April 1980 Vol. Thirteen No. Two

FLUORIDE

OFFICIAL QUARTERLY JOURNAL

OF

INTERNATIONAL

SOCIETY for

FLUORIDE

RESEARCH

Vol. Thirteen No. Two April 1980 Pages 46-95

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.

MANUSCRIPTS for publication should be submitted in English, double-spaced with generous margins. References should be arranged according to the order in which they are cited in the text, and written as follows: Author, title, journal, volume, pages and year. Each paper must contain a summary of not more than 12 lines.

FLUORIDE is listed in
Current Contents Agricultural
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EDITORIAL

FACTORS AFFECTING TOLERANCE TO FLUORIDE

The development of resistance to a toxic agent after prolonged exposure is a phenomenon common to many environmental poisons. With respect to fluoride Roholm, a keen observer, noted as long ago as 1937 that, in cryolite workers with skeletal fluorosis, nonskeletal symptoms such as gastritis, enteritis, etc. which occur early in the disease disappear as the illness progresses. He states:

"The rule is that for a period of some few days to some few weeks after starting at the factory, the worker suffers from these acute gastric attacks, whereafter they disappear, especially the nausea and vomiting. Thereafter some of the workers tolerate the dust without observing these symptoms" (1).

It has been demonstrated repeatedly that subjects with advanced skeletal fluorosis may be free of systemic symptoms. Grimbergen (2) observed the development of a tolerance to fluoride in individuals in the early stage of fluoride poisoning. In addition to the development of tolerance following repeated or prolonged exposure to fluoride, it is well known that there are wide variations in tolerance from person to person. Certain subjects can tolerate relatively large amounts of fluoride in their system without evidence of harm whereas, at the same level of intake, others are liable to show serious adverse effects.

Similarly, in plants, tolerance to fluoride has been demonstrated repeatedly not only in an individual plant but also among plants of the same species compared to other species. Certain plants such as roses and gladiolas are very susceptible to damage whereas others, such as chrysanthemums, can accumulate larger amounts of fluoride without visible damage (3, 4). Indeed even different parts of certain plants show marked differences in sensitivity to fluoride (5).

Upon inquiring into the reasons for this phenomenon, we find that a wide variety of factors are responsible, some known and others so far unexplored. For instance, Oeschlager, Moser and Feyler (6), have shown that adequate water intake of a plant tends to protect it from fluoride damage. Others (7) have shown that calcium and magnesium, present both in the soil and in the plant, tend to prevent adverse effects. Bovay (8) demonstrated that boron greatly modifies a plant's tolerance to fluoride by increasing its uptake.

In humans, resistance and tolerance to fluoride intoxication are undoubtedly related to uptake, transport, and excretion of the halogen. Obviously a person with impaired kidney function is more susceptible to damage because he or she accumulates more fluoride. Similarly, impaired liver function and its concomitant effect on many

enzyme systems, is likely to reduce the body's ability to cope with fluoride. One suspects that the alcoholics who developed skeletal fluorosis after long-term intake of wine adulterated with fluoride reported by Soriano (9), became more susceptible to the disease because of existing liver damage. Like in plants, deficiency of fluoride-binding electrolytes in the bloodstream, particularly calcium, magnesium, and other metals are known to affect the susceptibility to fluoride toxicity.

Whether or not the general state of nutrition in a population, especially vitamin intake, enters into this problem has not yet been sufficiently elucidated. Certain food habits of populations such as excess consumption of tea, seafood, and chicken are bound to alter the susceptibility to fluorosis. Jolly (10) has shown that the presence of certain trace elements in soils and water are other contributing factors. A genetic trend, perhaps conditioned by the ancestors' long-term consumption of fluoride is undoubtedly another factor in the determination of tolerance to fluoride. This may be significant in explaining the different response of individuals to fluoride intake in areas of endemic fluorosis. Thus a combination of causes seems to determine whether or not a particular person can or cannot tolerate fluoride at a magnitude of intake that might be harmful to others.

Two major features involving the susceptibility to fluoride intoxication have emerged, namely the acidity of body fluids (stomach content, urine, saliva, etc.) and the ability of a cell to retain or eliminate fluoride after the ion has penetrated the cell membrane.

Whitford and Pashley (11) demonstrated that fluoride is absorbed through the epithelium of the urinary bladder in rats at a ratio inversely related to the acidity of the urine. At a pH range of 1.85 to 5.50, 70% to 5% fluoride respectively was absorbed after 15 minutes. Formation of HF, which readily permeates tissue, appears to be involved in the process of absorption. The irritating action of HF in organs is bound to affect the response of an individual to the toxic action of fluoride.

The other development concerns experiments by Repaske et al.(12). When the fluoride concentration in growing mouse fibroblasts was increased by 5 ppm each month until a maximum of 70 ppm was reached, they developed resistance. Their growth rate did not differ from that of normal cells. A decrease in intracellular fluoride concentration was the major factor involved in the development of the resistance. This decrease of fluoride in the cell could result from either lesser permeability of the cell membrane to fluoride or from active transport of fluoride out of the cell into the culture medium. The investigators demonstrated that the latter mechanism, namely an active pumping of fluoride out of the cell, maintains the low fluoride level inside of the cell in the presence of high fluoride concen-

tration of the extracellular fluid.

These new developments now provide the rationale for clinical observations made by Roholm more than four decades ago. In the presence of continued fluoride intake, some individuals build up resistance to certain adverse effects of the halogen.

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TRACE ELEMENTS IN ENDEMIC FLUOROSIS IN PUNJAB

by

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SUMMARY: No cases of genu valgum syndrome, of the kind reported in Andhra Pradesh, have been encountered in the endemic fluorosis villages of Punjab. No appreciable change in manifestations of fluorosis has taken place since our previous description of the disease in 1971. Residents of Punjab consume trace elements molybdenum, copper and zinc in lesser quantities than in Andhra Pradesh. Villages of Punjab with endemic fluorosis consume less molybdenum and zinc, while copper consumption is higher. Total and calcium hardness exert a protective influence in the genesis of skeletal fluorosis, which is in accordance with earlier reports by Jolly et al. The protective role of calcium in the diet is emphasized in the prevention of this syndrome.

Introduction

A lively interest had been evoked by the change in the disease pattern of fluorosis in the endemic villages of Andhra Pradesh, particularly the occurrence of the genu valgum syndrome. The present study, therefore, has been undertaken to determine the incidence of the genu valgum syndrome in the villages of Punjab where chronic fluoride intoxication is endemic and to elucidate the possible role of trace elements in drinking water and a staple diet, in the etiology of the genu valgum syndrome.

For this purpose seven endemic fluorotic villages from Districts Sangrur and Bhatinda were surveyed and compared with an equivalent number of nonfluorotic villages of Patiala. Thirty samples of drinking water, ten of wheat and five of soil were collected from each village surveyed and analyzed for the trace elements fluoride, molybdenum, copper and zinc. In addition other chemical constituents of water were analyzed. A comparative and statistical study of the results in the fluorotic and nonfluorotic villages was carried out.

In the Punjab, the manifestations of fluorosis are quite consistent. No cases of genu valgum syndrome were detected in the endemic fluorotic villages of Punjab.

The genu valgum syndrome was first identified in endemic fluorosis of Andhra Pradesh by Krishnamachari et al. (1). This syndrome affects young adults and adolescents and poses several socio-economic

implications. It was not in existence earlier since no such cases have been described by Siddiqui et al. (3), who had worked in the same villages. The recent change in disease pattern has been attributed to the construction of Nagarjunasagar Dam which raised the subsoil levels of water. This in turn elevated the alkalinity of soil which promotes uptake of molybdenum in plants grown in this area. Excessive molybdenum ingestion through cereals increases the urinary excretion of copper according to Doesthale and Gopalan (2). This factor is blamed for the osteoporosis of bones which accounts for the genu valgum syndrome.

In Punjab, despite the construction of Bhakra Dam, the disease pattern of fluorosis remains unchanged and genu valgum is virtually absent in the endemic fluorotic villages.

Materials and Methods

An intensive epidemiological survey has been carried out in seven fluorotic villages of Punjab and compared with seven nonfluorotic ones. Fluorotic villages were surveyed from the endemic belt of districts Sangrur and Bhatinda in the villages of Khera, Rajiia, Salabatpura, Bajakhana, Bhikhi, Gurney Kalan and Ganja Dhanaula. Children below fifteen years were examined for evidence of dental fluorosis. Particular attention was paid to young adults and adolescents (5-30 years) with respect to evidence of genu valgum in the form of knock-knee deformity which was strikingly absent. Dental mottling was fairly common in all age groups and grading was adopted according to Jolly et al. (4). The criterion for skeletal fluorosis was based mainly on clinical data namely, on limitation of spinal movements and on bony exostoses. Old bedridden persons were examined in their homes for evidence of neurological manifestations such as paraplegia or quadriplegia.

Drinking water samples from hand pumps were collected from every fifteenth house ensuring thereby that all regions of the village were covered. About ten samples of dry food (mainly wheat), and four to five samples of soil were obtained in different areas of every village and analyzed. A similar survey was carried out in seven nonfluorotic villages of the district of Patiala. The villages surveyed were Kalyan, Rakhra, Sanaur, Kauli, Lachkani, Sidhuwal and Khera. An equivalent number of water, food and soil samples were collected from these villages for comparison.

Water samples were analyzed for trace elements and other chemical constituents including chloride, alkalinity, total hardness, calcium hardness and total solids. Food samples were analyzed for trace elements while fluoride could be analyzed only from soil samples. The methods employed for analysis were as follows:

a) Fluoride: (i) In water: Alizarine visual method (Standard Methods for Analysis of Water and

Waste Water) (5).

(ii) In food and soil: Fluoride Ion Electrode Method (6).

b) Copper: Sodium diethyl ditio carbamate method (7).

c) Molybdenum: Thiocyanate Method (8).

Molybdenum could not be analyzed in water even after concentrating 1000 cc to 1 cc. Thus the subsequent analysis for molybdenum in water was abandoned.

All other constituents were analyzed according to "Standard Methods for Examination of Water and Waste Water".

d) Zinc: Dithizone Method (Ref. 5, p. 359)
e) Total hardness: Edta Titrimetric Method (5, p. 179)
f) Calcium hardness: Edta Titrimetric Method (5, p. 84)
g) Chloride: Argentometric Method (5, p. 96)
h) Alkalinity: Mixed Indicator Method (5, p. 52)
i) Total solids: Evaporatory Method (5, 288)

Results

Dental Fluorosis: Children up to fifteen years of age were examined for dental mottling. Depending upon severity grading was adopted as described earlier. In all seven villages, 1893 out of 3086 children had dental fluorosis of various grades. The deciduous teeth were hardly involved. In different villages, the incidence ranged between 48.4 to 83.4% as shown in Table 1.

Table 1
Incidence of Dental Fluorosis

S. No.	Name of Village	Mean Water F ppm	Total No. Exam.	% of Childred ages 5-15 Grade I %	Grade II %	Grade III %	Total %
1.	Khara	9.95	546	20.6	44.6	18.2	83.4
2.	Ganja Dhanaula	3.24	405	21.2	33.4	13.4	68.0
3.	Rajia	3.7	432	14.0	29.8	21.4	65.2
4.	Bajakhana	3.3	630	13.6	24.2	20.6	58.4
5.	Salabtpura	14.14	336	15.7	16.7	22.6	55.0
6.	Bhikhi	4.27	507	13.2	22.6	15.4	51.2
7.	Gurney Kalan	3.38	230	11.5	16.4	20.5	48.4

The incidence bears no constant relationship to the mean water fluoride levels of the village.

Table 2
Incidence of Skeletal Fluorosis (Above 21 years)

S. No.	Name of Village	Mean Water F ppm	Total Incidence %	Males Examined	Males Incidence %	Females Examined	Females Incidence %
1.	Khara	9.95	66.34	225	82.5	168	44.7
2.	Salabatpura	14.14	64.5	185	76.4	135	48.2
3.	Rajia	3.7	44.7	248	56.4	210	31.0
4.	Bajakhana	3.3	44.5	318	49.8	235	37.4
5.	Bhikhi	4.27	40.6	280	48.7	165	27.0
6.	Ganja Dhanaula	3.24	19.6	192	26.5	228	13.8
7.	Gurney Kalan	3.38	16.3	265	19.6	132	9.8

Table 3
Molybdenum Levels of Food

	Fluorotic	Nonfluorotic
	µgm/100 gm	
Mean	12.75	17.34
Range	3.6-30.45	9.45-32.55
S.D.	±2.9	±4.00
S.E.	±0.59	
"t" value	7.77	
At p 0.05	Significant	

Molybdenum content of water was untraceable (less than 1 µgm/liter)

Skeletal Fluorosis: The incidence of skeletal fluorosis in adult population ranges from 16.3 to 66.3% above age 21 as shown in Table 2.

Out of a total of 2986 adults examined, 1713 were males and 1273 females. The incidence of fluorosis is higher in males than in females. In some cases radiological examination of forearm bones revealed ossification of the interosseous membrane even in mild cases. Genu valgum was conspicuously absent in all people of the endemic fluorotic villages of Punjab regardless of their socio-economic status.

Trace Elements

Molybdenum: In the water samples the molybdenum content was untraceable (i.e. less than 1 µgm/liter). However, in samples of food from fluorotic villages, it ranged from 3.6 to 30.5 µgm/100 gm with the mean levels of 12.8 µgm/100 gm of food. In nonfluorotic villages, the mean levels in food were 17.3 µgm/100 gm (Table 3).

Table 4
Copper

	Water µgm/liter		Food mg/100 gm	
	Fluorotic	Nonfluorotic	Fluorotic	Nonfluorotic
Mean	10.3	10.50	9.96	0.54
Range	1.17-42.4	2.5-45.6	0.49-1.4	0.28-1.06
S.D.	±5.7	±5.9	±0.23	±0.24
S.E.	±0.56		±0.03	
"t" value	0.3		14.0	
At p 0.05	Insignificant		Significant	

Table 5
Zinc

	Water mg/liter		Food mg/100 gm	
	Fluorotic	Non-Fluorotic	Fluorotic	Nonfluorotic
Mean	0.26	0.37	1.62	2.36
Range	0.05-0.75	0.05-0.6	0.5-5.00	0.5-4.8
S.D.	±0.15	±0.14	±0.46	±1.00
S.E.	±0.01		±0.13	
"t" value	11		5.69	
At p 0.05	Significant		Significant	

The statistical analysis reveals that the molybdenum content of cereals grown in nonfluorotic villages is higher, signifying thereby that consumption of molybdenum is lower in the fluorotic belt of Punjab than in the nonfluorotic villages.

Copper: As obvious from Table 4, the mean water copper levels are almost the same in samples of fluorotic and nonfluorotic villages, namely 10.3 and 10.5 µgm/liter respectively. But the copper content of wheat in fluorotic villages is significantly higher than in nonfluorotic villages. This finding indicates that consumption of copper in fluorotic villages is greater which may be a protective factor to the genesis of the genu valgum syndrome.

Zinc: The zinc content in water as well as in food samples of fluorotic villages is significantly lower than in nonfluorotic villages as shown in Table 5. Thus the residents of the fluorotic belt consume less zinc in their daily diet.

Table 6 presents other chemical constituents of water in fluorotic compared to nonfluorotic villages.

Table 6
Statistical Analysis of Constituents of Water

Number of Samples 210		Fluoride ppm	Chloride mg as $CaCo_3$	Alkalinity mg as $CaCo_3$	Total Solids gm	Hardness mg as $CaCo_3$	Calcium Hardness mg as $CaCo_3$	Magnesium Hardness mg as $CaCo_3$
Fluorotic villages	Mean	6.00	176.0	554.5	1.77	147.8	53.0	97.1
	S.D.±	3.40	63.9	113.6	0.39	73.8	26.7	54.8
Nonfluor- otic villages	Mean	0.44	147.2	127.6	0.99	415.2	219.0	195.4
	S.D.±	0.23	63.2	31.3	0.36	99.6	69.6	86.2
	S.E.±	0.24	6.20	8.13	0.03	8.55	514.0	7.04
"t" value		23.16	4.64	52.5	26.00	31.2	32.2	13.96
Remarks		S	S	S	S	S	S	S

P 0.01 S=Significant

Table 7
Statistical Analysis of Fluoride in Soil

	Number of Samples	Mean	Fluoride (m.Mol/100 gm) S.D.	S.E.	t value	Remarks p=0.01
Fluorotic villages	35	261.4	±33.9	±7.06	26.16	Significant
Nonfluorotic villages	35	76.7	±24.5			

Thus there is a greater uptake of fluoride in cereals grown in the endemic fluorotic belt.

In the water obtained from fluorotic villages, the mean content of fluoride, chloride, the alkalinity and total solids are significantly higher while total hardness and calcium hardness is lower. The higher total hardness and the calcium content of water chelate fluoride and thus prevent the genesis of fluorosis in non-fluorotic villages.

Analysis of Soil: We were able to estimate only the fluoride content of soil samples from various villages. In fluorotic villages, the mean fluoride levels were 261.4 ± 33.9 m.Mol/100 gm or 49,666 ppm while it was 76.7 ± 24.5 m.Mol/100 gm or 14,273 ppm of soil in non-fluorotic villages (Table 7).

Discussion

The emergence of the genu valgum syndrome among residents of Andhra Pradesh adjoining Nagarjunasagar Dam has been highlighted by Krishnamachari et al. (1). In Punjab, however, despite the construction of the Bhakra Dam long ago, no cases of genu valgum were found in the fluorotic belt. The disease still exhibits the same manifestation as described earlier by Jolly et al. (4, 9). The incidence of skeletal fluorosis in the range of 16.3 to 66.3% in the various villages is almost the same as in the earlier findings. The diseases manifested in Punjab still represent one of the most severe forms leading to neurological complications such as quadriplegia and paraplegia.

Krishnamachari et al. (1) have attributed the genesis of genu valgum to excessive ingestion of molybdenum through cereals which leads to increased urinary excretion of copper. For this reason, we analyzed the molybdenum and copper contents of drinking water and of cereals in fluorotic and nonfluorotic villages of Punjab.

Molybdenum is untraceable in drinking water: the main source of intake of this element are cereals grown in the Punjab villages. Ingestion of molybdenum and zinc is lower, while that of copper is higher in the fluorotic belt of Punjab as compared to nonfluorotic villages. This is illustrated by a comparative study of trace elements in water and dry cereals of Punjab and Andhra Pradesh shown in Tables 8 and 9.

Table 8
Molybdenum, Copper and Zinc in Commonly Used Grains from Punjab and Andhra Pradesh

		Number of Samples	Molybdenum mg. 100 gm of food		Copper		Zinc	
Punjab wheat	Normal	70	0.0.73	0.004	0.54	0.14	2.36	1.00
	Fluorosis	70	0.0128	0.002	0.96	0.23	1.62	0.46
Andhra P. sorghum	Normal	47	0.75	0.039	4.03	0.202	10.7	1.12
	Fluorosis	52	1.20	0.057	4.80	0.222	19.4	0.76

The data of Andhra Pradesh were procured by Krishnamachari et al. (10). In wheat, obtained from Punjab, all trace elements are considerably lower than in sorghum from Andhra Pradesh. On the

other hand, the consumption of molybdenum and zinc is lower in fluorotic villages of Punjab, whereas that of copper is higher in these villages. This may be one of the reasons that the fluorotic individuals of Punjab have not developed genu valgum.

Table 9
Fluoride, Copper and Zinc in Water

		Number of Samples		Fluoride ppm	Zinc ppm	Copper µgm/liter
Punjab	Normal	210	Range	0.1-1.15	0.05-0.6	2.5-45.6
			Mean	0.44	0.37	10.50
	Fluorosis	210	Range	0.25-22.5	0.05-0.75	1.17-42.4
			Mean	6.00	0.26	10.30
Andhra P.	Normal *					
	Fluorosis	44	Range	3.0-13.0	0.1-10.0	0.01-0.1

* Figures not available

Krishnamachri et al. (11) postulated that the low calcium intake by residents of the Andhra Pradesh fluorotic belt is partly responsible for the genesis of genu valgum. The average daily calcium intake in these areas is 300 mg according to Siddiqui (3). However, in Punjab, the average daily intake of calcium from various sources exceeds 900 mg daily both in adults and in young subjects. The higher calcium intake undoubtedly contributes to the protection of Punjab residents from developing fluorosis.

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FLUOROSIS IN DAIRY CATTLE DUE TO HIGH FLUORIDE ROCK PHOSPHATE SUPPLEMENTS

by

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SUMMARY: Non-defluorinated rock phosphate which was used as a mineral supplement in the feed of twenty herd of dairy cows over a period of 6 - 12 months, induced clinical fluorosis in about 70 cows. The condition was characterized by lameness, exostoses in hind legs and ribs, and mild dental changes. Fluoride values were elevated in the blood sera, urine, milk and bones of affected animals. Serum fluoride levels returned to normal upon withdrawal of the contaminated rock phosphate whereas, urine concentrations remained elevated for several months.

Introduction

Fluorosis (chronic fluoride poisoning) in animals is caused by prolonged ingestion of excessive quantities of inorganic fluoride. The sources of the fluoride in cases of bovine fluorosis have been

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Presented at the Tenth Conference of the I.S.F.R., Oxford, England, September 16-19, 1979.

due to emission of fluorine-containing gases or dusts from industrial plants (1), to high levels of fluoride in the drinking water (1) or in soil (2), fluorine-bearing soils deposited on forage by wind and rain or high levels in mineral supplements (1). The mineral supplements involved usually consist wholly or partially of rock phosphate which often contains high concentrations of fluoride (up to 50,000 ppm) and invariably must undergo defluorination prior to incorporation into animal feed (3, 4).

Mineral supplementation of the diet of dairy cattle is implemented in Israel by the addition of dicalcium phosphate, manufactured to a standard which precludes high levels of fluoride in dairy feed. The present study details the consequence of the inclusion into dairy feed of refined rock phosphate instead of the conventional mineral supplement, causing the first case of clinical fluorosis in Israel.

Clinical Findings

Varying degrees of lameness were observed in high yielding dairy cattle on two adjacent farms. In the subsequent 3-4 months about 70 milking cows in 20 beighboring farms became similarly affected. Apart from the lameness no signs of ill-health had been reported in these cows and milk yields were normal.

In mild cases an intermittent generalized lameness was seen whereas in more severe cases the cows crossed their forelegs while standing (Fig. 1); they were reluctant to walk, or stood on their knees and had difficulty in rising from recumbency. Several cows showed a pain response on palpation of the digits and coronet. The more severely affected animals showed a reluctance to walk on concrete or other hard surfaces. Possible causes of lameness were eliminated in the differential diagnosis.

The sign of crossing of the forelegs was suggestive of fluorosis. Further examinations revealed prominent palpable exostoses on the medial surface of the metatarsals (Fig. 2) and to a lesser degree on the ribs. The teeth were examined and mild brownish discoloration of the corner incisors with some brown vertical lines on the other incisors were observed.

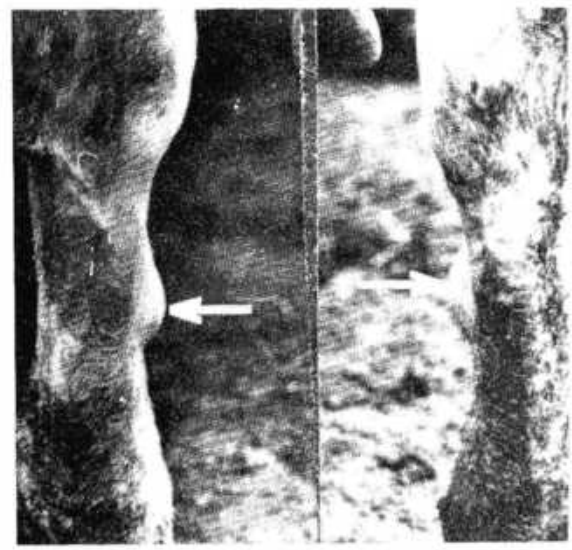
Investigations revealed a common source of dairy feed and water in all affected farms. The farms were situated in a rural area, with no possible contamination from industrial sources. Samples of drinking water and blood from 6 of the affected cows were taken for a fluoride analysis, some 6 months after the first case of lameness were seen.

Fig. 1

Fig. 2

Fluorotic Cow

Crossed-leg pose



Exostoses on metatarsal medial surfaces

Laboratory Findings

Fluoride determinations were made by means of a fluoride-specific electrode and standard techniques (5, 6). The urinary fluoride levels were corrected to a specific gravity of 1.030 (7). Levels of fluoride in the sera of the cows in one affected farm, were 0.4, 0.6, 0.7, 0.8, and 0.9 ppm; normal levels are below 0.3 ppm, threshold levels 0.3-0.4 ppm, moderately elevated levels 0.4-0.5 ppm and high fluoride levels greater than 0.5 ppm (8). These findings reinforced the clinical diagnosis of fluorosis. Levels of fluoride in the water were not abnormally high for livestock as recommended by the Environmental Protection Agency (9).

Subsequent inquiries revealed that undefluorinated rock phosphate had been added to the concentrates for a period of about six months prior to the appearance of the first case of lameness instead of the conventional dicalcium phosphate supplement. Samples of this rock phosphate were examined (10). Fluoride levels were elevated for incorporation into dairy feed. Analysis of batches of this rock phosphate revealed wide variations in the level of fluoride in the samples. After making these observations, the cows received a concentrate containing dicalcium phosphate in place of the rock phosphate supplementation. Further samples of blood, urine and milk were taken from lame cows after changing the concentrate (Table 1,2). In 4 of the 17 blood samples taken on 11 farms, fluoride concentrations were normal, whereas in 4 samples they were moderately and in 9 markedly elevated (8). In 5 of the 18 urine samples taken, normal (up to 8 ppm) (8) or slightly elevated (up to 13 ppm) levels of fluoride were found. In the remaining 13 samples, the fluoride values were

markedly elevated (Table 1). In 3 of 6 milk samples, the fluoride levels were normal (up to 0.12 ppm) (8), 2 showed a slight increase in fluoride and one was markedly elevated (Table 1). After removal of the contaminated rock phosphate from the diet, the serum fluoride levels returned to normal (Table 2), whereas urinary fluoride remained elevated for a prolonged period (Table 3,4).One of the cows was unable to rise and had to be slaughtered. Another 3 cows (one of them in-calf), were culled over a period of 6 months for reasons unconnected with fluorosis. Fluoride concentrations in their bones and in those of their fetuses (11) were high, especially in the fetuses (Table 5). The remaining cows, over a period of weeks, made uneventful recoveries.

Table 1

Fluoride Concentrations (in ppm) in Blood (Serum), Urine* and Milk of Fluorotic Cows

Farm	Cow	Blood	Urine	Milk
A	1	0.74	63	0.2
	2	0.88	44	0.34
	3	0.33	28	-
	4	0.47	29	0.18
B	1	0.32	21	
	2	0.17	7	
C		0.54	24	
D	1	0.47	47	
	2	-	25	0.13
E	1	0.36	23	
	2	0.32	12	
F		0.13	10	0.09
G		0.7	12	
H		0.13	19	
I		0.25	13	
J		0.5	64	0.08
K	1	0.45	24	
	2	0.45	28	

Table 2

Changes in Blood (Serum) Fluoride Concentrations (in ppm) in Fluorotic Cows

	Days after removal of dietary rock phosphate	
Cow No.	0	30-50
1	0.88	0.21
2	0.7	0.21
3	0.9	0.13

* Corrected to specific gravity of 1.030

Normal values

F in blood < 0.30 ppm)
F in urine < 8.04 ppm) Shupe, 1966
F in milk < 0.12 ppm)

Discussion

Rock phosphate-induced fluorosis is not uncommon despite the discovery of its potential harmful effects nearly 50 years ago (12). Fluoride levels in rock phosphate (4) vary from 20,000 to 50,000 ppm (average 35,000) depending on its source. Most feed phosphates originate from rock phosphate which must undergo defluorination to reduce the fluoride levels to meet phosphorus requirements of the animal without causing any adverse effects (4). The fluoride in rock phosphate is less soluble and thus is about half as toxic as that in

sodium fluoride, the form most commonly used in experimental fluorosis (4, 13). The safe level of fluoride derived from rock phosphate (13) in the total diet for dairy cows was established at 60-100 ppm (13). The fluoride concentrations found in the rock phosphate in this outbreak were from 1330-8490 ppm (Table 5) which after incorporation into the concentrates would provide from 13-89 ppm. The high yielding dairy cows ingested about 15 kg of concentrates daily. Thus, a 500 kg cow would ingest as much as 1.35 g/F/day or 2.67 mg/F/kg body weight. This is above the tolerated dose which was reported as 1.5 mg/F/kg (14) when rock phosphate is the source. However, insufficient data are available to determine an accurate tolerance based on the fluoride concentration present in various phosphate supplements (4).

Table 3
Fluctuations in Urinary Fluoride Concentrations* (ppm) in Fluorotic Cows

	Days After Removal of Dietary Rock Phosphate				
Cow No.	1-30	31-60	61-90	91-120	130
1	44	19	-	17	9
2	99	28	20	10	-
3	17	23	29	17	-
4	19	10	18	11	-
5	29	24	-	10	10
6	28	28	-	28	10
7	-	11	11	-	-
8	-	-	14	16	-
9	-	-	12	12	-
10	15	-	-	10	-
11	-	13	-	16	

* Corrected to specific gravity of 1.030

Table 4
Fluoride Concentration in Bones from Cows with Clinical Fluorosis

Farm			
A	Metacarpus	10500 ppm	(Fat-Free Ash)
B	"	13485 ppm	Metacarpus Calf Embryo: 202 ppm
C	"	6200 ppm	
D	Metatarsus	7850 ppm	
	Ribs	2424 ppm	

Table 5
Regulations Governing Fluoride Level in Mineral Supplements

Supplement	Maximum F^- Permitted	Country
DCP (Dicalcium phosphate)	1700 ppm	Israel
DFP (Defluorinated Rock Phosphate)	1800 ppm	Israel
Mineral Supplement	2000 ppm	EEC
Rock Phosphate	800 ppm	Canada
Defluorinated Phosphate	1 part of fluoride to 100 parts of phosphorus	U.S.A.

Fluoride Levels in Rock Phosphate Fed to Fluorotic Dairy Herds in Israel 1300, 1780, 3600, 3800, 8940 ppm

The diagnosis of fluorosis in this case was established on clinical and laboratory findings. Lameness (7, 15, 16) is usually the first abnormality to be noticed in fluorosis. General intermittent ("shifting") lameness has been observed elsewhere in cases of fluorosis whereas the crossing of the forelimbs has occurred virtually exclusively in Great Britain, in cases of industrial (airborne) fluorosis (17). This acute severe lameness is associated with fracture of the pedal bone (3rd phalanx), due to its increased fragility as a result of pathological changes induced by the accumulation of fluoride (15, 16). The crossing of the forelegs as seen in this outbreak was an attempt by the animal to shift weight from bilaterally fractured pedal bones. Crawling on knees, apparently an attempt to avoid pain from the fractured pedal bones, has also been seen in other cases of fluorosis (18). After removal of the contaminated mineral supplement the lameness in the cows steadily diminished and no fresh cases were seen.

Exostoses are commonly observed in fluorosis and usually do not cause lameness (6). The medial surface of the metatarsus is a typical site (19). The dental changes were mild, especially in view of the high levels of fluoride in the urine and bones. Because adult cattle were involved, the permanent teeth had already been formed before exposure to high levels of fluoride. Blood levels of fluoride were initially high, indicating intake of excessive fluoride (20), but after the concentrates were changed, the fluoride levels returned to normal. Similar findings have been reported elsewhere (21). The extremely high urinary fluoride levels are at variance with the literature concerning fluoride concentrations - whether they are corrected to a particular specific gravity (usually 1.030 or 1.040)(16, 17), or correlated with creatinine values in the urine (22). In addition there appear to be some discrepancies regarding normal fluoride concentrations in urine. Burns and Allcroft(16) reported levels 1-6 ppm as normal but generally values below 10 ppm appear to be nor-

mal (corrected to specific gravity 1.030). In adults (4-6 years old), Shupe (8) considered 8.04 ppm normal. In chronic fluorosis no adverse effects were found associated with urinary fluoride levels as high as 11 ppm (corrected to specific gravity 1.040) (8). Griffith-Jones (23) described fluorosis, characterized by chronic lameness induced by ingestion of high concentrations of fluoride in a high phosphorus (probably rock phosphate) supplement. Urinary fluoride levels (corrected to specific gravity 1.030) of 3.3, 3.9, 5.0, 11.7 and 12.7 were "abnormally high" (23). In six fluorotic herds Hillman et al. (24) found that urinary fluoride averaged 5.31 ppm, ranging from 1.04 to 15.7 ppm. Shupe (8) classified concentrations found by us as "severe fluorosis ..". Whereas blood fluoride levels are related to current fluoride intake, urinary concentrations remain elevated for prolonged periods while stored fluoride is being removed from bones (25).

Bone fluoride concentrations were also high, ranging from 6200-13485 ppm. In 4-6 year old cows normal values in bones reached 1221 ppm whereas in chronic fluorosis with no adverse effects levels were up to 2794 ppm (8). Values found by us were classified by Shupe as "severe fluorosis" (8). The fluoride concentration in the metacarpus of the 7 month embryo was also high (202 ppm) as compared with normal values for newborn calves (11 ppm) (24), indicating that fluoride was transferred across the placenta. This finding has been experimentally established elsewhere (20).

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BIOCHEMISTRY OF FLUOROSIS - METHODS FOR EVALUATING FLUORIDE IN BLOOD SERUM. A CRITICAL AND COMPARATIVE STUDY

by

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SUMMARY: Using pooled blood serum from a community in which the drinking water contained 0.95 ppm fluoride, the authors conducted a comparative study of the conventional methods for determining fluoride levels in order to evaluate the discrepancies in blood serum fluoride values reported in the literature. The data revealed the following facts:

(a) Fluoride exists in two forms: as free or inorganic fluoride, and combined or organic fluoride.

(b) Free fluoride can be evaluated by selective electrodes without prior separation treatment.

(c) After deproteinization with trichloroacetic acid or through microdiffusion, under mild conditions, values obtained were similar to those using the former method.

(d) In order to determine the amount of total fluoride prior mineralization is indispensable, for which purpose the "dry" method with alkaline fixing is recommended.

(e) With the combined fraction there exists a labile fraction which becomes ionized and separated depending on the intensity of the treatment to which the sample is submitted in order to separate total fluoride. With the use of perchloric acid such fraction represents approximately 20% of the amount of total fluoride.

Existing discrepancies in fluoride values should therefore take into account the methodology used, which will liberate to a greater or lesser extent, the labile potentially ionizable fraction.

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Introduction

In studies of endemic fluorosis, it is obviously necessary to know the concentration of fluoride in blood serum and in other organ fluids. The literature reveals numerous contradictory findings covering the levels of fluoride in blood serum, with different investigators (1,2,3) supporting their widely differing values.

In seeking an adequate method for such determination, we undertook a comparative study of the techniques which are most commonly used. Our findings indicate that the reason for such discrepancies lies in the method employed for the separation of fluoride in blood serum, not in the procedure used for the final assay.

Material and Methods

Our working material consisted of pooled blood serum from individuals residing in a community with 0.95 ppm of fluoride in drinking water. The pooled serum was subjected to different methods for the liberation of fluoride. In every case the final evaluation was made with the Orion 94-09 selective ion electrode for fluoride ion, connected to an Orion 701 Digital pH/mv meter (Orion Research).

The following methods were studied:

a) Direct measurement of serum fluoride with the ion selective electrode (4)
b) Serum deproteinization with 25% trichloroacetic acid, followed by measurement with the ion selective electrode (5)
c) Fluoride separation by microdiffusion techniques in a system with no more acidity than that due to the presence of a hydrochloride solution of silicone fluid followed by determination with the ion selective electrode (6)
d) Previous deproteinization of the blood serum with perchloric acid followed by microdiffusion, in keeping with the variants of the techniques described by Taves (7), Paez et al. (8) and Singer and Armstrong (9)
e) Calcination of the serum in the presence of an alkaline fixative, followed by microdiffusion in accordance with the techniques described by Bianchi, Dapas et al. (10) and Taves (2), or followed by distillation as described by Singer and Armstrong (11)

Results

Table 1 records the results obtained. In order to avoid subjective errors, each of the values represents the average of at least three readings by different investigators who were unaware of the results obtained by the others when carrying out their analysis. The data thus gathered permit the following conclusion:

The methods based on direct determination of fluoride in blood serum by means of ion selective electrodes yield similar values to those in which previous deproteinization with 25% trichloroacetic acid or microdiffusion at low acidity (4,5,6) were used.

Deproteinization undertaken with perchloric acid produced higher values (7, 8, 9).

The values are even greater when calcination techniques with alkaline fixing are used in order to liberate all fluoride present in blood serum (2, 10, 11).

Discussion

It is clear that the discrepancies in values referred to above originate in the method used for the separation of fluoride prior to the actual fluoride determination.

According to our present knowledge, two forms of fluoride are present in blood serum, i.e. free or inorganic fluoride and combined or organic fluoride. Both forms together represent the total fluoride content.

In order to evaluate the free fluoride, it is sufficient to use an ion selective electrode, without any prior separation treatment. In this respect we disagree with those who maintain that the serum proteins interfere with the normal function of the electrode (12). Our values are comparable both by direct determination and by measurement after deproteinization or microdiffusion methods (Table 1).

The determination of total fluoride requires prior mineralization which we performed by calcination with an alkaline fixative. A labile fraction can be detected in the combined or organic fraction depending on the intensity of the treatment applied in order to obtain the separation of total fluoride. The values will be higher or lower according to the method used to make the separation and the intensity of the treatment.

If perchloric acid is used in the microdiffusion technique for the separation of fluoride (7, 8, 9), the resulting values are higher than those obtained when the liberating agent is only the acidity resulting from the hydrochloric solution of silicone fluid (6). Thus there is a third fraction which we term the combined labile fraction. This fraction represents approximately 20% of the total fluoride according to the working conditions described by Taves (7), Paez et al. (8), Singer and Armstrong (9). We feel that such labile fraction is responsible for the discrepancy of values reported in the literature since fluoride becomes separated in different amounts from the combined or organic fraction and appears as a free or ionizable fraction depending upon the treatment used.

Actually the determination of such a labile fraction does not serve any useful purpose. Therefore we recommend employing methods which do not run the risk of partially liberating it. The inorganic or free fluoride, therefore, should be evaluated directly with the ion selective electrode or else by low acidity microdiffusion (6).

Table 1
Comparison of Methods for Measuring Fluoride Concentration in Blood Serum

	Method	Author(s)	Results (ppm)
Ionic Fluoride	Direct method with selective electrode	Dapes, Paez (4)	0.064
	Serum deproteinization with 25% trichloroacetic acid	Barnes, et al. (5)	0.055 *
	Microdiffusion with silicone fluid	Fry, Taves (6)	0.064
Labile Fraction	Serum deproteinization with 6.8 M perchloric acid and microdiffusion with silicone fluid (3 h at room temp.)	Taves (7)	0.098
	Serum deproteinization with 50% perchloric acid and microdiffusion with perchloric acid and silicone fluid (3 h at room temp.)	Paez et al. (8)	0.099
	Serum deproteinization with perchloric acid and microdiffusion 22 h at 55-60° C	Singer, Armstrong (9)	0.108
Total Fluoride	Serum fractional calcination (2 h) and microdiffusion with perchloric acid and silicone fluid (3 h at room temp.)	Bianchi, Dapas et al. (10)	0.145
	Serum calcination at 600° C. (3) and microdiffusion with perchloric acid and silicone fluid (3 h at room temp.)	Taves (2)	0.149
	Serum fractional calcination (2 h) and distillation with perchloric acid (145-150°C)	Singer, Armstrong (11)	0.144

* This value, which is 14% lower than the other two in the series, may be due to fluoride retention.

Subsequent estimation should be established with the ion selective electrode or spectrophotometry. Total fluoride determinations require prior calcination, followed by ion selective electrode or spectrophotometric determination after microdiffusion techniques.

Recent studies carried out by our group, which will be the subject of a future publication, enabled us to calculate the amount of total fluoride in blood serum on the basis of the application of a mathematical formula to the values of ionic fluoride, with a precision estimated at $p<<0.01$.

Recent publications support our observations in regard to the labile fraction. Taves (13) states that non-ionized fluoride is mainly bound to proteins, whereas Singer and Armstrong (12) in an experiment based on ultrafiltration speak of a dialyzable fraction which is not the inorganic one, nor is it bound to proteins of molecular weight over 25,000.

Acknowledgment

This investigation was possible thanks to the aid of Secretaria de Estado de Ciencia y Tecnologia, Argentina.

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Addendum: The paper by Singer, L. and Ophaug, R.: Concentrations of Ionic, Total, and Bound Fluoride in Plasma. Clin. Chem. 25:523-525, 1979, which appeared after this paper was submitted for publication supports the existence of the two fluoride fractions in blood serum.

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BIOCHEMICAL VARIATIONS IN CHANNA PUNCTATUS (BLOCH.)* DUE TO SODIUM FLUORIDE TREATMENT

by

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SUMMARY: Channa punctatus (Bloch.) maintained in the medium containing sodium fluoride (10 ppm) showed the following biochemical changes at 15° C as well as at room temperature (30.22± 0.54° C) as compared to the controls.

1. Hyperglycemia was observed upon treatment with sodium fluoride at room temperature.
2. Cholesterol and sodium contents increased both temperatures.
3. Potassium and calcium showed an increase at room temperature and a decrease at 15° C.
4. Total protein also increased on treatment with sodium fluoride. The significance of the above results is discussed.

Introduction

Alterations in various physiological and chemical parameters have been observed in most of the teleosts under stress (1, 2 and 3). Although pollution of rivers and lakes by industrial effluents is known to damage the aquatic species, the effect of specific chemicals and the physiological variations caused by them are not yet well established.

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* Channa Punctatus (Bloch.) belongs to the family known as snakehead - a type of fish which breathes air and lives in very polluted water.

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The present study is designed to evaluate biochemical variations of the effect of sodium fluoride, a potential agent for ecological damage (4), on Channa punctatus (B.).

Material and Method

Healthy fish were obtained from a local fish market and acclimatized to the laboratory conditions for one week (5). The length and weight of the fish were noted. First the Lc 50 concentration (NaF 10 ppm) were observed both at 15°C (maintained in BOD, thermostat) and at room temperature (30.22°±0.54°C). The mean weight of the fish both at room and cold temperatures were 102.43±2.93 gms and 118.80±4.23 gms and their length 19.90±0.41 cms and 20.42±0.68 cms respectively. Fish were acclimated to chemically-treated media for a week and analyzed on the eighth day. Blood from fish was collected in the heparinized vials by severing the caudal fin and analyzed. Control samples were also maintained simultaneously similar to the experimental samples. Alimentary canals were examined for parasitic infestation; fish which were infected were excluded from the study. A part of the blood was used for the estimation of glucose (5) and cholesterol (6): the remaining sample was spun at 3000 rpm to collect clear plasma which was analyzed for total protein, albumin and globulin (5, 7).

Plasma ionic contents were determined by means of a flame photometer (Elico model C120).

Results

An increase in plasma total protein at room temperature and a decrease at 15°C (Tables 1, 2) was observed when fish were subjected to treatment with NaF. A significant decrease in the mean albumin content was observed in both sexes and at both temperatures. A significant increase in globulin content was found only at 15°C. The albumin/globulin ratio decreased in both sexes at 15°C and in females acclimated only to room temperature and decrease at 15°C. Cholesterol and sodium contents were found to increase at both temperatures when compared to controls. However the potassium and calcium levels increased at room temperature and decreased at 15°C (Tables 3, 4).

Discussion

Alterations in various physiological and chemical parameters were reported in teleosts when subjected to different stresses (1, 2, 3). Following the abrupt transfer of the gold fish to low temperatures, mortality increased and plasma chlorides were reduced (9). Likewise Prosser (10) reported an initial decrease in plasma sodium and chloride levels in Salmo gairdnerii after abrupt transfer to low temperature.

Table 1

Alterations in the Levels of Plasma Proteins in Channa Punctatus Exposure to Sodium Fluoride at Room Temperature

Parameter	CONTROL No	Mean	SE	ACCLIMATED No	Mean	SE	t value	d.f.
Total Protein gm/100 ml								
Male	15	1.98	0.21	10	2.55	0.11	2.09	23
Female	21	2.29	0.90	11	2.36	0.11	0.45	30
Pooled	36	2.78	0.31	21	2.45	0.15	-1.55	55
Albumins gms/100ml								
Male	15	1.57	0.15	10	1.69	0.24	0.48	23
Female	15	1.57	0.15	11	1.09	0.13	-2.27*	24
Pooled	30	1.57	0.15	21	1.37	0.27	-0.27	49
Globulins gms/100ml								
Male	15	1.27	0.10	10	0.99	0.18	0.62	23
Female	15	1.27	0.10	11	1.34	0.15	1.21	24
Pooled	30	1.27	0.16	21	1.18	0.24	0.82	49
Albumin/Globulin (Ratio)								
Male	15	2.15	0.32	10	2.74	0.93	0.69	23
Female	15	2.15	0.32	11	1.05	0.38	-2.18*	24
Pooled	30	2.15	0.32	21	1.85	0.97	-0.70	49

SE = Standard Error; d.f. = Degrees of Freedom; * = P<0.05

Table 2

Alterations in the Levels of Plasma Proteins in Channa Punctatus Exposure to Sodium Fluoride at 15°C

Parameter	CONTROL No	Mean	SE	ACCLIMATED No	Mean	SE	t value	d.f.
Total Protein gms/100ml								
Male	12	1.56	0.12	12	2.29	0.12	-4.06**	22
Female	8	2.10	0.09	12	2.20	0.18	0.43	18
Pooled	20	1.77	0.19	24	2.25	0.21	3.20**	42
Albumin gms/100ml								
Male	12	1.93	0.13	12	1.23	0.23	-2.62**	22
Female	8	1.88	0.16	12	1.42	0.14	-2.14	18
Pooled	20	1.91	0.21	24	1.32	0.27	-3.92	42
Globulin gms/100ml								
Male	12	0.16	0.01	12	1.06	1.29	3.08**	22
Female	8	0.29	0.07	12	1.11	0.23	2.81**	18
Pooled	20	0.39	0.25	24	1.09	0.45	3.38**	42
Albumin/Globulin (Ratio)								
Male	12	10.83	0.43	12	5.53	2.66	-1.97	22
Female	8	9.43	2.45	12	3.44	0.49	-2.66	18
Pooled	20	10.27	2.04	24	4.48	2.80	0.94	42

SE = Standard Error; d.f. = Degrees of Freedom; ** = P<0.01

Table 3

Alterations in the Glucose, Cholesterol and Ionic Contents in Channa Punctatus on Exposure to Sodium Fluoride at Room Temperature

Parameter	CONTROL No	Mean	SE	ACCLIMATED No	Mean	SE	t value	d.f.
Glucose (mg/100ml)								
Male	15	16.60	3.01	10	26.70	5.63	1.72	23
Female	21	43.69	5.79	11	42.19	7.66	0.26	30
Pooled	36	21.60	7.33	21	34.28	9.68	2.13*	55
Cholesterol (gms/100ml)								
Male	15	2.84	0.13	10	7.41	1.13	4.96**	23
Female	21	3.12	0.10	11	6.60	1.08	4.43**	30
Pooled	36	4.03	0.19	21	6.99	1.56	3.19	55
Sodium (meg/lit.)								
Male	15	532.00	15.49	11	697.00	19.77	0.87	24
Female	21	208.00	75.05	11	685.00	19.75	4.54**	30
Pooled	36	343.53	2.54	22	977.55	151.37	6.97**	56
Potassium (meg/lit.)								
Male	15	25.66	7.17	10	227.50	90.65	1.93	23
Female	21	91.71	1.38	11	113.82	6.27	4.54**	30
Pooled	36	78.65	11.88	21	117.95	86.40	2.44*	55
Calcium (meg/lit.)								
Male	15	1.01	0.22	10	1.68	0.58	1.23	23
Female	21	1.68	0.53	11	0.94	0.26	0.98	30
Pooled	36	1.33	0.54	21	1.29	0.61	0.08	55

SE = Standard Error; d.f. = Degrees of Freedom; *=P<0.05; ** = P<0.01

Table 4

Alterations in the Glucose, Cholesterol and Ionic Contents in Channa Punctatus on Exposure to Sodium Fluoride at 15°C

Parameter	CONTROL			ACCLIMATED			
Glucose (mg/100ml)							
Male	12	25.71	6.48	12	17.08	3.91	-1.24	22
Female	8	40.34	7.35	12	25.89	6.29	-1.86	18
Pooled	20	31.56	5.92	24	21.48	2.81	-2.21	42
Cholesterol (gms/100ml)								
Male	12	4.18	0.45	12	7.11	0.85	3.66	22
Female	8	3.20	0.46	12	7.49	0.70	1.09	18
Pooled	20	3.42	0.67	24	7.30	1.09	5.79	42
Sodium (meg/lit.)								
Male	12	342.50	77.40	12	691.25	16.15	4.43**	22
Female	8	478.78	38.10	12	712.50	18.98	3.26**	18
Pooled	20	397.00	101.38	24	701.87	143.80	0.32	42
Potassium (meg/lit.)								
Male	12	344.50	49.19	12	116.25	20.62	-4.29**	22
Female	8	307.00	22.68	10	110.00	6.49	-9.89**	16
Pooled	20	329.50	22.01	22	113.13	21.58	-7.08**	40
Calcium (meg/lit.)								
Male	12	16.50	9.59	12	0.82	0.28	-0.28	22
Female	8	3.20	0.35	12	0.48	0.11	-8.66	18
Pooled	20	5.30	2.29	24	0.65	0.22	-4.48	42

SE = Standard Error; d.f. = Degrees of Freedom; ** = P<0.01

It is known that when fish are acclimated to low temperatures their whole body metabolism or oxygen consumption declines (11). Their osmoregulatory ability can also be reduced in cold.

These studies suggest that the physiological process of most teleosts appears to be refractory or diminished as a result of stress. Similar physiological reorganization was observed in Channa punctatus (B.)upon treatment with sodium fluoride at different temperatures following biochemical changes as a compensatory mechanism. The decrease in protein content suggests that the energy is substituted by protein degradation or that there may be depletion in protein synthesis.

The low glucose content suggests a possible need for high energy under stress condition leading to an increase in glycolytic activity. The increased amount of potassium also suggests cell deterioration, since potassium levels act as an indicator for cell damage. The increase or decrease in the plasma ionic content suggests there might be disturbance in the osmoregulation in Channa punctatus (Bloch) due to sodium fluoride treatment.

Acknowledgement

The authors are grateful to Professor P. Ramachander Rao, Head Department of Zoology, Osmania University for providing necessary facilities and to the University Grants Commission for the financial assistance.

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UPTAKE OF FLUORIDE BY MAGNESIUM TRISILICATE

by

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SUMMARY: Earlier studies in this laboratory on the uptake of fluoride by serpentine indicated the proxy of fluoride ions for hydroxyl ions. The present paper seeks to elucidate the behavior of magnesium trisilicate from the standpoint of its uptake of fluoride ions in aqueous solutions under varying experimental conditions.

One gm of magnesium trisilicate (MTS) I.P. (Boots Company India Ltd.,) can reduce the fluoride content from 1000 µg to 800 µg in 100 ml of solution. Data comparing serpentine with MTS are given in order to elucidate the similarities and essential differences.

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* Chemical Division

Introduction

Naturally occurring serpentine, reported to take up fluoride from aqueous solutions is usually a mixture of minerals (1); the contribution of each of the minerals in the assembly to the total fluoride uptake could not be ascertained due to difficulties involved in their separation. Inasmuch as the serpentine group of minerals are hydrated magnesium metasilicates, it is of interest to examine the uptake of fluoride by synthetic magnesium silicate. Attempts to synthesize magnesium silicate by slow hydrolysis of silicon tetraethoxide and magnesium ethoxide following Bremer and Steinberg (2) failed to yield encouraging results. Subsequently, aqueous solutions of magnesium salts and sodium silicate were mixed under various conditions of CO_2 partial pressure and concentrations of the salts. In each case, a gel-like product of indefinite composition, rich in magnesium oxide, was obtained thus making further work in this direction difficult.

It was therefore desirable to determine whether MTS, which is readily available and extensively used as an antacid, might possibly possess defluoridating properties. The chemical composition of MTS (Boots Company Ltd. 'Gastomag') employed in this study is given in Tables 1 and 2. For comparison, data on serpentine (1) are also given.

Experimental Data

One gram of MTS powder was added to 100 ml of sodium fluoride solutions of different concentrations in polythene bottles. The bottles were shaken for about 30 minutes and then allowed to stand for 24 hours at which time some of the material remained in suspension whereas the major amount had settled at the bottom of the bottle. The suspended material passed through Whatman No. 42 filter paper. In order to obtain clear solutions for the estimation of fluoride, the suspensions were kept in a cataphoresis tube and electrolyzed by applying 12 volts D.C. The suspended material settled near the anode indicating a negative charge on the colloid. Fluoride was estimated in the clear solution by standard methods (3-6).

By carrying out blank runs, we ascertained that the observed decrease in the fluoride content was not within analytical errors. The uptake of F by MTS with varying F concentration is shown in Table 3.

Action of Water on MTS

A known quantity (1g) of MTS was allowed to react with 100 ml of distilled water for 24 hrs. The material reacted with water releasing some ions as shown in Table 4. These observations are similar to those obtained in the case of serpentine reported earlier.

Table 1
Chemical Composition

Constituents	MTS %	Serpentine
SiO_2	50.21	44.39
Al_2O_3	0.93	0.55
Fe_2O_3	0.10	1.07
FeO	0.02	0.06
TiO_2	nil	nil
CaO	0.90	3.50
MgO	20.51	35.87
Na_2O	0.61	0.04
K_2O	0.18	0.05
F	0.20	0.17
H_2O+	12.26	9.57
H_2O-	14.55*	0.66
SO_2	--	0.16
CO_2	--	4.03
	100.47	100.12
MgO : SiO_2	3:5	5:6
MgO : H_2O	4:3	5:3

* Since MTS was absorbing moisture continuously from the atmosphere, the material was heated in a platinum crucible at 110°C and weighed immediately after 5 minutes of cooling in the desiccator.

Table 2
Trace Metal Content

Trace Elements	MTS(ppm)	Serpentine (ppm)
CU	6	20
Pb	5	75
Zn	250	<5
Co	6	<5
Ni	5	<5
Mn	50	200
P	<5	<5

Table 3
F^- Removal by MTS from Solutions With Varying F^- Concentrations (as NaF)

F^- (ppm) in Original Solution	F^- (ppm) After Treatment
0	0.3
2	1.5
4	2.8
10	8
100	80
1000	800
5000	4400
10,500	8600
17,500	14,000

Note: MTS added 1 g, volume of solution: 100 ml; Time of contact: 24 hrs. Temp. 27°C

Uptake and Release of Fluoride

Experiments were carried out for the uptake of fluoride from solutions ranging in concentration from very dilute to a saturated solution of sodium fluoride at 27°C (Table 3). Experiments for the release of fluoride taken up by MTS by the dilution method were conducted in the following manner: To 100 ml of saturated sodium fluoride solution (17,500 gF/ml) 1g of MTS was added and the mixture was shaken for 1 hr. After allowing it to stand for 24 hrs. 25 ml of the solution in equilibrium with MTS was taken out and the fluoride was estimated. To the remaining solution (75 ml) 25 ml of distilled water was added and the mixture was shaken for 1 hr. This

procedure was repeated at 24 hr. intervals and the fluoride content estimated. The results of both uptake and release of fluoride by MTS are shown in Table 5.

Table 4
Change in the Quality of Distilled Water in Contact with MTS

Time in hrs.	pH	Sp.Cond.* m.mhos. cm at 20°	CO_3	HCO_3	CI	F	Ca	Mg
0	6.5	8	..	..	..	..	..	..
24	8.5	249	12	138	7	0.3	1	14

Weight: 1 g passing 200 mesh; volume of water: 100 ml.
* Specific Conductance (in) moles.

Table 5
F^- Uptake and Release with Variation of F^- Concentration

Uptake			Release		
F^- concentration in solution	F^- Uptake by MTS		F^- concentration in equilibrium solution	F^- remaining with MTS	
g/ml (A)	g/g (B)	B/A	g/ml (A')	g/ml (B')	B/A
1.5	50	33	14,500	300,000	21
3.0	100	33	11,600	227,500	20
8.0	200	25	9,000	197,500	22
8.0	2,000	25	7,050	167,500	24
800	20,000	25	5,600	136,250	24
1600	40,000	25	4,450	111,250	25
4100	90,000	22			
8400	160,000	19			
14,500	300,000	20			

Amount of MTS: 1g; total volume of solution: 100 ml; temp.: 27°C

Effect of Heating MTS to Various Temperatures in Relation to Its Ability for Uptake of Fluoride

One gram of material was heated in each case to 100°, 600°, and 1100°C. MTS heated up to 600° C slowly absorbed water from the atmosphere. Its defluoridating property was not destroyed. But when MTS was heated up to1100°C the material no longer absorbed moisture from the atmosphere. It also lost its defluoridating property. Complete dehydration of MTS deprived it of its defluoridation property.

Since such lesions in FAC and strychnine poisoning can be identical, they cannot be used for differential diagnosis. Therefore, performance of confirmatory toxicological tests is essential.

The literature concerning pathological lesions in poisoning from the above agents is incomplete. In a description of the pathology of strychnine poisoning in dogs, Kamel and Ahlamy noted few characteristic findings (5). They reported generalized congestion diffusely distributed throughout all the splanchnic organs, a sign of asphyxia. However, they did not mention congestion, ecchymosis or petechiation of the thymus and pancreas. In one textbook on toxicology, FAC is listed as a poison that causes gastroenteritis (6). This finding, however, was not characteristic of our series, although diffuse congestion of the gastric mucosa was found in experimental poisoning of dogs that had ingested FAC from poisoned sheep carcasses (9). We also noted that, in strychnine poisoning, the dog's stomach is not empty

Table 2
Laboratory and Post-Mortem Findings in Fatal FAC Poisoning (Dogs)

Case No.	Hemorrhages in Thymus	Hemorrhages in Pancreas	Other Findings	a) Inoculation test in mice* (with stomach content)	b) TLC examination for strychnine in liver	c) Citric acid** (µg/g in guinea pig kidney after injection of extract of heart & kidney from dogs)
1	+	+	Pulmonary edema, empty stomach			188
2	+	-	Hemorrhages in epicardium	Tonic-clonic convulsions	* * * All Negative * * *	600
3	+	+	None	(1 died)		188
4	+	-	Empty stomach			221
5	+	-	Empty stomach			176
6	+	+	Pulmonary edema, empty stomach			188
7	+	-	Hemorrhages in myocardium	No effect		341
8	+	+	Empty stomach			255
9†	+	-	None	No effect		212

† cat; * Evaluated up to 15 minutes after injection; ** Citric acid concentration in kidneys of normal guinea-pigs is less than 70 µg/g (4).

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FLUORIDE BRIEFS

The brush-tailed possum (T.Vulpecula) of Western Australia is nearly 150 times more resistant to fluoroacetate intoxication than the same species from South Australia. However both animals show similar capabilities to inhibit aconitate hydratase activity in the liver by fluorocitrate and to defluorinate fluoroacetate by glutathinone, a dependent enzyme mechanism which results in the formation of free fluoride ion and S-carboxymethylcysteine.

Mead, R.J., Oliver, A.J., and King, D.R.: Aust. J. Biol. Sci., 32:15-26, 1979.

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Analysis of the aluminum content of several proprietary antacids by titration with sodium fluoride by means of the fluoride electrode compared favorably with the official method. Less sample manipulation, more rapid procedure and easier end point detection are some of the advantages of this method.

Cooper, M.E., Ballatine, J., and Woolfson, A.D.: J. Pharmacol. 31:403-405, 1979.

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In freshly collected human erythrocytes, the uptake of 51 Cr-chromate was inhibited by 90 mM sodium fluoride. The sensitivity of the cells to uptake of chromate increased by storage of the cells in vitro for 5 weeks. By means of gel chromatography, two forms of Cr were detected within the cell namely, unchanged chromate and another form apparently bound to macromolecules such as hemoglobin.

Holland, R.I.: J. Inorg. Biochem., 11:1-6, 1979.

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SPECIAL REPORT

Kizilcaoren -- A Health Survey in an Endemic Fluorosis Village

by

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SUMMARY: The fluoride content of the water supply in Kizilcaoren has ranged between 3.8 - 4.9 ppm presumably for about 650 years. A survey conducted by the Anadolu University yielded data concerning the historical background, the socio-economic and the health status of the residents.

In 1959 radioactivity was detected by plane in Kizilcaoren Valley where a village by the same name is located about 36 kms north of the state road between Ankara and Askisher (Fig. 1) in central Anatolia. Fifteen years later Huseyin Kaplan, a geologist, and his team investigated the geological structures of the area. They found a large number of elements including calcium fluoride, barium sulphate and barium carbonate, pseudomerase (MnO_2), iron sulfate, thorium oxalate (composed of 0.35 - 0.06% Th), and uranium oxide complexes.

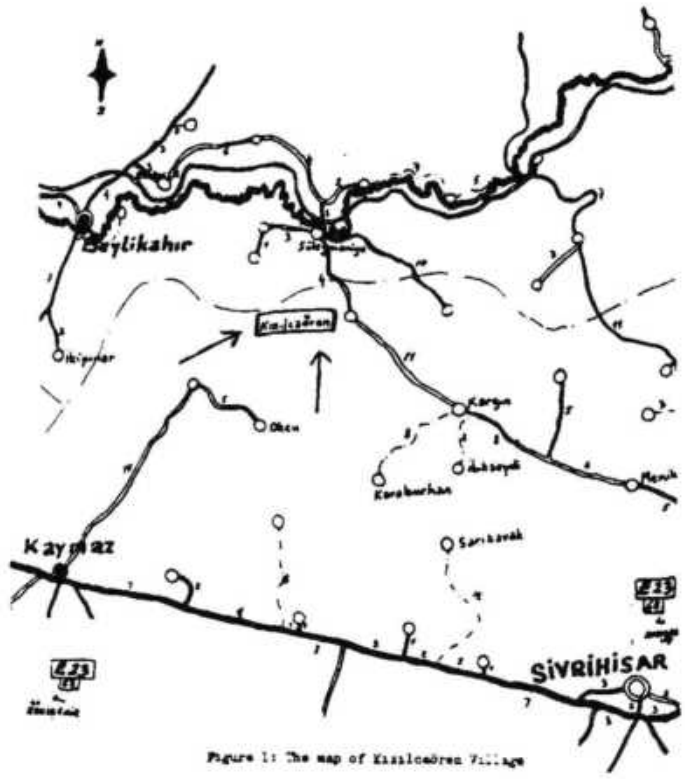


Figure 1: The map of Kizilcaören Village

From the Anadolu University Medical Faculty, Eskisehir, Turkey.

Kizilcaoren's water supply is derived from three hills, Karaburun Sivri, Kusuk Koyklu and Koca Yayla. Its chemical analysis showed a low salinity, no radioactivity and a high fluoride content (3.8, 4.2 and 4.9 ppm) (1). The inhabitants, it is believed, have been drinking this water for about 650 years.

Historical Background

At a very large, old cemetery in the center of the area we found a tomb with an unwritten headstone which, we learned, belonged to Musa Bey. Other relics of interest are a scriptured marble in the Latin alphabet at the entrance to this tomb, a large earthenware jar near the cemetery, a Turkish bath called Dogrul, built by Dumlu Bey for his daughter Duriye about 3 kms from the village. An old resident stated that the ruin of a castle at the top of the crescent-shaped hill, now totally destroyed, has been used as an observation tower by the people of old Kizilcaoren. In ancient times the village was believed to have been located along a caravan line and its people were engaged in making large earthenware jars. According to the villagers, the inhabitants formerly enjoyed excellent health and reached an advanced age. A striking feature of these people was their yellow colored teeth; inhabitants of other villages called them "men with yellow teeth".

Method of Survey

In June 1978 the medical faculty of Anadolu University, having learned about the presence of endemic fluorosis in the village, made a survey to determine the extent of the illness and the socio-cultural level of the inhabitants. Two teams were engaged in the study. The first one consisted of three interns from the Department of Community Medicine and four residents from the Departments of Pediatrics, Internal Medicine, Orthopedics and General Surgery. The second team of four technicians was headed by the author. Through a house-to-house survey, the number of inhabitants, their ages and sexes were determined. The children at the primary school of the village were examined by the pediatricians. Three hundred thirty-seven persons of the total population of 361 were examined, x-ray films were taken by a portable outfit in the village.

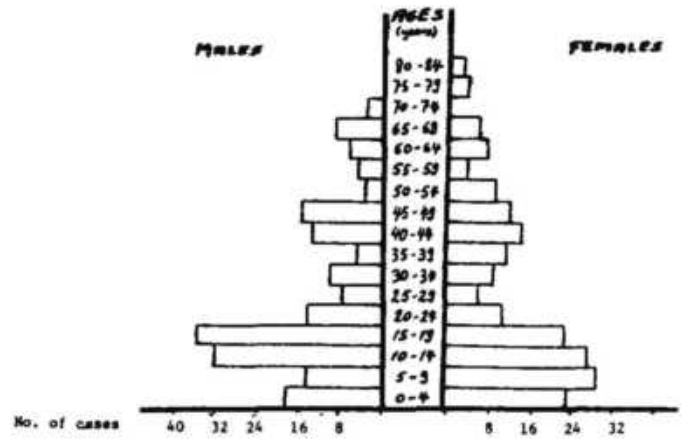
Socio-economic State

According to our survey, the number of persons per family averaged 5.5 compared to 5.2 in Eskisehir in 1967 and to 5.5 in Etimesgut, Ankara (2). The percentage of abortions among the 78 married women was 22.9%, only six (7.6%) gave a history of stillbirth, premature birth and sterility.

The basis of the population pyramid (Fig. 2) was narrow for the boys in the age group 0-9 years, and for the girls 0-4 years of age. Age groups of 15-19 and 20-29 years were also small for females. We

assume that this was the effect of marriage and migration, and of deaths during pregnancy. The difference in age between men and women, 60-69 years in men and 55-59 years in women was difficult to explain. Of all inhabitants, 38.8% were 0-14 years of age; 48.8% were in the age group 15-49 and 12.4% were 50 or more years old. The mean age of all residents was 34(Turkish standard 52 years)(3). The rate of illiteracy was 26.9% compared with 33.3% throughout Turkey.

Figure 2: Population Pyramid Age Distribution of the Kilzilcaoren



The yearly income of twenty-seven families (41.5%) was below 10ooo TL (500 U.S. dollars). Thirty-six families (55.4%) earned between 10-30ooo TL (500-1500 U.S. dollars) annually, and only two families more than 30ooo TL (over 1500 U.S. dollars). The average size of fields and planting area per family was $60.65 \times 10^3 m^2$. Seven families (10.7%) had no fields and twenty-nine families (44.6%) had only a parcel of $1-49 \times 10^3 m^2$. The remaining twelve families (18.4%) had between 100-149 and over $150 \times 10^3 m^2$. Some of the fields were irrigated from boreholes, otherwise dry farming methods were used. Twenty-nine families (44.6%) had between 1 and 49 head of sheep and only five families (7.7%) had between 100 and 150 sheep. Thirteen families (20%) had no animals. Very few people owned cattle and poultry.

Seventy-one percent of the buildings were one story high; 74.1% were built of stone. The others were made of stone-brick and sun dried bricks. Most houses (68%) were covered with soil. The others had tile roofs. The average house had three rooms; two persons had to live in the same room. Only 2% of the houses had regular bathrooms, 17.5% had kitchens, 60.7% had toilets of which only 2.6% were considered sanitary. The lighting was satisfactory in 18.2% of the houses, 19.5% had no windows. Most homes (80.1%) used petrol lamps as a source of light at night, 81% used shrubwood for cooking and 96.6% for heating. Sheepcotes were located within 50 of the houses; 13.3% were underneath and 36.7% were attached to the homes.

No special place was provided for garbage; the feces were collected in an open pit near each household. Flies were abundant in all houses, mice in 74.2% and mosquitos in 50%. Only 37% of the families showed any concern about these pests.

The average marrying age was 19 for boys and 17 for girls. The village people considered three the ideal number of children in a family but, as stated above, the number of children per family averaged 4.3. The villagers desired birth control but no health center was available in the area to provide proper counselling.

Health Status

The water with natural fluoride content was obtained from four fountains that were distributed throughout the village. Dental defects were observed in 267 persons (74%) of the total population. On the other hand, nine children between 1-10 months of age (mean 5.6 months) (2.4%) had, as yet, no teeth. Thirty-three women with no dental problems had come from other villages. All children seven years of age and above, born and raised in Kizilcaoren, had dental problems; below that age they were insignificant.

Bone and joint complaints (29.6%) and epigastric pain (25.8%) were problems second and third in importance. Respiratory disease occurred in 12.7% of the total population and kidney stones in 2.2%. In Kizilcaoren Village, 41.6% of the people were under the care of dentists and physicians.

The physical examinations performed on 337 persons of the total population of 361 revealed that seventy-three of the 142 children (52%) and 64 of 195 adults (33%) were ill in addition to their dental problems. The following illnesses were recorded in the children: dermatitis (17), parasitosis (14), healed rickets (6), conjunctivitis (5), sinusitis (4), convulsions (3), deformity of the head (3), acute infectious diseases (3), acute rheumatic fever (3), heart murmur (3), severe anemia (2), enuresis nocturia (2), scabies (2), corneal opacity (1), epigastric pain (1), hemangioma (1), hearing defects (1), hypospadiasis (1).

In adults the findings were: epigastric pain (17), heart disease (7), hypertension (6), spastic colitis (6), diabetes (3), sinusitis (2), hemorrhoids (2), hernia (2), lipoma (2), rheumatic arthritis (1), diabetes insipidus (1), Bell's palsy (1), vaginitis (1), kidney disease (1), eczema (1), pathology in the right hip (1).

In 91 of 157 children aged 0-15 years, x-rays of the left arms were negative; forty-seven of 91 children (52%) had retarded bone age. The knee, chest and pelvic x-rays in adults showed evidence of fluorosis. Approximately half of the 166 adults (75 or 45.2%) had advanced fluorosis.

Therefore, in addition to their low socio-economic and socio-cultural level endemic fluorosis was the major factor involved in the unhealthy state of the Kizilcaoren inhabitants.

Acknowledgement

We are thankful to all persons who contributed to this research, especially to Dr. Yusuf Ozkan, member of Parliament.

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FLUORIDE BRIEFS (Cont.)

Phenobarbitol pretreated rats exposed to 1% halothane for two hours under conditions of hypoxia showed within 24 hours extensive necrosis of the central lobes of the liver. There was also an increase of serum glutamic pyruvic transaminase(SGPT) and a decrease of hepatic microsomal cytochrome P-450. Glutathione levels in the liver were unchanged. In the rats exhibiting centrilobular necrosis, the 24-hour urinary excretion of fluoride increased 2.6 fold. Animals not pretreated with phenobarbitol and those given larger amounts of oxygen showed less or no liver damage. Halothane is likely to be metabolized to hepatotoxic intermediates by a reductive or nonoxygen dependent cytochrome P-450 dependent pathway.

McClain, G.E., Sipes, I.G., and Brown, B.R., Jr.: Anesthesiology 51:321-326, 1979.

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Children from fluoridated Basel (1 ppm F) showed twice as much fluoride in the superficial enamel of deciduous teeth as children in nonfluoridated (0.14 ppm) Hamburg.

Straubig, W., and Ghulzow, H.J.: Dtsch. Zahnaerztl. Z. 34:480-483, 1979.

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TREATMENT OF PRIMARY OSTEOPOROSIS WITH FLUORIDE AND CALCIUM
CLINICAL TOLERANCE AND FRACTURE OCCURRENCE

by

Riggs, B.L., Hodgson, S.F., Hoffman, D.L.,
Kelly, P.J., Johnson, K.A. and Taves, D.

(Abstracted from JAMA 243:446-449, 1980)

The authors studied 36 patients, twenty-nine women and seven men, with primary osteoporosis whom they treated with sodium fluoride, calcium supplement and Vitamin D. Twenty-four were post-menopausal (mean age 64.3 years), five prior to the menopause; the mean age of the seven men was 49.2 years. All had normal values for serum calcium phosphorus and alkaline phosphatase and urinary calcium. They received 40-65 mg per day of sodium fluoride. The dosage was adjusted to maintain a serum concentration between 5 and 10 µM. The daily supplement of calcium (1 to 1.5 gm) was given as calcium carbonate in divided doses. Twenty-four patients received 50,000 units of Vitamin D twice weekly. Twenty-six of the 36 patients were observed 4-6 years. In 10, the study was discontinued mainly because of adverse reactions, and intercurrent disease. In one individual, death due to respiratory failure was caused by multiple severe compressions of the thoracic vertebrae.

In 42% (15 patients) adverse reactions occurred, mostly rheumatic illness. Synovitis occurred in 6 patients with pains in ankles and knees, tenderness and swelling. Another kind of joint involvement was a plantar fascial syndrome with diffuse pain and tenderness of the plantar surface of one or both feet.

In two patients recurrent vomiting was controlled when the dose of sodium fluoride was reduced. In five patients, iron deficiency (anemia) developed with hemoglobin levels less than 10 g/dL. One of these individuals had gastric ulcer. In the other four, barium examination of the stomach and duodenum were normal. Seven patients had dyspepsia, mild pains and stiffness in joints and developed alopecia.

Four developed neurological disturbances, cerebral demyelination, Parkinson's disease, peripheral neuritis and retinal macular degeneration. Six patients, who received Vitamin D, showed hypercalcemia and/or hypercalciuria which required reduction of the dose or discontinuance of the vitamin.

Before treatment was instituted an average of four vertebral fractures occurred per patient. Of the total of 51 new vertebral fractures, 23 (45%) occurred during the first year of therapy. In addition, four patients had fractures of other bones mainly ribs and

metatarsals; one had a spontaneous hip fracture. In addition, several patients had bone spurs of the calcaneus and of the tibia and osteophyte formation of the spinal column. One patient had dense decalcifications of the ligamentum flavum.

The authors stated that "despite adverse effects ---- the combined fluoride and calcium regimen remains a promising approach to treatment of osteopenic bone disorders". Nevertheless they concluded their paper as follows: "Clearly more data are needed. Until these are available, we believe that this regimen should continue to be restricted to investigational use."

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THE EFFECT OF FLUORIDE AND LEAD IONS ON CHROMOSOMES IN HUMAN LEUKOCYTES IN VITRO

by

D. Jachimczak and B. Skotarczak
Szczecin, Poland

(Abstracted from Genetica Polonica, 19:350-358, 1978)*

The authors observed marked chromosomal alterations during metaphase from the addition of fluoride and lead ions to human leukocyte cell cultures. They obtained blood from male donors of the Medical Academy of Szczecin. The cells were cultured from the blood of each donor for 96 hours. Fluoride and lead were added 24 hours before the completion of each culture. The cells were then subjected to hypotonic shock, fixed with methanol and the chromosome preparations were stained. Photographic analysis and classification were made of metaphase aberrations, according to standard procedures. The concentrations of fluoride ion (from sodium fluoride) were 0.6, 6.0 and 60 ppm and the lead ion concentrations (from lead acetate) were 2.1 and 210 ppm. Controls were treated in the same manner except for the absence of fluoride and lead salts.

The chromosomal aberrations observed included structural anomolies involving both chromatid and isochromatid gaps and breaks, a-

* For an earlier, less detailed abstract, see Fluoride, 12:212,1979.

centric fragments, aneuploids and polyploids. The effects of fluoride were most pronounced at the highest concentrations (60 ppm) but even at concentrations of 0.6 ppm they were still significantly greater than in the controls. On the other hand the defects due to lead ions were not related to the concentration. Aberrations were more frequent at the lower concentration (2.1 ppm) than at the higher (210 ppm). In the culture containing 60 ppm fluoride, the authors noted extremely few mitoses suitable for the analysis. At this concentration the number of chromosome aberrations was high and exceeded markedly that found in both lower concentrations. Whereas there was no significant difference in the extent of alterations at the 6.0 ppm and 0.6 ppm fluoride concentrations, the difference between these concentrations and the controls was highly significant($p=0.001$).

In conclusion the authors pointed to the fact that the 0.6 ppm concentration is being used in drinking water in Szczecin for prevention of dental caries. They registered "apprehension regarding the aptness of this treatment".

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CENTRAL NERVOUS SYSTEM MEDIATION OF FLUORIDE HYPERGLYCEMIA IN THE RAT

by

E. L. McGown and J. W. Suttie
Madison, Wisconsin

(Abstracted from Toxicol. and Applied Pharmacol. 48:205-211, 1979)

Hyperglycemia, not uncommon in acute fluoride intoxication, has been shown to be mediated by the release of epinephrine from the adrenal medulla. This response could be the result of increased activity of the sympathetic nervous system due to the calcium binding ability of fluoride. However there is some evidence that fluoride induces release of epinephrine by direct action on the adrenal medulla. McGown and Suttie demonstrated experimentally that the fluoride-induced release of epinephrine and the resulting hyperglycemia is mediated primarily by splanchnic impulses arising in the central nervous system.

In male rats the greater splanchnic nerves, which release epinephrine resulting in hypertension and hyperglycemia, were severed im-

mediately below the diaphragm. In addition sodium fluoride (0.5μmol in 10 μl) or saline (10 μl) was injected into the ventricle of the brain in order to determine the possible adrenal stimulatory effects of fluoride upon the central nervous system.

Fluoride-induced hyperglycemia was mediated by elevated concentrations of epinephrine in the blood plasma. Bilateral splanchnicotomy prevented the fluoride-induced increase of both epinephrine and blood glucose. These studies therefore suggest that the stimulating effect of fluoride on the adrenal medulla is mediated primarily by splanchnic impulses arising in the central nervous system.

When the ganglion were blocked by means of a combination of hexamethonium (20 mg/kg iv) plus atropine (5 mg/kg iv), in the unanesthetized animal, the fluoride-induced hyperglycemia decreased.

Injection of fluoride directly into the ventricle produced marked hyperglycemia which was partially blocked by prior treatment with hexamethonium and atropine.

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FLUORIDE BRIEFS (Cont.)

To separate gaseous and particulate fluorides, gaseous fluoride is trapped by an alkaline treated filter and postsampling heat treatment of the filters promotes desorption of gaseous fluorides from particulate ones. The authors reported a high degree of comparability between this method and other standard impinger methods for collection of HF.

Einfield, W., and Horstman, S.W.: Amer. Industr. Hyg. Assoc. J. 40:626-632, 1979.

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During and following enflurane anesthesia of less than 2.0 MAC per hour, the peak serum fluoride levels of 24 markedly obese patients (weight 127.6±6.0 kg) were higher and the rate of increase of fluoride in the serum was more rapid than in nonobese control subjects weighing 67.3±1.2 kg. There was no gross evidence of nephrotoxicity in either group. Possible reasons for the increased enflurane metabolism in obese persons are differences in fluoride ion kinetics, of hepatic delivery and penetration of volatile anesthetics and altered microsomal enzyme activity in the liver.

Bentley, J.B., Vaughan, R.W., Miller, M.S., Calkins, J.M., and Gandolfi, A.J.: Anesth. Analg. 58:409-412, 1979.

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BOOK REVIEW

Continuing Evaluation of the Use of Fluorides, edited by Erling Johansen, Donald R. Taves, and Thor O. Olsen. AAAS Selected Symposion 11. Pp. 321 + xxviii, 71 figures, 47 tables. Published for the American Association for the Advancement of Science by Westview Press, 5500 Central Avenue, Boulder, Colorado 80301, June 1979. Hardcover, $20.00.

Since its first symposium on "Fluorine and Dental Health" held in Dallas, Texas, in December 1941, the American Association for the Advancement of Science has, from time to time, sponsored similar meetings dealing with dental and medical aspects of fluorides and fluoridation. In February 1977 such a session took place in Denver, Colorado, and was summarized shortly afterward in Fluoride (1). With their appearance in book form, the papers presented at this symposium, plus additional material, are now conveniently available to a wide audience.

The volume is comprised of 13 chapters contributed by 16 specialists in various dental and biomedical areas of fluoride research. The first four chapters consider past and present applications of fluorides to the prevention and arrest of dental caries and to the treatment of osteoporosis. The next six chapters are more biochemical in nature and are concerned with the determination of fluoride in blood, the estimation of dietary fluoride intake, and the physiology of fluoride in relation to body distribution, effects of pH on absorption, toxicity, and excretion, acquired cell resistance, and the metabolism of fluorinated anesthetics. The last three chapters deal with problems and reports of toxic effects associated with the use of fluoride tablets or drops and the fluoridation of drinking water.

In these respects the book is obviously a welcome addition to the fluoride literature. Moreover, many valuable findings and concepts are brought out in it. Among those that might be singled out are: (a) the importance of adequate intake of calcium in conjunction with fluoride therapy for osteoporosis, a treatment still considered experimental because of unsolved problems of side effects and uncertain improvement in bone strength (J. Jowsey, B.L. Riggs and P.J. Kelly, Ch. 4); (b) the lower average fluoride levels in human blood (W.S. Guy, Ch. 5) and in food (D.R. Taves, Ch. 6) as determined by the ion-selective electrode compared with values derived by nonspecific colorimetric methods; (c) the close parallel between ionic serum or blood fluoride concentration and the fluoride level of the drinking water, urine, and skeleton, but not soft tissues (D.R. Taves and W.S. Guy, Ch. 7); (d) the marked effect of pH on the transport of fluoride in body tissues, with increased retention and toxicity at lower pH (greater acidity) and increased urinary excre-

tion at higher pH (lower acidity) (G.M. Whitford and D.H. Pashley, Ch. 8); (e) the enhanced resistance of cells to toxic effects of fluoride resulting from an acquired ability to maintain a relatively low intracellular fluoride concentration against an externally much higher one (M. G. Repaske and J.W. Suttie, Ch. 9); (f) the deleterious effects on bone of elevated serum fluoride resulting from waterborne fluoride and renal impairment (W.J. Johnson, D.R. Taves, and J. Jowsey, Ch. 12).

In addition to these topics, the symposium also dealt with a number of more controversial issues but with an obviously overriding commitment to a profluoridation viewpoint. Thus, although they acknowledge that dental fluorosis is significantly increased by fluoridation, neither R.F. Sognnaes (Ch. 1) nor H.C. Hodge (Ch. 11) concedes any other health hazard from it. In fact, Sognnaes even suggests that "it may well be that the amount of fluoride provided by water fluoridated at the 1 ppm level may be too little rather than too much for long term preventive effects on the skeletal and vascular systems in adult man." (Emphasis in original.) At the same time he calls attention to adverse oral soft tissue and systemic effects that have been reported from the use of topical fluoride preparations, concluding that "the high concentrations of [fluoride in] some products may be neither biologically desirable nor clinically necessary."

For his part, Hodge cautions that "the widely recommended dosage regimen which gives infants 0.5 mg F daily for the first two years of life" is too high and should be reduced to 0.25 mg/day because of the increased risk of dental fluorosis at the higher level of intake, but he too discounts evidence of any other toxic effects. For example, he states (incorrectly) that "No documentation was provided" by Feltman and Kosel for the systemic ill effects from fluoride tablets which they reported in 1961, and for which they provided conclusive proof with placebo controls. Moreover, Hodge does not cite the additional, fully confirmatory findings reported by Shea et al. in 1967 (2), although they are cited by Taves in Ch. 13.

Hodge further contends that in temperate regions of the United States waterborne fluoride below 2 ppm causes nothing more serious than "mild" dental mottling, which he does not consider objectionable --even though it is often quite noticeable. But again he speaks incorrectly, because even the data he cites reveal the occurrence of the more severe degree of "moderate" dental fluorosis in an artificially fluoridated New England community (Table 2, p. 258). Moreover, he fails to mention that Dean's original surveys disclosed the presence of "moderate" mottling in midwestern cities with less than 2 ppm fluoride in the drinking water(e.g., in Elmhurst and Galesburg, Ill.). Incidentally, in Table 2 on p. 258 the percentage of "mild" dental fluorosis in Clovis, N.M., should read 35.5, not 3.5%. Also, the figures for the group index of fluorosis in the work of Aasenden and Peebles cited in that same table were incorrectly calculated in

the original paper. The correct figures, according to Dean's method used for the other entries, are: Group I (F supplement), 1.22 (not 0.88); Group II (control), 0.11 (not 0.07); Group III (1 ppm F water) 0.61 (not 0.40).

Far more serious than these errors, however, is Hodge's mistaken assertion that "accidental ingestion of 20 to 100 tablets containing 1 mg F each would be followed by signs and symptoms no more drastic than nausea and vomiting, perhaps by abdominal pain and prostration". He has evidently overlooked the fact that acute poisoning in the form of gastrointestinal hemorrhaging has been documented in infants and children receiving even the recommended daily doses (2,3). Moreover, deaths in children resulting from the accidental ingestion of fluoride tablets, even fewer than 100, are now on record (4).

In his review of systemic and topical anti-caries effects of supplemental fluoride (Ch. 2), T.M. Marthaler likewise emphasizes supporting data with little or no reference to contrary findings. Chapter 3 by E. Johansen and T.O. Olsen on the use of topical fluorides in the prevention and arrest of dental caries presents a similar viewpoint but contains a greater wealth of information and is far more objective in dealing with shortcomings and health risks in such prophylactic procedures.

This same unevenness of treatment is also evident with other topics. On p. 180 Taves and Guy state that "the serum fluoride concentration necesssary to produce increased urine flow rates is about 50 μm [0.95 ppm] in the rat and in the human" (see also p. 295). Yet Fig. 2 on p. 155 shows that this very effect--albeit in mild form--was observed in Taves himself with 1 ppm fluoridated water and a serum plasma fluoride concentration presumably below or at least not much above 1 μm (0.02 ppm). And of course there is significant laboratory evidence (5), which is not cited, as well as much clinical evidence (6), that clearly demonstrates polydipsia-polyuria from artificially fluoridated water.

This neglect of extensive contrary data is also seen in the discussion of dietary fluoride intake and the sources of error in the determination of fluoride in food products (Ch. 6). As pointed out in a recent Canadian National Research Council report (7), foods and beverages processed with fluoridated water are used extensively in Canada and the United States. On the average, they now contain 0.5 ppm more fluoride than when they were prepared with nonfluoridated water. Additional comparative studies cited by the CNRC report provide further evidence that dietary fluoride intake increases with the use of fluoridated water for food preparation and cooking, just as is shown in Fig. 2 on p. 155 (Δ entries).

The closing chapter by Taves deals with various clinical, laboratory, and epidemiological reports of nondental adverse health effects of fluoridated water. This reviewer was given an opportunity

to comment on this important chapter, and he is pleased to find that many of the changes he suggested were made, although he still sees serious shortcomings. Foremost among these is the seemingly arbitrary and, in effect, pejorative, labeling of reports of harm from fluoridated water as "claims" and treating them as though they have little or no validity. Taves' criteria for judging these reports (properly controlled experiments designed to eliminate bias, independent confirmation, and consistency with other knowledge) are reasonable enough, but the manner and extent to which they are applied are not.

Despite much discussion and rationalization, Taves' negative view of the fluoride origin of the many reported adverse effects of fluoridated water rests mainly on the fact that they are often not reported in circumstances in which one might expect to find them. This is indeed an intriguing problem, which has recently been considered at length (6), but it is not the primary point at issue. The fundamental, strictly scientific question is whether any agent other than fluoride can in fact account for the observed results. This is the central issue that must be addressed and resolved before any other.

With respect to the clinical evidence, reversible illness is found to occur after the start of fluoridation or when the patient moves to a fluoridated community. The symptoms disappear spontaneously when the patient uses otherwise identical nonfluoridated water for drinking and cooking, only to return when the use of fluoridated water is resumed. Such findings have been independently confirmed by blind and double-blind tests. The symptoms are an attenuated form of acute fluoride intoxication as well as those of chronic fluorosis, all with well- established biochemical bases. Therefore, in the absence of any more plausible explanation, the logical, scientific conclusion is inescapable that they do indeed originate from fluoride, whether mediated by other factors or not.

The failure of certain investigators to find or report adverse nondental effects of fluoridated water does not mean that such effects, which have been found repeatedly by others, did not occur or were not present. The symptoms (headache, muscular weakness, paresthesia, excessive tiredness, gastrointestinal distress, etc.) are so common that their origin from fluoride, when this happens to be the case, is easily overlooked. But this fact does not disprove that they can be caused by fluoride, any more than the failure of leading physicians in the last century to "confirm" the findings of Semmelweis can be said to have disproved what he observed concerning the origin of puerpural fever. A public health measure is not safe simply because it is widely accepted and adopted. The ill-fated swine-flu vaccination program of 1976 is a stern reminder of this fact.

Reports of an association of fluoride with the occurrence of Down's syndrome (mongolism), mutagenic effects, and cancer take up

the remaining portions of this final chapter. Again, Taves is skeptical of the validity of these various findings, but the evidence he offers is far from sufficient to dismiss them, especially when particular details of the work which are omitted from his discussion are considered. For example, in regard to Down's syndrome, he fails to mention that Rapaport, in his original report in 1956, noted only a very small increase in the prevalence of such births after relatively short periods of fluoridation, just as was subsequently found by others without mention of this fact. In Illinois, Rapaport also discovered that the association of Down's syndrome births with natural fluoride water appeared to hold for smaller cities of 5,000 to 10,000 population as well as the larger ones mentioned by Taves. When these and other data are brought into the picture, an association of fluoride in drinking water with the occurrence of Down's syndrome, at least in the U.S. studies that have been reported, becomes far less questionable. Even Taves concedes that the present data "cannot rule out an increase for long-term exposure, particularly in younger mothers."

A related situation occurs in Taves' discussion of findings concerning mutagenesis and cancer. After reviewing available data and considering problems of experimental protocol in various laboratory studies, he concludes that fluoride may be a "weak" mutagen in certain animals, but with reference to humans, such results "should not be given too much weight," he grudgingly states. Likewise, he questions the validity of a large-scale investigation by Taylor and Taylor demonstrating both <u>in vitro</u> and <u>in vivo</u> tumor growth acceleration by fluoride in the 1 ppm range on grounds that certain dose-response relationships he would expect to see were evidently not found. Relative constancy of effects by fluoride over a wide concentration range is not without various precedents, however (6), and therefore this fact is not a sufficient basis on which to discount these findings, especially since similar tumor-promoting effects were found by these same investigators with bromide and iodide, although at somewhat higher concentrations. If what was found for these two halogens was valid, why should not the same be true of fluoride?

The problem of fluoride and cancer in human populations is a very difficult one, and Taves himself has obviously devoted a great deal of effort to it. His own results, based on the standardized mortality ratio approach with decennial period data, suggest at most a 1% to 2% increase in cancer death rate associated with fluoridation. Even this small an increase, if real, would be far from negligible. However, an overall enhancement of 4% to 5%, as found by the direct method and by a broader-based SMR approach, has not been disproved either. Moreover, much of the previous work which claims to show no association between fluoride water and cancer incidence or cancer mortality is found, on closer examination, to favor just the opposite conclusion (6).

It is clear, therefore, that in this volume the AAAS has once again lent its prestige and support to a continuing effort to sustain fluoridation with a clean bill of health. Even though the work contains a large amount of valuable as well as recent information, the fact remains that the underlying viewpoint of the symposium speakers and the organizers was to minimize and discount evidence of serious adverse effects of low-level fluoride intake. Under these circumstances it is not surprising that the gravely mistaken notion that fluoridation is safe for everyone continues to be so widely propagated. The AAAS has much for which to answer.

A.W.B.

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