

by

R.F. Baltazar, M.M. Mower, R. Reider,
M. Funk and J. Salomon
Baltimore, Maryland

(Abstracted from Chest, 78:660-663,1980)

A 25-year old colored man was admitted to the emergency room about 2.5 hours following intentional ingestion of rat poison, identified as sodium fluoride. The examination was essentially unremarkable except for tachycardia with 160 beats per minute and the presence of gallop rhythm, slight hemoconcentration with a hematocrit value of 40 percent, and hemoglobin level of 16.4g per 100 ml.

Forty-five minutes later, the ECG showed considerable peaking of the T-wave. Approximately one hour after admission, the patient developed ventricular arrhythmia which failed to respond to intravenous administration of lidocaine and repeated defibrillation. The patient developed profuse drainage of bright red blood from the nasogastric tube. At autopsy, marked congestion of the lungs and liver, mild left ventricular hypertrophy and marked hyperemia of the serosa of stomach and esophagus were noted.

In two mongrel dogs, which were given 500 mg sodium fluoride as 0.8M solution in 5% dextrose water during a 45-minute period, peaking of T-waves in both standard and precordial leads were reproduced. Immediately prior to death, transient ST sagging in the anterolateral leads was quickly followed by respiratory arrest, bradycardia and ventricular fibrillation. Serial electrolyte determinations revealed a progressive rise in potassium level in the absence of any evidence of an acid-base disturbance. The final potassium levels were 6.9 and 6.1 mEq/l respectively in the two experimental animals.

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