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ABSTRACTS

DISCUSSION

A VIEW OF THE BEIJING CONFERENCE

The XXth Conference of the International Society for Fluoride Research (ISFR), in Beijing, China, was the largest in the history of the Society, being attended by 200 Chinese scientists and almost 100 scientists from 15 other countries. The Conference was co-sponsored by the Chinese Ministry of Health and the World Health Organization (WHO). The latter organization had set certain conditions for its co-sponsorship, including automatic representation at the Conference of WHO nominees. In view of the main theme of the Conference - the wide range of serious health problems caused by widespread fluorosis in China - it was surprising to some of us that the two WHO nominees who attended were from dental research institutions: one from USA and one, representing the International Association for Dental Research, from New Zealand (both competent scientists, however - the latter presented valuable information on defluoridation methods).

Perhaps less surprising, in the circumstances, was the conflicting evidence presented in the various conference papers. One of the WHO-nominated dental scientist's Invitation Lecture at the Conference's opening session, on a study in China supported by a grant from the USA government's National Institute for Dental Research (NIDR), presented results of a study of selected populations indicating that fluoride reduced the risk of dental caries, and that nutritional deficiencies did not alter the detrimental effects of fluoride intake. On the other hand, a large amount of evidence, from India, China, and elsewhere, indicated that nutritional influences have a marked effect on the extent and severity of fluorosis. Another USA NIDR-supported study presented data to "demonstrate that long-term exposure to fluoride in drinking water, even at an elevated level, does not have genotoxic effects in humans." However, other researchers, from China, presented evidence of human genotoxic effects from fluoridated drinking water.

Indeed, a revelation to many at the Conference was the extraordinarily wide range of research into fluorosis conducted by Chinese scientists. Among the detrimental effects of fluoride which they reported, sometimes at relatively low levels of intake, were: bone fragility and slow healing of fractures, cardiovascular disease and early electro-cardiograph abnormalities, cerebro-vascular disease and lowered intelligence, muscular damage and malfunction, chromosomal aberrations and birth defects, and cancer. Death rates associated with many of the above were also significantly higher in some endemic fluorosis areas. Also reported from China were variable synergistic effects of fluoride and aluminum, fluoride and arsenic, and fluoride and selenium. These Chinese reports confirm many earlier findings from India on skeletal and non-skeletal effects of fluoride.

An outstanding contribution was the paper by Gritsan, Miller and Shmalkov on abnormal plant development in the Ukraine. They reported that, among the 16 environmental pollutants studied, fluoride was the most damaging.

A highlight of the Conference was a tour, for the foreign delegates, to an endemic fluorosis area around the city of Langfang, southeast of Beijing. Defluoridation plants in three villages were visited. The warm friendly welcome of local inhabitants, and the disturbing cases of crippling fluorosis presented, made a deep impression on us all. At the last village visited, the schoolchildren, born since the water fluoride concentration had been reduced to 0.8 ppm, paraded in welcome. Their teeth were proudly made available for our inspection. However, every child

I and others examined displayed the bilaterally distributed chalky white opacities along the growth lines of the enamel, which are unmistakably characteristic of dental fluorosis. The reductions in water fluoride concentrations to levels around the "optimal" 1 ppm level - still officially endorsed and recommended by WHO - have brought great improvements, but toxic effects are still clearly evident. At the Conference Professor A K Susheela, of the All-India Institute of Medical Sciences, stressed that the aim should be to reduce fluoride concentrations to the lowest possible level.

At the conclusion of the Conference a "Round Table" meeting of Chinese and foreign experts was convened by the Director of the Endemic Diseases Control Department of the Chinese Ministry of Health.. Again, there was conflicting advice to the Chinese from foreign experts. Professor Susheela from India, supported by other ISFR members, stressed the importance of educational efforts involving the common people, as well as experts from disciplines in addition to dentistry and medicine, to tackle the fluorosis problem. The WHO nominees, on the other hand, tended to play down the value of such an approach. One of them even suggested to the Chinese that in future they turn to organizations other than the ISFR for help with their problems. The fact remains that the International Society for Fluoride Research is the only organization which brings together fluoride researchers and provides a forum for interchange of ideas and findings. As such, it is willing to publish the varying views, and interpretations of data, of its members. But, unlike some other professional organizations, ISFR does not endorse or oppose any political policies concerning fluoride, nor try to influence or persuade its members on such matters. What I and others find disturbing is the possibility that money for research grants could influence research findings toward the interests of donors.

The XXth ISFR Conference in Beijing was a great success and a credit to its organizers. In his opening Conference address the Society's President, Professor Humio Tsunoda of Japan, thanked Professor Cao Shouren and his co-workers on the Organizing Committee for their careful and detailed preparations.

Another co-sponsorship condition of the WHO was: "any document containing a report of the meeting should be reviewed and cleared by WHO before it is circulated in either a restricted or public form." The WHO's conditions were accepted by the Conference's Organizing Committee. This Editorial, however, is not an official report of that Committee, but is a personal view of this editor. Other viewpoints are welcome.

John Colquhoun

[Note: This issue of the Society's journal contains one paper and one abstract presented at the Conference. Other papers and abstracts will appear in future issues.]

NEW ISFR OFFICERS AND FUTURE CONFERENCES

New Officers of the Society are listed inside the front cover. Members are grateful to the retiring President, Professor Humio Tsunoda, for his devoted service and generous financial support. In accordance with Society policy, the retiring First and Second Vice Presidents became the new President and First Vice President respectively. Professor N B K Yoshitake of Japan was elected new Second Vice President. The next Conference will be in Hungary in 1996, and probably in Washington State, USA, in 1998.

FLUORIDE ABSORPTION *IN VITRO* BY THE GASTROINTESTINAL TRACT OF THE RAT

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SUMMARY: Fluoride absorption by the gastrointestinal tract of the rat was studied with the everted sacs. Duodenum, proximal jejunum, distal ileum, and colon absorbed fluoride proportionally to luminal concentration (0.5 - 10 mM), whereas absorption by the stomach was curvilinear and lower than that observed in the intestines. In the stomach and duodenum, a net flux of fluoride directed towards the mucosa was observed when 0.5 mM NaF was initially present on both sides of the everted sacs.

Key words: Fluoride absorption; Gastrointestinal tract; *In vitro*; Rat.

Introduction

Absorption of fluoride by the stomach has been studied *in vivo* in relation to gastric pH and generally measured as the appearance of the halogen in plasma (1,2). A few studies were done on intestinal absorption of fluoride (3,4). Fluoride is thought to be passively transported (4) mainly as HF (2). To our knowledge, a comparative study along the gastrointestinal tract is lacking in the literature. The objective of this paper is to compare net fluoride absorption at different levels of the gut prepared as everted segments. This method, initially introduced by Wilson and Wiseman (5), is also suitable to determine if a substrate could be transported against a chemical gradient.

Material and Methods

Male Sprague-Dawley rats with body weight from 190 to 220 g were purchased from Iffa-Credo (L'Arbresle, France). The animals were fed a standard rat food and tap water *ad libitum*. The food was removed 48 hours prior to experiments.

Transport of fluoride in the rat gastrointestinal tract was studied *in vitro* by the everted gut sacs method described by Wilson and Wiseman (5). The animals were anaesthetized and the entire gut was excised and freed from the mesenteric attachments. Everted sacs were constructed from stomach, duodenum, proximal jejunum, distal ileum, and proximal colon (about 8 cm long). The sacs were precisely filled with 0.5 ml (intestinal and colon segments) or 1.5 ml (stomach) of saline and incubated at 37°C in a buffer saline (pH 7.4). The saline was chosen so as to minimize water absorption; it contained 25 mM NaCl, 5 mM KCl, 10 mM Tris-HCl, 10 mM Tris-base, 20 mM $NaHCO_3$, 140 mM mannitol and varying concentrations of NaF (0.5, 2, and 10 mM) for kinetics study. Next the sacs were incubated in a beaker containing about 200 ml of the same buffer without NaF and continuously oxygenated (95% O_2 + 5% CO_2) for one hour at 37°C. After an incubation period, the sacs were removed, blotted dry, and emptied; the serosal fluid (inner) was taken for volume and fluoride concentration measurements.

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The volume of the mucosal fluid (outer) being large (200 ml) in relation to that of the inner solution (0.5 or 1.5 ml), the mucosal volume may be considered an infinite reservoir, and the appearance of fluoride in the serosal fluid can be directly transformed to equivalents of fluoride transported per hour per gram tissue (wet). To determine whether fluoride could be transported against its gradient, everted duodenum and stomach were incubated in the same conditions as previously described but with 0.5 mM NaF present on both sides of the sacs.

Fluoride present in the serosal fluid was determined by direct potentiometric method (6) with fluoride ion-selective electrodes (Orion Research 96.09.00).

Calculated values of experimental group were expressed as mean ± s.e.m. The significance of the difference between means was determined by analysis of variance (ANOVA). Linear functions were derived by regression analysis and statistical significance determined with ANOVA.

Results

When NaF was present at varying concentrations on the mucosal side, fluoride was absorbed along the entire gastrointestinal tract as shown by the net flux directed towards the serosal compartment of the everted sacs (see Table). In the stomach, net fluoride flux was not linear with respect to external concentration of NaF (departure from linearity with $P < 0.001$), whereas in intestinal segments (duodenum, jejunum, ileum) and colon, the net fluxes were significantly linear ($P < 0.001$). Comparisons between duodenum, jejunum, and ileum did not show any significant difference between the net fluxes, so a pooled regression line could be calculated with a slope equal to 0.17 $\mu Eq.h^{-1}.g^{-1}.mM^{-1}$.

At the three concentrations tested, absorption of fluoride by the stomach was significantly lower than that measured in the other segments of the gut ($P < 0.001$). When the mucosal concentration of NaF was 0.5 mM, absorption of fluoride by the proximal colon was lower than that measured in duodenum, jejunum, and ileum segments ($P < 0.001$).

When 0.5 mM NaF was present on both sides of the everted sac, net fluoride flux was in contrast directed towards the mucosa of the stomach and duodenum (see Figure). The disappearance of fluoride from the serosal fluid increased ($P < 0.001$) from 0.079±0.007 to 0.160±0.013 $\mu Eq\ h^{-1}.g^{-1}$ when the volume of the filling solution was raised from 1.5 to 2 ml, but the rate of removal was practically unchanged (from 11 to 14%). In the everted duodenum filled with 0.5 ml, the rate of disappearance attained 39.6%.

Discussion

The experimental results show that fluoride is absorbed by all parts of the rat gastrointestinal tract when it is present only on the mucosal face. This absorptive capacity of the entire gut explains the relatively low elimination of soluble fluoride in the faeces reported earlier (7).

The linear kinetics of fluoride absorption by small intestine and colon suggests a passive diffusion of fluoride at pH 7.4 through the digestive wall. Absorption of fluoride by epithelial cells is thought to occur mainly in HF form (8-10). When NaF was initially present on both sides of the everted duodenum, net fluoride flux was directed towards the mucosa. The leaky nature of the duodenum (11) may be

TABLE. Fluoride absorption at different levels of the gastrointestinal tract of the rat.

Everted sac	Luminal NaF (mM)		
	0.5	2.0	10.0
Stomach	0.057 ± 0.004	0.258 ± 0.07	0.801 ± 0.049
Duodenum	0.095 ± 0.005[ab]	0.353 ± 0.019[a]	1.814 ± 0.083[a]
	y = 0.181x; r = 0.98		
Jejunum	0.098 ± 0.006[ab]	0.329 ± 0.014[a]	1.548 ± 0.070[a]
	y = 0.152x; r = 0.98		
Ileum	0.086 ± 0.007[ab]	0.357 ± 0.26[a]	1.767 ± 0.070[a]
	y= 0.176x; r = 0.95		
Colon	0.068 ± 0.004[a]	0.320 ± 0.11[a]	1.548 ± 0.069[a]
	y= 0.155x; r= 0.98		

Results are expressed as μEq F^- transported. $h^{-1}.g^{-1}$ tissue (n=8). Data obtained with stomach show a significant deviation to linearity ($P<0.001$). Slopes of regression lines in the other segments are statistically significant ($P<0.001$). [a] significantly different from stomach ($P<0.001$); [b] significantly different from colon ($P<0.001$).

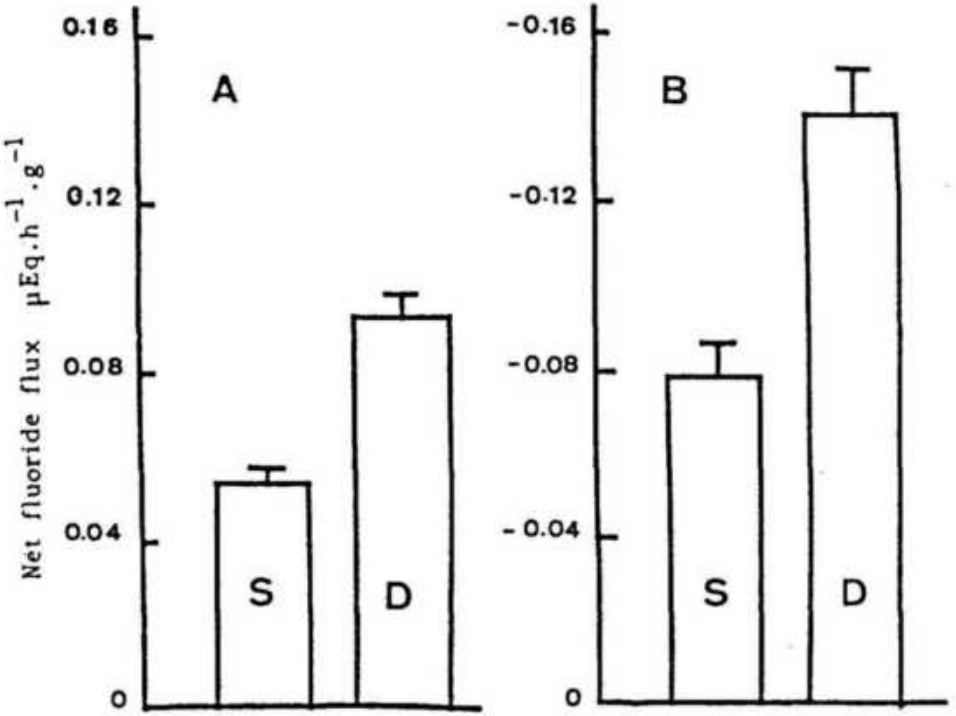

FIGURE. Net fluoride fluxes across the everted stomach (S) and duodenum (D) of the rat. NaF 0.5 mM was present on the luminal side only (A) or on both sides of the sac (B). Serosa to mucosa net flux is indicated by negative values and mucosa to serosa net flux is indicated by positive values (n = 7-8). Handle bar represents one s.e.m.

responsible for a high rate of free fluoride secretion if compared to the stomach; in this context the role of the paracellular pathway merits further investigation.

The kinetics of absorption of fluoride by the everted stomach was not linear in the range of concentrations tested. A secretion of fluoride was observed when NaF was present on both sides of the stomach. The rate of secretion was independent of the level of distension of the stomach or serosal hydrostatic pressure. Secretion of fluoride may explain the nonlinear kinetics of fluoride absorption. At a high concentration (*i.e.* 10 mM), fluoride was transported from the mucosal fluid during the incubation period. When its serosal concentration reached a certain level, a back diffusion took place leading to a noticeable decrease in the net rate of absorption.

In conclusion, fluoride absorption seems to proceed via a passive diffusion. Regional differences were observed between stomach, small intestine, and colon. The nonlinear absorption of fluoride by the stomach may be due to back diffusion of the anion.

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STUDIES ON SURAL NERVE BIOPSIES IN ENDEMIC SKELETAL FLUOROSIS

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SUMMARY: Sural nerve biopsies from 13 patients with radiologically confirmed skeletal fluorosis were studied for myelinated fibre densities, frequency distribution of their diameters, and single teased nerve fibre preparations. It was observed that most of the biopsies showed a marked reduction in myelinated fibre densities with more than half of them involving the smaller fibres of less than 7 μm diameter. Teased fibre measurements of internodal lengths and internodal diameters point to myelinated fibre dropout being due to axonal degeneration with secondary demyelination. The selective loss of small fibres is unlikely to be due to an entrapment neuropathy alone, and possibility of primary toxic injury needs to be considered.

Key words: Fluorosis; Neuropathy; Sural nerve.

Introduction

Neurological changes associated with skeletal fluorosis have been attributed in part to compression radiculomyelopathy (1). There are also reports suggesting anterior horn cell involvement in fluorosis (2,3). Non-skeletal toxic effects in experimental fluorosis in various organs, especially skeletal muscle and spinal cord, have also been studied (4,5). Studies on peripheral nerves in patients with established skeletal fluorosis hardly exist, except one electrophysiological study (6) which confirmed the myopathy being secondary to compression myeloradiculopathy. We therefore felt it necessary to study the peripheral nerve (sural nerve) pathology in patients with radiologically confirmed skeletal fluorosis.

Materials and Methods

Thirteen patients (eleven men and two women) between 20 and 60 years of age, from endemic districts of Andhra Pradesh in Southern India, were included in this study. Their urinary fluoride levels ranged from 3 ppm to 7 ppm. All except one male patient had clinical evidence of cervical cord compression at about C_2-C_4 levels. Lower limbs were apparently unaffected. Minimal and patchy sensory deficits were observed in a few, and the levels corresponded to the levels of cervical cord compression. There was no evidence of pain or muscle weakness in the upper limbs. There was as such no evidence of peripheral nerve entrapment. Electrophysiological aspects unfortunately could not be studied in these patients.

An informed consent was obtained from these patients after the objectives of the study were explained. A sural nerve biopsy at level of the ankle was performed under local xylocaine infiltration anesthesia. There were no significant post biopsy sequelae apart from numbness over the little toe.

The excised nerve was processed in three different ways for study:

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1. Myelinated fibre densities and diameter frequency distribution.
2. Histological changes.
3. Internodal lengths and diameters on teased fibre preparations.

The methodologies used were standard established methods for studying myelinated fibres on paraffin section (7, 8) and single nerve fibre preparations (9). Routine histological stains like Haematoxylin and Eosin, Masson's trichrome, and Holme's stain for axons were used to study qualitative changes (10). Myelinated fibre densities and diameters were measured manually on photomicrographs. Areas were estimated by planimetry while internodal measurements were done with an ocular screw micrometer and mechanical stage vernier. Fibre densities were expressed as fibres per sq. mm cross-sectional endoneural area. Myelinated fibres were classed according to their size as < 1 μm, < 2 μm, *etc* as diameter measurements were made based on the concept of lesser fibre diameter (11). These were grouped into two categories: those with diameters less than 7 μm (small fibres), and those with diameters of 7 μm and more (large fibres). Functionally, these correspond to slow conducting and fast conducting myelinated fibres, respectively (12). The correlation coefficients (r values) were determined to establish the relationship (linear) between internodal lengths and internodal diameters (in 7 subjects).

Results

Myelinated fibre densities were compared with normal values for sural nerve reported in the literature (7), which were also determined by identical methodology (Table 1). This procedure was unavoidable because we could not obtain similar data owing to non-availability of suitable autopsy material.

TABLE 1. Myelinated fibre densities in normal sural nerves (7)

Age	Density in thousands/sq.mm Mean ± SD
17 to 39	6.13 ± 1.11
40 to 59	5.78 ± 0.90
60 to 80	4.78 ± 1.08

O'Sullivan and Swallow, 1968 (7)

Values of myelinated fibre densities in sural nerves as shown in Table 2 ranged from 2,494 to 5,433 fibres per sq. mm endoneural area, which were significantly lower (-2 SD) in 10 out of the 13 cases compared to normal values for age (Table 1). The diameter of these fibres, which normally show a bimodal distribution with equal number of small (<7 μm) and large (>7 μm) fibres, exhibited an unequal distribution with larger fibres constituting 60% or more in six out of thirteen nerves studied (Table 2 and Figure 1). This predominance was found to be statistically significant ($P<0.05$) using a students 't' test for differences in proportion. Only one subject, however, showed predominance of small myelinated fibres. Clusters of axons suggesting regenerating axonal clusters were seen in all cases.

Table 2. Myelinated fibre densities and diameters in sural nerve biopsies from cases of fluorosis.

Patient	Age in years	Mean fibre density**	C.V.	Sample size ***	Myelinated fibres Percentage	
					< 7 µm	> 7 µm
MRIH	40	3451	7.40	433	79.5	20.5
MYSH	45	3427	8.44	518	51.7	48.3
SKMO	56	3422	0.99	397	35.3	64.7
JRMNA	50	2795	2.10	331	33.8	66.2
KSTR	50	4500	3.38	497	47.9	52.1
NRSR	20	3469	9.31	354	37.1	62.9
NRSH	45	2494	23.83	394	43.4	56.6
NRSL	60	3076	22.59	400	34.7	65.3
PPNA	38	2867	22.06	553	46.6	53.4
SBIH	57	5433	3.85	784	49.7	50.3
RMIH	35	3699	9.22	337	23.7	76.3
MLMA*	50	3461	19.88	471	44.4	55.6
MLMA*	40	4168	16.72	644	40.0	60.0

* Female C.V. Coefficient of variation
** Mean of three bundles
*** Number of fibres measured for diameters

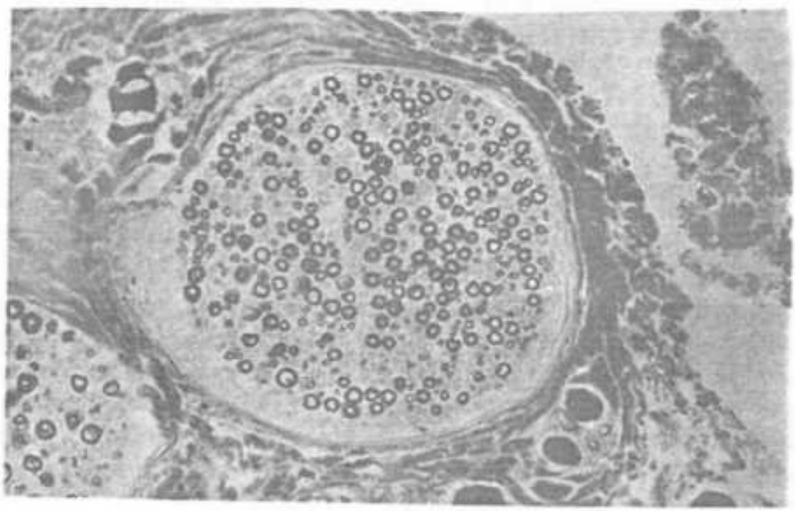

FIGURE 1. Photomicrograph of a sural nerve fascicle showing decreased density of myelinated fibres with prominent large diameter fibres. Kulthchitsky's Haematoxylin x 100.

Teased fibres

The correlation coefficient (r), which is an index of myelin/axon damage in single nerve fibre preparations, ranged between 0.35 and 0.54 which is well below the normal values for any age reported in the literature (9), and indicates damage and repair of myelin and axons over time. There was, in addition, a greater scatter of the individual values resulting in a loss of the linear relationship between internodal lengths and corresponding diameters. Evidence of paranodal demyelination and myelin wrinkling suggestive of axonal atrophy was also evident (Figure 2).

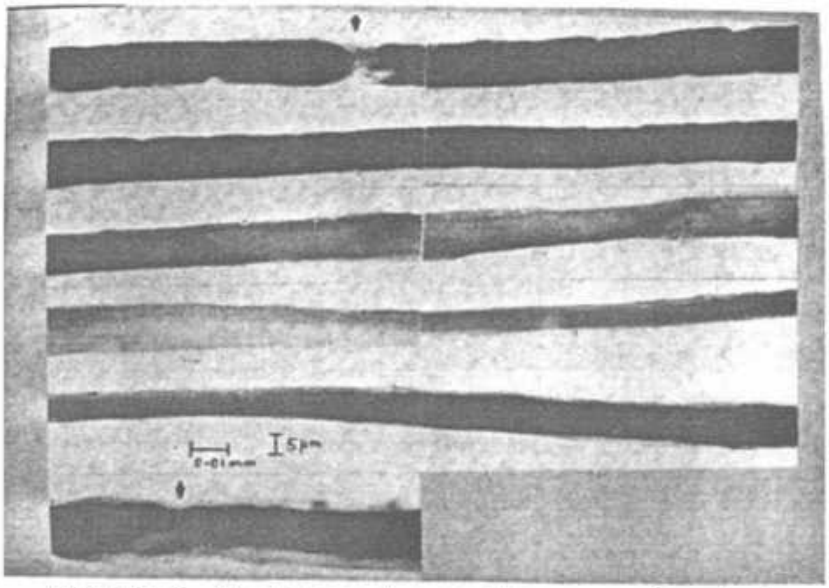

FIGURE 2. Single teased nerve fibre preparation. Arrows indicate Nodes of Ranvier. There is evidence of paranodal demyelination, wrinkling and demyelination in mid-segment.

Discussion

Myelinated fibre densities in the sural nerves were very much reduced, indicating a dropout, probably due to axonal degeneration or demyelination or both. It is, however, unusual that there was a relative sparing of larger fibres, which is not the case in most compression neuropathies.

It is also possible that due to a partial loss of fibres, especially the smaller and slow conducting myelinated fibres with intact larger and fast conducting fibres, no significant reduction in conduction velocities could be observed in earlier electrophysiological studies (6). The present data suggest a fairly selective damage to small myelinated fibres or their neurones due to fluorosis rather than compression neuropathy alone. Toxic neuronal injury due to excessive ingestion of fluoride needs to be considered.

Acknowledgements

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A METHOD FOR ESTIMATING INDIVIDUAL PREDISPOSITION TO OCCUPATIONAL FLUOROSIS

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SUMMARY: The occupational fluorosis risk factors were estimated in a three-stage study among the workers of aluminum and cryolite plants using dermatoglyphics as a genetic marker. This study helped: 1) to establish the existence of genetic predisposition to fluorosis and develop criteria for estimating it, and 2) to prove that predisposition to fluorosis was associated with the same dermatoglyphic features in the workers of both industrial groups. Multifactorial analysis of the set of 15 genetic and non-genetic factors was performed with the help of pattern recognition methods, and demonstrated reliable (90-100%) discrimination between two groups of workers: those who had developed fluorosis and those who had not. Each of the 15 risk factors under study was examined for the degree and the direction of influence. A PC software program was developed in the course of the study, making possible the estimation of individual predisposition to the disease. The method was used to investigate 397 disease-free workers in the electrolysis shop of an aluminum plant. Predisposition to fluorosis was discovered in 22 of them (5.5%).

Key words: Occupational fluorosis; Predisposition.

Introduction

Occupational fluorosis presents a serious problem, particularly in the aluminum and cryolite industries. In Russia this disease is estimated to constitute up to 70% of all occupational diseases in these industries.

The main trend in the prevention of occupational fluorosis is maximum possible elimination of fluorine compounds from the working environment. However, this approach has technological limitations, which make important another, additional, approach: to reduce the risk of this disease, even at high fluoride concentrations, by reducing the susceptibility of personnel to it. The latter approach is supported by the fact that the levels of contamination at the existing aluminum and cryolite plants cause fluorosis in a small proportion of workers. The majority of them retire without any marked specific pathology. The above situation suggests an attractive method for detecting particularly fluorosis-susceptible workers or job applicants, which would permit radical improvement of preventive measures.

Materials and Methods

Earlier we developed a methodology to establish criteria for evaluating individual predisposition to occupational diseases (8), and successfully tested it with reference to various pneumoconioses (5,7,8). It seemed reasonable to undertake a similar study for occupational fluorosis. The subjects were selected among the workers of two cryolite and two aluminum plants located in the Urals (Russia).

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In accordance with the methodology, the study was carried out in three successive stages. The first stage consisted of the complex analysis of all the known risk factors. To this end two cohorts were formed from the workers of the cryolite (cohort 1 = 376 workers) and aluminum (cohort 2 = 412 workers) plants who were working in the main production shops on January 1, 1970, and who had no pathological changes characteristic of fluorosis. For each of them we gathered information on 14 basic risk factors for fluorosis (Table 2).

The cohorts were divided into 2 classes: 1) those who did not develop fluorosis: cohort 1 = 245 persons, cohort 2 = 261 persons. 2) those who developed fluorosis: cohort 1 = 131 workers, cohort 2 = 151 workers.

Multifactorial analysis, with the help of the mathematical method of pattern recognition, was aimed at:

1) reliably describing the differences between the two classes of workers;
2) estimating the degree of influence (informativity) produced by each factor;
3) determining the direction of influence for each factor.

The first objective was achieved using the variant of discriminant analysis known as "training by tutor", which consists of selecting at random a sample of 10-12% of observations from the entire set covering both classes for an "exam". The other observations are used to teach the computer, which then will search for relevant discriminating rules. The quality of the rules found is checked by the percentage of correctly recognized observations in the "exam" sample.

The degree of influence (informativity) of each factor was estimated by measuring the Euclidean distance between the centers of the class under consideration. The direction of influence was determined by the rate of occurrence of the factors in these classes.

All of the above problems were solved with the help of the KVAZAR software package (4) developed at the Institute of Mathematics and Mechanics of the Ural Division of the Russian Academy of Sciences.

At the second stage we studied the role of the genotype in the development of fluorosis, using dermatoglyphics as a genetic marker. This choice was determined by our experience of studying genetic predisposition to silicosis, coronary heart diseases and cancer (5,9,10), and literature data on the use of dermatoglyphics for estimating predisposition to endemic fluorosis (1). Our dermatoglyphic methodology, however, was different from the commonly used one.

First of all we had to eliminate the effect of non-genetic factors found to be most influential at the first stage: 4 factors for the cryolite plants (the duration of exposure to fluorine, time of life in the region of fluorine pollution, occupation, and nationality) and 5 for the aluminum plants (occupation, breaks in occupational contact with fluorine, diseases of locomotor system in personal history, alcohol abuse, and nationality) (11,12).

Secondly we analyzed a set of 59 dermatoglyphic features determined for each individual in accordance with the dermatoglyphic nomenclature (6), also using the pattern recognition method mentioned above.

Based on these principles we selected 60 pairs at the cryolite plants and 90 pairs at the aluminum plants matched for the above factors.

At the final third stage we added one more factor, genetic predisposition, to the 14 factors characterizing each individual included in the cohort. Its alternative values ("no" or "yes") were based on estimates obtained at the second stage of the study. Mathematical treatment was carried out in the same sequence as at the first stage.

Results and Discussion

Stage 1

Since the first stage was essentially a preliminary study, and its results have already been published (11,12), we here summarize the most important findings. The best results of pattern recognition in the "exam" sample were obtained in both studies while taking into account all the 14 factors:

Cohort 1
group without fluorosis = 81.4% of correct answers;
group with fluorosis = 66.6%;
Cohort 2
group without fluorosis = 78.2%;
group with fluorosis = 83.3%.

Each of the 14 factors was estimated for its degree and direction of influence.

The results obtained suggest that this set of factors contains sufficient information to provide a satisfactory description of predisposition to fluorosis. To achieve these results, however, we had to use all the selected factors, which confirms the hypothesis that predisposition to fluorosis depends on a multitude of factors. At the same time the impossibility of obtaining better recognition results on the basis of the available information on the risk factors indicates that the set under study lacks some important factors. These missing factors were assumed to be directly related to the genotype.

Stage 2

At this stage we considered the following questions:

1) Is there genetic predisposition to occupational fluorosis at all?

2) Are there differences in the character of predisposition between the workers of the aluminum and cryolite plants? The second question arose in connection with clinical differences in fluorosis between the workers in these two industries (2).

First we estimated the informativity of each of the 59 dermatoglyphic features. Then all the available information about these features was considered for sufficiency for describing differences in the dermatoglyphic picture of those who had developed fluorosis and those who had not. For the cryolite plant reliable discriminating rules (83% of correct recognitions at the "exam") were obtained using 7-10 most informative features (Table 1) (11).

For the aluminum plants the best results (100% correct answers) were obtained with 10 dermatoglyphic features taken into account.

Table 1. Most informative dermatoglyphic features

No.	Name of feature
1	Width of palm lines on right hand
2	Direction of main palm line D on left hand
3	Ball pattern of finger 1 of right hand
4	Palm ridge count ab of right hand
5	Ball pattern of finger IV of right hand
6	Pattern in zone between fingers IV and V of left hand
7	Palm ridge count cd of left hand
8	Ball pattern of finger I of left hand
9	Direction of main palm line B on left hand
10	Palm ridge count ab on left hand

The direction of influence of each of these features was also found to match in both cohorts. The possibility of obtaining reliable discriminating rules using subsets of 7-10 features only (out of 59) points to significant differences in the dermatoglyphic pattern between workers who had developed occupational fluorosis and those who had not.

Agreement between the results obtained for the workers of the industries under consideration permitted us to discard the hypothesis that there are differences in the nature of genetic predisposition to fluorosis between the workers of the aluminum and cryolite plants. As regards clinical differences between the "cryolite" and "aluminum" fluoroses reported in the literature, this fact may be explained by the action of other "non-fluoride" industrial factors which are different for these industries.

Stage 3

At the final stage we examined each member of the experimental cohorts for the presence or absence of genetic predisposition to fluorosis using the best discriminating rules. Each subject was thus characterized by a set of 15 genetic and non-genetic factors.

First we determined the degree of influence produced by each of these factors. Table 2 shows that the phenotype of predisposition to fluorosis ranks first in both studies. Thus, the contribution of the genetic component is sufficiently high even against the action of many other factors. The features characterizing fluorine exposure rank high as well.

Next we checked whether the information on the above 15 risk factors was sufficient for ensuring the discrimination between patterns of predisposition and non-predisposition to fluorosis. Best recognition of the "exam" patterns was achieved using 14 most informative features for cohort 1 (100% correct answers for both groups) and 13 features for cohort 2 (93.75% of correct answers for those who had not developed fluorosis and 100% for the cases of fluorosis).

Comparison of these results with those obtained at stage 1 demonstrates that the inclusion of information on genetic predisposition ensured higher problem-solving reliability.

Examination of each feature for the nature of its influence clearly demonstrated that in both industries genetically predisposed individuals (as detected dermatoglyphically) are more likely to develop fluorosis.

Predisposition to fluorosis does not mean, however, that this disease is unavoidable. Its selective development in different individuals depends on a particular combination (favorable or unfavorable) of genetic and non-genetic factors. The effect of the group of features characterizing fluorine exposure is very important as well.

Table 2. Significance of various factors for development of fluorosis in workers of cryolite and aluminum industries

Factor	Aluminum		Cryolite	
	Rank	Informativity	Rank	Informativity
Genetic predisposition	1	1.00	1	1.00
Personal small-holding	2	0.654	5	0.251
Duration of living in fluorine-contaminated area before occupational exposure	3	0.584	4	0.256
Duration of occupational exposure to fluorine	4	0.482	2	0.643
Occupation	5	0.316	6	0.123
Smoking	6	0.158	13	0.016
Nationality	7	0.139	10	0.069
Diseases of locomotor system in personal history	8	0.102	9	0.077
Availability of modern housing conditions	9	0.102	3	0.593
Alcohol abuse	10	0.086	12	0.024
Diseases of liver in personal history	11	0.082	15	0.001
Diseases of kidneys in personal history	12	0.055	14	0.004
Breaks in occupational exposure to fluorine	13	0.023	7	0.115
Diseases of endocrine system in personal history	14	0.006	11	0.053
Age at beginning of occupational exposure to fluorine	15	0.002	8	0.098

Analysis of the factor "duration of occupational exposure to fluorine" for the direction of its influence demonstrates a two-phase character of dependence. The risk of fluorosis grows throughout the initial period of exposure from 1 to 15 years, going down afterwards. This can be explained by the accumulation of fluorine in the organism, which increases the probability of developing this disease. During the second phase the most susceptible individuals have to leave the industry, and the remaining workers stay healthy, exhibiting greater resistance despite the continuing exposure, which accounts for the decrease in the risk of developing fluorosis.

The duration of living in a fluorine-contaminated area was observed to have a direct effect on the development of fluorosis, which may be attributed to the exposure to additional fluorine.

All the workers of the cryolite plants were divided professionally into two groups: 1) those working in direct contact with both hydrogen fluoride (HF) and fluorine salts; 2) those exposed predominantly to HF. The first group exhibited a higher probability of developing fluorosis due to their higher exposure to fluorine, and due to the specific toxicokinetic properties of gaseous and aerosol fluorine compounds that are responsible for their retention in the organism.

All the workers in the electrolysis shops at the aluminum plants were divided into three groups: 1) auxiliary personnel; 2) anode workers; and 3) electrolyzers. The risk of fluorosis was found to be highest for the third group and lowest for the first group.

Breaks in the exposure to fluorine reduces the risk of fluorosis, which is in agreement with the findings of other researchers (3).

Social factors contribute greatly to individual predisposition to fluorosis. Thus, smoking, alcohol abuse, unsatisfactory housing conditions and the availability of a personal small-holding increase the risk of fluorosis as the direction of their influence demonstrates it. As regards the last factor in the above list of social factors, we think that its negative role may be explained by additional intake of fluorine compounds from vegetables grown on small holdings located within fluorine-contaminated areas.

Biological factors were found to make an additional contribution to the predisposition to fluorosis. Thus, first occupational exposure at the age of 18-26 accounts for a higher risk of fluorosis than that at the age of 30-40. Young people demonstrate greater susceptibility to toxic effects of fluorine compounds, which is likely to be due to functional immaturity of their protective mechanisms.

Of the two basic ethnic groups inhabiting the Ural region - Russians and Turks - the former demonstrate higher susceptibility to fluorosis.

Thus, studies carried out in two fluorosis-hazardous industries show good agreement as regards the effect of genetic and non-genetic risk factors. This fact proves that the methodology used was adequate to the problem under study.

The discriminating rules elaborated at stages 2 and 3 may be used for detecting fluorosis-susceptible individuals among both employees and applicants.

At the end of 1993 we used this method at one of the aluminum plants to investigate 397 electrolysis workers who did not exhibit any signs of fluorine

intoxication at the time of examination. Of these, 22 workers (5.5%) demonstrated higher individual susceptibility. We are planning to investigate all the workers (about 2000) employed in the electrolysis shops of this plant. A follow-up study of this group will permit us to estimate the actual accuracy of this method in the estimation of individual predisposition to fluorosis.

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INFLUENCE OF ESSENTIAL PHOSPHOLIPIDS (EPL) ON SELECTED BIOCHEMICAL PARAMETERS OF LIPID METABOLISM IN RATS CHRONICALLY EXPOSED TO AMMONIUM FLUORIDE VAPOURS

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SUMMARY: The influence of essential phospholipids (EPL) on selected lipid parameters in rats chronically exposed to ammonium fluoride vapours was studied. The content of total lipids, cholesterol, and triglycerides in serum, and the content of cholesterol and triglycerides in liver homogenate were measured. An advantageous influence of EPL on disorders in lipid metabolism due to ammonium fluoride was found.

Key words: Ammonium fluoride; Essential phospholipids; Fluoride intoxication, Lipid metabolism.

Introduction

Lipid disorders are known to be one of the major risk factors in premature atherosclerosis. They have become an object of interest for multidisciplinary studies since lipids and lipoproteins are important in basic metabolic processes.

Lipid metabolism in the organism may be affected not only by disease processes but also by exogenous factors such as pollution of the environment by fluorine compounds. The effect of fluoride on fatty acid changes can be studied from different aspects, *e.g.*, absorption, bioavailability, oxidation and "de novo" pathway synthesis (1). In turn, essential phospholipids (EPL), through their effects on the activity of key enzymes needed in lipid transformations, are also able to regulate lipid metabolism. Moreover, phospholipids are one of the substrates in the esterification of cholesterol and they take part in the transport of lipids in plasma. Therefore, the decision was made to investigate if, and to what extent, the essential phospholipids (EPL) influence lipid metabolism in animals chronically exposed to ammonium fluoride vapours. Among many fluorine compounds used for animal intoxication, ammonium fluoride was chosen due to its ability to penetrate cells (2).

Materials and Methods

Sixty male Wistar rats of initial 300 g body wight were used in the study. The animals were divided into 6 groups of 10. Essential phospholipids (EPL) (Rhone-Poulenc Rorer, Nattermann Group).

Group I: control.

Group II: received EPL at 30 mg/kg of body weight.

Group III: received EPL at 100 mg/kg of body weight.

Group IV: intoxicated by ammonium fluoride (NH_4F).

Group V: intoxicated by ammonium fluoride and receiving EPL at 30 mg/kg of body weight.

Group VI: intoxicated by ammonium fluoride and receiving EPL at 100 mg/kg of body weight.

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During the entire duration of the study the animals were allowed free access to water and standard type of feed. EPL in definite doses was added to the balls of feed which were given to the animals every day prior to location in the intoxication chamber. Humidity, temperature and ventilation (10 m^3/hr) conditions within the chamber were controlled. Ammonium fluoride was passed into the chamber as an aerosol in a concentration of 2 mg F^-/m^3 of air. The concentration of fluoride ions in the chamber was controlled through the use of an ion-selective fluorine electrode (PN-83/2-04093,07). The rats were exposed for 5 hours daily, 5 days a week for 6 months. The content of total lipids, cholesterol and triglycerides in serum, and the content of cholesterol and triglycerides in liver homogenate were measured.

Results and Discussion

The results of these studies are presented in Tables 1 and 2. In the experiment described it was concluded that the amount of total lipids in rat serum clearly rises after intoxication with NH_4F and falls slightly under the influence of essential phospholipids (EPL) administration. In the exposed groups which were at the same time protected by EPL administration, the total lipid content fell to values obtained in the control.

Cholesterol content in serum rises under the influence of fluoride intoxication and is normalized after EPL administration. Triglycerides content in serum falls under the influence of essential phospholipids in non-exposed animals, and rises significantly after NH_4F exposure. Administration of EPL at 100 mg/kg body weight decreases the content of triglycerides in serum.

Measurement of liver homogenate contents showed decreasing cholesterol after chronic exposure to NH_4F vapours and a normalizing tendency of cholesterol content due to essential phospholipids (EPL). Amounts of triglycerides in liver homogenate decreased after administration of EPL at 100 mg/kg of body weight and increased significantly in exposed animals. Simultaneous administration of NH_4F and EPL at 100 mg/kg body weight returned triglycerides to the initial values.

The observed increase of total lipids in serum under chronic exposure to fluorine compounds has a proven biochemical basis. As mentioned above, fluorine influence on lipid metabolism is complicated. It can be studied under the aspects of bioavailability, metabolism and synthesis. Bioavailability was defined by Ebersdobler (3) as the usage of foodstuffs after digestion and absorption. The bioavailability of fluorides is influenced mainly by their absorption in the digestive tract, which is significantly increased in the presence of food fats (1). This may be indicative of the lipophilic character of the fluoride ion.

The weakening of lipid metabolism by fluorides is due to repression of the activity of a number of enzymes responsible for lipid transformation: triglyceride lipase (4,5), some nonspecific esterases (6,7), and the complete blockage of pyrophosphatase activity (8,9), which causes repression in the oxidation of fatty acids. During chronic exposure to ammonium fluoride vapours, administration of essential phospholipids (EPL) clearly improves biochemical lipid parameters, decreasing the content of total lipids to their initial values. This is consistent with observations by other authors (10). The observations can be explained by the activating influence of

EPL on lipoprotein lipase (LPL) (11) and its liver fraction (HTGL) (12), an enzyme which facilitates lipoprotein uptake from the plasma by the lipid tissue.

On the basis of available publications it is difficult to ascertain a significant increase in the content of cholesterol in serum under the influence of fluoride ions. Administration of EPL normalizes these values. It seems that the cause is the activating action of EPL on lecithin-cholesterol acetyltransferase (LCAT) (13), an enzyme which through esterification of cholesterol regulates its metabolism and circulation. The content of triglycerides in serum decreases under the influence of EPL. The increase of triglyceride levels due to NH_4F is caused, among other reasons, by blockage of triglyceride lipase activity (4, 5).

TABLE 1. Influence of EPL on some lipid parameters in serum of rats chronically exposed to NH_4F vapours

PARAMETERS	Group I $\bar{x} \pm SD$	Group II $\bar{x} \pm SD$	Group III $\bar{x} \pm SD$	Group IV $\bar{x} \pm SD$	Group V $\bar{x} \pm SD$	Group VI $\bar{x} \pm SD$
TOTAL LIPIDS (mg/dL)	270 ± 13,7	263 ± 8.68	256 ± 11.7	333 ± 16.9*	256 ± 17.9	252 ± 8.67
TOTAL CHOLESTEROL (mmol/L)	1.158 ± 0.11	1.410 ± 0.14	1.305 ± 0.17	2.582 ± 0.1*	1.378 ± 0.1	1.462 ± 0.06*
TRIGLYCERIDES (mmol/L)	0.73 ± 0.059	0.62 ± 0.09	0.68 ± 0.06	1.08 ± 0.028*	1.03 ± 0.06*	0.68 ± 0.069

$\bar{x}$ mean value
SD standard deviation
* statistically significant ($p < 0.01$) compared to control

TABLE 2. Influence of EPL on some lipid parameters in liver homogenate in rats chronically exposed to NH_4F vapours

PARAMETERS	Group I $\bar{x} \pm SD$	Group II $\bar{x} \pm SD$	Group III $\bar{x} \pm SD$	Group IV $\bar{x} \pm SD$	Group V $\bar{x} \pm SD$	Group VI $\bar{x} \pm SD$
CHOLESTEROL (mg/L g of tissue)	2.39 ± 0.21	2.18 ± 0.15	2.36 ± 0.17	1.79 ± 0.17*	1.90 ± 0.11*	2.09 ± 0.11
TRIGLYCERIDE (mg/L g of tissue)	12.7 ± 0.95	12.3 ± 0.84	7.0 ± 1.09*	15.3 ± 2.11	15.3 ± 2.11	11.9 ± 1.68

$\bar{x}$ mean value
SD standard deviation
* statistically significant ($p < 0.05$) compared to control

Conclusions

1. Increases in the contents of lipid fractions in serum of animals exposed to ammonium fluoride vapours show disorders in lipid metabolism.
2. Essential phospholipids (EPL) have an advantageous effect in chronic intoxication with ammonium fluoride, normalizing the lipid metabolism.

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TRANSIENT AND REVERSIBLE FLUORIDE TOXICITY IN SOME SOFT TISSUES OF FEMALE MICE

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SUMMARY: The effects of NaF ingestion (10 mg/kg body weight) and possible therapeutic effects of vitamin C (25 mg/animal/day) and/or calcium (25 mg/animal/day) were investigated on some soft tissues of female mice (*Mus musculus*). The decrease in protein levels in liver, muscle (pectoralis and gastrocnemius), and small intestine suggested an alteration in metabolism by fluoride and a possible change in the osmotic balance. The decline in the succinate dehydrogenase activity in muscle elucidates disturbances in oxidative metabolism and contractility. The significant accumulation of glycogen levels in muscle and liver led to decreased glycogen turnover, probably due to the reduction in activity of phosphorylase. The Ca^{++} levels of muscle were elevated, which would create an ionic gradient across the sarcolemma affecting muscle contraction. The Na^{+} and K^{+} levels in the kidney were decreased significantly, indicating an electrolyte imbalance. However, the cholesterol levels in the ventricle were not affected. The administration of ascorbic acid and calcium to NaF-treated mice revealed marked recovery from fluoride toxicity in all above parameters, showing that fluoride toxicity is reversible and transient with ameliorative effects of ascorbic acid and calcium alone and/or in combination. The recovery was more pronounced in the animal group treated with both ascorbic acid and calcium, thus indicating their synergistic action.

Key words: Ascorbic acid; Calcium; Fluoride; Mice; Reversibility; Soft tissues; Synergistic effect; Toxicity.

Introduction

Fluoride toxicity is increasingly becoming a matter of grave concern as many countries have been declared endemic for fluorosis. This makes it imperative for scientists to focus on the precise toxic effects of fluoride on various soft tissues, so that therapeutic agents can be effectively used. The toxicity of fluoride compounds administered orally differs from species to species. Every phase of metabolism can be affected critically by fluoride under certain conditions. Though the mechanism of fluoride toxicity is not known clearly, generally it is shown that fluoride kills in acute poisoning by blocking the metabolism of cells, either by inhibiting the enzyme or by intervening with the nerve impulse. Impairment of organ function is observed, due to cell damage and necrosis.

The non-essential role of fluoride in reproduction was observed by Tao and Suttie (1). NaF treatment to mice, rats, rabbits and guinea pigs caused significant alterations in the structure and function of testis, internal milieu of epididymis, vas deferens, and also affected the morphology and metabolism of their contained spermatozoa (2-12). Human spermatozoa also lost their mobility after 20 minutes incubation *in vitro* with 250 mM of NaF (13). However, there is a paucity of data on effects of fluoride in the females.

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In this study the effects of fluoride on some soft tissues of female mice, and the possible reversibility of the induced toxicity, by ingestion of ascorbic acid (AA) and/or calcium (Ca^{++}) as possible therapeutic agents, were investigated.

Materials and Methods

Healthy, adult female albino mice (*Mus musculus*) of Swiss strain, weighing about 35–45 gm, were housed in animal cages under standard conditions, maintained on standard diet, and given water *ad libitum*.

The animals were divided into five groups. All animals except the controls were subjected to NaF at a dose of 10 mg/kg body weight/day, selected on the basis of the previous work on rodents (2,3) as well as the LD_{50} value of fluoride which is 51.6 mg F^-/kg body weight in female mice (14).

The experimental protocol was as follows:

Experimental Protocol

Group	Treatment and dosage	No of animals	Duration (days)	Day of autopsy
I	Control	15	-	Sacrificed along with NaF treated
II	NaF treated (10 mg/kg body weight)	15	30	31st
III	NaF treatment as in group II, withdrawal after 30 days and from day 31st administration of AA (25 mg/animal/day) for another 30 days	15	30	61st
IV	NaF treatment as in group II, withdrawal after 30 days and from day 31st administration of Ca^{++} (25 mg/animal/day) for another 30 days	15	30	61st
V	NaF treatment as in group II, withdrawal after 30 days and from day 31st administration of AA + Ca^{++} (dosages same as in Groups III and IV)	15	30	61st

At the end of each treatment, the animals were sacrificed by cervical dislocation and the liver, muscle (gastrocnemius and pectoralis), small intestine and kidney were dissected out, blotted free of blood and utilized for the following biochemical estimations:

Protein: The protein levels in liver, muscle and small intestine of control and treated mice were determined by the Biuret method (15) and expressed as mg protein/100 mg fresh tissue weight. Protein present in the tissue homogenate reacts with Biuret reagent to give a bluish-violet colour which can be measured spectrophotometrically at 540 nm.

Phosphorylase: Phosphorylase activity in liver and gastrocnemius muscle of control and treated mice was assayed by the method of Fiske and Subbarow (16) as modified by Cori, Cori and Green (17).

The phosphorylase enzyme catalyses the substrate (glucose-1-phosphate) used under standard assay conditions which brings about the liberation of inorganic phosphorus. This inorganic phosphorus reduces the molybdic acid to phosphomolybdate in the presence of reducing agent, 1:2:4 aminonaphthol sulphonic acid (ANSA) which gives a blue coloured solution. The intensity of the blue colour is directly proportional to the amount of inorganic phosphorus released. The enzyme activity was expressed as mg phosphorus released/100 mg fresh tissue weight/15 minutes.

Glycogen: Glycogen content in tissue was estimated by the method of Seifter *et al* (18). Glycogen is precipitated and converted into glucose which gives a green colour on boiling with anthrone reagent. The intensity of the green colour could be measured on % transmittance scale. The levels were expressed as µg glycogen/100 mg fresh tissue weight.

Succinate dehydrogenase: The succinate dehydrogenase activity in the pectoralis muscle of control and treated animals was assayed by the modified tetrazolium reduction reaction method (19) using INT [2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl tetrazolium] as an electron acceptor. The electrons released by the action of the enzyme from the substrate, sodium succinate, are accepted by the electron acceptor (INT) which is reduced to a red coloured formazan. The resultant formazan was extracted in ethyl acetate and the colour intensity was measured on a 103 colorimeter at 420 nm. The activity was expressed as µg formazan formed/15 minutes/100 mg fresh tissue weight.

Na^+, K^+, and Ca^{++} levels by flame photometry: The Na^+ and K^+ levels in the kidney and Ca^{++} levels in the muscle were estimated by using the Systronics flame photometer, digital type 125, according to the method of Dean (20). The solutions under analysis were sprayed as a fine mist into a nonluminous flame which becomes coloured according to the characteristic emission of the metal. A very narrow band of wavelength corresponding to the element being analysed was selected by a light filter and allowed to fall on a photometer which is a measure of the element obtained on the digital display. The levels were expressed as ppm.

Cholesterol: Cholesterol in the ventricle of control and treated animals was estimated by the method of Pearson *et al* (21). Cholesterol present in the homogenate reacts with acetic anhydride in the presence of sulphuric acid to give a brownish green coloured complex which can be measured colorimetrically. The concentration of cholesterol was expressed as mg/100 mg fresh tissue weight.

Results

Protein: The protein levels in liver, muscle, and small intestine of mice decreased by NaF treatment (group II). The decrease was significant in liver ($p<0.001$) and gastrocnemius muscle and pectoralis muscle ($p<0.001$), respectively. Significant recovery was obtained ($p<0.01$) in each tissue when animals were administered ascorbic acid as compared to calcium (group III and IV), respectively. On the other hand, administration of ascorbic acid and calcium in combination as in group V showed a synergistic effect, and recovery was almost complete as levels were almost same as in control (Table 1).

Glycogen: A significant ($p<0.001$) accumulation in glycogen concentration occurred in both liver and gastrocnemius muscle after NaF treatment. A significant ($p<0.001$) recovery was obtained in glycogen levels after ascorbic acid treatment as compared to calcium administration (groups III and IV), respectively. The recovery was most pronounced in group V in which animals were fed ascorbic acid + calcium (Table 2).

Phosphorylase: A decrease in phosphorylase activity in liver and muscle in group II animals (NaF treated) was observed. The decrease in muscle phosphorylase in NaF treated animals was more significant ($p<0.001$) than in liver phosphorylase ($p<0.01$). The activity of phosphorylase was recovered by AA and calcium treatments (groups III and IV respectively) ($p<0.01$). The combined treatment of AA and calcium resulted in more significant recovery rather than AA and calcium alone in the activity of phosphorylase (liver $p<0.01$; muscle $p<0.001$) (Table 3).

Succinate dehydrogenase (SDH): SDH activity in pectoralis muscle of NaF treated mice decreased significantly ($p<0.001$) as compared to control. Administration of AA alone caused greater recovery than calcium administration. However, combined treatment with AA + calcium resulted in a synergistic effect for the recovery of SDH activity (Table 4).

Na^{+}, K^{+} and Ca^{++} levels: Sodium and potassium levels in kidney decreased after NaF treatment whereas calcium levels in the present study were enhanced in muscle as compared to control ($p<0.001$). However, sodium and potassium levels of animals fed with AA were almost as similar to control as those animals fed with calcium. But combined treatment of AA along with calcium caused much better recovery than individual treatments ($p<0.001$) (Table 5).

Cholesterol: Cholesterol in ventricle was not significantly affected, but a significant decrease ($p<0.001$) occurred in its level in group IV which were treated with calcium during the recovery period (Table 6).

Discussion

The aim of the study was to investigate the effects of fluoride toxicity and its reversal, if any, through ascorbic acid and/or calcium administration on some soft tissues of female mice, *Mus musculus* of Swiss strain, in the light of earlier data.

The results revealed decreases in protein levels which were attributed to impairment of polypeptide chain inhibition (22), weak incorporation of amino acids into proteins, and abnormal accumulation (23) or possibly inhibition (24) of DNA synthesis. Irrespective of high (50 mg NaF/kg body weight) or low (10 mg NaF/kg

TABLE 1. Levels of protein (mg protein/100 mg fresh tissue weight) in liver, muscle (gastrocnemius and pectoralis) and small intestine of control and treated groups

TISSUE	GROUPS				
	I	II	III	IV	V
Liver	26.48±0.18	22.20±0.45	25.05±0.79	23.15±0.76	26.10±0.61
Gastroc-nemius muscle	28.76±0.83	17.71±0.62	22.47±1.29	20.85±0.58	28.21±0.73
Pectoralis muscle	32.30±3.14	19.36±0.88	28.26±0.39	25.43±0.43	30.80±0.37
Small intestine	16.90±0.49	15.08±0.17	16.60±0.41	14.70±0.92	16.52±0.61

TABLE 2. Showing concentration of glycogen (µg/100mg fresh tissue weight) in liver and gastrocnemius muscle of control and treated groups (I - V)

TISSUE	GROUPS				
	I	II	III	IV	V
Liver	1332.46±12.32	1735.91±20.51	1540.81±30.25	1613.33±46.47	1382.49±29.74
Muscle	901.67±33.17	1255.64±22.11	1052.87±22.29	1170.13±32.25	919.01±28.07

TABLE 3. Showing activity of phosphorylase (mg phosphorus released/100 mg fresh tissue weight/15 min) in liver and gastrocnemius muscle of control and treated groups (I-V)

TISSUE	GROUPS				
	I	II	III	IV	V
Liver	26.49±0.92	20.52±0.81	25.77±0.82	22.45±1.51	26.68±0.96
Muscle	44.18±0.96	17.42±0.52	30.05±1.11	26.96±1.77	41.05±2.53

TABLE 4. Showing activity of SDH (µg formazan/100 mg fresh tissue weight/30 min) in pectoralis muscle in control and treated groups (I - V)

TISSUE	GROUPS				
	I	II	III	IV	V
Pectoralis muscle	223.62±4.69	129.00±2.15	184.96±4.20	169.13±2.90	189.76±2.12

TABLE 5. Showing levels of Na^+, K^+ from kidney and Ca^{++} in muscle (ppm) of control and treated groups (I-V)

PARA-METERS	ORGANS	GROUPS				
		I	II	III	IV	V
Na^+	Kidney	4.95±0.75	2.33±0.19	3.06±0.26	3.15±0.45	3.66±0.18
K^+	Kidney	3.45±0.25	2.50±0.10	3.00±0.20	2.80±0.55	3.20±0.15
Ca^{++}	Muscle	3.20±0.10	4.70±0.17	3.80±0.30	4.05±0.35	3.50±0.10

TABLE 6. Showing concentration of cholesterol (mg cholesterol/100 mg fresh tissue weight) in ventricle in control and treated groups (I - V)

TISSUE	GROUPS				
	I	II	III	IV	V
Ventricle	0.71±0.09	0.87±0.08	0.64±0.05	0.42±0.03	0.63±0.05

TABLES 1-6.
For all the above Tables: Values are mean ± S.E.
Group I Untreated control.
Group II NaF treated (30 days).
Group III NaF treatment as in Group II, withdrawal after 30 days and administration of ascorbic acid from day 31st for another 30 days.
Group IV NaF treatment as in Group II, withdrawal after 30 days and administration of calcium from day 31st for another 30 days.
Group V Combined treatment as in Group III and IV.

body weight) doses of NaF administration, collagen biosynthesis in NaF treated animals was reduced as compared to control (25), affecting the contractile properties of muscle. The results obtained in this study also revealed that the concentrations of protein in muscle (gastrocnemius and pectoralis), liver, and intestine were reduced after NaF treatment. The muscle protein was more significantly affected than liver. A similar decrease in the protein levels in various reproductive tissues as well as in liver and muscle of fluoride intoxicated experimental animal models (male) has also been reported (2,3,5,7-9,26). This was attributed to the decline in protein synthesis and its metabolism. Induction of some "stress proteins" have also been found in testis and epididymis of rat (27).

Dramatic changes occurred in carbohydrate metabolism after fluoride ingestion. Significant accumulation of glycogen in liver might be due to inhibition of glycolysis either by enolase mediated inhibition (28) or by decrease in isocitrate dehydrogenase. Hence accumulation of citrate occurs which is a negative modulator of phosphofructokinase (29). Alterations in catecholamine levels could also be responsible for the accumulation of glycogen and disturbed carbohydrate metabolism (2,3). Another factor causing accumulation of glycogen might be the inhibition of phosphorylase activity. In the present study, phosphorylase activity showed a significant decrease in muscle and a moderate decline in liver. These results concur with data earlier reported from our laboratory (2).

Ingestion of fluoride produces a specific metabolic alteration in rapidly growing cells by decreasing the cellular ATP and to a lower level than other metabolic inhibitors. Secondly, the extracellular calcium may be lost due to the formation of insoluble salts of calcium fluoride, calcium thus becoming unavailable for phosphorylase activation. The fluoride induced effects could also be due to internal injury to the cell membrane, resulting in receptors failing to receive hormone-mediated signals. In another study of fluoride toxicity from our laboratory a significant decrease in inner-membrane phospholipids (phosphatidyl serine, phos-

phatidyl ethanolamine, and phosphatidyl inositol) levels were obtained in testis and cauda epididymis after NaF treatment. This would affect the activity of membrane-bound enzymes like Na^+/K^+-ATPase by disturbing membrane fluidity and its integrity. Since the phosphatidyl inositol is involved in hormone receptor interaction, the androgen target organs would be affected in fluoride treated animals (27).

An oral dose of NaF (10 mg/kg body weight) caused a significant decrease in the activity of muscle SDH. Chinoy *et al* (26) have also reported inhibitory action of fluoride on muscle SDH. As SDH is known to be a mitochondrial enzyme involved in oxidative metabolism in the muscle, it is likely that the structure of mitochondria of muscle fibres might be altered by NaF, resulting in a change in the enzyme and thereby the contractile properties of muscle. Further ultrastructural studies in this direction are needed.

In the present study, the calcium levels were enhanced in pectoralis muscle, which might be due to transport of extracellular Ca^{++} to the intracellular region due to elevated catecholamine levels in the muscle. This may create an ionic gradient across the sarcolemma, thus hampering the overall muscle contraction. Calcium also inhibits the enzyme, phosphodiesterase, thus elevating C-AMP levels and disturbing Ca^{++}/C-AMP interaction in regulating mechanisms of muscle contraction (26).

According to McIvor (30), fluorosis is known to produce a marked K^+ efflux from intact cells. Clearance of F^- decreases as damage to the kidney increases (31). The fall in Na^+ and K^+ levels in the kidney and their subsequent rise in the urine was reported earlier (2, 3) and could be attributed to change in electrolyte balance in the intercellular and intracellular fluids. Suketa and Terui (32) also reported disturbances in Na^+ and K^+ levels in urine and serum of fluoride-intoxicated rats. Recently, Das and Susheela (33) reported that the fluorotic human population and NaF treated animals suffer from adrenal hypofunction. The loss of electrolytes brings about a decrease in body weight due to loss of water along with salts. Similar data have been obtained in fluoride-treated male rats, mice and fluorotic human populations (2,9).

Saralakumari (34) observed unaltered serum cholesterol levels in rats supplemented with sodium fluoride in drinking water for two months. Ectopic calcification of aorta in rabbits chronically treated with sodium fluoride is also known (35). On the other hand, in testis and ovary of sodium fluoride treated rats and rabbits, as well as in serum of fluorotic individuals, it has been reported (2-5) that, at least in the initial stages of fluoride toxicity, the animals or individuals do not suffer from marked alterations in cholesterol metabolism nor steroidogenesis in the gonads. Chronic treatment, however, causes alteration in testicular steroidogenesis and Leydig cell functions (11). In the present study, cholesterol levels in the ventricle were not significantly affected after 30 days of treatment.

All the above alterations in the various parameters were transient, and 30 days of oral treatment with AA and calcium along with NaF feeding reversed the toxic effects almost to control levels. AA administration was more potent than calcium administration, but the combination of AA and calcium caused almost complete recovery, suggesting a synergistic effect. Cholesterol levels in the ventricle

decreased within the recovery period after administration of calcium, suggesting restoration of cardiac lipid metabolism. The combined use of ascorbic acid and calcium as therapeutic agents, to help ameliorate fluoride toxicity, concurs with our finding that the combination reversed induced fluoride toxicity. The almost complete recovery appears to be due to their active participation in several metabolic processes, growth, and in overcoming stress conditions (2,3,36).

It is known that vitamin C and calcium deficiency, especially under fluoride toxicity, poor nutrition, and hard labour, aggravate endemic fluorosis (37,38). Data obtained from dietary surveys suggested that inadequate ascorbic acid and calcium were related to the severity of fluorosis (39). That calcium and vitamin C are necessary for amelioration of fluoride toxicity has been reported (7,10,26).

The present study makes clear that calcium and ascorbic acid have a significant role in overcoming fluoride toxicity, and have a synergistic effect on the recovery from NaF induced alterations in female mice, in line with earlier studies on males from our laboratory. Vitamin C, due to its active antioxidant as well as detoxification properties, is a promising and potent agent in suppressing fluoride toxicity. Similarly, calcium is known to form insoluble complexes with fluoride, and reduce its absorption and thereby its action. In addition, both these agents are involved in various metabolic processes. The synergistic effect of ascorbic acid and calcium might be due to inhibition of phosphodiesterase (which is a known inhibitor of C-AMP) and resulting augmentation of C-AMP levels, which is involved in activation of several kinases. We believe high priority should be given to the combined use of these two agents for fluoride endemic populations, at least for the children. However, monitoring of doses is necessary because high concentrations of these agents, particularly calcium, can be harmful.

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SISTER CHROMATID EXCHANGES: A STUDY IN FLUOROTIC INDIVIDUALS OF NORTH GUJARAT

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SUMMARY: The purpose of this preliminary investigation was to compare the genotoxic effect of fluoride in human individuals directly exposed to high concentrations of drinking water fluoride (1.95 to 2.2 ppm) with those in individuals exposed to concentrations (0.6 to 1.0 ppm) within the WHO permissible limit. Sister chromatid exchanges (SCE) and cell cycle proliferative index were studied in whole-blood cultures of fluorotic individuals and compared with normal controls of age-matched groups. The results suggest that the SCE rate in persons exposed to fluoride in the endemic (1.95-2.2 ppm) areas of North Gujarat is significantly higher than that of those living in Ahmedabad (0.6-1.0 ppm). There is no significant difference in the cell cycle proliferative index. Further investigations are needed to confirm the findings.

Key words: Fluorosis; Genotoxic; Mutagen; Sister chromatid exchanges.

Introduction

Fluoride is ubiquitously distributed in our environment. It is present in air, water, and fertilizer residues, and traces have been reported in almost every edible material that we consume in our daily life. Water is ordinarily the principal source of fluoride intake by the human population. Continuous exposure to higher than permissible levels of fluoride (> 1.0 ppm) in drinking water leads to crippling fluorosis. More than a million people in India are afflicted with skeletal and dental fluorosis. Numerous studies have been carried out in relation to the influence of fluoride on the environmental and biological systems, but there is a great deal of controversy regarding its potential genotoxic effects in mammals (1,2). The present study was undertaken with a view to investigate the genotoxic effect of fluoride, if any, on the endemic population of Gujarat.

Materials and Methods

Peripheral blood was collected from 100 individuals, mainly from Mehsana District of North Gujarat, and 21 from Ahmedabad. The inhabitants of these villages were examined for apparent mottled teeth, back pain, stiffness of back and joints, and other clinical manifestations including skeletal problems prior to blood sampling. Most of these individuals were unable to bend due to a stiff back, which indicated that they were afflicted with fluorosis. The clinical history was recorded in a standard proforma. Water samples of the villages were collected for the estimation of fluoride levels.

Metaphase chromosome preparations from the blood samples of the fluoride-affected individuals and control members were carried out by routine phytohaemagglutinin (PHA) stimulated cultures. 5-Bromodeoxyuridine (BrdU) was added at a final concentration of 10 µg/ml at 0 hours.

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The fluorescence plus Giemsa method was used for the scoring of sister chromatid exchanges, and the procedure of Perry and Wolff (3) was followed with slight modifications. All the slides were randomized with code numbers and were scanned by a single individual. The chromosomes were examined under a Nikon light microscope. The influence on the cell cycle proliferative index was evaluated by recording the percentage of cells in the first (M1), second (M2), and third (M3) cell divisions. A minimum of 100 cells were analysed from each culture. The cells in M2 phase were further examined and only the cells with well spread chromosomes were scored for sister chromatid exchanges (SCEs). A total of 25 cells from each culture were examined for SCE frequency. The Students 't' test was used for statistical analysis of SCE data.

Fluoride concentrations from the serum as well as from the water samples were determined with an Ion Selective Electrode Orion Model 701A.

Results

The frequencies of sister chromatid exchanges in fluoride affected individuals and in normal controls are shown in Table 1. The values are the average (mean) number of SCEs observed in minimum 25 M2 cells each with 46 chromosomes. Statistical analysis indicated a significant increase ($p<0.05$) in the SCE/plate in the study group when compared to the normal controls. The influence of fluoride toxicity on cell cycle proliferative index was also carried out. Cultures from both the fluoride affected individuals and normal controls showed presence of cells in 1st, 2nd and 3rd cell cycles indicating the existence of all three cell cycles in both the groups of cells. No significant variation was observed in the cell cycle proliferative index of fluoride affected individuals as compared to controls (Figure 1).

The serum fluoride level was significantly elevated ($p<0.001$) in persons residing in Gujarat as compared to those examined in Ahmedabad (Table 2).

Discussion

A number of investigators have utilized the SCE test to study the genotoxicity of fluoride (1,4-12). In the present study, human populations directly exposed to fluoride in drinking water in endemic regions of North Gujarat were investigated to evaluate the possible effect of fluoride on SCE. To the best of our knowledge this is the first report on genotoxic effects following long-term fluoride intake in an endemic area in India. In this study, the resuts from cultured human lymphocytes indicate a significant increase in the frequency of SCE in the study group compared to the normal control. *In vitro* studies have yielded inconsistent findings with regard to SCE in white blood cells. In human lymphocytes exposed to doses up to 4 mM (11) and 3 mM (12) of NaF, no significant variation in the frequency of SCE was reported. *In vivo* studies carried out in mice maintained for seven generations on a diet containing 1.2 mM NaF indicated no increase in SCE frequencies in bone marrow cells (8). On the other hand, an increase in the incidence of SCE was observed in cultured Red Muntjac cells and in cultured Syrian Hamster embryo cells at dosage levels of 0.5, 1.0, and 1.9 mM, respectively (9,10).

TABLE 1. Frequencies of SCEs in control and study group

Sr.No.	Group	No. of cases investigated	No. of metaphases screened	No. of metaphases with 2nd cell cycle	SCE/ plate*
1	Control	21	2173	612	7.46 ± 0.52
2	Study	100	11005	3856	8.79 ± 0.26

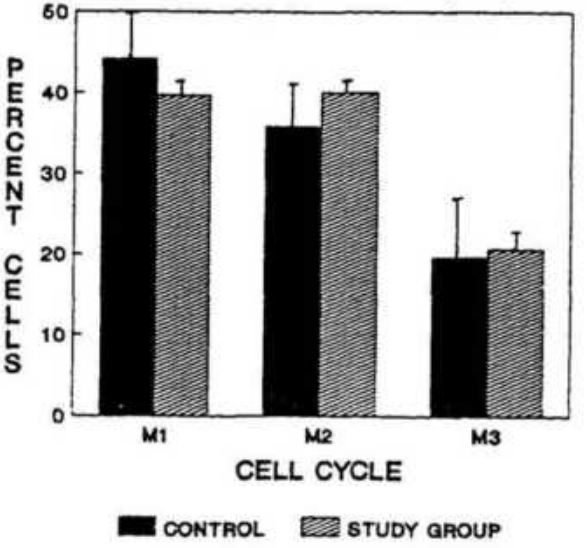
* = mean ± SE
The values are significantly different ($p < 0.05$).

TABLE 2. Fluoride concentration in drinking water samples and in serum of control and fluorotic cases screened for SCE

Area	Water Fluoride (ppm)	Serum Fluoride (ppm)
Control (Ahmedabad)	0.62 ± 0.02	0.04 ± 0.002
Range	0.60 - 1.04	0.03 - 0.05
n	24	25
Endemic Region (Mehsana District)	2.20 ± 0.05*	0.286 ± 0.02*
Range	1.949 - 2.211	0.177 - 0.539
n	36	24

Values are mean ± SE
n = number of samples
* = $p < 0.001$

FIGURE: Influence of fluoride on the cell cycle proliferation



Furthermore, no significant chromosomal damage was observed in lymphocytes of cattle with signs of chronic fluoride poisoning (13), in mouse oocytes at doses as high as 100 ppm, and ewe oocytes exposed to 10 ppm NaF (14). A significant increase in chromosome aberration was reported in human leukocytes (15), human fibroblasts (16,17), and in mice (18) maintained on fluoridated water.

It is, however, difficult to correlate the results of the present study with the earlier reports, since most of them have been carried out using either *in vitro* cultures or *in vivo* systems by treating the experimental animal with fluoridated compounds. Moreover, the doses of fluoride used for *in vitro* studies were much higher than those ever attained *in vivo* in body fluids of mammalian organisms (19), and the *in vivo* studies have been carried out at concentrations far in excess of those encountered in daily living (2). In the present study, the fluoride content in drinking water of the endemic villages (Table 2) was higher than the permissible level of 1 ppm, according to the WHO standards (20). Serum fluoride concentrations were also significantly higher in the fluorotic subjects of these areas, corroborating earlier data (21-23).

The results of the present investigation suggest that in fluoride-affected persons exposed to 1.95-2.2 ppm fluoride in drinking water chromosomal alterations as indicated by SCE frequency and chromosome aberrations were higher than in normal persons exposed to 0.6-1.0 ppm drinking water fluoride. However, analysis of samples from other endemic areas, and also use of a series of investigations including cytogenetic and mutagenic assays, are necessary to confirm the findings. Work is also needed on a large control group who are consuming 0 ppm or less than 0.2 ppm fluoride. However, the fluoride levels in drinking water in Ahmedabad and its vicinity range from 0.6 to 1.04 ppm (see Table 2).

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IMPACT OF ARTIFICIAL FLUORIDATION ON SALMON SPECIES IN THE NORTHWEST USA AND BRITISH COLUMBIA, CANADA

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SUMMARY: A review of literature and documentation suggests that concentrations of fluoride above 0.2 mg/L have lethal (LC_{50}) effects on and inhibit migration of "endangered" salmon species whose stocks are now in serious decline in the US Northwest and British Columbia. Fluoride added to drinking water, "to improve dental health", enters the fresh water eco-system, in various ways, at levels above 0.2 mg/L. This factor, if considered in "critical habitat" decisions, should lead to the development of a strategy calling for a ban on fluoridation and rapid sun-setting of the practice of disposal of industrial fluoride waste into fresh water.

Key words: British Columbia; Fluoride; Toxicity; Salmon species; US Northwest.

Introduction

In the US Northwest, species of salmon using the Snake-Columbia River system, are listed as "endangered". On the North Thompson River of British Columbia, Canada, sperm banks are being employed to preserve salmon species. Proposed water diversion on the Nechako River, in British Columbia, may threaten the internationally important Fraser River fishery. (See Map).

Joseph Cone, writing in the quarterly magazine, *The New Pacific*, in January 1994, reported that the annual migration of salmon in the Snake-Columbia River system had declined over the past century from an estimated 10-16 million to 2 million in 1991. He pointed out that "the problem is enormously complex - biologically, administratively and economically".

His article and reports in the media have stressed the problems with harvesting; loss of habitat through poor forestry practices, livestock and human settlement; and dams built for power and irrigation. Little emphasis is placed on the effects of pollution of water by toxic substances such as fluoride.

The aluminum industry is the chief beneficiary of power dams on the Columbia River system, and it is the fluoride wastes from smelters that first come to mind as sources of fluoride pollution. However, there is another potential source of contamination - the artificial fluoridation of community water supplies for the avowed purpose of improving dental health.

Fluoride and "critical habitat"

In discussions of "critical habitat" for endangered salmon species, *all* of the possible components must be evaluated. This study examines the possibility that artificial fluoridation of drinking water in communities along the course of salmon rivers is a factor to be included.

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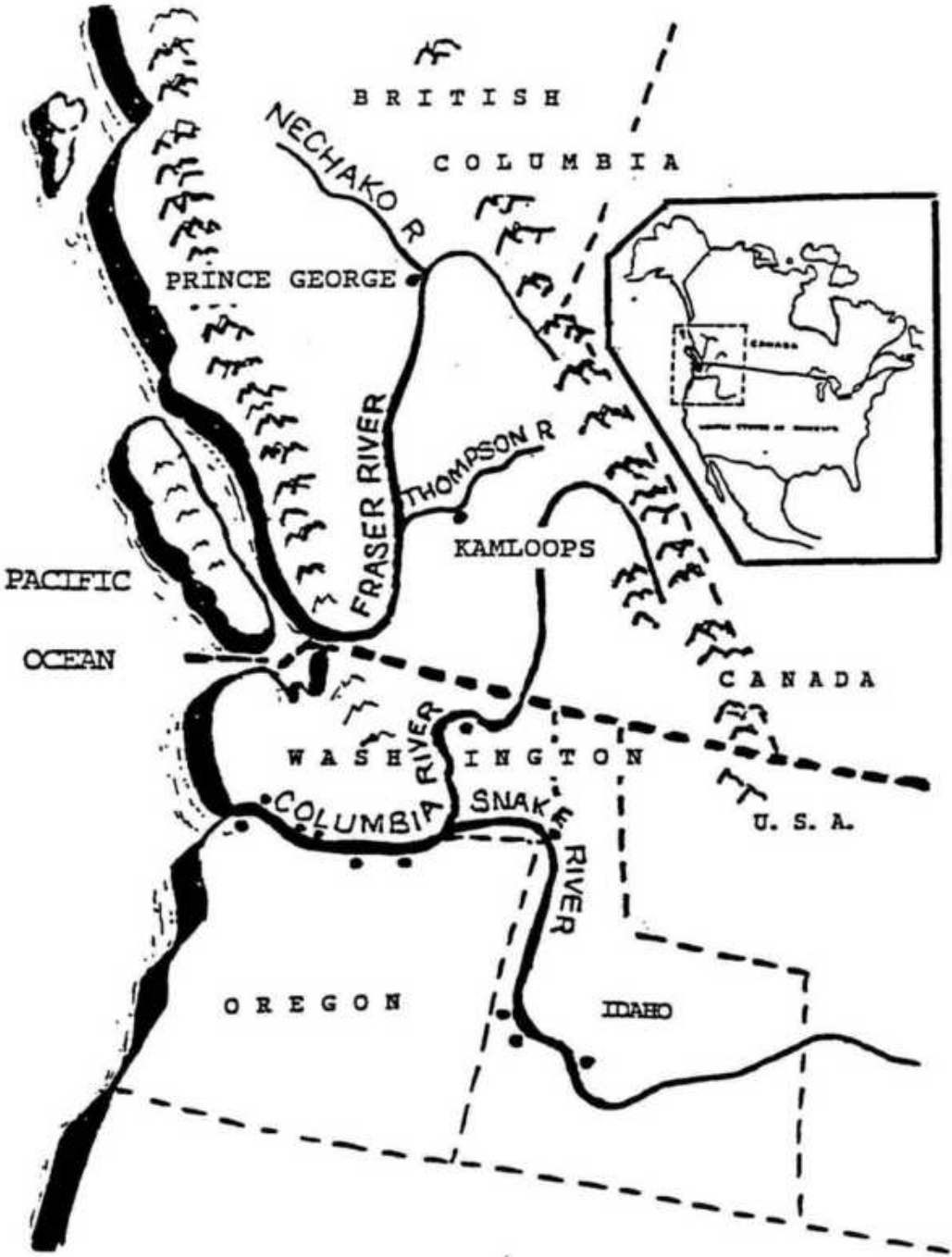
MAP OF THE AREAS



"Safe level" questioned

The US Environmental Protection Agency (EPA) (1) and the Province of British Columbia (BC) (2) adhere to a "permissible level" of 1.5 ppm (1.5 mg/L) for fluoride discharged into fresh water. BC's "recommended guideline" is currently 0.2 mgF/L; but this does not have the force of legislation. Neither the BC Ministry of the Environment nor the Washington State Department of Ecology requires fluoride estimations for sewer effluent permits as it is considered that fluoride is not significantly toxic to aquatic life in concentrations expected in discharges (3,4).

A review of the literature and other documents, such as court transcripts, reveals that levels below 1.5 mgF/L have been shown to have both lethal and other adverse effects on salmon.

"Evidence" presented by the EPA and other government bodies responsible for the environment suggests that harm can come to aquatic life only at concentrations that far exceed those in discharges from fluoridated cities. Both Groth (5) and Warrington (6) point out that many factors influence susceptibility of fish to fluoride: temperature; water hardness; pH; chloride concentration; and, the strain, age, and physiological and reproductive condition of the fish.

Groth points out that there are serious problems with "laboratory" experiments as opposed to "field" studies. In the former, "... many of the organisms tested for fluoride toxicity did not experience effects until levels of fluoride higher than those which might realistically be encountered in the environment were attained." Groth concluded that the finding can be misleading: the techniques of measurement may be inadequate to detect effects, and these may be at the population rather than the individual level (5).

There are studies showing the effect of temperature and hardness. Angelovic and others (7) showed lethal effects on rainbow trout related to temperature. Using sodium fluoride at the same degree of hardness (estimated at 44 by Warrington (6)), the 240-h LC_{50} at 7.2°C was found to be 5.9-7.5 mgF/L; at 12.8°C, 2.6-6.0; and, at 18.3°C, 2.3-7.3 mgF/L. Neuhold (8) reported the same result for 12.8°C and the same degree of hardness. Pimental and Bulkley (9), using a constant temperature of 12°C, found that the 96-h LC_{50} for rainbow trout with hardness levels, in mg/L, of 17, 49, 182 and 185 was associated with fluoride levels, in mg/L, of 51, 128, 140 and 193 respectively.

Warrington (6) in British Columbia, where the softness of major salmonid watercourses is the rule, combined the findings of Angelovic (7), and of Pimental and Bulkley (9) to calculate that the chronic threshold for rainbow trout at 12° and water hardness of 10 mg/L (calcium carbonate) is 0.2 mgF/L.

In a field study, Damkaer and Dey (10) demonstrated that high salmon loss (Chinook and Coho) at John Day Dam on the Columbia River, 1982-1986, was caused by the inhibition of migration by fluoride contamination from an aluminum smelter 1.6 km above the dam. The average daily discharge of fluoride in 1982 was 384 kg. This was associated, at the dam, with a fluoride concentration of 0.5 mg/L and a migration time of more than 150 hours and a 55% loss. In 1983, discharge was reduced to 107 kg/day. This was associated with a reduction of

concentration to 0.17 mgF/L and the migration time to less than 28 hours with a loss of 11%. In 1985, fluoride discharge of 49 kg/day was accompanied by a concentration of 0.2 mgF/L and a salmonid loss of 5%.

Damkaer and Dey confirmed the cause-and-effect relationship by means of a two-choice flume for fluoride gradient salmon behaviour tests. These determined that the "critical level" was 0.2 mgF/L.

It is interesting that the Damkaer and Dey study was not available at the time of Warrington's review.

There are other studies that indicate that fluoride at levels below 1.5 mg/L have lethal and other adverse effects on fish. Delayed hatching of rainbow trout occurred at 1.5 mgF/L (11); brown mussels died at 1.4 mgF/L (12); an alga (Porphyria tenera) was killed by a four-hour fumigation with fluoride with a critical concentration of 0.9 mgF/L (13); and, levels below 0.1 mgF/L were shown to be lethal to the water flea, Daphnia magna (14). These latter two studies suggest that salmon species may be affected by fluoride induced reduction of food supply.

Documents used in the Court case involving Meader's Trout farm in Pocatello, Idaho, in 1961 (15) contain evidence that between 1949 and 1950 trout damage and loss was related to fluoride contamination due to rain washing air-borne particles from leaves into hatchery water, at levels as low as 0.5 mgF/L.

Therefore, there is evidence that the "safe level" of fluoride in the fresh water habitat of salmon species is not 1.5 mg/L; but, 0.2 mg/L. Is this concentration exceeded by fluoridated communities on the banks of water-courses serving as salmon habitat?

Fluoride levels in water and sewer systems

In fluoridated areas, drinking water, obtained from surface water with an average fluoride concentration of 0.1-0.2 mg/L (16), is raised to the "optimal" level of 0.7-1.2 mgF/L by the addition of sodium fluoride, hydrofluosilicic acid, or sodium silicofluoride.

Fluoride, in community drinking water, enters the fresh water ecosystem in various ways. Surface run-off from fire-fighting, washing cars, and watering gardens may enter streams directly or through storm sewers at "optimal" concentration, 0.7-1.2 mgF/L. Most enters during waste water treatment.

Masuda (17) studied a large number of cities and calculated the concentrations in waste water that were *in excess* of the concentration present in the cities' water supplies. In raw sewage, this was 1.30 mgF/L; primary treatment reduced this slightly to 1.28 mgF/L; secondary treatment to 0.39 mgF/L. Singer and Armstrong (18) found 0.38 mgF/L in *unfluoridated* sewage and 1.16-1.25 mgF/L in *fluoridated* sewage.

It is clear that, in the case of artificially fluoridated communities, the concentration of fluoride in both surface run-off and sewer effluent exceeds 0.2 mgF/L.

The concentration of fluoride in receiving waters depends on a number of factors: background level (*i.e.*, concentration above effluent outlet); concentration of community water before fluoridation; amount of fluoride added; and, the rates of flow of production, discharge, and receiving water.

Studies show that elevated concentrations in fresh water receiving fluoridated effluent may persist for some distance. Bahls (19) showed that the effluent from Bozeman Montana of 0.6-2.0 mgF/L discharged into the East Gallatin River did not return to the background level of 0.33 mgF/L for 5.3 km. Singer and Armstrong (18) reported that a distance of 16 km was required to return the Mississippi River to its background level of 0.2 mg/FL after receiving the effluent of 1.21 mgF/L from Minneapolis-St Paul.

Although dilution reduces *concentration* over distance, the *amount* of fluoride in effluent is either deposited in sediment locally or is carried to the estuary where it may persist for 1-2 million years (16) or may re-contaminate if dredging were to take place.

Sewage sludge, a product of secondary treatment systems must contain high concentrations of fluoride. However, this is not measured, routinely, in the jurisdictions that were contacted for this study. This also, when spread on agricultural land, including forests, is a hazard in the "critical habitat" of salmon species.

During application, aerosols are created that may be ingested by animals or contaminate surface water. The sludge adds toxic substances to the soil. Fluoride can move into ground water and the run-off of soil particulates may enter streams that play a role in the life cycle of salmon.

Effluent from fluoridated cities is also discharged into tidal waters. Sea water has been shown to have a higher concentration of fluoride than unpolluted surface water (16). This concentration of 1.35-1.4 mgF/L is *total* fluoride. Ionic fluoride is 0.4-0.7-mgF/L and a similar amount is bound in ionic form to magnesium (20).

A more meaningful measure of fluoride pollution in sea water is the ratio of fluorine to chlorine (normally, 10^{-5}:1). Contaminated rivers flowing into an estuary, as well as direct discharge of effluent, can elevate the amount of fluoride. The possible effects on salmon species are left for future review.

Discussion

More research, especially field study, is required. However, from information that is available, 0.2 mgF/L in the fresh water ecosystem in the US Northwest and British Columbia appears to be the appropriate safe level for salmon species rather than 1.5 mgF/L currently accepted. Artificially fluoridated communities discharge fluoride into this ecosystem at levels that exceed this from surface run-off, sewage effluent and, probably, from the agricultural use of sludge.

Decreases in water volume and/or flow velocity have the potential to increase fluoride concentration. Increased water temperature will enhance fluoride toxicity.

Fluoridation deserves to be looked at as a component of "critical habitat" along with the more publicized factors.

A review of *Fluoridation Census 1985* published by the US Department of Health and Human Services (21) shows that along the course of the Snake River from the Idaho-Wyoming border to its junction with the Columbia River in Washington State, there are three water systems fluoridated at 1.0 mgF/L. Eight

artificially fluoridated water systems are located on the banks of the Columbia from the Canadian border to the mouth. That is, a total of 11 artificially fluoridated communities are located along the Columbia-Snake River system into which they release fluoride. Does this play a role in the catastrophic decline in salmonid stocks in this once highly productive ecosystem?

The declining salmon returns to the North Thompson, especially of Chinook and Coho, is threatening the existence of species. The City of Kamloops, which contributes run-off and sewage effluent to the North Thompson, is artificially fluoridated. Could this fluoride contribute to migration delay, as occurred at the John Day Dam? Could the decline be related to loss of basic feed or hatching abnormalities associated with toxic levels of fluoride? Effluent levels in Kamloops have been measured at 0.6-1.2 mgF/L by employees of the City (personal communication) but no field studies on the effect on salmon species have been carried out.

The Fraser River of British Columbia begins in the Rocky Mountains, north of the origins of the Columbia. The Fraser travels west to the City of Prince George, where it is joined by the Nechako River carrying water from the western portion of the Province. From there, it flows south to enter the Strait of Georgia after it is joined by numerous tributaries, the largest of which is the Thompson River.

Prince George, like Kamloops, is artificially fluoridated.

Does fluoride from Prince George contribute to reported declines in Chinook and Coho stocks in the Nechako? If the diversion of water from the Nechako River, as proposed in the "Kemano II" hydroelectric project, takes place and lowers the water level, slows the flow and raises the temperature of the Nechako-Fraser River system, will the fluoride from both Prince George and Kamloops be enhanced in its toxic effects not only on Chinook and Coho but on other salmon species such as the Sockeye upon which fishers of both the US and Canada depend?

Conclusion

The decline in salmon stocks, especially Chinook and Coho, is a major economic problem for both commercial and sport fisheries. "Critical habitat restrictions" are currently (April 1994) being formulated. Fluoride pollution should be included.

There are many questions. But, until evidence to the contrary, based on *impartially* conducted field studies, is available, the "criteria level" of fluoride, in fresh water, to protect salmon species in the US Northwest and British Columbia, should be 0.2 mgF/L. Acceptance of this level would condemn both the direct metering into fresh water of fluoride wastes from such activities as smelting and phosphate fertilizer manufacture and the entry of fluoride after its deliberate addition to community water supplies.

The strategy for eliminating unacceptable levels of fluoride from the "critical habitat" of Northwest Pacific salmon consists in the immediate banning of artificial fluoridation and the rapid sunsetting of the current disposal practices of fluoride-producing industries.

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SLOW-RELEASE SODIUM FLUORIDE IN THE MANAGEMENT OF POSTMENOPAUSAL OSTEOPOROSIS

C Y C Pak *et al*, *Annals of Internal Medicine 120* 625-632 1994
For Abstract see *Fluoride 27* (3) 172-173 1994

Critical Review by John R Lee MD

Dr Pak's recent interim report in the *Annals of Internal Medicine* (1) is an interesting example of why science sometimes seems to a bit out of touch with the real world. This randomized controlled trial of dosing postmenopausal women with slow-release sodium fluoride for the purpose of studying fluoride's effect on bones of postmenopausal women states that its objective is to test whether this treatment "inhibits vertebral fractures without causing fluoride complications." The bone effect of prolonged overdose of fluoride is, however, quite well known; it is a disease called osteofluorosis, also known as osteosclerosis, which is, according to Dorland's medical dictionary, "the hardening or abnormal denseness of bone." It is accompanied also by eventual calcifications in connective tissue such as ligaments, tendons, and peri-articular tissue. There are several points to be raised here.

The abnormal bone denseness resulting from fluoride is of poor quality and, while the increased density helps compression strength, it generally leads to weakness of tensile strength. Thus, previous tests (2-4) of fluoride "treatment" for osteoporosis finds a decrease in vertebral (compression) fractures but an increased incidence of hip and long bone fracture, compared to control patients, after four years or so of the treatment. Other researchers have advised abandoning fluoride as a legitimate treatment of osteoporosis for that reason and fluoride's toxicity (5). Dr Pak *et al* are intent on detecting a difference in vertebral compression fractures, no matter how minor and insignificant that is in treating osteoporosis.

In clinical practice, the occurrence of atraumatic minor compression fractures of vertebra is common in postmenopausal osteoporotic women and is frequently asymptomatic, being found only by radiographs though the patient may have noted a slight decrease in her height over time. The more morbid consequence of osteoporosis is hip fracture which has the potential for seriously disabling patients. Thus, the goal of osteoporosis research should be directed to prevention of loss of tensile strength of bones, not merely compression strength. The protocol for Dr Pak's USPH funded and FDA approved investigational new drug application for Mission Pharmacal Company seems limited to demonstrating the obvious, *i.e.*, that excessive fluoride causes osteofluorosis.

In his study, Dr Pak is administering about 25 mg of fluoride per day in a slow release form to postmenopausal women in order to raise their serum fluoride levels from 50 ng/ml to slightly over 100 ng/ml while avoiding fluoride's known gastric inflammatory effects such as mucosal erosions, ulcers, and bleeding which regularly accompany usual oral fluoride supplementation at this dosage. While the slow release form of administration will protect against these particular gastric symptoms of fluoride toxicity, it will likely have little or no effect on the later connective tissue abnormalities routinely resulting from fluoride intake at this level. In this report, the average duration of fluoride supplement exposure of the treated women is a little under two and one-half years. Since bone turnover time in postmenopausal women is relatively slow, one would not expect the decrease of bone tensile strength (and increase in incidence of hip fractures) to occur for several more years.

The one interesting finding in Dr Pak's interim report is the fact that fluoride supplementation did not cause any reduction in vertebral fractures in women on estrogen supplementation compared to controls. Among estrogen-treated women, the fracture-free rate of placebo (no fluoride) group compared to that of the fluoride group was 75.0% and 76.9% respectively, an inconsequential difference. Dr Pak *et al.* in commenting on this non-difference, rather obliquely state that "No statistical differences related to estrogen treatment were noted between the slow-release sodium fluoride and placebo groups in estrogen-treated patients, probably because of the small sample size." Unstated is the corollary that, in women on estrogen replacement therapy, fluoride treatment offers no possible benefit, regardless of how minor it is or regardless of the potential toxicity of fluoride.

Further, if one looks at the reasoning underlying the context of fluoride treatment for osteoporosis in the light of multiple trials in the US and world-wide, the idea of a therapeutic window for fluoride dosage appears quite remote. As mentioned above, prior studies of "therapeutic" fluoride supplementation found an increase in hip fractures in the treated groups compared to controls, and a number of good studies (6-9) have shown hip fracture incidence is statistically significantly correlated with even the lower fluoride levels as found in fluoridated communities, compared to non-fluoridated communities. To believe that some dosage range between these two ranges of fluoride intake will be effective in preventing osteoporotic hip fractures is a true stretch of the imagination. Certainly no one believes that osteoporosis is a disease of fluoride deficiency.

Finally, regardless of the ultimate outcome of this study, it obviously has no bearing on the question of water fluoridation.

In summary: Dr Pak's trial of inducing what is essentially a controlled form of osteofluorosis (osteosclerosis) in postmenopausal women, and attempting to prove its effectiveness by measuring the incidence of radiographically-identifiable vertebral fractures is inherently flawed and has little relevance to effective treatment of osteoporosis.

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MARKED DECREASE IN TRABECULAR BONE QUALITY AFTER FIVE YEARS OF SODIUM FLUORIDE THERAPY - ASSESSED BY BIOMECHANICAL TESTING OF ILIAC CREST BONE BIOPSIES IN OSTEOPOROTIC PATIENTS

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Abstract from *Bone 15* (4) 393-399 1994

Sodium fluoride has for more than 2 decades been a commonly used therapeutic agent for established osteoporosis because of a repeatedly documented anabolic effect on trabecular bone mass. Recently, however, three controlled trials have failed to demonstrate any therapeutic advantage of NaF over placebo with respect to vertebral fracture rate. Also, there have been several reports of an increased incidence of nonvertebral fractures during fluoride administration. Thus, the efficacy of fluoride therapy remains a controversial issue. The aim of this longitudinal study was to investigate the effect of sodium fluoride (40-60 mg per day), calcium (45 mmol), and vitamin D-2 (18,000 IU) on trabecular bone strength, assessed before and after 1 or 5 years of treatment for osteoporosis. Iliac crest biopsies were taken before and after 1 year of treatment in 12 patients, and before and after 5 years of treatment in 14 patients. Measurements were made of biomechanical, competence, ash content, and bone fluoride content, and bone strength parameters were normalized for ash content, thereby obtaining a measure of trabecular bone quality. Bone fluoride content was significantly increased after both 1 and 5 years of treatment, indicating that the administered fluoride had been ingested. After 1 year of treatment, no difference was observed in iliac crest trabecular bone ash content. A general trend for decreased bone strength and bone quality was observed, but this was insignificant. After 5 years of fluoride treatment, an insignificant decrease in iliac crest trabecular bone ash content was observed. A significant reduction of 45% was found in trabecular bone strength ($p < 0.05$), and an even more pronounced reduction of 58% was found in trabecular bone quality ($p < 0.01$). The results of this study indicate that long-term administration of sodium fluoride may be detrimental to bone quality, at least as measured in non-loaded iliac crest trabecular bone.

Key words: Biomechanical competence; Bone fluoride content; Fluoride therapy; Iliac crest biopsies; Osteoporosis.

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COMPRESSIVE PROPERTIES OF CORTICAL BONE: MINERAL ORGANIC INTERFACIAL BONDING

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Abstract from *Biomaterials 15* (2) 137-145 1994

Bone tissue is an anisotropic non-homogeneous composite material composed of inorganic, bone mineral fibres (hydroxyapatite) embedded in an organic matrix (type I collagen and non-collagenous proteins). Factors contributing to the overall mechanical behaviour include constituent volume fraction, mechanical properties,

orientation and interfacial bonding interactions. Interfacial bonding between the mineral and organic constituents is based, in part, on electrostatic interactions between negatively charged organic domains and the positively charged mineral surface. Phosphate and fluoride ions have been demonstrated to alter mineral-organic interactions, thereby influencing the mechanical properties of bone in tension. The present study explores the effects of phosphate and fluoride ions on the compressive properties of cortical bone.

Key words: Bone; Interfacial bonding; Mechanical properties; Mineral.
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EFFECTS OF FLUORIDE ON HUMAN BONE CELLS *IN VITRO* - DIFFERENCES IN RESPONSIVENESS BETWEEN STROMAL OSTEOBLAST PRECURSORS AND MATURE OSTEOBLASTS

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Abstract from *European Journal of Endocrinology 130* (4) 381-386 1994

The cellular effects of sodium fluoride (NaF) on human bone cells in vitro have been variable and dependent on the culture system used. Variability could be attributed to differences in responsiveness to NaF among different populations of cells at various stages of differentiation in the osteoblastic lineage. In this study we compared the effects of NaF in serum-free medium on cultures of more differentiated human osteoblast-like (hOB) cells derived from trabecular bone explants and on osteoblast committed precursors derived from human bone marrow, *i.e.* human marrow stromal osteoblast-like (hMS(OB)) cells. Sodium fluoride (10^{-5} mol/L) increased proliferation of hMS(OB) cells ($p < 0.05$, $N = 10$) but was not mitogenic to hOB cells ($p > 0.05$, $N = 10$). Alkaline phosphatase (AP) production increased in both hMS(OB) ($p < 0.05$, $N = 9$) and hOB cells ($p < 0.05$, $N = 9$). No significant effects on procollagen type I propeptide production were obtained in either culture. In the presence of 1,2 5-dihydroxycholecalciferol (10^{-9} mol/L), NaF enhanced alkaline phosphatase ($p < 0.05$, $N = 8$), procollagen type I propeptide ($p < 0.05$, $N = 7$) and osteocalcin ($p < 0.05$, $N = 7$) production by hMS(OB) cells but not by hOB cells. Our results suggest that osteoblast precursors are more sensitive to NaF action than mature osteoblasts and that the in vivo effects of NaF on bone formation may be mediated by stimulating proliferation and differentiation of committed osteoblast precursors in bone marrow.

Key words: Human bone cells; *In vitro*; Osteoblasts.
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EXPOSURE TO HIGH FLUORIDE CONCENTRATIONS IN DRINKING WATER IS ASSOCIATED WITH DECREASED BIRTH RATES

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Abstract from *Journal of Toxicology and Environmental Health 42* 109-121 1994

A review of fluoride toxicity showed decreased fertility in most animal species studied. The current study was to see whether fluoride would also affect human birth rates. A U.S. database of drinking water systems was used to identify index counties with water systems reporting fluoride levels of at least 3 ppm. These and adjacent counties were grouped in 30 regions spread over 9 states. For each county, two conceptionally different exposure measures were defined, and the annual total fertility rate (TFR) for women in the age range 10-49 yr was calculated for the period 1970-1988. For each region separately, the annual TFR was regressed on the fluoride measure and sociodemographic covariables. Most regions showed an association of decreasing TFR with increasing fluoride levels. Meta-analysis of the region-specific results confirmed that the combined result was a negative TFR/fluoride association with a consensus combined p value of .0002-.0004, depending an the analytical scenario. There is no evidence that this outcome resulted from selection bias, inaccurate data, or improper analytical methods. However, the study is one that used population means rather than data on individual women. Whether or not the fluoride effect on the fertility rate found at the county level also applies to individual women remains to be investigated.

Key words: Birth rates; High fluoride exposure.

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IN VITRO FLUORIDE TOXICITY IN HUMAN SPERMATOZOA

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Abstract from *Reproductive Toxicology 8* (2) 155-159 1994)

Effects of sodium fluoride (NaF) on washed, ejaculated human spermatozoa at doses of 25, 50, and 250 mM were investigated *in vitro* at intervals of 5, 10, and 20 min. Sodium fluoride (NaF) did not affect the extracellular pH of sperm, except that a slight acidification was caused by the 250 mM dose only. The treatment caused a significant enhancement in acid phosphatase (ACPase) and hyaluronidase activities after 5 and 10 min. However, the decrease in the lysosomal enzyme activity after 20 min treatment could have been due to the gradual increase in

fluoride accumulation by spermatozoa leading to membrane damage. Silver nitrate staining of sperm revealed elongated heads, deflagellation, and loss of the acrosome together with coiling of the tail. Sperm glutathione levels also showed a time-dependent decrease with complete depletion after 20 min, indicating rapid glutathione oxidation in detoxification of the NaF. The altered lysosomal enzyme activity and glutathione levels together with morphologic anomalies resulted in a significant decline in sperm motility with an effective dose of 250 mM.

Key words: ACPase; Forward progression; GSH; Human sperm; Hyaluronidase; *In vitro*; Morphology; NaF; pH.
Reprints: N J Chinoy, Department of Zoology, Gujarat University, Ahmedabad 380009, Gujarat, India

CHRONIC ALUMINUM FLUORIDE ADMINISTRATION. 1. BEHAVIORAL OBSERVATIONS

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Abstract from *Behavioral and Neural Biology 61* (3) 233-241 1994

This study examined the behavioral effects of chronic ingestion of various monofluoroaluminum complexes in drinking water. Forty young adult male Long-Evans rats were divided into four groups of 10 rats each. The groups received different concentrations of AlF_3 in the drinking water from three sample solutions having a total Al concentration of 0.5, 5.0, and 50 ppm, respectively, or double-distilled deionized water on an ad lib. basis for 45 weeks. General decline of bodily appearance was observed in the lowest concentration AlF_3 group, and animals in this group succumbed in greater numbers during the course of the study than those in any other group. Examinations of performance in an open field, an analysis of walking patterns, and a balance beam test did not find any difficulties indicative of motor disorder. Indeed, on the initial trial on the balance beam, the AlF_3-treated animals exhibited superior performance. No group differences were found in behavior assessed by spontaneous alternation or by a modified Morris water maze test. When retested in the Morris maze after a low dose of scopolamine (0.4 mg/kg), the control animals took longer to reach the platform while the AlF_3-treated rats were not affected. In an olfactory preference test, the AlF_3-treated animals failed to show preferences exhibited by the controls, indicating a possible olfactory impairment. The level of Al in the brains of the AlF_3-exposed rats, as determined by direct current plasma analysis, was almost double that of the control animals. There was a similar trend for the Al content found in the kidneys.

Key words: Aluminum fluoride; Behavior.
Reprints: R L Isaacson, State University of New York Binghamton, Department of Psychology, Binghamton, NY 13902 USA.

COMPARISON OF THE EFFECTS OF FLUORIDE ON THE CALCIUM PUMPS OF CARDIAC AND FAST SKELETAL MUSCLE SARCOPLASMIC RETICULUM

Evidence for tissue-specific qualitative difference in calcium-induced pump conformation

C Hawkins, A Xu and N Narayanan
London, Ontario, Canada

Abstract from *Biochimica et Biophysica Acta - Biomembranes 1191* (2) 231-243 1994

Comparison of the effects of fluoride (NaF, 1-10 mM) on the catalytic and ion transport functions of the Ca^{2+}-ATPase in sarcoplasmic reticulum (SR) vesicles isolated from rabbit cardiac and fast-twitch skeletal muscles revealed similarities as well as striking tissue-specific differences depending on the experimental conditions employed. Short preincubation (3 min at 37 degrees C) of cardiac or fast muscle SR with fluoride in the absence of Ca^{2+} and ATP prior to initiating enzyme turnover by simultaneous addition of Ca^{2+} and ATP to the assay medium resulted in a strong inhibitory effect of fluoride on ATP-energized (oxalate-facilitated) Ca^{2+} uptake and Ca^{2+}-ATPase activity. On the other hand, when turnover was initiated by the addition of ATP to SR preincubated with fluoride in the presence of Ca^{2+} but in the absence of ATP, fluoride caused concentration-dependent stimulation of active Ca^{2+} uptake by fast muscle SR with no appreciable change in Ca^{2+}-dependent phosphoenzyme (EP) formation (from ATP) or Ca^{2+}-ATPase activity but inhibition of active Ca^{2+} uptake by cardiac SR with concomitant inhibition of EP formation and Ca^{2+}-ATPase activity. Exposure of cardiac or fast muscle SR to fluoride in the presence of both Ca^{2+} and ATP resulted in concentration-dependent stimulatory effect of fluoride on Ca^{2+} uptake with no change in EP formation or Ca^{2+}-ATPase activity; this effect diminished substantially at saturating oxalate concentration in the assay. Assessment of the effects of deferroxamine (1 mM) and exogenous aluminum (10 μM) did not indicate a requirement for aluminum in the inhibitory or stimulatory effect of fluoride. These results suggest that (a) the Ca^{2+} and ATP-deprived (E^1/E^2) but not the Ca^{2+} plus ATP-liganded (CaE^1ATP) conformation of the SR Ca^{2+}-ATPase is susceptible to inhibition by fluoride in bath cardiac and fast muscle; (b) the Ca^{2+}-bound conformation (CaE^1) of the SR Ca^{2+}-ATPase is susceptible to inhibition in cardiac muscle but is refractory to fluoride in fast muscle; and (c) the stimulatory effect of fluoride is largely secondary to its ability to mimic the action of oxalate in intravesicular Ca^{2+} trapping when the fluoride-resistant enzyme is turning over normally. Fluoride inhibited phosphorylation of the Ca^{2+}-free enzyme by P-i in cardiac and fast muscle SR indicating that fluoride sensitivity of the phosphorylation site of the SR Ca^{2+}-ATPase is similar in cardiac and fast muscle. In cardiac SR, disruption of the functional interaction between Ca^{2+}-ATPase and its regulatory protein phospholamban, through phosphorylation of the latter (by cAMP kinase) did not alter the fluoride sensitivity of the Ca^{2+}-bound enzyme (CaE^1). These results, coupled with the refractoriness of CaE^1ATP to fluoride in cardiac and fast muscle SR, suggest that a tissue-specific difference in the accessibility (reactivity) of the nucleotide binding site to fluoride upon Ca^{2+} binding to the enzyme may account for the observed difference in fluoride sensitivity of the cardiac versus fast muscle enzyme - *i.e.*, when the ATPase is in CaE^1 conformation, its ATP binding site is 'fluoride-reactive' in the cardiac enzyme but is 'fluoride-resistant' in the fast muscle enzyme.

Key words: Calcium pump; Cardiac muscle; Fast skeletal muscle; Fluoride; Sarcoplasmic reticulum.

Reprints: N Narayanan, University of Western Ontario, Department of Physiology, Medical Sciences Building, London N6A 5C1, Ontario, Canada

EFFECTS OF PLASMA FLUORIDE AND DIETARY CALCIUM CONCENTRATIONS ON GI ABSORPTION AND SECRETION OF FLUORIDE IN THE RAT

G M Whitford
Augusta, GA USA

Abstract from *Calcified Tissue International 54* (5) 421-425 1994)

This 30-day balance study with weanling rats was designed to determine the effects of plasma fluoride and dietary calcium concentration and their interaction on the absorption, balance, and tissue concentrations of fluoride. The three major groups differed according to the total exposure and plasma concentrations of fluoride. One group received fluoride only in the diet and the other two received additional fluoride by continuous infusion from miniosmotic pumps implanted S.C. Each group was divided into two subgroups with dietary calcium concentrations of 0.4% or 1.4%. Fluoride intake with the diet did not differ among the groups. Fecal fluoride excretion was directly related to plasma fluoride concentration. The absorption and balance of dietary fluoride were inversely related to plasma fluoride concentration. These effects were greatest in the groups fed the 1.4% calcium diet. The interactions of plasma fluoride and dietary calcium on these variables were highly significant ($P < 0.0001$). The balance of dietary fluoride was negative in the four groups that received additional fluoride by infusion. In the two groups that received fluoride only in the diet, the plasma and bone fluoride concentrations were 41% and 59% lower, respectively, in the 1.4% dietary calcium group. The findings indicate that net fluoride secretion into the GI tract can occur when plasma fluoride concentrations and calcium intake are elevated. They suggest that elevated plasma fluoride levels and calcium intake are factors that may diminish the effect of oral fluoride treatment in osteoporotic patients.

Key words: Balance; Dietary calcium; Metabolism; Osteoporosis; Secretion.
Reprints: G M Whitford, Medical College of Georgia, Department of Oral Biology, Augusta, GA 30912 USA.

EFFECT OF A COMBINED CHLORHEXIDINE AND NaF MOUTHRINSE - AN *IN VIVO* HUMAN CARIES MODEL STUDY

B N Ullsfoss, B Ogaard, J Arends, J Ruben, G Rolla and J Afseth
Oslo, Norway

Abstract from *Scandinavian Journal of Dental Research 102* (2) 109-112 1994

Chlorhexidine (CHX) is probably the most widely used and the most potent chemical plaque inhibitory agent, whereas fluoride (F^-) is the only truly accepted anticaries agent available at present. As they have discrete mechanisms of action, a combination effect of these agents on human dental caries may exist. The inhibitory effect of CHX on the formation of, and acid production in, plaque may reduce

a relatively extreme cariogenic challenge sufficiently for it to be overcome by the local F^- concentrations achieved by brushing or rinses. The aim of this study was to evaluate the possible caries inhibitory effect of combining 2.2 mM CHX mouthrinses used twice daily with daily 11.9 mM NaF rinses in an *in vivo* human caries model using plaque-retaining bands on premolars scheduled for extraction. Nine subjects (a total of 28 teeth) were fitted with the bands for 4 wk. Saliva and plaque samples were collected before and after the study period for bacterial cultures, and the tooth surfaces were analyzed by microradiography after careful tooth extractions. The combination of CHX and F^- rinses resulted in enamel mineral loss only slightly higher than that observed in "sound" enamel and clearly less than with F^- rinses alone. Both total plaque bacteria and Streptococcus mutans were reduced by CHX rinses, confirming the discrete mechanisms of action.

Key words: Chlorhexidine; Dental caries; Dental plaque; Fluorides; Mutans streptococci; NaF.

Reprints: J Afseth, University of Oslo, Rikshosp, Medinnova Sf, N-0027 Oslo, Norway

FLUORIDE UPTAKE IN HUMAN DENTINE FROM GLASS-IONOMER CEMENT *IN VIVO*

M Mukai, M Ikeda, T Yanagihara, G Hara, K Kato, H Nakagaki and C Robinson
Nagoya, Japan and Leeds, England

Abstract from *Archives of Oral Biology 38* (12) 1093-1098 1993

The purpose was to examine F uptake and distribution in dentine from a F-containing glass-ionomer cement *in vivo*. Nine volunteers were selected from dental students who were scheduled for extraction of their third molars. Two cavities were prepared on the same occlusal surface of the third molars for each subject; one was restored-with glass-ionomer cement (Virtabond), the other with zinc phosphate cement as a control. After 3 months the teeth were extracted. F profiles in the dentine from the cavity floor to the pulpal surface were determined in tissue immediately adjacent to the restorations. An abrasive micro-sampling technique was used. The F concentration of the dentine was highest immediately beneath glass-ionomer cement filling, decreasing towards the pulpal surface. Overall F concentrations were greater in the dentine beneath the glass-ionomer cement than in that beneath the zinc phosphate cement. It was concluded that the glass-ionomer cement markedly enhanced fluoride uptake by underlying dentine *in vivo*.

Key words: Cement; Dentine; Fluoride; Glass-ionomer; *In vivo*.

Reprints: M Mukai, Aichi Gakuin University, School of Dentistry, Department of Operative Dentistry, Chikusa Ku, 2-11 Suemori Dori, Nagoya, Aichi 464, Japan.

DENTAL TISSUE EFFECTS OF FLUORIDE

O Fejerskov, M J Larsen, A Richards and V Baelum
Aarhus, Denmark

It is now well-established that a linear relationship exists between fluoride dose and enamel fluorosis in human populations. With increasing severity, the subsurface enamel all along the tooth becomes increasingly porous (hypomineralized), and the lesion extends toward the inner enamel. In dentin, hypomineralization results in an enhancement of the incremental lines. After eruption, the more severe forms are subject to extensive mechanical breakdown of the surface. The continuum of fluoride-induced changes can best be classified by the TF index, which reflects, on an ordinal scale, the histopathological features and increases in enamel fluoride concentrations. Human and animal studies have shown that it is possible to develop dental fluorosis by exposure during enamel maturation alone. It is less apparent whether an effect of fluoride on the stage of enamel matrix secretion, alone, is able to produce changes in enamel similar to those described as dental fluorosis in man. The clinical concept of post-eruptive maturation of erupting sound human enamel, resulting in fluoride uptake, most likely reflects subclinical caries. Incorporation of fluoride into enamel is principally possible only as a result of concomitant enamel dissolution (caries lesion development). At higher fluoride concentrations, calcium-fluoride-like material may form, although the formation, identification, and dissolution of this compound are far from resolved.

It is concluded that dental fluorosis is a sensitive way of recording past fluoride exposure because, so far, no other agent or condition in man is known to create changes within the dentition similar to those induced by fluoride. Since the predominant cariostatic effect of fluoride is not due to its uptake by the enamel during tooth development, it is possible to obtain extensive caries reductions without a concomitant risk of dental fluorosis.

Key words: Dental caries; Dental fluorosis; Dose-response relationship; Enamel; Post-eruptive effects; Pre-eruptive effects; TF index.

Reprints: O Fejerskov, Royal Dental College, Faculty of Health Sciences, University of Aarhus, Vennelyst Boulevard, DK-8000 Aarhus C, Denmark.

ERUPTION OF DECIDUOUS TEETH: INFLUENCE OF UNDERNUTRITION AND ENVIRONMENTAL FLUORIDE

Venkata Ranga Rao Kodali, K A V R Krishnamachari and J Gowrinathsastry
Osmania, Hyderabad, India

A house to house survey in six rural villages in South India was conducted to asses the influence of undernutrition and environmental fluoride on deciduous dental eruption. Three villages surveyed were from the endemic fluorosis area where the estimated fluoride concentration was 5 ± 1.2 ppm. Oral cavities of all the children in the age group 5-48 months were examined and a tooth was marked erupted when it was visible emerging through the gingiva. Undernutrition, as classified by Gomez classification, was widely prevalent among preschool children in the surveyed area (n = 708; normal: 5.1%; grade-I: 29.1%; grade-II: 53.1% and grade-III: 12.7%). Children in the severe grade of malnutrition possessed fewer teeth at a given age. Analysis of variance revealed that fluoride has significant ($P < 0.005$) detrimental effects on dental eruption among children in the 18-30 month age group. The efficacy of Bailey's formula (age in months = number of teeth erupted + 6) in indica-

ting the chronological age was evaluated in the 5-24 month aged children (n = 347). Bailey's formula failed to assess the age correctly in 87%, with underassessment in 58% and overassessment in 29%. Its efficacy did not differ between the endemic and non-endemic areas. We conclude that i) undernutrition is a prevalent problem in rural areas in South India, ii) age calculation by Bailey's formula did not indicate the chronological age in the majority of children and hence is not useful in undernourished populations, and iii) undernutrition compounded by high water fluoride may delay the eruption of teeth.

Key words: deciduous dentition; dental health surveys; fluoride; India; nutritional status; nutrition surveys; preschool child; tooth eruption.

Reprints: Venkata R R Kodali, Consultant Diagnostician, Apollo Hospital, Muthukur Road, Nellore, Pin-524 004, Andhra Pradesh, India.

WATER FLUORIDATION, TOOTH DECAY, AND CANCER

John A Yiamouyiannis
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Abstracted from a paper presented at the XXth Conference of the International Society for Fluoride Research, Beijing, China, September 1994

It has become widely accepted among dental and public health professionals that fluoridation reduces tooth decay by one-half to two-thirds. However, recent large-scale studies in New Zealand, Canada, and the United States have reported similar or lower tooth decay rates in nonfluoridated areas as compared to fluoridated areas. Moreover, findings in the United States and worldwide show that, over the last 25 years, reductions in tooth decay rates in nonfluoridated areas are comparable to those in fluoridated areas.

In 1977, epidemiological studies by Dr Dean Burk and me showing a link between fluoridation and cancer were the subject of full-scale Congressional Hearings. As a result, Congress mandated that the US Public Health Service conduct animal studies to determine whether or not fluoride causes cancer under laboratory conditions. The USPHS retained the Batelle Memorial Institute in Columbus, Ohio, to perform two studies, one on mice and another on rats. The most significant finding was the occurrence of an extremely rare form of liver cancer, hepatocholangiocarcinoma, in fluoride-treated male and female mice. In 1989 Batelle released the results of their rat study which showed a dose-dependent relationship between oral squamous cell metaplasias and fluoride in both male and female rats. Similar results regarding oral cell dysplasias were reported in a Proctor and Gamble study. In addition, the Batelle rat study showed a dose-dependent relationship between fluoride and the number of male rats with tumorous or cancerous oral squamous cells - and also between oral squamous cell metaplasias and tumors/cancers in female rats. In male rats it was found that osteosarcomas, a rare form of cancer, were confined to rats in the two high fluoride groups.

Finally, using three different data bases, we found that, in humans, the bone cancer incidence rate, mostly osteosarcoma, was around 50% higher in males living in fluoridated areas (and many times higher in those under 20), and that the incidence of oral and pharyngeal cancers was 30-50% higher in fluoridated areas.

Key words: Cancer; Dental caries; Fluoridation.

Reprints: Dr John A Yiamouyiannis, Safe Water Foundation, 6439 Taggart Road, Delaware, Ohio 43015, USA.

AN ANALYSIS OF THE CAUSES OF TOOTH DECAY IN CHILDREN IN TUCSON, ARIZONA

Timothy Jones, Cornelius Steelink and Jeanne Sierka
Tucson, Arizona USA

Abstracted from a paper presented at the Annual Meeting of the American Association for the Advancement of Science, San Francisco USA, February 22 1994

This revealing study by University of Arizona researchers adds to the growing list of comprehensive surveys which cast doubt on the validity of past claims for a dental benefit from low water fluoride levels. It is unique in its thorough assessment of a wide range of possible causative factors.

The genesis of the study was a 1992 report of a citizens' advisory committee, chaired by Emeritus Professor of Chemistry Cornelius Steelink, to study the benefits and risks of water fluoridation. The committee, from the evidence available, reported that there was no obvious relation of water fluoride content to the prevention of tooth decay in Tucson. In fact, a positive correlation seemed to be present. As Professor Steelink stated (*Chemical and Engineering News*, July 27 1992): "the more fluoride a child drank, the more cavities appeared in its teeth." Despite that finding, the City Council decided, on the advice of public health officials, to fluoridate the water supply.

After that decision, Steelink and others from the University of Arizona undertook a more thorough investigation of factors affecting tooth decay in children. His colleagues were an anthropologist and a public school nurse. Funds for the study came from the Departments of Anthropology and Chemistry.

Tucson had a unique data base for such an epidemiological study. There existed a compilation of dental records for all (26,000) elementary school children for the year 1987-1988. Detailed demographic statistics were accessible, in the Anthropology Department, for the city population of 500,000. Behavioral patterns had been recorded for this same population by the Garbage Project, a unique research division of the University of Arizona Anthropology Department. Finally, hydrologic data were available for different areas of the city, where fluoride content of the municipal water varies with the location of the wells.

They succeeded in gathering the following data on city households: income, ethnicity, children in school, education level of parents, fluoride content of neighborhood drinking water, mean DMFTs for children in school, sugar consumption, candy consumption, toothpaste usage, school fluoride mouthwash frequency, soda pop consumption, antibiotic usage, and a few other items. Many of the above factors are referred to in the literature as "dentally aware" practices. The garbage analysis of the households indicates that, contrary to popular dogma, poor people are not "dentally unaware". They practice the same types of dental prophylaxis as do middle class residents.

The goal was to find correlations between caries in children (DMFTs) and all of the above factors. The results indicate that fluoride in drinking water appears to play a variable role. If one plots the total population caries rate versus fluoride content, the relationship is direct, thus confirming Steelink's preliminary finding. In some subpopulations the relationship is inverse and in others (*e.g.*, in this case, Hispanic) it is direct. Ethnicity seems to play the dominant role in Tucson. Household incomes and level of education are also strongly correlated.

Key words: Dental caries; Fluoridation; Garbage project; Tucson, Arizona.
Correspondence: Professor Cornelius Steelink, Department of Chemistry, University of Arizona, Tucson AZ 85721, USA.

FINAL REJOINDER

G Neil Jenkins
Newcastle upon Tyne, England

[Editor: This discussion started when Professor G Neil Jenkins was asked to submit an article in support of fluoridation. He responded by submitting a critique (*Fluoride* 27 No.1 1994 pages 37-44) of my published papers, to which I replied in the same issue. Dr Moolenburg contributed (*Fluoride* 27 No.2 1994 page 123) and the exchange continued in our last issue (*Fluoride* 27 No.3 1994 pages 174-179). In his latest covering letter Professor Jenkins writes: "I very much welcome your making *Fluoride* open for discussions as this is urgently needed." I in turn am grateful for his critique and subsequent participation, which enabled the issues to be examined in greater detail. I have invited him to have the last word. His rejoinder follows.]

I have never disputed that surveys during the last decade or so on the effect of fluoride on caries do show a smaller general effect than earlier results, the probable explanation being that fluoride toothpastes have reduced caries in the unfluoridated controls. But fluoridation is still justified, at least in Britain, where it has been shown to reduce the difference in caries experience between the different social groups - for references see Murray *et al* 1991 (1). The new survey among Indian children (2) refers to residents of an area with very high levels of fluoride in their water (the mean was 4.19 ppm with a note pointing out that intakes increase 2 to 4 fold during hard physical work in a hot climate). It has been known for many years that intakes of fluoride much above the optimum of 1 ppm in temperate climates increase caries (3) so the recent Indian results (2) fit into the known pattern.

I have nothing to add to my suggested explanation for the choice by Dean of the '21 cities'.

The data of Weaver, from which he concluded that fluoride postpones, rather than prevents, caries (4) were based on small numbers of women in whom 85% of the DMF indices represented the M fraction. This high extraction rate makes the DMF unreliable as a measure of caries in adults in whom many extractions would be for periodontal, prosthetic or surgical reasons (especially in the 1940s). The data of Murray (5) minimised this difficulty in several ways and found that the effect of fluoride was still detectable up to the age of 65.

The observation that early caries could be remineralized was made in the Tiel-Culemborg fluoridation scheme (6), so that 'white spots' *were* recorded and found to fall in numbers, in that study. Jackson (6) produced cogent evidence that the decline in caries *began* before fluoride toothpastes were widely used, indicating the existence of at least one unidentified factor, but his later figures show a marked increase in the decline as toothpaste usage increased (as the percentage of fluoride toothpastes rose from 71 in 1975 to 96 in 1980 the mean dmf fell from 4.17 in 1974 to 2.16 in 1980).

Uniformity of early results

Colquhoun is simply contradicting the facts in stating that recent surveys report 'no benefits' from fluoridation (though it would be correct if he had said 'recent selected surveys'). He ignores, for example, the British surveys published since the

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mid-1980's still showing a substantial benefit (although admittedly smaller than 20 years ago) from fluoridation - reviewed by Murray *et al* 1991 (1). In the results of the 113 fluoridation studies worldwide, 37% of the reductions are above the average and 24% below - a reasonable normal distribution for a complex disease process. See reference (1) below for full analysis and graphs. I have nothing to add to my defence of the use of these 113 schemes.

Diesendorf was mistaken in saying that the same large decreases were claimed whether the difference was between test and control or between the test group at different times. The two comparisons are plotted in reference (1) and show that the results were not quite the same. The test vs control graph was slightly skewed towards lower reductions whereas the 'before and after' results were more symmetrical about the mean.

Conclusion

It would seem that John Colquhoun and I will have to agree to differ over the effectiveness of fluoridation. I have enjoyed investigating his doubts, re-examining the evidence for my own conclusions and exchanging views in a calm and scientific manner.

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Dear John

The explanation of fluoridationists for the important finding of the Teotias in India - that water fluoride *causes* dental caries - is that the children examined by the Teotias drank water containing high fluoride levels (over 4 ppm on average) and that it has long been known that water fluoride levels much above the "optimal" 1 ppm increase caries.

According to Professor Burt (*Fluoride* July 1994 page 180), fluoridationists have also long known that fluoridated water prevents caries primarily by a local ("topical") effect, rather than by any systemic effect. Dean's famous 21-city graph - indicating that caries decreased as the water fluoride level increased from 0 ppm to 3 ppm - is now claimed, apparently, to be a topical effect. We are also now told that after the fluoride level reaches 4 ppm caries starts to increase. One wonders: a topical or a systemic effect? The early advocates of fluoridation always claimed that badly fluorosed teeth from high-fluoride water levels were "remarkably free of caries" - leading to the quest for an "optimal" level.

Fluoridationists also claim that fluoride toothpastes (which some children swallow, and which has very much higher fluoride levels - up to 1000 ppm) prevent caries, and are the reason for the lack of difference in caries rates between fluoridated and nonfluoridated communities. The rationale for the fluoridation theory has indeed become complex! One wonders also how fluoridationists will try to explain the recent Arizona finding that low water fluoride levels also appear to increase caries in most Tucson children.

Tohru Murakami MD
Maebashi-shi
Gunma-ken
Japan

[Dr Murakami is Secretary-General of the Japanese Society for Fluoride Research]

Dear John

Thank you for the copy of the recent journal with the Lee-Burt letters. There is one point from Dr. Lee's second letter, reinforced by your editor's footnote, which needs clarification. (In an effort to lift the tone of these discussions, I will ignore the gratuitous swipe in his "seemingly unaware" comment.)

I had stated in my first letter that fluoridated water, at concentrations around 1.0 mg/L, is accompanied by about 12% prevalence of the mildest forms of fluorosis. This figure comes predominantly from Dean's studies, at a time when water-borne fluoride was virtually the only significant source of fluoride for humans. I did not say, nor intend to imply, that fluorosis prevalence today in fluoridated areas is 12%; the prevalence of fluorosis, of course, has increased substantially since Dean's day in both fluoridated and non-fluoridated areas. As you pointed out in your editor's note, the study by Dr Szpunar and me here at the

University of Michigan is one of several in recent years which showed that prevalence levels of fluorosis are considerably higher in the United States and Canada than they once were. This can only be because infants and young children are now ingesting more fluoride than they used to because they are now exposed to more sources of fluoride. For interested readers, I have discussed this issue more fully in the three referenced papers:

1 Burt BA. The changing patterns of systemic fluoride intake. *Journal of Dental Research 71* 1228-1237 1992.
2 Burt BA. The increase in fluorosis in the United States: should we be concerned? *Pediatric Dentistry 15* 146-151 1993.
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Brian A Burt BDS MPH PhD
Professor and Director
Program in Dental Public Health
School of Public Health
University of Michigan

Dr Lee responds:

Dear John

I am gratified that Dr Burt has clarified his position concerning dental fluorosis incidence secondary to fluoridation, explaining that his previous statement in the July 1994 *Fluoride* referred to conditions of half-century ago and not today's, and agreeing that the incidence of dental fluorosis "has increased substantially since Dean's day in both fluoridated and non-fluoridated areas." Further, he agrees that this is evidence that "young children are ingesting more fluoride than they used to because they exposed to more sources of fluoride." What remains unclear is whether Dr Burt regards dental fluorosis as a visible sign of systemic fluoride toxicity and, if so, what should be done about it?

For example, if dental fluorosis exists in a non-fluoridated area, why would anyone want to add more fluoride by way of water fluoridation in that area?

If Dean's recommendation that an acceptable incidence of dental fluorosis for optimal fluoridation need never exceed 10% is correct, does it not mean that the present dental fluorosis incidence of > 20% in fluoridated areas indicates that the water-borne fluoride is not only redundant but excessive and should be discontinued?

We are left to wonder how Dr Burt explains the "topical cariostatic effect" of ingested water with 1 ppm fluoride. What mechanism could explain this unique ability of water that would not also apply to other sources of fluoride ingestion?

John R Lee MD
Sebastopol CA
USA

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