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ABSTRACTS

EDITORIAL

FLUORIDE THERAPY AND OSTEOPOROSIS

In 1948 Black, Kleiner and Bolker (1) reported what they considered to be encouraging results by the use of sodium fluoride tablets in doses of 80 mg 4 times a day for the treatment of leukemia and cancer. Rich and Ensinck (2) and subsequently numerous other investigators have utilized this approach in the treatment of osteoporosis in an effort to enhance calcification (3-6). The augmentation of bone mass was believed to strengthen or, at least stabilize, the skeleton. Shambaugh (5) applied the same method to patients with otospongiosis (otosclerosis) and reported considerable benefit in several thousand patients. Other competent investigators have noted marked subjective and objective improvement in osteoporosis. At the 1978 Fribourg conference of the International Society for Fluoride Research, Franke (7) and Reutter et al. (8) reported good results after long-term observation.

It is well established that fluoride increases the size of the apatite crystals. The above therapy often induces a positive calcium balance and enhanced calcification of bone mass. Under certain circumstances, however, the widening of mineralized osteoid occurs. The newly formed bone fails to mineralize and osteomalacia ensues. Jowsey (9) tried to overcome this problem by supplementation with calcium, vitamin D, or both. Nevertheless, in many cases this treatment resulted in increased fractures.

In a 1972 study by Riggs et al. (10) of 36 patients afflicted with primary osteoporosis, 24 patients received 40 to 65 mg NaF, 1 to 1.5 gm elemental calcium, 50,000 units of vitamin D twice weekly for 4 to 6 yrs; 45% of the new vertebral fractures occurred during the first year of therapy. In six patients, vitamin D was discontinued because of hypercalciuria and hypercalcemia with no difference in incidence of new fractures.

Recently, in October 1980, a 76-year old female, who received 50 mg NaF per day for 12 months, had 6 spontaneous fractures in 4 months for which the authors held NaF therapy responsible; the bone biopsy showed abnormal porosity due to NaF (11).

In the same month a 61-year-old female with severe osteoporosis and vertebral compression received 50 mg vitamin D daily and 600 mg calcium gluconate twice daily by mouth. After 2 months, because of the patient's intolerance, the calcium supplements had to be discontinued. The authors stated that bone pain increased and three additional vertebral compression fractures occurred (12). According to Stein and Granik (13) at higher fluoride levels, hard tissue in humans is weaker in static tests than bone containing less fluoride.

Most studies on high doses given to animals on an otherwise unchanged diet revealed either no alteration or a decrease in bone strength.

Aside from such effect on the skeletal tissue, other organs have also been adversely affected. Black, Kleiner and Bolker (1), who administered 80 mg NaF four times daily to cancer patients, noted that certain individuals developed gastrointestinal disturbances from this treatment;

amphogel had to be administered as an antidote. Rich observed arthral-
gia,and stomach and bowel upsets (14): Geall and Beilin (15) reported
optic neuritis from 20 mg NaF three times daily for six weeks. In the
Riggs study (10), synovitis, blood loss anemia and recurrent vomiting
were reversed in 84% of the patients on discontinuation of the drug.
Duffey et al. (16) observed giant monocytoid cells suggestive of reticu-
loendothelial malignancy in the bone marrow of three patients who re-
ceived 16 to 150 mg NaF for 1 to 36 months.

One cannot rule out the possibility that other late manifestations
of toxicity will not become evident after many years. Could fluoride,
because of its strong affinity for calcium, settle in other organs, par-
ticularly in the aorta which tends to calcify in advancing years? Fluo-
ride seems to accumulate in the aorta independently of its calcium con-
tent (17).

The tendency of fluoride to settle in ligaments, connective tissue
and joint capsules, which has long been recognized, suggests that fluo-
ride does not necessarily find its way, as desired, into the osteoporo-
tic bone but is deposited in connective tissue as well, where it gives
rise to calcification and induces arthritis (18). This result would
be especially distressful and hazardous to elderly persons who are not
otherwise prone to this disease.

Fluoride treatment for osteopenia was given careful attention at a
conference in Nyon, Switzerland (Oct. 9-12, 1977) sponsored by the Zymo
Corp., which produces fluoride tablets, and in the U.S.A. by the Ad Hoc
Committee, Strategy Workshop for Osteoporosis Research on February 8-10,
1978, at the conference at the National Institutes of Health, Bethesda,
Maryland.

The conclusion of the Bethesda conference was summarized the follow-
ing October in a JAMA editorial (19) as follows: "However,   studies in
humans have shown increased incidence of microfractures and macrofrac-
tures." The committee warned of possible   "unanticipated late mani-
festations of toxicity that will not become evident for many years", be-
cause of the "long lag times required to induce noticeable skeletal
changes." "Outside of investigational setting," they cautioned,"fluoride
should not be prescribed for generalized or localized osteopenia until in-
vestigations have documented the efficacy of high doses without unaccept-
able toxicity."

The AMA editorial compared the above treatment with the prophylac-
tic use of fluoride for prevention of dental caries "the only use of flu-
oride approved by the Food and Drug Administration." "The doses of flu-
oride ion used for treatment of both otospongiosis and generalized os-
teoporosis have been ten to 100 times those effective for prophylaxis of
dental caries."

In 1966, in reviewing data then available, the Food and Drug Adminis-
tration found no substantial evidence that prenatal fluoride preparations
are beneficial to tooth development in the fetus or in prevention of dent-
al caries in offspring (20). More recently, Glenn (21) and others (22)

have reported positive results in children with prenatal exposure to sup-
plemental fluoride similar to those recorded previously by Feltman and
Kosel (23). The latter team (23),however,observed adverse effects such
as stomach and bowel upsets in 1 percent of 1100 pregnant women and young
children to whom fluoride tablets were administered in doses correspond-
ing to those supposedly obtained from the average daily consumption of
fluoridated water.

As an example of the risk encountered in the dental prophylactic use
of fluoride, the American Dental Association in 1978 (24) cited evidence
that fluoride in the conventional doses being given to infants may consti-
tute a hazard with regard to mottling and stated that NIH had recommended
lowered extra-dietary fluoride doses. Shea et al. (25) have reported
gastrointestinal hemorrhages from such doses in infants. Waldbott (26)
encountered severe hemorrhages in a 9-year old boy requiring gastrectomy
from tablets containing 1 mg NaF. In a recent book, Waldbott described
numerous cases of nonskeletal fluorosis due to fluoridated drinking wa-
ter (27).

In view of all these facts, extreme caution concerning any increase
in the fluoride body burden and especially with respect to administering
fluoride in any form for therapeutic and prophylactic purposes is war-
ranted.

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PERIODONTAL DISEASE, ORAL HYGIENE AND FLUORIDE CONTENT
OF DENTAL DEPOSITS IN ALUMINUM WORKERS

by

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SUMMARY: In workers exposed to high concentrations
of fluoride, a clinical evaluation of periodontal
health and oral hygiene was carried out. Moreover
the relationship between periodontal condition and
the concentration of fluoride in dental plaque was
investigated.

The results point to a prevalence of ad-
vanced periodontal disease (mean PI = 5.06-5.92)
and to generally unsatisfactory oral hygiene (mean
OHI-S = 4.07-4.98). It was found that the progress
of periodontal disease is related to age as well as
to the length of employment.

A significant relationship between the con-
centration of fluoride in dental plaque, measured
with the ion-selective fluoride electrode, and the
condition of periodontal tissues was established.

KEY WORDS: Aluminum workers, periodontal disease, fluoride in dental plaque

Introduction

Little information is available concerning the effect of fluoride on
periodontal health and dental deposits due to industrial air pollution.
Studies on workers exposed to fluoride at high concentrations in various
industrial facilities have revealed advanced periodontal disease. Some
authors hold the view that, in addition to bad oral hygiene, fluoride is
the main cause of this alarming situation (1-9).

Histopathological studies of the mucosa in the upper respiratory
tract of workers exposed to fluoride in aluminum plants revealed inflam-
matory and hypertrophic lesions which eventually turn into atrophy of the
mucosa (10-12). It has been suggested that similar changes may appear in
the gingival mucosa.

The aim of the present study was to evaluate periodontal health and
oral hygiene in aluminum workers and to establish a relationship between
their periodontal condition and the content of fluoride in dental plaque.

Material and Methods

The study involved 158 aluminum workers in central Poland, aged 20
to 60, who had been exposed to fluoride compounds at high concentrations.

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Analysis of urine, previously performed on the subjects, revealed a flu-
oride content exceeding normal values (13).

The periodontal state was assessed clinically as well as on the ba-
sis of Russell's clinical Periodontal Index (14). Oral hygiene was es-
timated by the Simplified Oral Hygiene Index according to Green and Ver-
million (15).

The workers, who were subjected to the examination, were divided -
according to age and length of employment in the plant - into the follow-
ing three groups:

    Group A - junior workers, below 35 years of age with relatively brief
              employment (up to 2.5 years).
    Group B - junior workers, below 35 years with relatively long employ-
              ment (more than 2.5 years).
    Group C - senior workers, more than 35 years old with relatively long
              employment (more than 2.5 years).

Data on the average age and period of employment as well as the num-
ber of workers in the particular groups are given in Table 1.

Table 1

Mean Age and Employment Periods

	Group A	Group B	Group C
Age (years)	$\bar{X}$ 26	$\bar{X}$ 31	$\bar{X}$ 42
	(s=3.5)	(s=3.0)	(s=6.0)
Employment (months)	12.0	80.0	90.5
	(s=8.0)	(s=26.0)	(s=18.5)

The uniformity of the distribution of the mean index values obtained
for groups A, B and C, was statistically checked by means of the Mann-
Whitney test. The significance level of 0.05 and 0.01 was accepted.

Moreover, 44 workers were randomly chosen for sampling dental plaque.
The samples were collected at least 18 hours after discontinuing work.
The men were instructed to refrain from cleaning their teeth until the
plaque samples were taken. The fluoride concentration of the dental
plaque was determined by the use of the fluoride-ion specific electrode
made by Orion, USA (15-17).

The degree of the dependence of the periodontal disease on the fluo-
ride content in dental plaque was established by the correlation coeffi-
cients of the two variables. We also determined the parameters of the
linear correlation function, as well as the polynomial curves of the sec-
ond and third degrees for the coefficients. The appropriate curve corre-
lation coefficients were likewise determined.

In view of the similarity of the values of correlation coefficients,

for the purpose of description the second degree curve was chosen, the "η^2" correlation ration being the highest in that case, and the residual variance reaching its lowest value. The significance of the correlation coefficient was evaluated by the F-Snedecor-Fisher test for p=0.05.

Results

In the subjects under study, an advanced form of periodontal disease was frequently encountered with prevailing dystrophic features such as an obvious recession of the gingiva. The gingival tissue was pale and firm, showing no tendency to bleeding. Deep pockets were found which coincided with the high values of the Periodontal Index. For the groups under investigation, the index amounted to 5.06, 5.28 and 5.92 respectively (Table 2).

Table 2

Mean Periodontal and Oral Hygiene Index Values *

	Group A	Group B	Group C
PJ	5.06	5.28	5.92
OHJ-S	4.07	4.37	4.98
CJ	1.66	1.84	2.26
DJ	2.42	2.54	2.72

* Arrows point to statistically significant differences.

A comparison between the scores obtained for junior workers with short employment and senior workers with long employment (groups A and C) showed statistically significant differences in the mean values of the Periodontal Index. Differences were also found when comparing results for groups B and C, i.e. the junior and senior workers with long employment. However no significant difference was found between the results for junior workers with short and those with long employment(groups A and B) although the mean Periodontal Index score was high in both groups. It is worth noting that the variable coefficient was lowest in Group C.

Oral hygiene was found unsatisfactory in the workers from all three groups (Table 2). However, groups A and C differed in the amount of dental deposits and workers from group C displayed significantly higher values of the Oral Hygiene Index and of both components. Similarly, significant differences were found also between groups B and C, both with long employment: the mean values for Oral Hygiene Index and for its component Calculus Index were significantly higher in group C.

The measurement of fluoride content in dental plaque revealed unexpectedly high values of this element ranging widely from 273 to 9808 parts/10^6F$^-$ in the particular samples (Table 3). The values of the Pe-

riodontal Index in the men from whom the plaque samples were taken ranged
from 3.27 to 8.0. A study of the relationship between the periodontal
condition and the fluoride content of the dental plaque revealed an in-
terdependance of the two variables. The regression curve indicates a de-
terioration of the state of the periodontium associated with an increase
in the concentration of fluoride in dental plaque (Fig. 1).

Table 3

Values of Periodontal Index and F⁻ Content in Dental Plaque

Periodontal Index Values		F⁻ in Dental Plaque (parts/10^6 F⁻)	
Lowest	Highest	Lowest	Highest
3.27	8.00	273	9808

Figure 1

Regression Curve of Independence Between Periodontal
Index and Fluoride in Dental Plaque
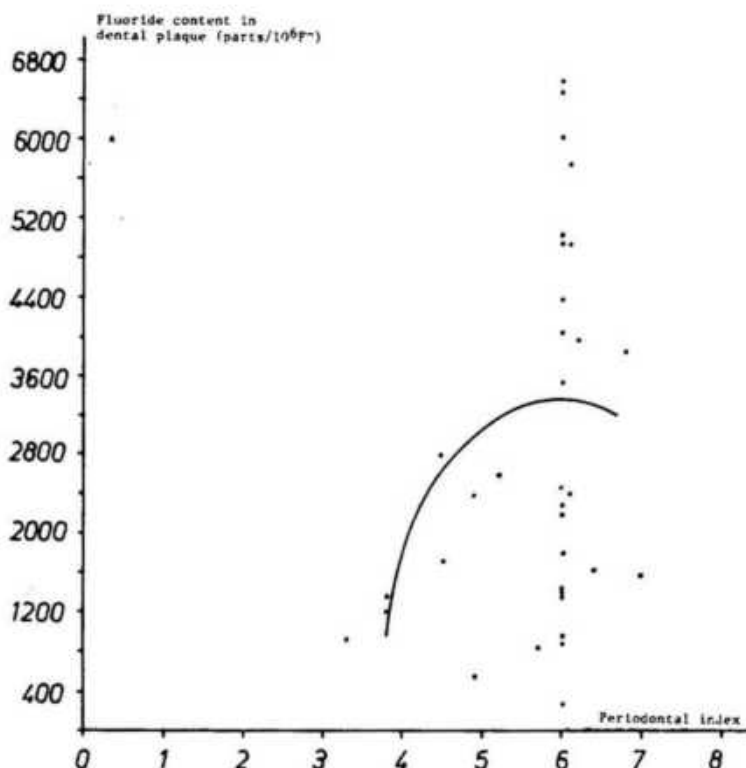
Discussion

A clinical assessment of periodontal tissues permits the conclusion that the high concentrations of fluoride specifically affect periodontal tissue, probably through dental deposits. A comparison between mean Periodontal Index values for the three groups of workers under study indicates that senior workers with long employment are most severely affected by periodontal disease. Our results suggest that the state of periodontal tissues depends not only on the fluoride content of the plaque but also on tissue resistance, which decreases with advancing age. The relatively low variability coefficient in group C, as compared with that of groups A and B, indicate the presence of some common factor which contributes to a similarity in clinical appearance of periodontal tissues. The advanced stage of periodontal disease in the junior workers, with relatively short employment, is also noteworthy. The question arises as to the length of time required for the occurrence of the typical changes in periodontal tissues frequently found in the workers of the aluminum plant. The observations indicate that, even during the first months of employment, distinct changes in periodontal tissues are apparent. It is also interesting to note that tooth mobility, in consequence of periodontal disease, was relatively rare despite the values of Periodontal Index as high as 8.0. This fact seems to be characteristic of the course of the disease in conditions of exposure to high fluoride concentrations.

Oral hygiene in the workers under study was generally unsatisfactory, and the damaging effect of dental plaque was doubtless enhanced by its high fluoride content. Harmful factors at the workplace, mainly hydrogen fluoride and fluoride-containing dust, may damage the oral mucosa and break down its resistance. It should also be borne in mind that high concentrations of fluoride are bound to affect the dental plaque bacteria by disturbing their metabolism, inhibiting their growth, and even killing them (19-24). The death of the microorganisms may, in turn, hasten the calcification of the plaque, which in turn might be partially responsible for the abundant deposits of calculus among the workers (25-26).

In conclusion, the results presented here point to the injurious effect of high concentrations of fluoride on periodontal tissues in industrial workers.

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MEASUREMENT OF SPINAL CANAL BODY RATIO IN FLUOROTIC SPINE

by

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SUMMARY: Twenty-five normal individuals all above thirty years of age were studied to establish the standard normal values of spinal canal body ratio. They were compared with age-matched patients with skeletal fluorosis visiting the Rajendra Hospital, Patiala. An attempt has been made to establish the critical saggital diameter and spinal canal body ratio in the cervical spine in fluorotic patients at which the spinal cord compression is likely to occur.

The mean canal body ratio was higher in fluorosis at every level of the vertebra as compared to that of normal controls. In other words, the spinal canal was narrow at every level in fluorosis as compared to normal con rols. The normal anteroposterior and interpredicular diameters in the Indian population were slightly less than in European countries.

The critical anteroposterior diameter in the cervical spine in fluorosis in the present series at which the spinal cord compression will occur is 9 mm and the critical spinal canal body ratio is 1:2.98.

The normal spinal canal body ratio was minimal at C_2 (1:1.08) but gradually increased up to D_{10}(1:3.94), then slightly decreased up to L_1 (1:3.62) and again increased to L_5 (1:4.64). The higher the spinal canal body ratio, the narrower is the bony spinal canal.

KEY WORDS: Fluorosis, spinal canal compression; Fluorosis, skeletal

Introduction

Fluorosis is a disease affecting, primarily, the skeletal system leading to secondary involvement of the nervous system. It has been proven to result from high fluoride content of drinking water in certain fluoride belts in India and in other parts of the world. Neurological symptoms in the form of myelopathy are due to narrowing of the spinal canal as a result of sclerosis of spinous processes, transverse processes, bodies of vertebrae and calcification of various ligaments, especially the posterior longitudinal ligament and exostosis reported by Siddiqui (1) in Nalgonda District, Hyderabad Deccan, and Singh and Jolly in 1961 from Punjab (2).

Singh et al. in 1962 (3) measured the bony spinal canal of a skeleton of a person affected by fluorosis and pointed out that the reduction was mainly seen in the anteroposterior diameter at every level while the transverse diameter remained almost the same except from C_7 to D_3 as compared to the normal individual skeleton.

Material and Methods

A clinical and radiological study was carried out on twenty-five patients with skeletal fluorosis attending the Rajendra Hospital, Patiala. A control group consisted of twenty-five normal subjects matched for age who visited the hospital for problems unrelated to the spine. A detailed clinical and radiological examination was done. The radiological examination consisted of anteroposterior and lateral views of the cervical, dorsal and lumbar spine using standard radiological techniques.

Additional views.namely open mouth for second cervical vertebrae, lateral views of the cervico-dorsal region for upper dorsal vertebrae, were also taken. All the radiographs were studied and measurements were made. The spinal canal body ratio was calculated according to the method prescribed by Jones and Thompson, Bristol, England in 1968 (4).

Interpedicular Diameter: The interpedicular diameter was measured in the anteroposterior views at each level from C_2 to L_5 vertebra, marked A in the radiography,

Anteroposterior Diameter: The anteroposterior diameter of the spinal canal from C_2 to L_5 vertebra was measured in the lateral radiograph from the middle of the posterior surface of the vertebral body to the point of fusion of corresponding laminae and spinous processes marked B in the radiograph (Figure 1). In cases of fluorosis,with calcification of the posterior longitudinal ligament, the measurement was done from the posterior surfaces of the calcified posterior longitudinal ligament.

Figure 1

Spinal Canal Body Ratio AB:CD
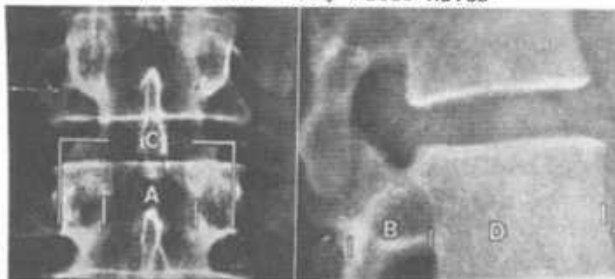
Measurement of interpedicular distance (A), anteroposterior diameter of spinal canal (B), transverse diameter of vertebral body (C) and anteroposterior diameter of vertebral body (D).

Similarly, the transverse and anteroposterior diameter of the middle of the adjacent vertebral body was measured in anteroposterior and lateral views marked C and D respectively. The product   of anteroposterior and interpedicular diameter of the spinal canal was related to the product of the anteroposterior and transverse diameter of the adjacent vertebral body. This ratio AB:CD is called spinal canal body ratio.

Observations

In all cases of skeletal fluorosis (Figure 2), the spinal canal body ratio was higher as compared to normal controls at each level of the vertebra; in other words, the spinal canal was narrow at every level of the vertebra.

Clinical Features: The most common complaints were progressive stiffness of the entire body, weakness of upper and lower limbs, pain radiating along nerve roots and quadriplegia. Neurological deficit was noted in twelve cases.

Radiological Findings: The various radiological findings are listed below:

Sclerosis of spine	25 cases
Osteophytes	25 cases
Posterior longitudinal ligament calcification	16 cases
Disc space narrowing	12 cases
Scoliosis of dorsal spine	14 cases
Kyphosis of dorsal spine	17 cases
Exaggerated lumbar lordosis	13 cases
Marked spinal canal narrowing	12 cases

The normal standard values of the above-mentioned measurements   for Indian individuals were derived from normal skiagrams of asymptomatic patients. The study of interpedicular distance (diameter) revealed a gradual increase in the mean interpedicular distance from C_2 (27.60 mm) to C_6 (29.52 mm) vertebra. A sharp fall followed up to D_5 (19.44 mm) that remained almost constant up to D_7 beyond which the interpedicular distance increased up to L_5 vertebra (32.04mm).

In twenty cases of skeletal fluorosis, the interpedicular distance decreased mainly from C_7 to D_3. Otherwise, at all other levels, the interpedicular distance was almost the same as that of normal individuals (Fig. 3, 4).

Anteroposterior Diameter: The study of anteroposterior diameter in twenty-five normal cases revealed that the mean anteroposterior diameter was maximum at C_2 (19.08) mm, at C_3 it was 16.64 mm. It increased gradually up to C_7 (17.16 mm), beyond which it continued to decrease up to D_5 (12.28 mm) where again there was a slow and gradual increase in anteroposterior diameter up to L_1 (18.88 mm) beyond which the anteroposterior diameter continued to decrease up to L_5 vertebra (16.60 mm).

The anteroposterior diameter in the 25 cases of skeletal fluorosis was reduced at each level of the vertebra as compared to normal controls. It is very much reduced in twelve cases of fluorosis showing spinal cord compression symptoms (Fig.5-9).

The spinal canal body ratio in normal controls was minimum at C_2 (1:108) and gradually increased up to D_{10} (1:3.94). Again it started decreasing gradually up to L_1 (3.62) beyond which it gradually increased in the spinal canal body ratio up to L_5 vertebra (1:4.64). In no case was the spinal canal body ratio in normal controls more than 1:1.95 in the cervical region, 1:5.20 in the thoracic region and 1:6.00 in the lumbar region.

In cases of skeletal fluorosis, the canal body ratio was higher than in controls at each level of the vertebra. The mean canal body ratio was 1:1.88 at C_2, 1:5.98 at D_{10}, 1:5.97 at L_1 and 1:8.50 at L_5. The spinal canal body ratio was further increased at each level of the vertebra in twelve cases of fluorosis showing myelopathy at C_2(1:2.27), at D_{10} (1:6.42) at L_1 (1:6.29) and L_5(1:8.71) (Tables 1,2,3). The critical anteroposterior diameter in the cervical spine in fluorosis patients with myelopathy was either 9 mm or less at any level of the vertebra and the spinal body ratio was 1:2.98 or more (Tables 4,5).

Figure 2

Interosseous Membrane and Sclerosis of Bones of Forearm

Fig.3,4 Interpedicular Distance in Cervical Spine and Sclerosis of Vertebrae

Figure 5

Anteroposterior Diameter
of Cervical Spine and
Calcification of Poste-
rior Longitudinal Liga-
ment Marked .. and
Marked Osteophytes and
Sclerosis of Vertebrae
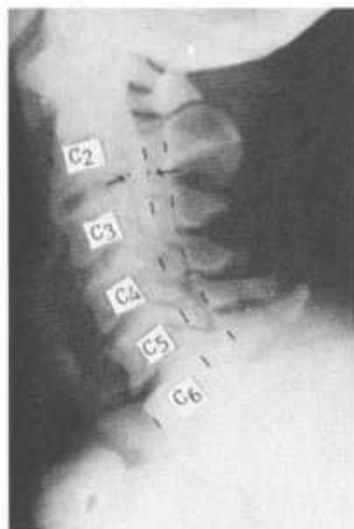
Figures 6 and 7

Measurement of Interpedicular and Anteroposterior
Diameter of Thoracic Spine and Sclerosis of Verte-
brae, Scolosis, Osteophytes and Kyphosis
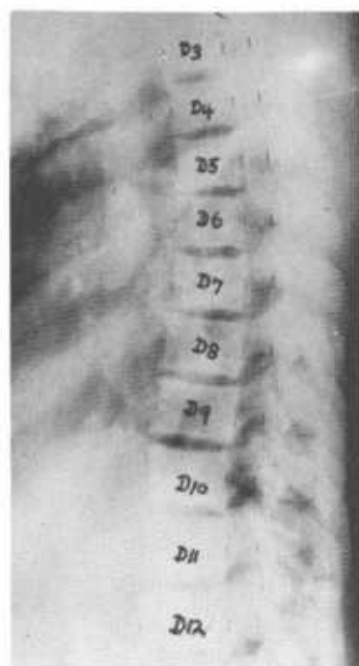
Figure 8

Measurement of
interpedicular
and anteropos-
terior diameter
of lumbar spine,
sclerosis of
vertebrae, marked
osteophytes and
calcification of
interspinous and
posterior longi-
tudinal ligament.

Figure 9

Table 1

Comparative Spinal Canal Body Ratio in Cervical Spine

Vertebral Level	Ratio in 25 normal individuals		Ratio in 25 fluorotics		Ratio in 12 fluorotics with myelopathy	
	Range	Mean	Range	Mean	Range	Mean
C_2	0.86-1.34	1.08	9.96-4.04	1.88	1.46-4.04	2.27
C_3	1.03-1.50	1.27	1.32-4.04	2.35	2.00-4.04	2.86
C_4	1.00-1.50	1.26	1.39-4.09	2.53	2.11-4.09	3.29
C_5	1.03-1.58	1.28	1.50-3.83	2.31	1.55-3.83	2.88
C_6	1.01-1.61	1.30	1.49-3.88	2.25	1.61-3.88	2.71
C_7	1.25-1.95	1.48	1.72-4.83	2.59	1.84-4.83	3.13

Table 2

Comparative Canal Body Ratio in Thoracic Spine

Vertebral Level	25 Normals		25 Fluorotics		25 Fluorotics with Myelopathy	
	Range	Mean	Range	Mean	Range	Mean
D_1	1.51-2.58	1.93	2.11-5.30	3.18	2.56-5.30	3.46
D_2	1.77-3.12	2.25	2.49-6.22	3.67	3.33-6.22	4.04
D_3	1.79-3.21	2.45	2.63-6.66	4.03	3.56-6.66	4.34
D_4	2.02-3.62	2.75	2.70-6.51	4.29	3.66-6.51	4.62
D_5	2.11-4.02	2.98	2.70-6.61	4.58	3.79-6.61	4.53
D_6	2.16-4.46	3.20	2.90-7.40	4.85	4.12-7.40	5.14
D_7	2.22-4.61	3.54	2.86-7.92	5.13	4.61-7.92	5.47
D_8	2.30-4.93	3.74	3.10-8.98	5.41	4.24-8.98	5.70
D_9	2.39-5.20	3.87	3.40-9.23	5.64	5.15-9.23	6.03
D_{10}	2.39-5.00	3.94	3.75-9.92	5.98	5.72-9.92	6.42
D_{11}	2.70-5.00	3.91	4.02-9.98	6.02	4.94-9.98	6.35
D_{12}	2.59-4.57	3.75	4.06-8.50	6.00	5.09-8.50	6.32

Table 3

Comparative Canal Body Ratio in Lumbar Spine

Vertebral Level	Ratio in 25 normal individuals		Ratio in 25 fluorotics		Ratio in 12 fluorotics with myelopathy	
	Range	Mean	Range	Mean	Range	Mean
L_1	2.49-4.33	3.62	3.80-8.40	5.97	4.70-8.40	6.29
L_2	2.60-4.57	3.82	4.00-9.85	6.26	5.21-9.85	6.60
L_3	2.91-5.29	4.09	4.91-10.18	6.80	6.19-10.18	7.11
L_4	3.26-5.98	4.29	5.08-12.66	7.39	6.65-12.66	8.04
L_5	3.57-6.00	4.64	5.90-13.21	8.50	6.85-13.21	8.71

Table 5

Canal Body Ratio in 12 Cases of Skeletal Fluorosis with Myelopathy in Cervical Spine

Case No.	C_2	C_3	C_4	C_5	C_6	C_7
1	1.61	2.25	2.98	2.30	2.02	2.28
3	3.23	4.03	3.52	3.52	3.45	3.98
5	2.18	2.66	3.45	3.08	2.62	2.85
6	1.95	2.45	2.60	3.06	2.60	2.98
7	3.12	2.73	2.11	1.55	1.61	1.84
8	1.62	2.91	3.49	3.02	2.80	3.04
15	2.40	3.17	2.91	2.72	2.71	3.45
18	4.04	4.04	3.62	3.49	3.03	3.81
19	1.46	2.00	3.20	2.69	2.76	2.43
20	1.76	2.88	4.09	3.83	3.88	4.83
22	1.70	2.47	3.07	2.37	2.37	3.09
25	2.16	2.75	3.47	2.68	2.68	3.00
Range	1.46 to 4.04	2.00 to 4.04	2.11 to 4.09	1.55 to 3.83	1.61 to 3.88	1.84 to 4.83
Mean	2.27	2.86	3.29	2.88	2.71	3.13

Table 4

Anteroposterior Diameter in Cervical Spine: 12 Cases of Fluorosis with Myelopathy in mm

Case No.	C_2	C_3	C_4	C_5	C_6	C_7
1	16	12	9	14	14	14
3	10	8	9	9	10	10
5	14	11	9	10	13	14
6	13	10	10	9	11	12
7	8	9	12	15	16	16
8	12	10	8	9	11	12
15	11	8	9	10	11	11
18	7	7	8	9	11	11
19	20	14	9	11	11	14
20	18	11	8	9	9	8
22	14	10	9	11	12	11
25	14	11	9	11	13	14

Discussion

Fluorosis, a disease prevalent in the southwest region of Punjab and in other parts of India is due to the high fluoride content of drinking water. People residing in these areas, especially farmers and laborers doing strenuous work in the fields and consuming more water during the summer season, suffer from a severe crippling form of the disease.

The mean spinal canal body ratio in the lumbar spine in the normal Indian population in the present series was more than that in the European counterparts as reported by Jones and Thompson (4) and less than that reported by Kakrala and Subba Rao(5) from southern India. The upper limit of canal body ratio in the normal lumbar spine was almost the same as that reported by Sandhu and Lakhanpal (6).

The upper limits of the interpedicular distance in the present normal series are almost the same as in the cervical and thoracic spine but somewhat less in the lumbar spine as reported by Elseberg and Dyke (7).

The mean anteroposterior diameter in the cervical spine in the Indian population, in the present series, was little less than that in the European counterparts as reported by Wolf (8). It was more than that in the Japanese but was almost the same as that reported by Bhalla, et al. (9) in India.

The spinal canal body ratio was higher at every level in cases of skeletal fluorosis as compared to normal controls. The reduction in interpedicular distance in cases of skeletal fluorosis was similar to that reported by Singh et al. (3) on the skeleton of a patient with skeletal fluorosis.

The anteroposterior diameter in patients of fluorosis was reduced at every level of the vertebra. However, the reduction was maximum in twelve cases showing spinal canal compression symptoms. These findings are similar to those reported by Singh et al. (3) in a skeleton.

The critical anteroposterior diameter in the cervical spine in fluorosis, at which the spinal cord compression symptoms are likely to occur, in the present series was 9 mm a little less than that reported by Wolf (8) who described the critical diameter to be 10 mm in cases of cervical spondylosis. The critical spinal canal body ratio, at which the spinal cord compression symptoms are likely to occur, was 1:2.98 in the cervical spine.

We, however, feel that the critical level in fluorotics is a separate entity and should not be used for evaluation of spinal canal measurement in patients, in general, who present symptoms of cervical spondylosis. In skeletal fluorosis, the vertebral canal is converted into a rigid pipe and the thoracic spine generally becomes fixed in kyphosis with the cervical spine in variable degrees of extension. Shortening of the anteroposterior diameter continues but the cord does not show signs of compression until a very late stage since the movements of the spine are nil or very much restricted. At this stage, the delicate cord lies

in a rigid pipe which has just enough space to accommodate it. The anteroposterior diameter mainly becomes smaller and smaller until it can no longer accommodate the cord in its physiologically functioning condition. At this point the cord gradually starts showing signs of compression. The spinal cord in skeletal fluorosis can yield to pressure, to some extent, before its functions are impaired, as the movements in the cervical spine in fluorosis were severely reduced or nil.

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IN HEAVY TEA AND COFFEE DRINKERS "NUTRITIONAL STATUS" OF FLUORIDE IN RELATION TO THAT OF OTHER MINERAL ELEMENTS

by

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SUMMARY: Fluoride "nutritional status" or loading
was determined by urine test in heavy drinkers of
tea and coffee. The level of soluble fluoride was
analyzed simultaneously in the brews of teas and
coffees. These investigations suggest that intake
of tea and coffee which contain large amounts of
fluoride can, in certain cases, represent a threat
to health.

KEY WORDS: Fluoride intake from tea; Fluoride from coffee

Introduction

Numerous analyses of nutritional status of mineral elements show that
the level of intake and body saturation of calcium, magnesium, iron, zinc,
manganese, silicon, copper, iodine and chromium is below the established
physiological requirements (1-4). All these mineral elements are impor-
tant for normal bone structure. It is being stressed more and more in the
literature that many natural substances have anti-nutritive effects: for
example, phytic acid, oxalic acid, thiocyanates The research of other
authors as well as that by us demonstrates that similar effects can be
attributed to artificial substances such as DDT, EDTA, fluoride and lead
which are widespread in our environment. All these substances reduce uti-
lization of mineral elements from the diet resulting in low mineral ele-
ment body saturation, transmineralization and biochemical changes in tis-
sue (3, 5).

The diet may be marginal with respect to adequate intake of several
micro-nutrients. Frequently a loading of macro-nutrient and environment-
al contamination such as fluoride in water, lead in the air are observed.
A natural high level of fluoride, especially in widespread use of tea and
coffee, causes a loading of fluoride.

In the present work, with reference to the above-mentioned changes
in mineral consumption and body saturation, the average intake of fluo-
ride with frequently consumed beverages and loading of fluoride in heavy
drinkers of tea and coffee was analyzed by testing urinary fluoride ex-
cretion.

Material and Method

The determination of fluoride in biological materials was conducted
with the electro-chemical method by the use of ion selective electrode
Crytur CSSR (6).

From the National Research Institute of Mother and Child Department of
Nutrition, Warsaw, Kasprzaka, Poland.

Levels of fluoride in beverages were determined in 19 varieties of tea and 6 brands of coffee. Extracts of tea and coffee were prepared according to routine brewing procedures.

Urine samples were collected from 47 heavy drinkers of tea and coffee, all of whom were more than 20 years of age.

Results

Tables 1 and 2 show the results of determination of soluble fluoride in condiments. The fluoride level, introduced with water, was subtracted. The results are shown in comparison with an average 1.7 mg daily level for fluoride.

Levels of fluoride in daily urine of men and women in our study and that by other authors are presented in Table 3.

Table 1

Soluble Fluoride in Condiments mg/kg Dry Matter

Tea	58 - 210
Coffee	28 - 72
Herb tea	16 - 60

Table 2

Beverages - % of Fluoride "Daily Requirements" 1.7 mg (2)

	1 Glass or cup (200 cm^3)
Tea	20 - 50%
Coffee	40 - 85%
Herb tea	3 - 15%

Discussion

It is necessary to balance body intake of fluoride from the entire diet, which now contains more fluoride than formerly due to industrial contamination, and altered nutritional habits. Not only the beverages tea and coffee (Tables 1 and 2), but also fish products and water introduce significant amounts of fluoride into our diet (6-7).

F⁻ Intake from Tea and Coffee

Table 3

Urinary F⁻ Excretion

Sex	F^- mg/24 hrs.	Authors
F	0.3-12.8	Own (1979)
M	4.5-9.4	Own (1979)
F	15	Schreiber (1964)
M	3.0-5.25	Schreiber (1964)
F& M	1.0-8.0	Wojnar (1960)

Nowadays specialists in nutrition have revised early data for daily fluoride requirements (1-2); some designate fluoride a nonessential toxic element in our diet because many other elements such as silicon and manganese may be more efficient for physiological ossification. Simultaneously, research of others shows that dental caries prevention can be achieved by microbiological immunization against two kinds of bacteria — Streptococcus sanquis and Streptococcus mutans (8). For this reason, the requirement for fluoride is controversial and open for discussion.

We conclude that heavy drinkers of tea and coffee (Table 3) have a loading of fluoride similar to that of endemic or industrial fluorosis; on an average the level is higher than 4 ppm for 24-hour urine (9-10).

On the basis of considerable experimental data and theoretical premises it is reasonable to assume that an improvement in nutritional habits and food pattersn can lower the incidence of many diseases arising from loading or imbalance of various constituents in the entire diet. Our investigations suggest that intake of tea and coffee containing high amounts of fluoride can, in certain cases, constitute a threat to health.

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FLUORIDE INGESTION DURING PREGNANCY AND LACTATION:
MORPHOLOGICAL EFFECTS ON MATERNAL RAT BONE

by

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SUMMARY: Anorganic preparations of femurs from
female rats, given 150 ppm fluoride in the drink-
ing water for ten weeks prior to and during one
subsequent pregnancy and lactation period, were
examined by SEM and compared to control rats. The
major difference seen was a marked increase in
endosteal resorption. Presumably a decreased
serum calcium concentration, caused by bone flu-
oride deposition along with a high demand on cal-
cium under the influence of lactation, resulted
in increased bone mineral mobilization from the
endosteal surface.

KEY WORDS: Bone, maternal rat; Fluoride, effect on bone; Fluoride in
pregnancy; Bone, morphology in pregnancy

Introduction

It is known that the ingestion of fluoride in drinking water alters
the processes of bone formation, mineralization and resorption in the rat
(1) and that part of the mineral requirements of lactation is met by bone
resorption (2,3). Studies on the effects of fluoride during pregnancy
and lactation have dealt primarily with the fluoride concentration in ma-

ternal and fetal blood, in the placenta and fetus, and in urine (4-10).
To date, the effects of fluoride ingestion during pregnancy and lactation
on maternal bone morphology are unknown. Since most reports on how fluo-
ride affects the skeleton have focused on nonpregnant adults, the present
study was to determine the morphological effects of high fluoride inges-
tion on the skeletal system of rats subjected to the calcium-depriving
regimens of pregnancy and lactation.

Materials and Methods

Adult female Sprague-Dawley rats were divided into control and exper-
imental groups of 14 animals each. The control group was given distilled
drinking water; the experimental group, distilled drinking water to which
150 ppm sodium fluoride was added.   All animals were maintained on Puri-
na rat-chow pellets and appropriate water ad libitum throughout the study.

At the end of an initial ten week period, seven rats from each group
were sacrificed by ether overdose. The femurs were dissected from both
extremities and prepared for scanning electron microscopy (SEM) as de-
scribed below. The remaining female rats were then bred and underwent
one pregnancy and a 21-day lactation period. Litter size was normalized
to eight pups on the day of delivery. Maternal femurs were collected at
the end of the lactation period.

In preparation for SEM, the proximal and distal epiphyses from all
femurs were removed by two transverse cuts with a rotary bone saw; the
diaphyses and distal epiphyses were sectioned longitudinally with a jewel-
er's saw. The organic matrix of each bone specimen was removed by immers-
ing the specimen in 5% sodium hypochlorite for four hours. The anorganic
bones were rinsed in distilled water, air dried, and mounted on stubs.
The diaphyseal specimens were mounted so that either the periosteal or
endosteal surface was exposed; the epiphyseal specimens were mounted to
expose the metaphyseal trabeculae. The specimens were coated with gold
and examined with a Philips 500 scanning electron microscope. All areas
of the diaphyseal specimens were examined except the areas of muscle at-
tachment sites on the periosteal surfaces and the areas opposite the mus-
cle attachment sites on the endosteal surfaces.

Results

Diaphyseal Periosteum: The periosteal surface of the femoral dia-
physis in nonpregnant controls is dominated by apposition areas which are
characterized by regularly shaped and closely packed mineralized segments.
The orderly arrangement of the mineralized segments represents the under-
lying pattern of the collagen fiber bundles. These features are charac-
teristic of areas of typical active bone formation and represent the cal-
cification front (Fig. 1). In nonpregnant, fluoride-treated animals, the
periosteal surface consists of irregularly arranged mineralized segments
separated by gaps. These features suggest that the calcification fronts
have not completely reached the limits of the collagen fiber bundles. The
loosely packed mineralized segments produce a frayed appearance of woven
bone formation (Fig. 2).

Figure 1
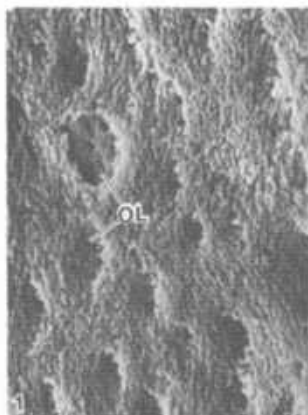
Figure 2

Periosteal surface of femoral diaphy-
sis, control, nonpregnant rat. Osteo-
cyte lacunae (OL), in the process of
being enveloped in bone matrix x640.

Periostel surface of femoral diaphysis,
fluoride-treated nonpregnant rat. Back
walls of osteocyte lacunae (OL) are
composed of irregularly arranged miner-
alized segments x 640.

Figure 3
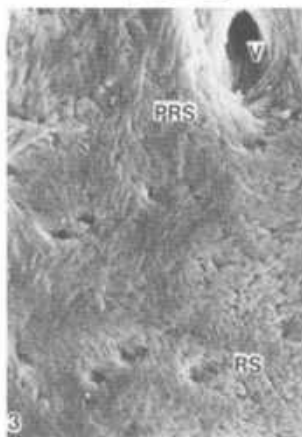
Figure 4

Periosteal surface of control rat femur
after pregnancy and lactation, charac-
terized by resting (RS) and prolonged
resting surfaces (PRS). V, vascular
channel x 640.
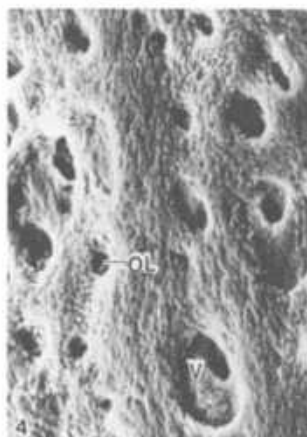
Periosteal surface of fluoride-treat-
ed rat femur after pregnancy and lac-
tation, composed of resting apposition
areas. V vascular channel; OL, osteo-
cyte lacuna x 640.

The periosteal surface of femurs from control animals after pregnancy and lactation is characterized by resting surfaces. Resting surfaces are typified by fully mineralized collagen fiber bundles. In addition, prolonged resting surfaces are observed around osteocyte lacunae (perilacunar bone) and vascular channels. On these surfaces, mineralization has progressed beyond the collagen fibers giving the surface a relatively smooth appearance (Fig. 3). In fluoride-treated animals after pregnancy and lactation, the periosteal surface appears to be composed of resting apposition areas, where mineralization has ceased before being completed; numerous forming osteocyte lacunae are visible (Fig. 4).

Diaphyseal Endosteum: The endosteal surfaces of femurs from control and fluoride-treated nonpregnant animals are similar in appearance. These surfaces are dominated by fully mineralized areas although some areas of bone formation are present. The fully mineralized areas have a smooth texture since the arrangement of the bone crystals follows that of the collagen fiber bundles. The bundles are uniform and deposited in parallel rows exhibiting a highly ordered pattern (Fig. 5). Evidence of resorption on this surface is rarely seen.

After pregnancy and lactation, the femoral endosteal surface of control animals shows prominent depressions indicating areas of bone resorption. These depressions, or Howship's lacunae, represent the former position of osteoclasts on the endosteal surface. The resorptive areas are regularly outlined and generally uniform in depth. Shallow ridges en-

Figure 5

Endosteal surface of femur, fluoride-treated, nonpregnant rat. The fully mineralized surface, similar in control, nonpregnant rats. V, vascular channel x 640.

Figure 6
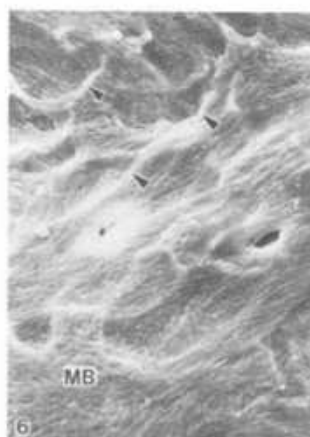
Femoral endosteal surface of control rat after pregnancy and lactation. The surface is characterized by fully mineralized bone (MB) and resorption areas. The resorption area relatively flat, showing a similar depth throughout and is subdivided by ridges (arrowhead) x 640.

circling individual surface depressions subdivide the smooth bottom of the
resorption areas (Fig. 6). These characteristics are indicative of either
a previously resorbed area which is now inactive, or an area in which the
resorption process is proceeding slowly. In contrast, the endosteal sur-
face of femurs from fluoride-treated animals after pregnancy and lactation
is characterized by extensive areas of active bone resorption. The resorp-
tion of endosteal lamellae at different levels is reflected in deep How-
ship's lacunae which are irregular in shape and surrounded by sharp ridges
(Figs. 7,8).

Figure 7   Figure 8
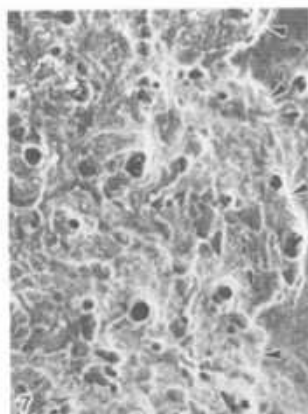
Endosteal surface of fluoride-treated
femur after pregnancy and lactation.
Resorption areas are larger and deep-
er than those seen in control animals
after pregnancy and lactation. Arrow-
heads indicate a border of a large
resorption area x 160.
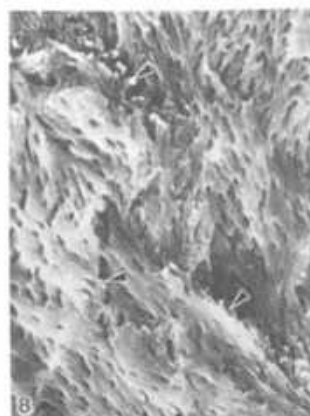
Endosteal surface of fluoride-treated
rat femur after pregnancy and lactation.
The individual depressions within re-
sorption area surrounded by sharp, ir-
regular ridges (arrowheads). Extensive
resorptive process has exposed endosteal
lamellae at different levels x 1250.

Metaphyseal Trabeculae: The metaphyseal trabeculae from nonpregnant,
control rats are smooth, cylindrical, and regularly shaped (Fig. 9). Typi-
cal areas of fully mineralized bone, apposition areas and resorption areas
are seen (Fig. 10). In contrast, the trabeculae from fluoride-treated,
nonpregnant rats are rough and irregularly shaped (Fig. 11). Although
some apposition and resting areas can be seen, resorption areas predomi-
nate indicating an extensive resorption process. Some of the Howship's
lacunae are deep and have prominent edges, whereas others are shallow and
poorly defined (Fig. 12).

After pregnancy and lactation, the metaphyseal trabeculae of control
animals are dominated by resorption areas, although resting and apposition

Figure 9

Metaphyseal trabeculae from non-
pregnant control, smooth, cylin-
drical and regularly shaped
x 270.

Figure 10
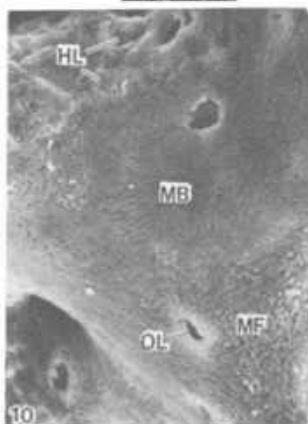
Metaphyseal trabeculae from nonpregnant
control. Areas of fully mineralized bone
(MB), a resorption area with Howship's
lacunae (HL), a mineralization front
(MF) with osteocyte lacunae(OL) x 540.

Figure 11

Metaphyseal trabeculae from flu-
oride-treated nonpregnant rat,
rough, irregular in shape x 270.

Figure 12
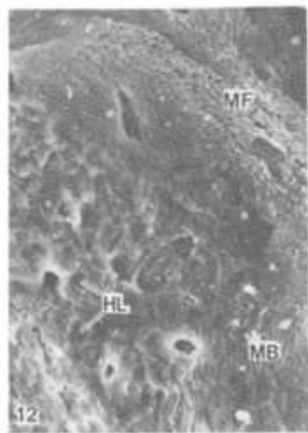
Metaphyseal trabeculae, fluoride-treated
nonpregnant rat. Surface of trabeculae
dominated by large resorption areas with
Howship's lacunae (HL), some areas of
fully mineralized bone (MB) and mineral-
ization fronts (MF) x 540.

Figure 13
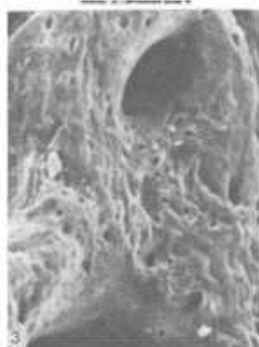
Metaphyseal trabeculae from control after pregnancy and lactation, cylindrical in shape. Surfaces dominated by resorption areas x 270.

Figure 14
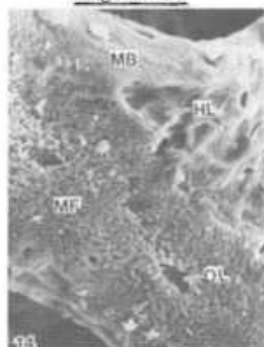
Metaphyseal trabeculae from control after pregnancy and lactation. Fully mineralized bone (MB), a mineralization front (MF) with osteocyte lacunae (OL), and Howship's lacunae(HL) x 540.

Figure 15
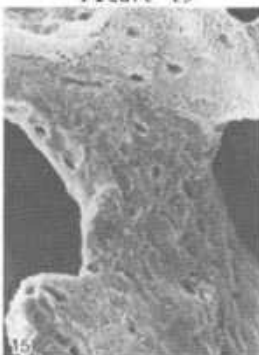
Metaphyseal trabeculae, fluoride-treated rat after pregnancy and lactation. Trabeculae similar to control animals after pregnancy and lactation except for areas of new bone formation covering previously resorbed zones x 270.

Figure 16
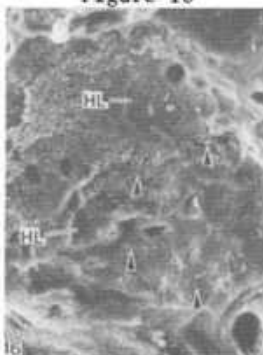
Metaphyseal trabeculae from fluoride-treated rat after pregnancy and lactation. Newly formed mineralized segments (arrowheads) cover previously resorbed areas containing Howship's lacunae (HL) x 540.

areas are present. While the trabeculae are similar to those found in non-pregnant, fluoride-treated animals, the resorption process in the control pregnant animals is not so extensive; consequently, the trabeculae retain much of their regular, cylindrical shape (Figs. 13,14). The trabeculae of fluoride-treated animals after pregnancy and lactation (Fig. 15) are

similar to those of pregnant control animals with one difference. Newly
formed mineralized segments are frequently seen on the surfaces of pre-
viously resorbed areas. These segments are irregular, frayed and appear
to have been randomly deposited (Fig. 16).

Discussion

The results of this study indicate that there are changes on the fem-
oral bone surfaces which can be attributed to the effects of fluoride, of
pregnancy and lactation, and of fluoride plus pregnancy and lactation .

Fluoride is known to increase crystal size and crystal perfection in
biological apatites, and to reduce mineral solubility (11). As a conse-
quence of these effects, the crystals are more stable and less reactive
in surface exchange reactions. These changes may account for the reduced
loss of calcium from bone with a high fluoride content (12). Presumably,
these changes also increase the resistance of bone to the actions of pa-
rathyroid hormone (13). Thus, the deposition of fluoride in bone appears
to interfere with the normal calcium homeostatic mechanism.

The surface features of the femurs from nonpregnant female fluoride-
treated rats seen,in the present,study are similar to those reported in
other studies in which male rats were treated with the same fluoride dos-
age (14,15). Thus it appears that the effects of fluoride ingestion are
not influenced by the sex of the animal. In both male and female rats,
the ingestion of 150 ppm fluoride in the drinking water for ten weeks re-
sults in an increase in periosteal matrix and bone formation, a decrease
in endosteal resorption, and an increase in metaphyseal trabecular bone
resorption.

It is well documented that, as a result of improved crystalline tex-
ture, fluoride promotes the stabilization of newly synthesized bone mat-
rix and inhibits bone resorption (12,16). Therefore, one might assume
that the decrease in endosteal resorption seen in the nonpregnant rats
was due to the formation of mixed fluorhydroxyapatite crystals in bone
laid down during the time of fluoride ingestion. However, the effects of
fluoride are too rapid (within ten weeks) to be significantly related to
the way in which new bone formation occurred during the period of flu-
oride ingestion. Thus one must look to some other property of fluoride
than its effect on crystals in newly formed bone. There is evidence that
fluoride inhibits numerous enzyme activities (17, 18). Therefore, the me-
tabolic function of bone resorbing cells might be inhibited when exposed
to high fluoride concentrations. Consequently, it seems more likely that
the reduction in bone resorption on the endosteal surface of the femoral
diaphysis is due to a direct toxic effect of fluoride on bone resorbing
cells.

The same principle would apply to the cells lining the trabecular
surfaces. However, cellular activity at the surface of trabecular bone
differs from that at the endosteal surface of cortical bone. At the en-
dosteal surface, bone surface cells are involved in bone remodeling which
mediates changes in bone shape and bone mass. In contrast, remodeling by

bone surface cells of trabecular bone is related to the homeostasis of calcium. The inhibition of metabolic function of these cells, when they are exposed to high fluoride concentrations, would lead to a fall in serum calcium and a compensatory increase in endogenous parathyroid hormone secretion. This rise in serum parathyroid hormone would stimulate the differentiation of bone progenitor cells into osteoclasts. As seen in the present study, the consequence appears to be an increase in trabecular bone resorption to just the degree necessary to compensate for the inhibition of homeostatic bone resorption, thereby maintaining the concentration of calcium in blood at or near the normal level. These findings are consistent with the concept that the initial effect of fluoride interaction with bone is primarily in those areas of bone which function to maintain basic calcium equilibrium between bone and blood (19). In the rat, this appears to be reflected first in areas of trabecular bone.

The increase in periosteal matrix and bone formation along with an inhibition of the process of mineralization at the periosteal surface agrees with histological studies using nonpregnant rats treated with fluoride in the drinking water (1,20,21). The deposition of woven bone on the periosteal surface may also be related to an increase in parathyroid hormone secretion since, in the skeleton of individuals with hyperparathyroidism, loss of synchronized collagen deposition is reflected by the loss of lamellar bone structure and its replacement with woven bone (22-24).

Pregnancy puts a strain on the mineral balance of the rat, but will not reduce the amount of bone mineral in the skeleton if calcium is fed in adequate amounts (25). In contrast, lactation represents a heavy drain on the calcium homeostatic mechanism and will, irrespective of the amount of calcium in the diet, reduce the stores of bone mineral (25,26). In the present study, the characteristic surface features of the femurs from untreated rats after pregnancy and lactation are an absence of new bone deposition on the periosteal surface and resorption on the endosteal and trabecular bone surfaces.

In fluoride-treated rats, pregnancy and lactation appear to result in no new bone deposition on the periosteal surface, extensive resorption on the endosteal surface, and resorption of metaphyseal trabeculae. The major difference seen with fluoride ingestion during pregnancy and lactation is the marked increase in endosteal bone resorption, the mechanism for which is uncertain. Secondary hyperparathyroidism has been observed both in fluoride-treated (27) and in calcium-deficient rats (28), and the mineral removal may be mediated through the action of parathyroid hormone probably by osteoclastic resorption. Rats are highly efficient in their retention of calcium (29) and mineral (calcium) turnover normally occurs by trabecular bone remodeling. However, it appears that remodeling of metaphyseal trabecular bone is insufficient to provide the needed calcium during pregnancy and lactation. Moreover, the period of fluoride ingestion was 16 weeks, so that conceivably the concentration of fluoride at the surface of the trabecular bone was high making it more resistant to resorption. Although evidence of resorption is seen on the endosteal surface of nontreated pregnant rats, the surface characteristics are indica-

tive of a slow resorptive process. In contrast, the areas of resorption
on the endosteal surface of fluoride-treated pregnant rats are more ex-
tensive and the surface features indicate an active resorptive process.
The calcium homeostatic mechanism is challenged by the calcium-depriving
regimens of fluoride ingestion plus pregnancy and lactation, and bone min-
eral mobilization in the rat appears to be accomplished primarily by in-
creased endosteal resorption.

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ANTIDOTES IN EXPERIMENTAL FLUOROSIS ON PIGS
MORPHOLOGICAL STUDIES

by

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SUMMARY: In pigs fed 5 mg/kg NaF daily for 1 year,
cortices of long bones became thicker. On the other
hand, in cancellous bone osteopenia resulted. Simul-
taneous application of high doses of Borax reduced
NaF effect on bone cortices, the addition of Mg meta-
silicate to a lesser extent. Mg oxide caused no such
reduction. The parathyroid showed increased activi-
ty morphologically following high dose NaF treatment
for 4 months.

KEY WORDS: Pigs, antidotes for fluorosis in; Fluorosis, antidotes; Anti-
dotes for fluorosis; Borax,antidote for fluorosis; Magnesium
oxide.

Material and Methods

Experimental studies were performed in pigs with varying   doses of fluoride with or without antidotes. In the first experiment, 48 yearling pigs were divided into 8 equal groups. Group 1 served as control. Groups 2 and 3 received 0.5 and 5 mg NaF/kg daily, group 4 received 0.3 mg/kg Borax daily, group 5, 0.5 mg NaF and 0.3 mg Borax. Pigs in groups 6, 7, and 8 received 5 mg of NaF, additional treatment included 3 mg of Borax, 6 mg of magnesium metasilicate or 2 mg of magnesium peroxide, respectively. The experimental period was 1 year.

In the second experiment, 32 eight-month old pigs were treated with larger doses of NaF and Borax bound to an ion exchanging substance. The experimental period was 4 months. When sacrificed, the left humerus, left radius and ulna, liver, heart, kidney, thyroids and parathyroids of all animals were preserved for histologic examination. Weight, length and volume of long bones were recorded. Measurements of the cortical thickness in all quadrants were obtained from a transverse section of the humerus mid-diaphysis. The width of the marrow cavity was also recorded. From histologic sections of the proximal epiphysis of the humerus, the amount of cancellous bone was determined by quantitative morphometry.

Results

Daily application of 0.5 mg or 5 mg of NaF/kg resulted in larger bones. Compared to control pigs and pigs treated only with Borax, long bones from both fluoride groups were longer and thicker (Table 1). This is in disagreement with Forsythe and co-workers (1) who found shortening of the long bones. The cranial cortex at the mid-diaphysis was markedly thickened, less so in the caudal and medial quadrants and not at all in the lateral one. The cranio-caudal diameter of the medullary cavity was decreased. Fluoride analyses by proton absorption spectroscopy showed that newly formed subendosteal and subperiosteal bone contained much more fluoride than the deeper bone; the values were 700 and 250 ppm respectively. This agrees with the findings of Jokl and Skinner (2) who studied the distribution of fluoride in growing sheep. Thickening of the cortex and narrowing of the medullary cavity were reduced in the high Borax group and less reduced in the low Borax and Mg metasilicate groups (Table 1, Fig.1). The differences were significant only for the high Borax group. These results were obtained from the first experiment. In the second experiment, using higher doses for a shorter time, no such effects were recorded.

Quantitative morphometry of the cancellous bone in the humerus epiphysis showed that low doses of sodium fluoride increased the area of trabeculae, whereas high doses decreased this area (Table 2); high doses, thus, induced osteopenia. Similar findings have been described in laboratory animals and in cattle and sheep (3). Treatment with high doses of fluoride for 4 months induced increased parathyroid activity as evaluated by the diameter of the nuclei (Table 3). Treatment with antidotes did not influence the parathyroid activity significantly. Similar stimulation of parathyroid activity has been observed by Faccini and Care (4) in lambs and by Faccini and Teotia (5) in man.

Figure 1

Transverse Sections of Humerus Mid Diaphysis
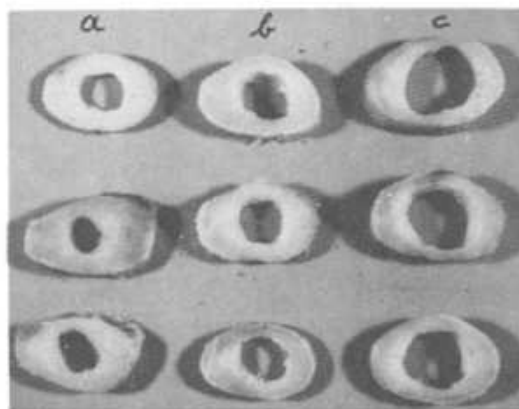
a:0.5 mg NaF;   b:5.0 mg NaF;   c:5.0 mg NaF + 3.0 mg Borax

Table 1

Cross Section of Humerus

	Length (mm)		Volume (mm)		Thickness of Diaphyseal Cortex(mm)*	Bone Marrow Cavity Dia- meter (mm)	Index Bone Marrow: Thick- ness of Cortex
	Humerus	Radius	Humerus	Radius			
Control	223	237	310	203	10.8	17.1	1.2
0.5 mg NaF	229	251	348	228	13.1	14.2	1.7
5.0 mg NaF	226	240	355	243	13.9	15.4	1.6
0.3 mg Borax	218	235	315	216	12.6	17.3	1.2
0.5 mg NaF + 0.3 mg Borax	229	252	328	227	12.2	19.4	1.0
5.0 mg NaF + 3.0 mg Borax	228	243	339	236	10.8	19.4	1.0
5.0 mg NaF + 6.75 mg Mg Metasilicate	221	236	310	211	12.1	18.4	1.1
5.0 mg NaF + 2.25 mg MgO	225	244	333	231	12.4	16.5	1.3

** - Cranial Quadrant

Table 2

Percentage of Bone Trabeculae in Cancellous Bone

I	Control	31.8
II	0.5 mg NaF	37.0
III	5.0 mg NaF	29.9
IV	0.3 mg Borax	30.0
V	0.5 mg NaF + 0.3 mg Borax	32.5
VI	5.0 mg NaF + 3.0 mg Borax	32.7
VII.	5.0 mg NaF + 6.75 mg metasilicate	31.4
VIII	5.0 mg NaF + 2.25 mg MgO	32.3

High fluoride ingestion resulted in slight enlargement of the thyroid gland and a reduction in height of the follicular cells. The results on the thyroid gland are preliminary and require further investigation.

Table 3

Parathyroid Activity

Groups		Cut Faces of Nuclei
I	Control	$20.18/\mu m^2$
II	1.5 mg NaF	$18.98/\mu m^2$
III	18 mg Borax	$20.48/\mu m^2$
IV	15 mg NaF + 18 mg Borax	$20.85/\mu m^2$
V	15 mg NaF + 0.5 A_1 (Boric Acid)	$23.82/\mu m^2$
VI	15 mg NaF + A_2 (Borax)	$22.27/\mu m^2$
VII	15 mg NaF + 20 mg Vit. C	$22.60/\mu m^2$
VIII	15 mg NaF + 1.25 g $Al_2(SO_4)_3$	$21.88/\mu m^2$

Conclusion

Feeding 5 mg/kg NaF daily for a period of 1 year to pigs resulted in thicker cortices of long bones. On the other hand, in cancellous bone, osteopenia resulted. The effect of NaF on bone cortices could be reduced by

simultaneous application of high doses of borax and, to a lesser extent, by addition of Mg metasilicate. Mg oxide caused no such reduction. The parathyroid showed morphologic indication of increased activity following treatment with high doses of fluoride for 4 months. The effects of high fluoride ingestion on the thyroid gland require further investigation.

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EFFECT OF HIGH FLUORIDE INTAKE ON CHICKEN PERFORMANCE, OVULATION, SPERMATOGENESIS AND BONE FLUORIDE CONTENT

by

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SUMMARY: Three levels, 150, 300 and 600 ppm, of NaF were added to the basal ration of Hisex breed male and female chickens (98 days old). Body weight gain, total feed consumption, feed conversion and mortality (until 158 days of age) were not influenced. At this age, increased fluoride content of the long bones of the treated groups were observed (P<0.05). Long bones of the male birds tended to accumulate more fluoride than did the long bones of the females.

Egg production started on the 157th and 158th day in all groups. Its rate during a period of 70 days showed a tendency to decrease as the level of added fluoride rose. Although ovulation, as reflected by egg laying, was not in-

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fluenced, initiation of spermatogenesis was de-
layed in the testes of the 600 ppm group   and
giant spermatid cells were observed. Breed var-
iation in the response of chicken to the added
level of fluoride was suggested.

Introduction

Fluoride is widely distributed in nature and in soil depending on its
geological formation. Phosphate fertilizers and certain industries are
additional sources of this element. Consequently, the fluoride content
of plants and water beds depends on the type of soil, fertilization regime
and its nearness to certain factories. Feed formulas supplemented with
raw rock phosphate are high in fluoride. Said et al. (1) used raw rock
phosphate, as phosphorus supplement, for growing pullets and layers   at
three levels: 0.6, 1.2 and 1.8% of the ration and found that the added
fluoride levels were 216, 432, and 648 ppm respectively.

Studies on the adverse effects of high fluoride intake on the health
of mammalian and avian species started early in the thirties of this cen-
tury. Hauck et al. (2) reported depressed appetite and weight gain   in
young chicks (less than two months old) after feeding them 1358 ppm fluo-
ride as sodium fluoride. However, such a dose had little effect on   the
pullets. Changes in the weight of several tissues were induced in young
chicks by supplementing the control ration with 150 ppm fluoride in   the
form of sodium fluoride (3). Similar treatment caused variations in   the
electrophoretic pattern and concentration of serum proteins of growing
chicks (4). In young turkeys, the effect of various levels of fluoride
(100, 200, 400, 800, and 1600 ppm) were studied by Anderson et al. (5).

In the present experiment the basal ration was supplemented   with
three levels of fluoride, namely 150, 300 and 600 ppm and effects on
chickens' performance, ovulation, spermatogenesis and bone fluoride con-
tent were observed.

Materials and Methods

One hundred-eighty male and 260 female chicks (98 days old) were tak-
en from Hisex breed parent stock. Birds of each sex were weighed and ran-
domly accommodated into four pens. From this age until 22 weeks old, the
chickens received a basic ration after which another ration was given un-
til termination of the experiment (Table 1). Both rations were supple-
mented with fluoride (as NaF) at levels of 0, 150, 300, and 600 ppm. Pens
of each sex were assigned, at random, to one of these levels, making four
groups of chickens, namely the control group, 150 ppm, 300 ppm, and 600ppm
group. All chickens were fed and watered ad libitum.

On day 105, 112, 119, 126, and 133, three birds from each pen were
sacrificed to observe the morphological development of the ovaries and to

Table 1

Ingredients and Analysis of Experimental Rations

Ingredients	from 14-22 weeks of age	from 23 weeks of age till end
Corn, yellow	40	38
Wheat	25	20
Barley	25	15
Meat Meal (50%)	8	8
Soybean Meal (50%)	--	12
Calcium Carbonate	1.5	6.5
Salt	0.5	0.5
Calculated Analysis:		
Crude Protein (%)	13.7	17.0
Metab. Energy (Kcal/Kg)	3000	2850
Calcium (%)	1.45	3.45
Phosphorus, available (%)	0.5	0.5

dissect out the testes. The latter were washed with normal saline solution and transferred into formalin-acetic acid fixative solution (6). Eosin-hematoxylin stained sections were prepared from the testicular tissue to examine for spermatogenesis following a procedure described by Humason (7). When chickens reached the age of 158 days, their final body weight was recorded and long bones of six birds from each group of both sexes were saved for determination of their fluoride content by a colorimetric method reported by Holub (8). At this stage of the study, namely 60 days after the starting date, the total feed consumption was calculated, body weight gain and feed conversion were determined, mortality was recorded.

The experiment was continued with the female chickens until day 228. Egg production was determined at specified intervals. Wherever necessary, data were subjected to statistical analysis using Tukey's significant difference procedure (9).

Results

The initial body weights of the birds, of the same sex, in all groups did not differ from each other as shown in Table 2. Table 2 also shows that feeding the basal ration supplemented with the used levels of fluoride did not induce variations in body weight gain of the groups of both sexes.

The three levels of added fluoride failed to induce statistically sig-

nificant differences in total feed consumption and feed conversion. Never-
theless, there was a tendency toward increased total feed consumption in
the fluoride-treated groups which was reflected on feed conversion.   Bet-
ter feed conversion was recorded in the 150 ppm group and 300 ppm   group
of the male and female respectively.   The number of birds which died dur-
ing   60 days did not suggest a relationship between fluoride in the ra-
tion and mortality (Table 2).

<u>Table 2</u>

Effect of F⁻ Added to Basic Ration on Body Weight,
Total Feed Consumption, Feed Conversion, Mortality
and F⁻ Content of Long Bones

	Sex	Treatments			
		0 ppm F⁻	150 ppm F⁻	300 ppm F⁻	600 ppm F⁻
Initial Body Weight (g)	M	$1790^a \pm 264$	$1862^a \pm 210$	$1700^a \pm 240$	$1868^a \pm 221$
	F	$1284^a \pm 256$	$1288^a \pm 183$	$1255^a \pm 117$	$1292^a \pm 147$
Weight Gain (g/60 days)	M	$890^a \pm 159$	$993^a \pm 144$	$1016^a \pm 149$	$919^a \pm 147$
	F	$694^a \pm 99$	$744^a \pm 127$	$771^a \pm 123$	$728^a \pm 106$
Total Feed Consumption (g/60 days/bird)	M	8751	8794	9565	9274
	F	5674	5880	5862	5982
Feed Conversion	M	9.83	8.85	9.00	10.10
	F	8.17	7.90	7.60	8.20
Mortality (birds during 60 days)	M	2	3	3	3
	F	2	2	3	2
Fluoride Content of Long Bones (ppm)	M	$660^a \pm 38.8$	$1484^b \pm 164.5$	$2173^c \pm 166.8$	$2456^c \pm 306.0$
	F	$616^a \pm 40.0$	$1371^b \pm 309.0$	$1413^b \pm 267.7$	$1970^c \pm 177.3$

Values given as means ± standard deviation.   Means of the same row with
different superscripts are significantly different (P<0.05).

Figures of fluoride concentration in long bones of the control   and
treated groups are presented in Table 2.   The fluoride content of   the
long bones was higher in fluoride supplemented birds.   Variations   were
noticed as fluoride levels increased.   The bones of males tended to accu-
mulate more fluoride than the bones of females.

Table 3 presents egg production of the four groups during the indi-
cated periods.   The mean percentage revealed that the 600 ppm fluoride
level had a negative effect of about 5%.

Table 3

Effect on Egg Production of F$^-$ Added to Basic Diet

Treatments

Periods	0 ppm F$^-$	150 ppm F$^-$	300 ppm F$^-$	600 ppm F$^-$
Apr.6*-13	2.19	2.43	2.44	2.44
Apr. 14-20	7.32	6.71	6.62	5.92
Apr. 21-27	18.63	16.92	16.38	16.03
Apr. 28-May 4	25.92	22.16	20.91	20.46
May 5-11	54.79	52.78	47.39	46.22
May 12-18	57.52	54.69	51.67	51.06
May 19-25	55.76	54.20	54.81	50.32
May 26-June 1	54.84	55.00	47.73	45.29
June 2-8	51.96	52.06	45.99	37.98
June 9-15	52.17	50.64	41.46	36.58
X	38.11	36.76	33.54	31.23
S.D. ±	22.12	21.93	19.82	18.47

* On the 6th of April the chickens were 157 days old

Structural Changes: Microscopic examination of stained testicular sections showed a similar structural picture in specimens of the control and treated groups at 105 days. Seminiferous tubules were without lumens: spermatogenesis was developed up to primary spermatocytes; Leydig cells were small in size and undeveloped. In testicular sections on day 119, lumens could be observed in the majority of the seminiferous tubules and sperms appeared in some of them. Leydig cells were very well developed. This description applies to testes of the control, 150 ppm, and 300 ppm groups. Testicular sections of the 600 ppm group showed opening of most of the seminiferous tubules, whereas spermatogenesis had reached the stage of spermatids. Furthermore, spermatid giant cells were observed in some tubules. Leydig cells were comparable, in their development, to those of other groups.

Spermatozoa were in most of the seminiferous tubules, lumens in testicular sections of the control, 150 ppm and 300 ppm groups at day 126. On the other hand, testes of the 600 ppm group at this age showed spermatozoa in some tubules and giant spermatid cells could still be observed. The structural picture of the testes was similar in all groups at day 133 as spermatogenesis was completed and giant spermatid cells were no longer seen in sections of the 600 ppm group.

Regarding development of the ovary, no morphological differences were observed between the control and treated groups. Ovulation, as in-

dicated by egg laying, took place in the control and treated groups a-
round day 157 and 158.

Discussion

Fluoride levels, 150, 300, and 600 ppm added to the ration of chick-
ens of this experiment, did not significantly influence growth rate com-
pared with that of the control animals. Depressed weight gain was ob-
served after feeding chicks a ration supplemented with 4300 ppm fluoride
(2). On the other hand, Said et al. (1) recorded growth depression at
20 weeks of age with 648 ppm added fluoride.

Data on body weight gain, total feed consumption, feed conversion
and mortality rate agreed with previous findings that poultry exhibits
greater tolerance to high fluoride intake than mammalian species. The
maximum safe dietary levels of 300-400 ppm fluoride as rock phosphate
was reported for growing chicks (10).

Egg production was affected by the supplementary fluoride, especial-
ly in the 600 ppm group. Decreased egg production also was observed af-
ter feeding laying hens a ration to which 4300 ppm fluoride was added(2).
Whereas the addition of 200 ppm fluoride had a positive effect on egg
production, a negative effect was observed when the fluoride supplement
was increased to 400-800 (11). According to Gerry et al. (10), 500-700
ppm fluoride was a safe dietary level for laying hens.

The results of the structural investigation indicated that the 600
ppm was the only added level of fluoride which impaired the initiation
of spermatozoa production in chicken. No previous report on the effect
of high fluoride intake on spermatogenesis in avian species has appeared
in the literature. In mammalian species, however, Ridha et al. (12, who
studied the effect in this laboratory, noticed impaired spermatogenesis
in which 125, 250, and 500 ppm fluoride was added. Under the conditions
of the present experiment, the added levels of fluoride neither influ--
enced the morphological aspects of the ovaries, nor initiation of egg
production. According to Said et al. (1), Leghorn hens came into produc-
tion 8-11 days later than controls following the addition of 648 ppm flu-
oride to their ration, starting from the fifth week of age. Several pa-
rameters of reproductive performance of Leghorn hens were not influenced
by adding 100 ppm fluoride to their drinking water (13). Response of
chickens to added fluoride, may be due to breed differences. In mammal-
ian laboratory animals, namely adult female mice, atretic follicles and
degenerating oocysts were manifest in the ovaries after consuming rations
supplemented with 250 and 500 ppm of fluoride for four weeks (14).

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FLUORIDE IN THE GROUNDWATER OF SELECTED LOCALITIES
IN THE DISTRICT OF COTTBUS

by

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SUMMARY: In a survey of the fluoride content of
groundwater deposits in the Cottbus district, areas
were selected with respect to emission loading by
brown coal power plants. From the typical hydroge-
ological formation of a glacial scene, the natural
fluoride content in the groundwater will be present-
ed. The depth of the groundwater level as well as
the anthropogenic influences on the fluoride level
is taken into consideration. In spite of consider-
able amounts of fluoride in the environment of the
Cottbus district, the average fluoride content in
the groundwater-bearing formation is actually rela-
tively low.

KEY WORDS: F^- in groundwater (Cottbus); Cottbus, F^- in

F^- in Cottbus Groundwater

Introduction

The fluoride content of groundwater deposits in selected areas of the Cottbus district were studied. The areas which were selected were correlated with atmospheric measurements related to emission loading by brown coal power plants. The three main areas were between 600 and 1000 square kilometers:

1. Area I,which is predominantly agricultural with comparatively little air pollution by fluoride,is the so-called "area of comparison."
2. In Areas II and III,fluoride emissions were comparatively high.

We endeavored to answer the following questions:

1. What is the maximum natural fluoride level in groundwater-bearing formations?

2. Are there anthropogenic influences on the fluoride level and, along the same line, differences in relation between the named areas and the depth of groundwater level?

The findings were divided into four categories of fluoride concentration.

According to Table 1,about 80% of the groundwater samples showed less than 0.2 mg F/1. This fact is based on the typical hydrogeological formations of a glacial fashioned scene throughout the Cottbus district.

Table 1

Distribution of F^- in Groundwater

Area	<0.1 mg/1	>0.1 – 0.2 mg/1	>0.2 – 0.4 mg/1	>0.4 mg/1
I (393 samples)	51.0%	43.3%	5.6%	1.0%
II (447 samples)	51.0%	30.6%	14.1%	4.3%
III (160 samples)	56.1%	29.7%	10.8%	3.4%
II and III (607 samples)	52.5%	30.5%	13.0%	4.0%

Area I = "area of comparison" (predominantly agricultural)
Area II, III = areas with comparatively higher emission loading of F^-.

A low natural fluoride content in groundwater is characteristic of a sedimentary rock stratification of the subsoil. Contrary to other rock for-

mations, the glacial fashioned sedimentary rocks form layers of sand, gravel, clay, marl and clay marl with low natural fluoride content. Therefore, in the investigated localities, the limit value for the natural fluoride content of groundwater is approximately 0.2 mg/1.

It follows that the fluoride level is probably influenced by anthropogenic factors. Furthermore, generally a distinction can be made between the findings in Area I and those in the other areas. In Area I, the percentage of groundwater samples with more than 0.2 mg/1 fluoride is distinctly lower than in other areas. A correlation between the level of emission versus the atmospheric level of fluoride in the investigated areas and the fluoride content in groundwater could be considered.

Area II showed the highest immission loading of gaseous fluoride compounds. For instance, according to atmospheric test values, the level of fluoride compounds in area II is four to five times higher than in area I. For this reason area I has been designated "area of comparison."

Furthermore, in all localities, intensive agricultural cultivation of the soil can be a fundamental reason for entry of fluoride ions in groundwater. Fluoride is chemically bound in phosphate fertilizers and is time-dependent for being released into the soil as water-soluble fluoride ion. Thus the excess in natural fluoride content in groundwater of area I could be explained by application of phosphate fertilizers. In particular, by comparing the sum total of researched towns or villages with the number of them with fluoride values above 0.2 mg/1, it can be shown that this difference between area I and the other areas is due to anthropogenic influences.

Table 2 shows that the maximum percentage of towns or villages with fluoride above 0.2 mg/1 are in area II; lowest percentage in area I. Furthermore it can be pointed out that, in area II in particular, the distribution of fluoride content in groundwater is significantly shifted to the higher values in comparison, for instance with an average in the Cottbus district (Table 3).

Table 2

Towns/Villages with 0.2 mg F/1

Area	Number Researched	Number with >0.2 mg F/1
I	61	19(=31%)
II	63	35(=56%)
III	28	13(=47%)

It is typical for towns or villages where the fluoride was more than 0.2 mg/1 that the groundwater samples came from shallow wells. In this connection the depth of groundwater level must be taken into consideration:

F⁻ in Cottbus Groundwater

Table 3

Percentage of Towns or Villages with
Various Levels of F⁻ in Groundwater

mg F/l	Area II	Average in the Cottbus District
≤0.1 mg/l	30.8%	48.7%
>0.1-0.2 mg/l	31.7%	39.2%
>0.2-0.4 mg/l	28.8%	9.5%
>0.4 mg/l	8.7%	2.6%

It is characteristic for area II that the most shallow water-bearing formation is less than one or two meters underground. In comparison with area I, the four or five times higher loading by gaseous fluoride compounds in connection with the low depth of groundwater and a relatively high permeability of the sedimentary rock stratum for water and fluoride ions would explain the results in area II. Consequently, generally speaking, there is a relation between the natural fluoride content, the anthropogenic influenced fluoride content of groundwater, the depth of groundwater level and the depth of well-filters. A comparison of the fluoride distribution in groundwater from deep and shallow wells makes this fact clear (Tables 4 and 5). Water samples from central water supply plants represent groundwater from wells which as a rule are between 15 and 20 meters deep.

Table 4

F⁻ in Raw Water of Central Supply Plants
(Deep Well Systems)

Area	<0.1 mg/l	>0.1 - 0.2 mg/l	>0.2 - 0.4 mg/l	>0.4 mg/l
I (132 samples)	25.0%	70.4%	3.8%	0.8%
II (55 samples)	25.5%	61.8%	12.7%	-
III (62 samples)	37.1%	58.1%	4.8%	-
Total I - III	28.1%	65.5%	6.0%	0.4%

The following can be pointed out:

As the depth of the groundwater level increases the respective depth of well-filters is connected with a slight increase in natural fluoride content. This observation has already been described in the literature(5, 6) for glacial fashioned hydrogeological formations.

Table 5

F⁻ in Raw Water of Small Supply Plants
(Predominantly Shallow Wells)

Area	≤0.1 mg/l	>0.1 – 0.2 mg/l	>0.2 – 0.4 mg/l	>0.4 mg/l
I (274 samples)	57.3%	34.3%	6.2%	2.2%
II (413 samples)	52.3%	29.7%	13.6%	4.4%
III (114 samples)	57.0%	27.2%	11.4%	4.4%
Total I – III	54.7%	30.9%	10.8%	3.6%

Therefore the deep well systems have a significant maximum in the concentration range of >0.1 mg/l to 0.2 mg/l. Nevertheless, on an average, the concentration range from 0.2 mg/l upwards is, in comparison with shallow wells, relative low. In general, shallow wells which are fitted with well-filters at a water-bearing depth of less than 10 meters are typically private wells for small water supply plants. On the other hand, water samples from deep well systems can be given a significant maximum in the concentration range equal to and less than 0.1 mg/l fluoride.

However, the concentrations from 0.2 mg F/l upwards are, in comparison with deep well systems, significantly higher. This result can be attributed solely to a greater efficacy of the anthropogenic factors.

The above reported findings must be considered preliminary.

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LEVELS OF MUSCLE AND LIVER TISSUE ENZYMES IN
CHANNA PUNCTATUS BLOCH EXPOSED TO NAF

by

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SUMMARY: The effect of NaF (10 ppm, LC50) on the
muscle and liver tissue enzymes of Channa punctatus
(Bloch) was studied at room temperature: 30.22±0.54°C.
and at 15°C.

Liver GDH and LDH decreased at both temperatures.
Increased activity of AchE, GOT and GPT in both liver
and muscle homogenates were significant. The results
are discussed in light of substrate concentration and
metabolic activity.

KEY WORDS: Channa punctatus, F^- in liver enzymes; Channa punctatus,
F^- in muscle enzymes; NaF, effect on Channa punctatus

Introduction

Laboratory experiments on the toxic effects of chemicals reflect the
physiological adjustment of the animals under stress, particularly their
enzyme mechanism of both exogeneous or endogeneous origin. Studies on
the effect of NaF leading to variation in enzymes have been performed by
many investigators on different animals (1-4). Studies on the fish popu-
lation are limited, especially concerning Channa punctatus (Bloch)(5,6).

The present work is undertaken to study enzyme variations in tissues
of Channa punctatus (Bloch) given 10 ppm NaF treatment at room tempera-
ture and at 15°C.

Material and Methods

Fish were acclimatized to laboratory conditions as described earlier
(5, 6), exposed to 10 ppm NaF (LC 50 concentration) for one week and sac-
rificed on the eighth day both at room temperature 30.22±0.54°C. and at
15°C. The mean weight of the fish was 102.43±2.93 gms, 118.80±4.23 gms
and their length 19.90±0.44 cms, and 20.42±0.68 cms respectively. Control
samples also were maintained simultaneously.

Liver and muscle tissues were removed from the fish in cold condition
and 10% homogenate was prepared using 0.05M sucrose. The homogenates were
processed to estimate the different enzyme levels, i.e. LDH (Lactate dehy-
drogenase, L-lactate NAD oxidoreductase E C 1. 1. 2 7 (7), SDH(Succinate
acceptor oxidoreductase E C 1. 3. 9 9) and GDH (Glutamate dehydrogenase
NDA (P), oxidoreductase E C 1. 4. 1. 3.) (7), Transaminases (Glutamate py-

Table 1

Effect of NaF on Tissue Enzymes at Room Temperature

Parameter with Tissue	Control (10 Fish) Mean	SE	Acclimated (10 Fish) Mean	SE	t value	d.f.
Muscle						
AchE	0.039	0.011	0.101	0.02	2.31	20
GOT	159.83	41.13	597.22	65.74	5.38**	20
GPT	287.60	65.33	331.46	30.80	0.65	20
SDH	0.045	0.009	0.101	0.02	2.36	20
GDH	0.016	0.002	0.042	0.01	1.55	20
LDH	0.019	0.006	0.056	0.03	3.50**	20
Protein mg/gm tissue	31.18	6.46	35.83	2.49	0.71	20
Liver						
AchE	0.025	0.008	0.026	0.008	0.06	20
GOT	145.38	23.90	399.03	67.44	-3.29**	20
GPT	276.91	36.52	346.93	60.77	0.95	20
SDH	0.085	0.01	0.217	0.01	1.49	20
GDH	0.085	0.01	0.053	0.01	1.24	20
LDH	0.088	0.016	0.065	0.004	1.02	20
Protein mg/gm tissue	50.79	2.55	41.79	8.31	0.95	20

Table 2

Effect of NaF on Tissue Enzymes at 15°C

Parameter with Tissue	Control (10 Fish) Mean	SE	Acclimated (10 Fish) Mean	SE	t value	d.f.
Muscle						
AchE	0.016	0.003	0.035	0.02	5.24**	20
GOT	125.52	13.40	169.37	13.09	3.1**	20
GPT	117.82	16.87	232.34	19.60	4.43**	20
SDH	0.025	0.002	0.145	0.05	2.07	20
GDH	0.028	0.009	0.146	0.03	3.90**	20
LDH	0.024	0.008	0.160	0.021	5.25**	20
Protein mg/gm	33.23	4.02	38.88	3.18	-1.102	20
Liver						
AchE	0.016	0.001	0.030	0.003	4.42**	20
GOT	135.46	10.47	500.44	124.09	2.93**	20
GPT	213.95	23.03	208.32	45.85	0.11	20
SDH	0.830	0.031	0.075	0.01	0.244	20
GDH	0.028	0.007	0.057	0.04	0.076	20
LDH	0.055	0.014	0.009	0.002	3.23**	20
Protein mg/gm tissue	50.15	3.96	48.87	4.89	0.26	20

SE = Standard Error;   d.f. = Degrees of freedom;   SDH, GDH and LDH = Moles of Formazen/mg protein/hr.;
GOT, GPT and AchE = IU/hr/mg protein.

ruvate transminases GPT: <u>E</u> <u>C</u> <u>2</u>. <u>6</u>. <u>1</u>. <u>2</u>.and Glutamate transaminase GOT
<u>E</u> <u>C</u> <u>2</u>.<u>6</u>.<u>1</u>. <u>1</u>.) (8), Cholinesterase (<u>E</u> <u>C</u> <u>3</u>.<u>1</u>.<u>1</u>.<u>7</u>.)(9) and total protein(10).

Results

The results are presented in Tables 1 and 2. The muscle homogenate
showed an increasing trend for AchE (Cholinesterase), SDH, GDH, LDH, GOT,
GPT and amount of total protein on treatment with sodium fluoride compared
to controls. The increase was significantly high in the GOT activity at
room temperature (Table 1) and AchE activity at 15°C. (Table 2). Whereas
in the case of liver homogenate an increase was observed in AchE, GOT,GPT,
and SDH levels at room temperature (Table 1), at 15°C. elevation was ob-
served in the levels of AchE, GOT, GPT (Table 2) and in the levels of GDH,
LDH and total protein at room temperature (Table 1). The values for GPT,
SDH, LDH and protein content decreased in comparison to controls (Table 2),
the decrease in LDH levels were statistically significant.

Discussion

Cellular malfunction and high levels of serum enzymes are usually as-
sociated with damage or death of the cell (12). When there is malfunction
in cell activity, the function of cell membrane is disturbed and as a re-
sult the enzyme flows into the serum, and levels of enzymes in serum are
high.

Slater and Bonner (1) observed that fluoride and phosphate separate-
ly exert a weak inhibitory effect on succinic dehydrogenase competing with
succinate for the enzyme. Low levels of LDH, GDH observed during the pre-
sent study in liver tissue at both temperatures suggest an indirect effect
of fluoride on these enzymes by way of blocking the supply of substrates.
Increased activity of AchE, GOT, and GPT in both liver and muscle homog-
enates and GPT, SDH, GDH and LDH in muscle tissue supports the elevated
amino acid metabolism; the possibility of increased gluconeogenesis lead-
ing to hyperglycemia during fluoride toxicity. The high elvels of enzyme
observed in the present study also indicate the energy compensation through
their activity since, under sodium fluoride treatment, oxygen consumption
is decreased resulting in a high metabolism rate (5, 6).

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COMPARISON OF METHODS USED TO ESTIMATE F⁻ IN PLANTS

by

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SUMMARY: The aim of this paper is to evaluate some
selected methods being employed to fix fluoride in
biological material.

KEY WORDS: Fluoride in plants; Analysis of fluoride; Comparison of
           methods.

Introduction

The participants at the 1979 Polish Symposium in Szczecin concerning
the metabolism of fluoride (1), discussed methods to estimate fluoride in
biological material. Different methods yield different values which makes
it difficult, often impossible to compare results. Some laboratories, es-
pecially small ones, do not have at their disposal, the fluoride selective
electrode which is recommended equipment in such quantitative analyses.

More or less popular methods to estimate fluoride described in the
literature are gravimetric, titration, colorimetric, nephelometric, flu-
orimetric, spectrographic, polarographic, amperometric, conductometric,
enzymatic, catalytic and radiochemical. In the following, five of these
methods will be assessed for their usefulness in laboratory work. ·

Material and Methods

In our experiments, the criterion was the sensitiveness in detecting fluoride in solution; in other words, the smallest quantity possible to be estimated in moles per dcm^3. We compared the following methods: 1) ion selective electrode; 2) colorimetric method using $Th/NO_3/_4$ - Alizarin S (2); 3) enzymatic method; 4) colorimetric method employing Fe/III - sulphosalicylic acid (3,4); 5) titration method resorting to $Th/NO_3/_4$ - Alizarin S (5,6).

NaF was used to prepare the standard solution for the calibration curve.   Peas were employed for comparing fluoride concentration in biological material measured by the above-mentioned method.   The sample of pea was ground and then alkalized with aqueous suspension of CaO (7), digested at 105°C and distilled as previously described (8).   With the enzymatic method, we exploited the fact that fluoride inhibits the activity of nonspecific esterase (enzyme classification EC 3.1.1.1).   The enzyme was prepared from a horse liver according to the method reported by Connors (9), 0.36 mg of enzymatic protein was incubated for 1 hour at 30°C in phosphate buffer pH 8.0.   The enzyme substrate was provided by ethyl butyrate.   After the enzymatic hydrolysis of ethyl butyrate in the presence of a different amount of fluoride, the released butyric acid was titrated by using 0.05 M sodium hydroxide, up to pH = 8.2.   From the amount of released butyric acid the grade of the esterase inhibition was calculated which is proportionate to fluoride concentration in the investigated solution.

Results and Discussion

Fig. 1 shows the range of methods employed in fluoride concentration whereas Table 1 presents the results achieved at the determination of fluoride in the pea by the stipulated methods.

Table 1

Results of Determination of Fluoride in Pea

Method	F⁻ ppm
Ion selective electrode	14.87
Colorimetric method using $Th/NO_3/_4$	15.96
Method using nonspecific esterase	19.14
Colorimetric method using Fe(III) sulphosalicylic acid	18.07
Titration method using $Th/NO_3/_4$ - Alizarin S	15.06

Figure 1

Ranges of Methods Employed
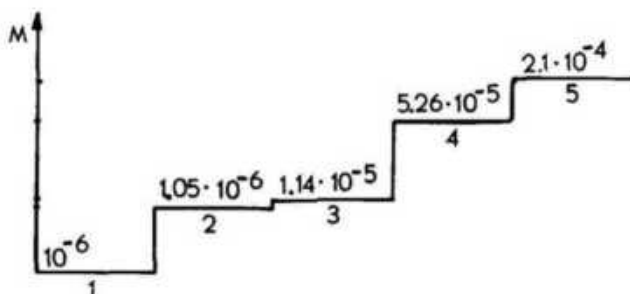
1. Ion – selective electrode
2. Colorimetric method using $Th(NO_3)_4$ – Alizarin S
3. Method using nonspecific esterase
4. Colorimetric method using $Fe(III)$ – sulphosalicylic acid
5. Titration method using $Th(NO_3)_4$ – Alizarin S

It is well-known in biochemistry that the enzymatic methods claim to be very sensitive. In this experiment, the enzymatic method fails to prove the rectilinear dependence below 10^{-5} M fluoride concentration. The ion selective fluoride electrode surpasses the enzymatic methods in the quantitative analysis of F^-. The ion electrode is more sensitive, more handy, faster and offers a large range of measurement with respect to all methods tested in this work, including the enzymatic one.

The chemical methods tested and recorded in this report show, in comparison with the ion electrode, a higher level of fluoride concentration: the titration method of about 1.3%, colorimetric methods with Th/NO3/4 plus Alizarin of approximately 7.3%, and colorimetric method with Th/NO3/4 plus Fe/III about 21.5% and the enzymatic method up to 28.7%. Among the chemical methods worthy of attention are titration and colorimetric, wherein Th/NO3/4 with Alizarin S was used. These 2 methods are relatively simple, fast and produce approximate as well as reproducible results.

Peas were selected for fluoride testing level in biological material because they are known to have a high level of fluoride (10).

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PHARMACOKINETIC INVESTIGATIONS AFTER APPLICATION OF SODIUM FLUORIDE TO CHRONICALLY EXPOSED PERSONS

by

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SUMMARY: Coated and uncoated sodium fluoride tablets were given in single doses to normal subjects and to those exposed to fluoride for prolonged periods. Twenty-four plasma profiles and 4-day urinary recovery rates were determined by means of a fluorine sensitive electrode. Significant differences were observed between the various preparations and the two groups of persons. For balance studies, the combination of plasma and urinary fluoride assays are the most useful.

KEY WORDS: Fluoride tablets, coated, uncoated; Plasma fluoride; Urinary fluoride

Introduction

During the past decade several authors have carried out pharmacokinetic investigations after application of fluorides. In most of them, how-

ever, uncoated sodium fluoride tablets were used (1-4). We proposed to
obtain data on to what extent coating affects absorption, incorporation
and elimination. Therefore we administered to unexposed persons, sodium
fluoride tablets without coating and two different NaF coated preparations
and compared the results with those found in persons chronically exposed
to fluoride.

Material and Methods

Our investigations were performed on 11 persons, 4 males and 7 fe-
males, with chronic fluoride exposure, aged 56 to 74 years (average 68.3).
All had been residing for decades in a district with high fluoride levels,
not of natural origin. Several industrial fluoride emittents formed a
large fluctuating increase of fluoride level in the drinking water and of
atmospheric pollution. In the area under consideration both industrial
(5) and neighborhood fluorosis (6), dust damage in plants (7), fluorosis
in cattle (8) and dental fluorosis in children (9) have been observed. Six
persons without any fluoride exposure (2 male, 4 female) ranging in age
from 30 to 72 years (average 56.5) served as controls.

All subjects were given a single dose of 40 mg uncoated sodium fluo-
ride. Each time, after one week, two more fluoride enteric-coated tablets
were administered in a single dose. Before giving the NaF tablets, basic
fluoride values in plasma and urine had been measured for three days. Fol-
lowing administration of fluoride tablets, fluoride analyses in blood plas-
ma were made after 1, 2, 4, 6, 12, and 24 hours. Simultaneously, fluoride
measurements in 24-hour urine were carried out for four days. Preliminary
investigations had shown that after a single dose of 40 mg NaF the urinary
fluoride excretion remained high for slightly more than 3 days (10). All
fluoride analyses were performed by means of a Czechoslovakian fluoride
sensitive (selective) electrode made by the firm Monokrystaly Turnov.

Results

Blood Plasma: Fluoride plasma levels increased rapidly in normal
persons after administration of uncoated NaF. They reached their maximum
value within two hours followed by a prompt decrease. With coated prep-
arations the plasma concentration increase was distinctly slower but the
value remained at a high level for several hours (Fig. 1). The surface
areas of plasma curves, as an approximate rate of the so-called bioavail-
ability, calculated by the aid of trapezium rule, were found to be signi-
ficantly higher with NaF enteric-coated tablets than with uncoated tablets.

In the subjects with chronic fluoride exposure we found significantly
higher basic values of fluoride in blood plasma (2.6 ± 1.5 $\mu mol/l \triangleq 0.05 mg/l$;
in nonexposed persons (1.1 ± 0.4 $\mu mol/l \triangleq 0.02$ mg/l). After application
of all three NaF preparations, the curves increased more rapidly, top
values were reached earlier and plasma fluoride concentrations were higher
in comparison with the data in nonexposed persons (Fig. 2). The surface
areas in the chronically exposed persons were also higher than in normal
persons after subtraction of different basic values (Table 1).

Figure 1

Plasma Fluoride Levels After Application of Various NaF Preparations
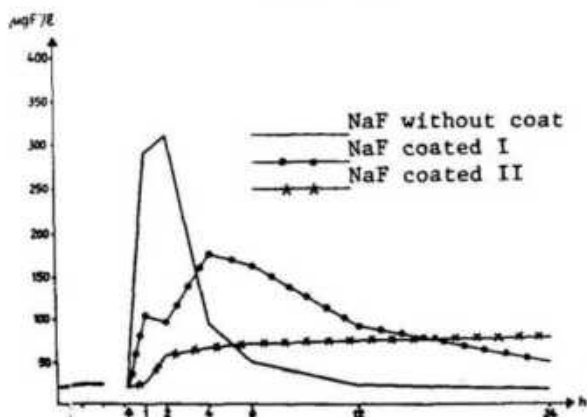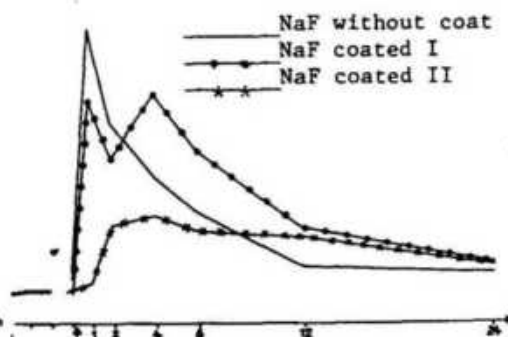
6 Controls

Figure 2

Chronic Fluorine Exposed Persons

Plasma Fluoride Levels After Application of Various NaF Preparations

Figure 1 (left): 6 Controls — NaF without coat, NaF coated I, NaF coated II

Figure 2 (right): Chronic Fluorine Exposed Persons — NaF without coat, NaF coated I, NaF coated II

Table 1

Surface Areas of Plasma Curves (Percentage)

	Normal Persons	Persons Chronically Exposed
NaF uncoated	100	182.7
NaF enteric coated I	203.4	271.5
NaF enteric coated II	130.7	150.9

Uncoated NaF is promptly absorbed and the plasma elimination takes place in both test groups within the first 6 hours according to an e-function. On half-logarithmic paper, the curves are straight lines of various declination. The half-time in normal persons consists of 1.7 hours. In chronic fluoride exposed persons, we found a higher half-time of 3.3 hours. Applied coated sodium fluoride did not show an e-function due to the multiple factors.

Urine: Urinary fluoride excretion is highest in normal persons after administration of uncoated NaF and reaches the maximum value on the second day after exposure. With the two coated preparations, the urinary fluoride excretion is lower and the maximum values are reached a little later (Fig. 3).

Figure 3   Figure 4

Fluoride Levels in 24-Hour Urine After Application of
Various Fluoride Preparations
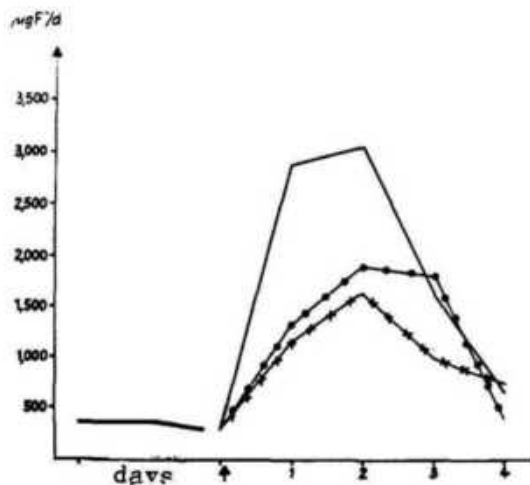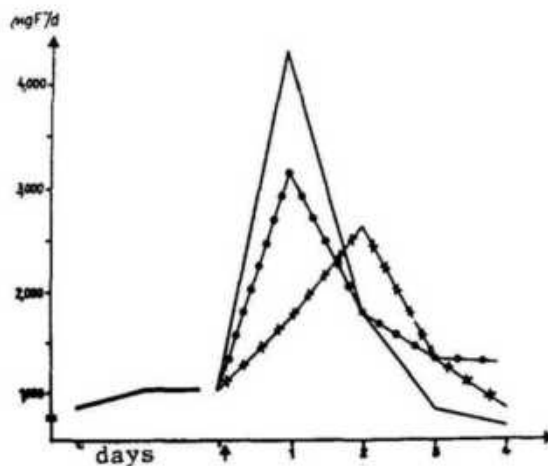6 Controls   Chronic Fluorine Exposed Persons

——————— NaF without coat
•—•—•— NaF coated I
——*— NaF coated II

In chronically exposed persons, the basic elimination is significant-
ly higher and consists of 51.6 ± 23.5 µmol/24h ≙ 1.1 mg/24 h (normal per-
sons: 19.2 ± 6.4 µmol/24 h ≙ 0.3 mg/24 h). After intake of sodium fluo-
ride, the major portion has been eliminated in urine on the first day
(Fig. 4.)

The 4-day recovery rate in urine, calculated by the daily fluoride
elimination over 4 days after subtraction of the basic excretions. shows
how much of the applied fluoride is eliminated by the kidneys. Table 2
shows that the urine recovery rate is higher in uncoated NaF than in coat-
ed preparations. Calculating the percentage of daily fluoride elimination
(Table 3), the main elimination in fluoride-exposed persons takes place on
the first day, shifting, in normal persons, to the 2nd or 3rd day.

Pharmacokinetic Investigations

Table 2

4-Day Recovery Rate of Fluoride in Urine Related
to the Applied Amount (in Percent)

	Normal Persons	Persons Chronically Exposed
NaF uncoated	37.4	22.2
NaF enteric coated I	23.7	22.5
NaF enteric coated II	18.2	12.5

Table 3

Daily Percentage of Fluoride Elimination in Urine

	Day	Normal Persons	Persons Chronically Exposed
NaF uncoated	1st	37.7	77.3
	2nd	39.6	23.4
	3rd	18.4	2.1
	4th	4.8	0
NaF enteric coated I	1st	22.8	55.6
	2nd	35.9	22.0
	3rd	34.1	12.2
	4th	7.1	10.1
NaF enteric coated II	1st	28.7	36.9
	2nd	40.0	40.1
	3rd	19.3	21.8
	4th	12.0	0.2

Discussion

After application of uncoated and coated fluoride preparations in
normal persons we found, in spite of similar amounts of NaF, different
plasma concentrations and elimination ratios. The curves after administra-
tion of uncoated sodium fluoride preparations correspond essentially to
those of other authors (1,2,10,11). According to the prompt absorption and
the intensive renal elimination which immediately follows, the plasma curve
shows a narrow-based peak with relatively low surface area. The enteric-
coated preparations yield, after slower absorption, delayed plasma value
increases and the surface areas lay above that in uncoated NaF. This fact
points to a higher bioavailability of enteric-coated preparations. On the
other hand, the elimination ratios in coated tablets are in agreement with
the literature (12), in spite of higher surface areas lower than in uncoat-

ed preparations. Thus one may conclude that administration of an uncoated preparation, because of rapid absorption, is followed by very high plasma concentrations and a high renal concentration gradient. Therefore, the result is an extensive elimination and - according to the only short-term increase of plasma fluoride value - a low incorporation ratio into the skeleton. On the other hand, coating causes not only better stomach compatibility but also a more continuous absorption. In our opinion respecting these preparations, lower renal elimination with better bioavailability does not point to reduced intestinal absorption but probably to a higher fluoride incorporation ratio into the skeleton. The plasma concentration as well as the time factor influence the skeletal installation (13).

Findings regarding persons chronically exposed to fluoride, after additional experimental fluoride administration, are significantly divergent. They differ quantitatively and time-wise in several points from those of normal persons. In the first group, maximum plasma values were reached earlier. Surface areas were distinctly higher after the levelling of the various basic values. Main renal elimination takes place earlier than in normal persons.

One can assume after longterm exposure that the fluoride binding structures (bones) are almost completely filled with fluorides (11) and, therefore, no primary flowing off into these 'stores' can take place. As a result, the concentration increase is steeper, plasma values higher and renal elimination more rapid.

In normal persons.in whose bones the maximum concentration has not been reached,the absorbed fluorides are soon taken up by the skeleton. Therefore,in this case, the surface areas are lower and the maximum plasma concentration as well as the top levels of elimination are reached later. The distinctly different half-times of the decrease in the fluoride level after administration of uncoated NaF favor this concept.

The enteric coated I NaF preparation shows two peaks in the curve of plasma concentrations, according to the tablet's structure. The first peak corresponds to the prompt delivery of sodium fluoride from the tablet's outside layer and the second one to the fluoride in the tablet's nucleus (14). The enteric coated NaF II shows the most distinct delay in absorption. The coexistence of delayed absorption and already present elimination involves only low plasma concentrations and later top values in urine.

According to our results, in balance studies, besides 24-hour plasma profiles the measurement of fluoride elimination in urine should be extended during 4 days because renal fluoride elimination of uncoated NaF, especially of enteric-coated preparations, requires longer than 24 hours. In balance studies,plasma level investigations alone are not sufficient for an exact determination.

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IONIC VARIATIONS IN TISSUES OF CHANNA PUNCTATUS BLOCH ON EXPOSURE TO NAF

by

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SUMMARY: Channa Punctatus (Bloch) exhibited the following physiological adjustment by altering its ionic content when treated with 10 ppm sodium fluoride (LC50) at room temperature. 30.22±0.54°C. and at 15°C:

1) Increased metabolic rate, 2) Onset of polyuria, 3) A decrease in the potassium level indicating that the cell integrity is under stress, 4) A marked variation in the ionic content which also suggests kidney dysfunction.

KEY WORDS: Channa punctatus, ionic variations from NaF; Sodium fluoride, ionic effects in Channa punctatus

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Introduction

Fluorine intoxication and ionic variations due to sodium fluoride treatment have been reported in rats (1,2). Such observations in aquatic fauna are very rare except for a few observations on the reproductive phase and on variations in blood parameters (3-6).

This paper describes the osmotic disruption in Channa Punctatus (Bloch) on exposure to 10 ppm sodium fluoride at room temperature and at 15°C through tissue electrolytes.

Material and Methods

Fish obtained from the local fish market and acclimated to laboratory conditions as described earlier (5,6) were exposed to 10 ppm sodium fluoride (LC50) for one week,both at room temperature 30.22±0.54°C,and at 15°C,and sacrificed on the eighth day. The mean weight of the fish both at room temperature and at 15°C was 102.43±2.93 gms, and 118.80±4.23 gms respectively; their length 19.90±0.44 cms and 20.42±0.68 cms respectively. Control samples were also maintained simultaneously under similar conditions. Liver, gill, muscle and kidney tissues were removed under cold conditions. A portion of the tissue was taken and 10% homogenate was prepared using 0.05 M sucrose for the estimation of total protein (7). Tissue ion-content was determined using a flame photometer (Elico Model Cl 20). To estimate the ionic content, 2% homogenate was prepared using deionized water to which one mililitre (1 ml) of 0.1 N hydrochloric acid was added.

Results

At room temperature as well as at 15°C, sodium levels increased in all tissues. Whereas the increase in the sodium level was significant in muscle and gill tissues at room temperature, at 15° it was pronounced solely in liver and kidney tissues. In contrast, potassium levels at both temperatures in all tissues showed a decreasing tendency. Whereas the increase in potassium content was significant in all the tissues at room temperature, at 15°C. it was pronounced solely in muscle tissue.

A decreasing trend was observed in the levels of calcium in muscle and liver at room temperature. Whereas levels in the gill showed a decreasing trend at both temperatures, the decrease in liver tissue was significant. Calcium increased significantly in muscle, liver, and kidney at 15°C. At both temperatures, the total protein content increased in muscle whereas, in the liver, gill and kidney, the protein content decreased.

Discussion

Fish are eminently capable to maintain a constant composition of the fluids that bathe their cells. Under stress, the homeostatic condition is affected mainly due to the change in ionic content of blood fluid as well as tissue cells. The present results show a balanced ratio of sodium potassium. Since Channa punctatus (B.) under stress showed a de-

Table 1

Alteration the Ionic and Protein Contents in Tissues of <u>Channa</u>
<u>Punctatus</u> (Bloch) Exposed to NaF at Room Temperature

Parameter with Tissues	Control (10 fish)		Acclimated (10 fish)			
	Mean	SE	Mean	SE	t value	d.f.
Sodium Meq/lit/gm						
Muscle	166.61	30.44	253.47	22.65	6.81**	18
Liver	166.60	15.31	254.47	40.65	1.88	18
Gill	170.33	29.38	296.14	34.74	2.68*	18
Kidney	61.52	16.82	256.49	81.15	2.16	18
Potassium Meq/lit/gm						
Muscle	134.55	11.29	29.84	2.83	9.78**	18
Liver	104.97	14.83	23.32	3.40	6.64**	18
Gill	51.79	9.72	18.93	1.89	3.63**	18
Kidney	67.27	5.78	25.17	0.29	5.18**	18
Calcium Meq/lit/gm						
Muscle	8.85	2.23	5.07	0.75	1.72	18
Liver	15.63	4.79	4.86	0.46	2.46	18
Gill	7.79	1.16	4.42	1.01	2.19	18
Kidney	7.06	0.63	11.36	4.32	0.89	18
Protein mg/gm tissue						
Muscle	31.18	6.46	35.83	2.49	0.71	18
Liver	50.79	2.55	41.79	8.31	0.95	18
Gill	47.17	2.73	38.51	9.77	0.78	18
Kidney	62.34	5.18	57.73	7.73	0.47	18

SE = Standard error; d.f. = Degrees of freedom

Table 2

Alterations in the Ionic and Protein Contents in Tissues of <u>Channa</u>
<u>Punctatus</u> (Bloch) Exposed to NaF at 15°C

Parameter with Tissue	Control (10 fish)		Acclimated (10 fish)			
	Mean	SE	Mean	SE	t value	d.f.
Sodium Meq/lit/gm						
Muscle	137.83	40.75	231.59	45.99	1.59	18
Liver	80.73	0.90	182.19	13.53	7.48**	18
Gill	121.00	1.94	182.74	61.32	1.00	18
Kidney	96.52	10.82	198.49	31.24	3.10**	18
Potassium Meq/lit/gm						
Muscle	177.60	14.46	79.67	13.18	5.01**	18
Liver	137.19	29.12	102.87	23.55	0.92	18
Gill	137.00	21.77	96.48	9.38	1.71	18
Kidney	188.50	19.13	104.91	39.58	1.90	18
Calcium Meq/lit/gm						
Muscle	3.14	0.42	4.98	0.49	2.87**	18
Liver	3.14	0.41	5.82	1.36	3.38**	18
Gill	13.47	3.90	7.06	3.05	1.29	18
Kidney	3.03	0.69	8.33	1.62	3.01**	18
Protein mg/gm tissue						
Muscle	33.23	4.02	38.88	3.18	-1.10	18
Liver	50.15	3.96	48.87	4.89	0.26	18
Gill	52.22	7.14	48.42	8.60	0.34	18
Kidney	44.75	3.66	37.68	1.86	1.72	18

SE = Standard error; d.f. = Degrees of freedom

creased oxygen consumption rate and a high rate of metabolism (5), the required energy must have been supplemented through the dissociation of sodium dependent ATpase through the excitation of the sodium pump. A decrease in potassium was observed in fluoride intoxicated rats by Suketa et al. (2) who associated the decrease in the overall energy metabolism with fluoride.

The resulting effect of fluorosis as reported by Kick et al. (1) is polyuria. It is characterized by increased urinary excretion of inorganic phosphate along with calcium, magnesium, potassium and sodium. The loss in the ionic content of the tissues, especially the amount of potassium observed during the present study, is suggestive of the onset of polyuria. The increase in total protein, solely in muscle, clearly shows the energy utilization by protein metabolism since muscle tissue plays an important role in the fish body.

The overall picture of ionic content also suggests the loss in cell membrane integrity under stress with the low level of potassium since cell deterioration is associated with an increased level of potassium. The observed variations also suggest kidney disfunction.

Acknowledgement

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THE EFFECT OF RENAL IMPAIRMENT ON FLUORIDE RETENTION
OF PATIENTS HOSPITALIZED IN A LOW-FLUORIDE COMMUNITY

by

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Kuopio, Finland

Some previous studies indicate extra fluoride retention in human bones caused by severe renal insufficiency. Plasma and serum fluoride concentrations may also be elevated in a fluoridated community. The results from low-fluoride areas are less consistent. The first aim of the present study was thus to test the relation between ionic serum fluoride concentration and renal function in patients with kidney disease in an area where there is only 0.1-0.2 ppm fluoride in the drinking water.

Earlier calculations indicate that a creatinine clearance lower than 16 ml/min induces extra fluoride accumulation. The threshold may be even lower. The second aim was therefore to relate daily fluoride excretion of each individual to his/her renal function capacity to find out at which level of renal impairment fluoride accumulation begins.

Thirdly, the earlier series concerning fluoride retention in renal patients were not properly selected. The present series was divided into two groups according to the place of residence of the patients to test the effect of the place of residence on the results.

The results showed a good linear inverse correlation between individual serum fluoride concentrations and log serum creatinine levels for both city residents and non-resident renal patients. If the serum creatinine levels had not been converted to their logarithms, the regression line would have been curved, as in the previous, less controlled study. Both groups of patients with renal disease had significantly elevated serum fluoride concentrations and excreted distinctly less fluoride than their controls.

The correlation between fluoride excretion in 24 hours and the serum creatinine concentrations indicates that renal insufficiency can quite easily give rise to fluoride retention. When the serum creatinine concentration exceeds 114 μmol/l, the fluoride excretion/24 hours becomes inhibited, especially in city residents. Thus the loss of even half of the renal excretory capacity may result in slight fluoride retention. Finally, the comparison of the results between the city residents and non-residents demonstrates very similar trends in both groups. The findings are very similar to those obtained earlier with the mixed series. It is therefore concluded that the only possible error caused by the material in the previous results is in the mean fluoride concentrations, which might have been slightly lower if the patients had been more carefully selected.

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                                        Author's Abstract

A SUBACUTE FLUORIDE INTOXICATION DURING TREATMENT OF
OSTEOPOROSIS WITH SODIUM FLUORIDE (NAF)

by

Van Kesteren, R.G., Duursma, S.A.*, and Van Der Sluys Veer, J.*
Utrecht, The Netherlands

A 57-year-old female with severe postmenopausal osteoporosis had been
treated unsuccessfully with calcium and nortestosterone. She was then given
sodium fluoride in combination with calcium and dihydrotachysterol. After
three months she complained of excessive thirst, whereupon it was determined
that her blood sodium and chloride levels were slightly elevated (146 and
113 mM, respectively) and her bicarbonate level was reduced (20.5 mM). Al-
though her serum potassium, calcium bilirubin, acid phosphatase, GOT, GPT,
total protein, and glucose levels were normal, her blood alkaline phospha-
tase had increased to 201 U/1, her γ-glutamyl transpeptidase from 11 to 39
U/1, her serum creatinine from 85 to 125 μM, her blood urea to 9.5 mM, and
her serum ionic fluoride from 0.01 μg/ml (0.01 ppm or 0.5 μM) to 0.75 μg/ml
(39 μM) instead of the expected 0.2 to 0.3 μg/ml (10.5 to 16 μM).

These findings suggested renal impairment with excessive retention of
fluoride. It was then discovered that for the last six weeks she had been
receiving an erroneous dose of 225 mg of sodium fluoride/day instead of the
prescribed 75 mg/day. Administration of sodium fluoride was immediately
stopped, and after six weeks the serum concentration had decreased to 0.07
μg/ml (3.7 μM); the serum creatinine, γ-glutamyl transpeptidase, and al-
kaline phosphatase had also decreased to normal. When sodium fluoride
therapy was again resumed, only 30-45 mg/day was required to maintain the
serum fluoride concentration in the desired range of 0.2 to 0.25 μg/ml
without increasing creatinine, γ-glutamyl transpeptidase, or alkaline
phosphatase levels.

The authors suggest that the elevated fluoride intake had temporarily
created renal impairment secondary to dehydration with increased skeletal
retention and reduced excretion of fluoride. They point to "the possible
toxic effects of the use of relatively large doses of fluoride."

KEY WORDS: Fluoride Intoxication, subacute; Osteoporosis, NaF treatment

A.W.B.

Abstracts

A STUDY ON FLUORIDE AS THE SOURCE OF ENDEMIC DIETARY FLUOROSIS IN GUIZHOU, CHINA

by

L. RiBang, T. JianAn, W. WuYi, and W. LiZhen
Beijing, China

The average fluoride content in drinking water of the endemic fluorosis region in GuiZhou is 0.07 ppm which is far below the standard for fluoride in drinking water published in 1976 in China.   We conclude, therefore, that endemic fluorosis in the area arises from fluoride in food ,not from fluoride in local drinking water.

The average fluoride level in corn and chili in the fluorosis region is 46.54 ppm and 466.73 ppm respectively, 9 and 19 times higher than that in healthy regions. Whereas the level of fluoride in other grains and vegetables, in the region where the disease is endemic, is quite close to that in healthy regions, fluoride in corn and chili baked with coal are respectively 9 and 99 times higher than F^- in unbaked corn and chili in the same place within the fluorosis region.

On the basis of these analyses, we conclude that the high fluoride level of corn and chili in the GuiZhou fluorosis region results from baking with coal and that.basically.fluorosis is caused by eating corn and chili baked with coal.

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FLUOROSIS IN A FLUORIDE AREA IN HENAN PROVINCE, CHINA

by

L. RiBang, T. JianAn, W. WuYi, and W. LiZhen
Beijing, China

The current report deals with the formation and distribution of fluorite and the relationship between fluorosis and fluorite in Henan province. The total fluoride content in fluorite was determined to be 68, 675 ppm. In the fluorite areas, fluoride levels in drinking water ranged between 1.60 ppm and 3.50 ppm. Both dissolved and total fluoride in the soil are higher than that in areas with less minerals.

Levels of fluoride in corn and wheat in the fluorite area are res-
pectively 3.60 ppm and 4.75 ppm. These levels are similar to those in
areas where the levels of minerals are lower suggesting that the trans-
mission route of fluoride in a fluorite area is from fluorite rock to
soil to drinking water to the human body, instead of from fluorite rock
to soil to food to human body. Drinking water is, therefore, the key
pathogenic link of fluorosis in a fluorite area.

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MECHANISM OF DEFLUORINATION OF ENFLURANE
IDENTIFICATION OF ORGANIC METABOLITE IN RAT AND MAN

by

Burke, Jr., T.R., Branchflower, R.V., Lees, D.E., and Pohl, L.R.
Bethesda, Maryland

Difluoromethoxydifluoroacetic acid ($CHF_2OCF_2CO_2H$) has been identified
as a metabolite of enflurance (CHF_2OCF_2CHCIF) in rat liver microsomes in
vitro and in human urine by gas chromatographymass spectrometry. The for-
mation of the metabolite in rat liver microsomes was dependent upon the
presence of NADPH and O_2, and was inhibited when SKF 525-A or CO/O_2 (8.2,
v/v) were present in the reaction mixture. When the C-H bonds of the
CHCIF group of enflurane or of the CHCI group of isoflurane ($CHF_2OCHCICF_3$)
were replaced with a C-Cl bond, virtually no fluoride ion was produced
from either derivative in rat liver microsomes. These results indicate
that cytochrome P-450 catalyzes the oxidative dehalogenation of
CHF_2OCF_2CHCIF at its CHCIF group to form $CHF_2OCF_2CO_2H$ and chloride and
fluoride ions. In contrast, the CHF_2 group does not appear to be appre-
ciably susceptible to metabolic oxidative dehalogenation. There results
can be used for the more rational design of new inhalation anesthetics
that would not be appreciably metablized to the potential kidney toxin F^-.

EFFECT OF FLUORIDE ON TISSUE MANGANESE LEVELS IN MICE

M. Singh
New Delhi, India

In view of the fact that manganese is an essential trace element, necessary for optimal growth and normal skeletal development, and a co-factor of many important enzymes of carbohydrate metabolism, manganese levels in liver, kidney and bone (femur) of mice were investigated. The mice were subjected for 16 weeks to levels of fluoride in drinking water ranging from 0 (control) to 200 ppm.

Manganese levels were significantly reduced in liver (in 50, 100, and 200 ppm groups) and in kidney (in 100 and 200 ppm groups). On the other hand, bone manganese levels were significantly elevated following ingestion of 25 ppm fluoride or more.

Abnormalities in cell function and ultrastructure, particularly in-volving mitochondria, are associated with manganese depleted soft tissues and with reduced activities of certain manganese activated enzymes. The results suggest that excessive fluoride ingestion disturbs manganese me-tabolism.

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EFFECT OF FLUORIDE ION ON PROLIFERATION OF VERO CELL LINE CELLS:
GROWTH ACCELERATION BY SODIUM FLUORIDE

by

A. Oguro, N. Koizumi, and K. Horii
Niigata City, Japan

The effect of fluoride ion (NaF) on proliferation was examined in Vero cells derived from kidney tissue of Cercopithecus aethiops. Fluo-ride ion concentrations in the growth medium of 0.1, 1, 10, and 100 ppm were employed in the first experiment and of 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, and 100 ppm in the second. Simplified replicate cultures were prepared and cultivated for about five days. After cultivation, cell growth was estimated by enumerating cell nuclei concentration. A rela-

tionship between cell proliferation and fluoride concentrations in the growth medium was confirmed statistically and details from control and experimental cell growth were investigated.

Cell growth was accelerated by 17-18% with increasing fluoride ion concentrations of 1-10 ppm in the first experiment. Maximum proliferation 22.6% occurred at 2 ppm in the second experiment. Thereafter with increasing fluoride ion concentrations cell growth was reduced. Near 100 ppm cell growth was completely stopped in both experiments.

KEY WORDS: Vero cells from kidney tissue; F^- effect on growth

Authors' Abstract

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AN EPIDEMIOLOGICAL STUDY OF CARIES AND ITS RELATIONSHIP TO FLUORIDE
CONTENT OF DRINKING WATER IN RURAL COMMUNITIES NEAR VARANASI

by

S.K. Ray, S. Ghosh, I.C. Tiwari, et al.
Varanasi, India

(Abstracted in Indian J. Prev. Soc. Med., 12:154-158, 1981)

The study was carried out in Ledhupur and Rustampur villages of Chiraigaon block, among 2117 residents examined for decayed, missing and filled (DMF) teeth, in relation to age, sex, caste, religion, occupation and fluoride content of drinking water. The overall prevalence was 49.1%. Since the DMF prevalence rate increased with age, 90% of residents above 48 years of age had dental caries. Females had a significantly higher rate than males ($P < 0.02$).

The caries rate increased as the fluoride levels rose to 1.1 ppm. Thereafter up to 1.5 ppm, the caries rate declined but rose again above 1.5 ppm. The minimum prevalence (33%) was observed at 0.2-0.3 ppm F^-, whereas the maximum (59%) was at 1.0-1.1 ppm.

KEY WORDS: India, dental caries and drinking water, fluoride in

Reprints: Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

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INSTRUCTIONS TO AUTHORS

"Fluoride", the official journal of the International Society for Fluoride Research (ISFR) is published quarterly (Jan., Apr ., July, Oct.). Its scope is the publication of papers and reports on the biological, chemical, ecological, industrial, toxicological and clinical aspects of inorganic and organic fluoride compounds. Papers presented at the annual ISFR conference are published in "Fluoride". Submission of a paper implies that it presents original investigations and relevant bio-medical observations. Review papers are also accepted.

Preparation of Papers

1.   General - No precise limit is given on the length of the paper; it should be written concisely in English, submitted in two copies, doublespaced with generous margins. Measures are given in metric system.

2.   Title - A concise but informative title should be followed by the name of author(s), the location and state (country) where the research was carried out. The name and address of the institution where the work was done should appear at the bottom of the first page.

3.   Summary - The paper should begin with a brief, factual summary.

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5.   Material and Methods - should be condensed; however if the methodology is new or developed by the author(s) it might be more detailed.

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7.   Discussion should deal with the general conclusions. Reference should be made to other work on the subject with an indication whether the experimental results are in agreement or in disagreement with previous work. In short papers, results and discussion can be stated together.

8.   Bibliography should be arranged according to the order in which the articles are cited in the text (not alphabetically). An example follows:

Fiske, C. H. and Subba Row, Y.: The Colorimetric Determination of Phosphorus. J. Biol Chem., 66: 375-400, 1925.

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