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## THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH

It is with great anticipation and excitement that we look forward to the XIXth Conference of the International Society for Fluoride Research to be held in beautiful Kyoto, Japan. Under the competent organization of Professor Y Yoshida and his committee we will meet September 8-11 1992 as scientists throughout the world representing such countries as Japan, Germany, Poland, India, France, Belgium, New Zealand, Hungary, China and USA. Hosted by the Osaka Medical College, scientists will meet and present scientific information on fluoride in sessions and poster exhibits. Lively discussions will encourage our total attention and participation. Students and young researchers will be able to discuss with renowned fluoride scholars and participate in sessions chaired by qualified experts.

There will be social programs for us and families to give the opportunity to view the spectacular architecture of temples and shrines and enjoy remarkable scenery. We will come in contact with the cultural environment of Kyoto and sample delicious cuisine. Our hosts have done all possible to make this stay valuable and enjoyable.

The International Society for Fluoride Research was organized 25 years ago with the purpose of advancement of research and dissemination of knowledge, pertaining to the biological and other effects of fluoride on animal, plant and human life. An individual holding a professional degree in any scientific profession is eligible for membership.

The first Conference of the ISFR was held in Frankfurt, Germany, in October 1967, followed by the second Conference in Barcelona, Spain, in January 1969.

In 1968 the picture of Dr Kaj Roholm first appeared on the cover as it has for the last 24 years. An editorial in 1968 detailed the contributions of this great scientist who carried out extensive animal experiments. He was the greatest authority of all times on the biological effects of fluoride. He published in the American Medical Association journals, took issue with the commission's conclusions on one of the most severe air pollution disasters which occurred in Belgium in 1930, and established the toxicant to be fluoride. His book *Fluorine Intoxication* written in 1937 remains one of the most sought after reference texts on fluoride.

Since the first ISFR Conference we have met 18 times in Germany, Spain, Holland, England, USA, German Democratic Republic, India, Japan, Switzerland and Hungary. Our present President, Professor Humio Tsunoda, is involved in human research on fluoride effects as is our Vice President, Dr M Bély. Professor Ming Ho Yu, the President Elect, studies the effects of fluoride on plants and animals. Professor Gene W Miller is Secretary and researches the physiological and biochemical effects of fluoride on higher plants. Members of the Advisory and Editorial Boards represent 13 countries of the world and as many scientific disciplines. This is the strength of the ISFR. Physicians, dentists, orthopedic surgeons, veterinarians,

biologists, chemists, biochemists, geologists, plant physiologists, toxicologists and others meet and disseminate scientific information, with the thread of fluoride providing the common interest and binding Society members together in a united and coherent forum. Many decades have elapsed since Roholm's article appeared, but unfortunately most of the data which he presented are as new to most scientists today as they were in 1937. In the last 50 years great advances have been made in fluoride research but there remains much to be done. Fluoride toxicosis is still an insidious disease that afflicts humans, other animals and plants throughout the world. Understanding its action on organisms; accumulation in air, water and land; measuring the presence of fluorine species in various substances; and controlling its emission are formidable problems that confront us. We must not only fully understand the toxic effects of fluoride, but be able to determine its value in usable non-toxic forms as therapeutic agents in the treatment of osteoporosis, dental decay and other biological diseases. Detrimental side effects must be weighed against any apparent benefit from using fluoride. The ISFR is not a political society nor does it endorse or oppose public views on fluoride. It can, however, provide the forum for scientific exchange and publication of sound research. It will hopefully for many years to come motivate scientists to research fluoride effects and report findings that may gain application to benefit humankind.

We have now entered a new era with the new editor of *Fluoride*, Dr John Colquhoun, replacing Mrs George L Waldbott who has served as editor since our founding member Dr George L Waldbott passed away. Many thanks are extended to past editors, including Professor Albert Burgstahler who have given of their talents, resources and time so unselfishly. We pledge Dr Colquhoun our full support and wish him well as our new editor.

Gene W Miller

#### NEWS OF CONFERENCE

Dr Koichi Kono of the 19th Conference Secretariat advises that three special lectures at the Kyoto Conference will be:

Professor Min-Ho Yu, Huxley College of Environmental Studies  
Western Washington University, USA  
FLUORIDE EFFECTS ON PLANTS  
with an emphasis on seed germination

Professor Emeritus Gene W Miller, Utah State University, USA  
THE EFFECTS OF FLUORIDE ON HIGHER PLANTS  
with special emphasis on early physiological and biochemical disorders

Professor Miklos Bély, National Institute of Rheumatology, Hungary  
THE STRUCTURE AND FUNCTION OF BONE TISSUE  
AND ARTICULAR CARTILAGE IN OSTEOFLUOROSIS

(The Conference timetable is on page 98)

## FLUORINE AND OTHER TRACE ELEMENTS IN HAIR BY X-RAY FLUORESCENCE ANALYSIS

H Watanabe, Y Yoshida, K Kono, M Watanabe, S Inoue,  
Y Tanioka, T Dote, Y Orita, K Umehayashi and H Nagaie\*  
Osaka, Japan

**SUMMARY:** We studied the effects of sampling areas, sex and aging, the difference between black and white hair, and the statistical distribution in concentrations of fluorine and 11 other elements (Ca, Mg, P, Na, K, Fe, Cu, Zn, Se, I, Al) of hair. The concentrations of elements in hair of 437 healthy inhabitants of rural areas and 31 healthy medical students were determined by X-ray fluorescence analysis. There was no difference of concentrations among the sampling areas. The concentrations of Ca, Mg, P, Na, K, Fe, Cu and Zn in black hair were higher than those in white hair. The concentrations of F, P, Cu, Se, Zn and I were distributed as a normal curve, and those of Ca, Mg, Na, K, Fe and Al were distributed as a log-normal curve. There were differences between male and female subjects, with fluorine levels in male hairs being significantly higher than those in female hairs. There were correlations in age in some elements of hair, and the concentration of fluorine in female hairs tended to increase with advancing age. The results suggest that such factors as sex, age, and color of hair must be considered carefully in the analysis of hair elements.

**Key words:** Aging; Hair color; Sex; X-ray fluorescence analysis.

### Introduction

It is widely accepted that human hair is a reliable indicator of exposure to toxic environmental elements (1). Additionally, human hair has a great potential for use in clinical diagnosis in view of its usefulness as biopsy material or as a chronic index. However, there is disagreement among researchers with regard to the effects of basic factors such as sex and age on the concentrations of elements in hair. It is necessary to determine normal concentrations of elements in hair and the effects of basic factors on these concentrations.

Yamagata *et al* described the application of X-ray fluorescence (XRF) analysis on the measurements of fluorine and 21 other trace elements, and suggested the usefulness of this method (2). The present report is concerned with the effects of sampling areas, sex, aging, the difference between black and white hair, and the statistical distribution of concentrations of fluoride and 11 other elements (Ca, Mg, P, Na, K, Fe, Cu, Zn, Se, I, Al) in hair by the XRF method.

### Materials and Methods

The hair specimens used for studying the effect of sampling areas were obtained from 22 male medical students (average age 23 years) and 9 female medical students (average age 22 years). About 30 strands of hair samples, 5 cm

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in length, were cut close to the scalp from the frontal, occipital, right and left temporal regions of the head. The hairs used for studying the difference between black and white hair were obtained from 61 healthy males (average age 58 years) and 20 healthy females (average age 72 years) living in the Osaka area of Japan. The samples used for studying the effects of sex and age, and the statistical distribution of concentrations of 12 elements were obtained from 115 males (average age 55 years) and 322 females (average age 57 years) living in rural areas of Japan.

The hair samples were washed with distilled water for 10 minutes followed by acetone for 10 minutes. The concentrations of 12 elements were determined by X-ray fluorescence analysis according to Yamagata's report (2).

## Results

Table 1 shows the concentrations of fluorine and 11 elements in hairs from four different regions of the head. There was no significant difference in all elements among the concentrations of the four regions according to analysis of variance.

Figure 1 shows the frequency distribution of fluorine concentration in hair of male and female subjects. The statistical distribution was not shown to be normal or logarithmic normal by means of the Kolmogorov test of fit (level of significance = 0.05) (3). However, it was judged that the distribution was approximately logarithmic normal.

Figures 2 and 3 show the frequency distributions of 11 other element concentrations in the hair of male and female subjects. The statistical distributions of K and Al in males, and Ca and Na in females were logarithmic normal by means of the Kolmogorov test of fit. No other elements are considered to be normal or logarithmic normal. However, the distribution of Cu, Zn, P, Se and I concentrations were considered to be approximately normal, and those of Ca, Mg, Na, K, Fe and Al concentrations were approximately logarithmic normal.

Table 2 shows the concentrations of 12 elements in black and white hairs. The levels of all elements were compared between black and white hairs from the same subjects. There was no significant difference in fluorine concentration between black and white hairs. The geometric mean concentrations of black hairs were higher than those of white hairs in most of the other elements. The differences in Ca, Mg, P, Na, K, Fe, Cu and Zn concentrations were especially significant at the 0.05 level according to the paired t-test.

Table 3 shows the arithmetic and geometric means of the concentrations of 12 elements in hair of male and female subjects. The levels of fluorine in male hairs were significantly higher than those in female hairs. The concentrations of Ca and Mg in female hairs were significantly higher than those in male hairs. The concentrations of P, Na and K in male hairs were significantly higher than those in female hairs. However, there was little difference in Cu and Se levels between male and female hairs.

Figure 4 shows the concentrations of fluorine in male and female hairs by age group. The level of fluorine in female hairs tended to increase with advancing age; however, there was little difference in fluorine levels in male hairs among the age groups.

Figure 5 shows the concentrations of Na, K, Ca and Mg in male and female hairs by age groups. The concentrations of Na and K tended to increase, and those of Ca and Mg tended to decrease with advancing age.



Table 1  
Element concentrations (geometric mean,  $\mu\text{g/g}$ ) in hair collected  
from four different regions of the scalp in the same person ( $n=31$ )

Element	Frontal		Right temporal		Left temporal		Occipital	
	Mean	95% range	Mean	95% range	Mean	95% range	Mean	95% range
F	20.97	8.56-51.38	22.51	10.34-48.98	19.24	8.75-42.32	23.08	10.01-53.26
Ca	278.09	150.50-513.85	278.08	142.05-544.41	270.12	126.85-575.20	290.61	139.34-601.90
Mg	30.18	14.61-62.37	26.37	13.76-50.56	26.65	12.16-58.12	29.22	14.47-59.00
P	144.73	88.99-235.39	134.05	90.53-198.48	136.37	94.41-197.01	138.63	97.74-196.62
Na	409.72	171.54-978.58	416.82	171.18-1014.94	424.65	196.30-918.65	402.77	189.30-856.98
K	57.85	26.56-126.04	60.24	28.90-125.56	60.77	28.48-129.83	59.01	30.24-115.17
Fe	33.13	28.85-36.79	32.83	29.88-36.07	32.61	29.96-35.93	32.87	29.44-36.72
Cu	20.66	18.78-21.74	21.41	18.25-25.10	21.24	16.63-27.13	21.72	16.91-27.55
Zn	178.26	160.23-198.32	178.45	155.28-205.07	174.48	142.58-213.52	181.38	158.16-208.01
Se	5.13	4.97-5.32	5.12	4.92-5.32	5.17	4.81-5.56	5.13	4.94-5.33
I	0.59	0.46-0.75	0.56	0.43-0.73	0.56	0.45-0.71	0.60	0.47-0.77
Al	3.11	1.22-7.89	2.91	1.20-7.03	2.77	1.11-6.92	3.14	1.09-9.03

Figure 1  
Frequency Distribution of Fluorine Concentrations in Hair

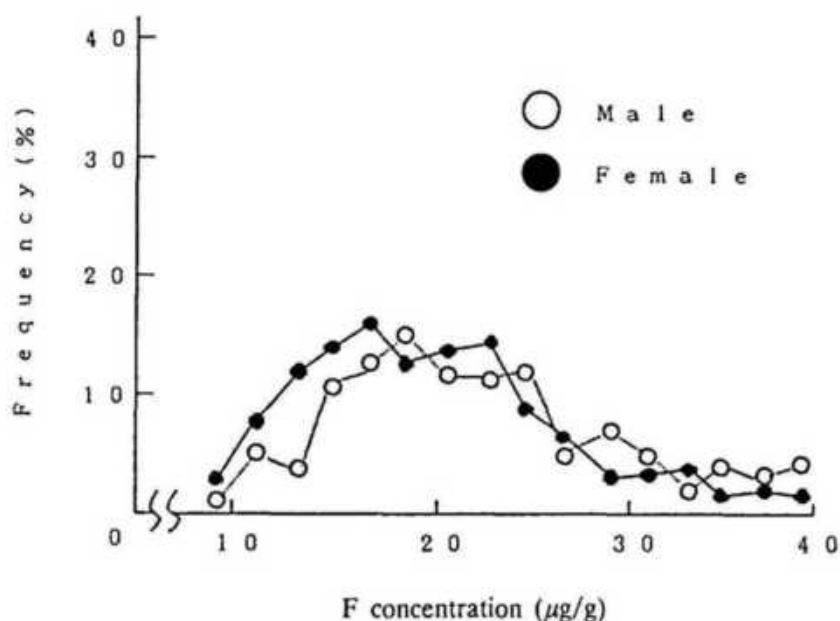


Table 2  
Element concentrations (geometric mean, µg/g) in black and white hair (n=81)

Element	Black hair		White hair	
	Mean	95% range	Mean	95% range
F	20.20	7.83 - 52.09	19.76	6.68 - 58.45
Ca	342.19	111.06 - 1054.00	116.95*	30.77 - 444.56
Mg	50.59	18.26 - 139.92	39.44*	16.55 - 93.95
P	160.71	97.78 - 264.14	115.69*	63.43 - 211.00
Na	634.96	149.62 - 2694.57	521.28*	135.82 - 2000.71
K	138.36	39.29 - 487.25	59.22*	27.45 - 127.63
Fe	35.59	26.86 - 47.17	33.42*	24.41 - 45.77
Cu	21.26	18.83 - 24.52	20.80*	17.77 - 24.35
Zn	165.89	105.98 - 259.67	151.29*	89.61 - 255.40
Se	5.01	4.79 - 5.24	4.93	4.55 - 5.35
I	0.53	0.33 - 0.85	0.51	0.29 - 0.89
Al	2.66	0.49 - 14.41	2.57	0.43 - 15.16

\*:  $P < 0.05$

Figure 2  
Frequency Distribution of Cu, Zn, P, Se and I Concentrations in Hair

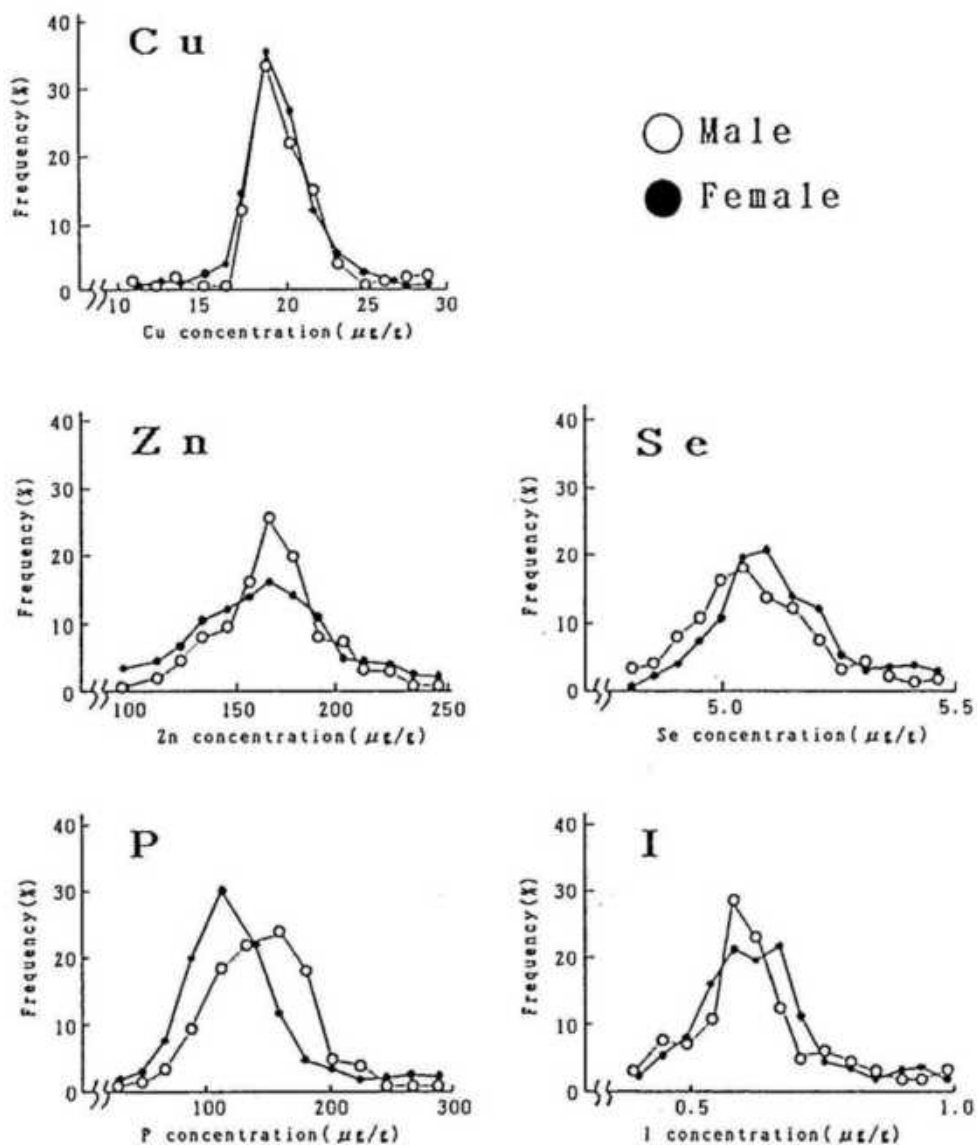


Figure 3  
Frequency Distribution of Ca, Mg, Na, K, Fe and Al Concentrations in Hair

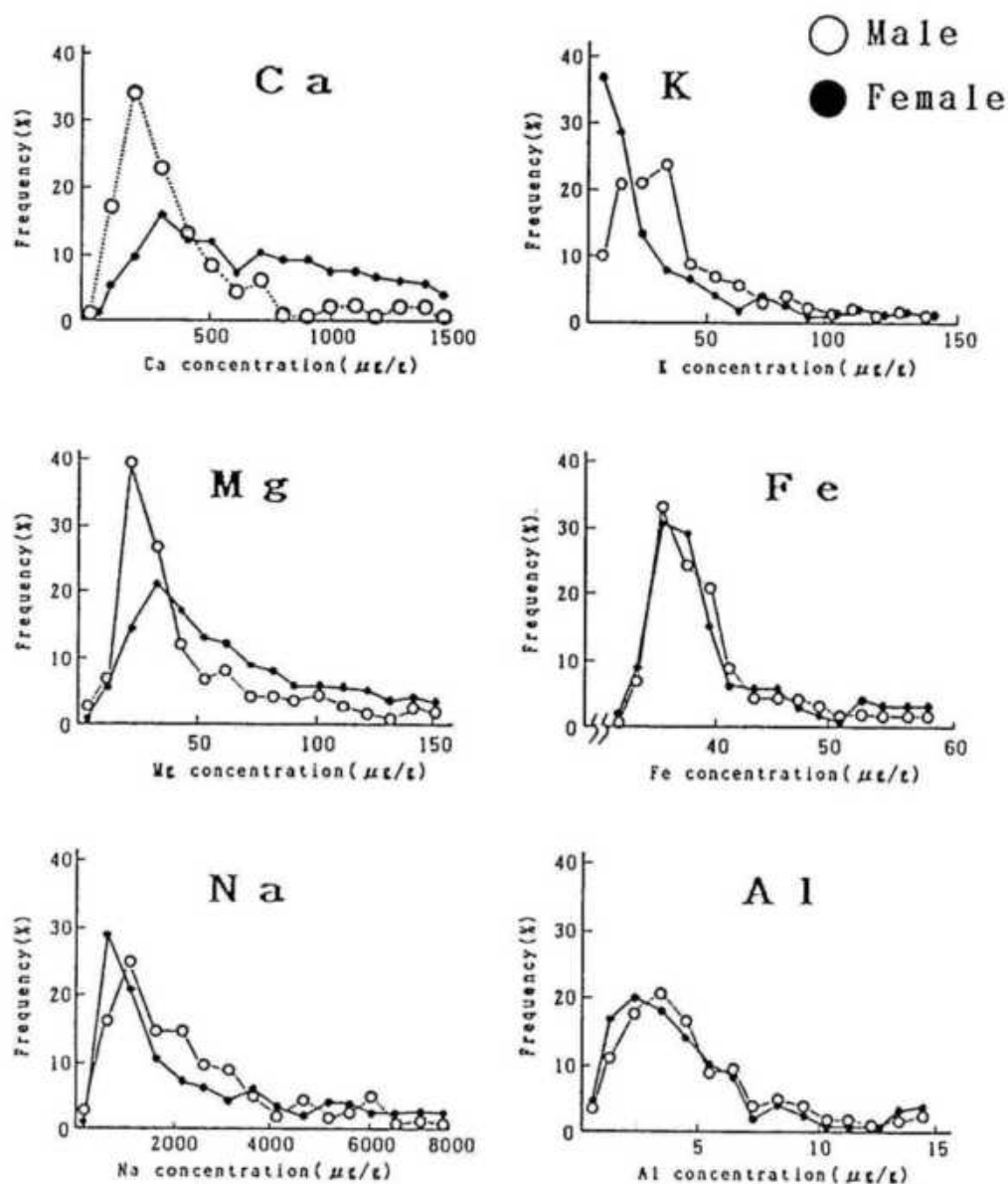


Table 3  
Concentrations of 12 elements in male and female hair ( $\mu\text{g/g}$ )

Element	Sex	Arithmetic		Geometric	
		Mean	S. D.	Mean	95% range
F	M	22.4	8.9	20.7	9.4 - 45.5
	F	19.3	6.2	18.3	9.8 - 34.2
Ca	M	368	232	324	123 - 851
	F	840	559	685	191 - 2448
Mg	M	38.7	22.6	34.5	14.0 - 84.8
	F	64.6	44.8	55.2	19.5 - 156.0
P	M	159	39.1	155	99 - 242
	F	136	30.6	132	102 - 260
Na	M	2238	1527	1822	531 - 6269
	F	1919	1726	1377	281 - 6758
K	M	328	228	267	71 - 994
	F	229	339	146	26 - 796
Fe	M	41.0	57.0	36.4	20.6 - 64.2
	F	46.4	96.6	37.8	18.1 - 78.7
Cu	M	21.7	4.4	21.5	16.2 - 28.4
	F	21.9	9.5	21.4	15.6 - 29.4
Zn	M	177	45.6	174	125 - 242
	F	183	52.0	177	113 - 277
Se	M	5.10	0.16	5.10	4.80 - 5.40
	F	5.14	0.21	5.14	4.78 - 5.52
I	M	0.59	0.11	0.58	0.38 - 0.89
	F	0.62	0.10	0.61	0.44 - 0.83
Al	M	4.54	2.96	3.76	1.14 - 12.4
	F	4.23	3.00	3.52	1.07 - 11.5

M: Male    F: Female

### Discussion

There are many studies of elements in hair by means of the ion-electrode method, inductively coupled plasma emission spectrometry, atomic absorption spectrometry and neutron activation analysis (4-6). However, it is important to determine the effects of sex, age and other factors on the concentrations of these elements for the application of the measurement of elements in hair for clinical diagnosis and evaluation of exposure to toxic elements.

This study indicated that the differences in fluorine and 11 elements among the concentrations of four regions of the scalp were not significant. These results might suggest that it is not necessary to consider the sampling areas carefully in the analysis of hair elements. Holzbecher *et al* reported that the difference in some elements among the concentrations of 9 regions were significant (6). However, these differences might result from surface contamination of hairs because unwashed hairs were measured in the study.

Yamagata *et al* reported that the frequency distribution of fluorine was logarithmic normal (2). This was not confirmed in the present study according to the Kolmogorov test. However, it was considered to be approximately logarithmic normal based on Figure 1. Kamakura (7) and Tsugane (8) reported

Figure 4  
The Concentration of F in Male and Female Hair by Age Groups

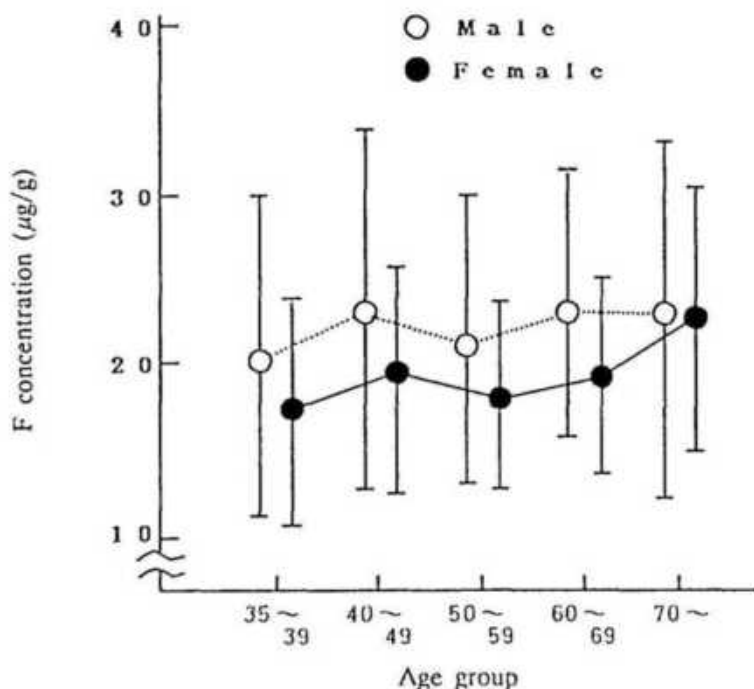
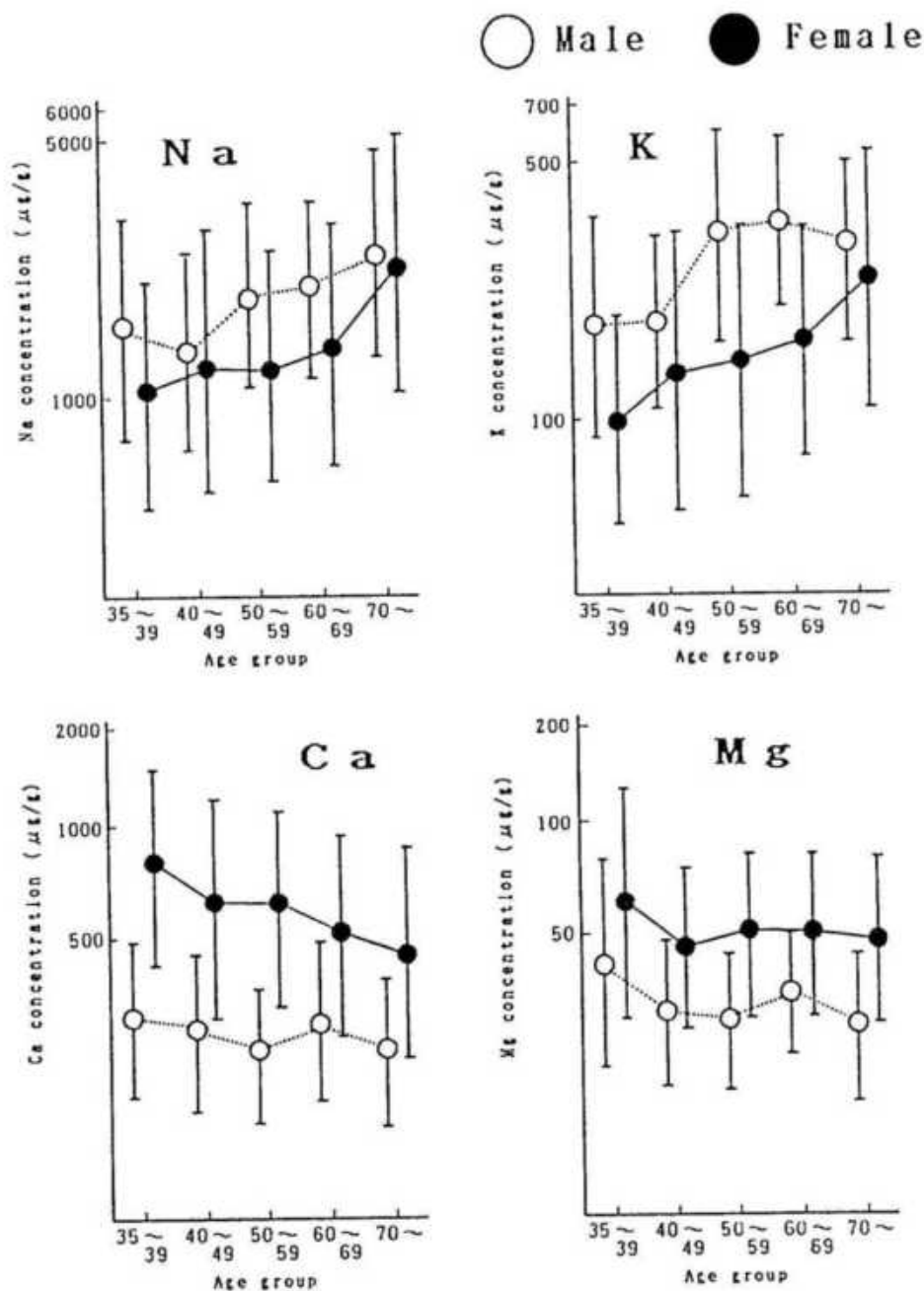


Figure 5

The Concentrations of Na, K, Ca and Mg in Male and Female Hair by Age Groups



that the statistical distribution of Ca, Mg, K, Fe and Al was logarithmic normal. Uchida *et al* found that the distribution of Se was normal (9). These reports are in agreement with the results in this study.

The first investigation of the relationship between hair color and trace elements contents was that of Schroeder and Nason (10). In their study there were differences of some elements among the concentrations in blond, brown, black and red hairs. However, it is possible that the differences resulted from the effects of sex, age and race, since their research was not matched for these factors. In our study the concentrations of many elements are considered to be affected by hair color because the element levels in black and white hairs from the same individual were compared. The concentrations of fluorine in hair were not affected by color.

There was no difference between concentrations of fluorine in male and female hairs in Yamagata's report. This is not in agreement with the present results. The reason might be that 95% of the females and 50% of the males in the present study used hair dyes or permanent hair wave chemicals. There are some studies which investigated the relationship between element levels and aging. Kamakura suggested that the concentration of Na tended to increase with age after age 20-29 years, and that the level of Ca tended to decrease after age 30 (7). Our results are in agreement with these findings. It is necessary to continue exploring the relationship between element contents and basic factors.

### Conclusion

We investigated the effect of sex, age, hair color, and sampling areas on the concentrations of fluorine and other elements in hair. Our findings suggest that many elements are affected by these factors. Sex, age and hair color in particular must be carefully considered in the analysis of elements in hair.

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## ASSOCIATION OF VITAMIN D DEFICIENCY WITH ENDEMIC FLUOROSIS IN INDIA

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**SUMMARY:** In India the clinical manifestations of fluorosis are reported at relatively low water fluoride levels. To investigate the role of nutritional factors, a study was conducted on 147 subjects of 2 villages known to be endemic for fluorosis. Ninety seven percent of the subjects had varying degrees of tooth mottling. Mean drinking water fluoride level was 3.3 ppm (range 0.55 to 11.2). The study group included 78 adults and 69 children. The radiological changes suggestive of fluorosis were present in 20 out of 21 patients for whom radiographs were taken. However, additional features of osteomalacia like osteoporosis (three), triradiate pelvis (two) and growth arrest line (two) were also present. In children in addition to tooth mottling a number of leg deformities were noted which included genu valgum (five), bowing of legs (three) and genu varum (one). Eight children who were subjected to radiological investigation had features of rickets like osteoporosis (five), growth arrest line and cupping of lower end of radius and ulna (one each). The radiological changes, raised serum alkaline phosphatase ( $237 \pm 107.6$  U/L) and a diet highly deficient in Vitamin D suggested coexistent nutritional rickets or osteomalacia which may be responsible for modifying the clinical picture of fluorosis in our subjects.

**Key words:** Fluorosis; India; Nutrition; Osteomalacia; Vitamin D.

### Introduction

Endemic fluorosis in India has two unique features: firstly, the clinical and radiological changes appear at relatively low water fluoride levels compared to western countries (1,2) and secondly, certain osteoarticular deformities like genu valgum and bowing of legs have been reported (3,4). A satisfactory explanation for these two features is lacking. Malnutrition which is common in India may modify the clinical picture of fluorosis. The effect of vitamin D deficiency may especially manifest in children because of increased requirement of nutrients during the period of rapid growth. Most of the published studies describe fluorosis in the adult population and there are only a few studies on pediatric fluorosis (3,5). In two villages, Deo Singh Khara and Marks Nagar of Uttar Pradesh, India, where fluorosis is endemic, a clinical and dietary survey was conducted.

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The comparison of the results in adults and children is presented with the aim of evaluating the role of nutrition in modifying the clinical picture of fluorosis

### Patients and Methods

The villages Deo Singh Khera and Marks Nagar are situated about 15 km from Lucknow. The population of these villages is 362 and 160 respectively. The staple diet of the villagers is cereals. Nonvegetarian food and dairy products are taken occasionally and in insufficient quantity. The sources of drinking water are shallow wells and handpumps. A door to door survey was conducted, medical histories were taken and physical examinations were done according to a fixed protocol. Tooth mottling was recorded on a I-III scale (6). Twenty one adults and 8 children were randomly selected and subjected to radiological examination which included radiographs of forearm including hand, pelvis, knee joint including leg, dorsolumbar spine in AP view, chest in PA, skull and cervical spine in lateral view. Haemoglobin, serum calcium, phosphorus, alkaline phosphatase, blood urea and serum creatinine were estimated. Urinalysis was also performed. Drinking water samples were analysed for fluoride content using concentration meter SA 270 and fluoride electrode 9409 BM from Orion USA. Ten families comprising 40 subjects were randomly selected and the nutritive value of the diet consumed by each family was calculated and compared with the recommendation of the expert group of ICMR (7). The results were calculated as the difference between per unit requirement and actual consumption.

### Results

Sixty nine children (12 years and under) and 78 adults were studied. The mean age of the children was 7.5 years (range 3-12) and mean age of the adults 33.9 years (range 13-90). The male female ratio was 2.4:1. In the adults, the symptoms and the physical signs were more frequent and more severe as compared to the children. In the adults pain and stiffness of joints (twenty nine), tingling and numbness in feet and hands (four) and weakness (three) were reported. The physical signs included tooth mottling (10 of grade I, 19 of grade II and 46 of grade III), 8 cases of stooping posture, 4 of bony outgrowths at the upper end of tibia, metatarsal bone and on the spinous process of thoracic vertebra resembling exostosis, 3 of generalised hyperreflexia of tendon jerks, and one of radiculopathy of right sixth cervical root. In the children tooth mottling grade I affected 24, grade II 22, and grade III 13. The leg deformities included 5 cases of genu valgum, one of genu varum and 3 of bowing of legs. The diet of the villagers was deficient in proteins by 28.6%, in calories by 42.7% and in vitamin D by 88.9%.

### *Radiological features*

The radiological features in the adults and the children are shown in the Table. In the adults the classical features of fluorosis were present: osteosclerosis 15, interosseous membrane calcification 17, calcification of musculotendinous attachment which leads to bony outgrowth pseudoxostosis 6, and osteophytes 11. Narrowing of cervical and lumbar canal were also present in 5. Osteoporosis of long bones (Figure 1) with coarse trabecular pattern were present in 3 patients although all of them had marked osteosclerosis in pelvis and thoracolumbar spine. In 2 females the features of osteomalacia, triradiate pelvis and growth arrest lines, were also present in addition to the changes suggestive of fluorosis (Figure 2). The radiological changes in the children included 4 with growth arrest line, 3 with bowing of tibia and fibula (Figure 3) and one each with cupping of lower end of radius and ulna and resorption in the metacarpal bones.

### *Biochemical features*

Serum calcium and phosphorus levels in the adults were  $9.39 \pm 1.38$  mg% and  $4.1 \pm 0.82$  mg%, in children  $9.87 \pm 1.39$  mg% and  $4.08 \pm 0.85$  mg% respectively which were normal but alkaline phosphatase levels were raised. Alkaline phosphatase was  $229.70 \pm 123.05$  U/L in adults and  $287.64 \pm 48.96$  U/L in children. Water fluoride level was above 2 ppm in 9 out of 10 samples examined in Deo Singh Kera. The mean value was 3.82 ppm (range 1.37-7.93). In Marks Nagar water fluoride was high in 2 out of 4 samples, the mean being 2.84 (range 0.55-11.2 ppm).

## **Discussion**

Fluorosis is endemic in both the villages Deo Singh Khera and Marks Nagar. Ninety seven percent of the subjects had varying degrees of tooth mottling. Twenty out of 21 adults whose radiographs were taken had membrane calcification or osteosclerosis or both which are characteristic of skeletal fluorosis. In 4 subjects features of osteomalacia - triradiate pelvis and growth arrest lines were also present. In the children the only evidence of fluorosis was tooth mottling. Seven out of 8 children whose radiographs were taken had radiological features of rickets. The secondary cause of rickets or osteomalacia like renal disease, malabsorption and anti-convulsant toxicity were excluded by clinical evaluation and relevant investigations.

The dietary deficiency of vitamin D suggests that nutritional osteomalacia and rickets coexisted with fluorosis in our patients. The association of genu valgum and osteoporosis with endemic fluorosis in adults has been reported from the state of Andhra Pradesh, India. Nutritional deficiency was suggested to be responsible for this syndrome although detailed dietary analysis was not performed (4). In children the radiological changes consistent with rickets, osteoporosis and hyperparathyroidism have been reported with fluorosis from another endemic area in India (3).

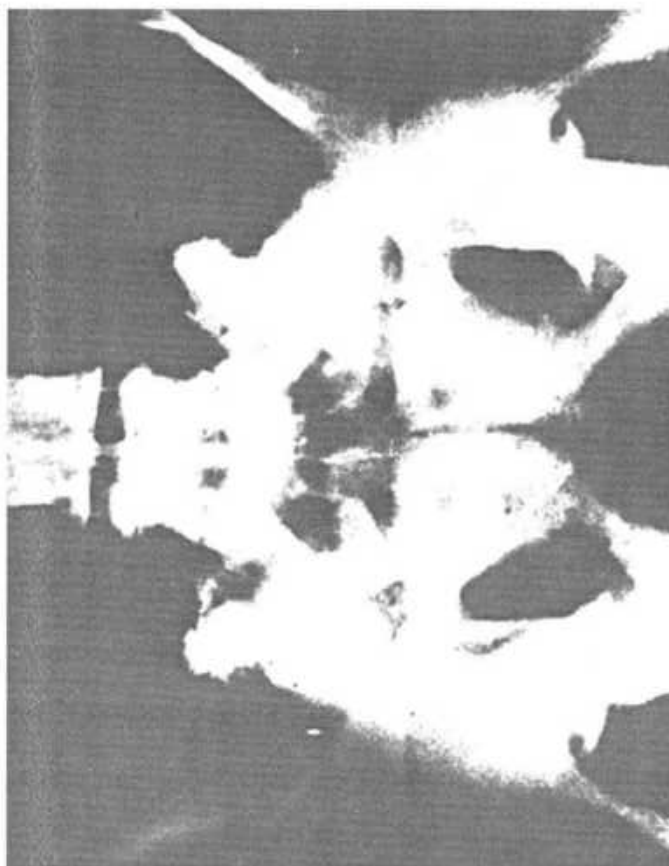
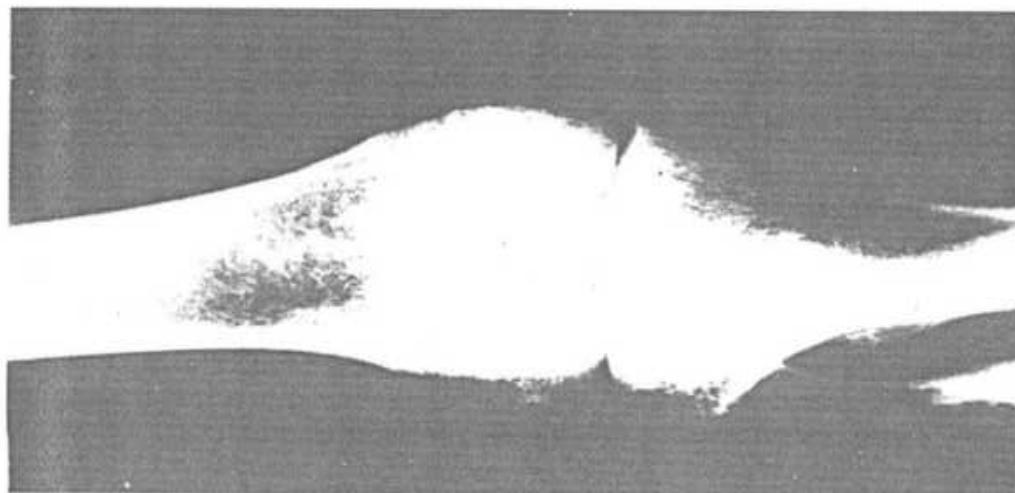


Figure 1 (left). Radiograph of knee joint AP view showing osteoporosis of tibia, fibula and calcification of interosseous membrane

Figure 2 (above). Radiograph of pelvis AP view showing triradiate pelvis and ligamentous calcification

Figure 3 (below, next page). Radiograph of leg lateral view of a fluorotic child showing bowing of tibia and fibula, coarse trabecular pattern and growth arrest lines





**Table**  
Radiological features in adults and children

Radiological features	Adults (N=21)	Children (N=8)
Osteosclerosis	15	-
Membrane calcification	17	-
Pseudoexostosis	6	-
Osteophytes	11	-
Osteoporosis	3	5
Tibial and fibular bowing	1	3
Growth arrest line	2	4
Coarse trabecular pattern	9	6
Triradiate pelvis	2	1
Cupping of lower end of radius and ulna	-	1
Subperiosteal bone resorption in metacarpal bones	2	1

The nutritional deficiency of vitamin D seems to be mainly responsible for the above mentioned clinical and radiological features; but fluorosis itself may be contributing. Fluoride produces metabolic changes in bone by enhancing both osteoclastic and osteoblastic activities. The former leads to osteoporosis and the latter to a state of calcium deficiency finally leading to rickets and osteomalacia (8). Fluoride also reduces absorption of calcium from the gut and enhances its removal from the bone (9). It is therefore likely that the leg deformities and the radiological changes in our patients may be due to an interaction of nutritional deficiency and complex metabolic factors. It is therefore possible that improvement of the nutritional status may reduce or even prevent the deformities associated with fluorosis. Further studies in this direction are needed.

### Acknowledgements

We thankfully acknowledge the help of Dr J K Dhaon, Assistant-Director, State Health Institute for his help in field study, Mr R P Batta and Mr A K Shrivastava of the Department of Dietetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences, for their help in dietary survey, Mr Anil Kumar of the Scientific Illustration Department of the Institute for illustrations and Mr V K Tripathi for secretarial help.

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## REVERSIBLE FLUORIDE INDUCED FERTILITY IMPAIRMENT IN MALE MICE

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**SUMMARY:** Sodium fluoride (NaF) fed to adult male albino mice at a dose of 10 mg and 20 mg/kg body weight, caused a significant decrease in sperm count and motility. Scanning electron microscopy and silver nitrate staining showed large numbers of deflagellated spermatozoa, with acrosomal, midpiece and tail abnormalities. The treatment caused loss of fertility rate when normal cycling female mice were mated with treated males. Withdrawal of treatment for a period of 2 months resulted in a significant recovery in sperm count and sperm motility as well as in fertility rate.

**Key words:** Acrosomal integrity; Fertility impairment; Mice; NaF; Sperm count; Sperm motility.

### Introduction

The human population are exposed to fluoride from various sources such as soil, water and air. Extensive research has been carried out during the past several decades on skeletal and dental fluorosis (1). However, the effects of fluoride on the reproductive organs leading to loss of fertility is incomplete and conflicting. Tao and Suttie (2) reported that fluoride had no essential role in reproduction of female mice. On the contrary, Messer *et al.* (3,4) found that low fluoride intake by female mice impaired their fertility and reproductive capacity although growth rate and litter size were not affected.

Chinoy and Sequeira (5) reported that reproductive organs of male mice were affected by 10 and 20 mg/kg body weight of NaF ingested for 30 days. The testis, epididymides and vas deferens showed more alterations in their histology and histoarchitecture than seminal vesicle and prostate. However, all induced effects on the structure of reproductive organs were reversible after treatments were discontinued for two months. Therefore, it was clearly demonstrated that the effects induced by NaF treatment were transient and reversible and hence no permanent damage occurred. The present study was undertaken to investigate the effects of fluoride on mouse sperm structure and motility as well as fertility.

### Materials and Methods

Sodium fluoride (10 and 20 mg/kg body weight) was administered orally to 20 healthy Swiss strain adult mice (20-30 g) in each group. The animals were maintained on standard chow; water was given *ad libitum*. They were housed in an air conditioned animal house at a temperature of  $26 \pm 2^\circ\text{C}$  and exposed to 12 to 14 daylight hours. The animals were divided into a control group and four treatment groups (Table 1). After treatment for 30 days, NaF

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was withdrawn for one or two months. The male mice were autopsied by cervical dislocation and the percent sperm motility and sperm count of the cauda epididymis from control, treated and withdrawal groups of mice were determined by using Neubauer chamber of the Haemocytometer of the Prasad *et al* (6) method and expressed as percentage and millions/mL respectively.

To conduct the fertility test, normal cycling females were cohabited with treated males on the 31st day after treatment and in the withdrawal group at the end of one or two months respectively in the ratio of 2:1. The vaginal smear was checked the following morning to observe the presence of sperm which indicated that mating had occurred; this was day "0" of pregnancy. The females were separated from the males and allowed to remain on a normal diet for 16 days after which they were autopsied. The uteri were opened longitudinally and the number of implantation sites in each uterine horn as well as the number of corpora lutea in the ovary were counted according to WHO protocol MB-50 (7).

The acrosomal integrity of the sperm from the cauda epididymis of control, treated and withdrawal group of mice was studied using the modified silver nitrate technique (8). The methods of Chinoy and Sanjeevan (9) and Chinoy and Chinoy (10) were used for scanning electron microscopic observations of mouse cauda epididymal spermatozoa, under normal as well as treated conditions.

## Results

**Sperm Motility:** The cauda epididymis sperm motility decreased significantly ( $p < 0.001$ ) after 30 days treatment with both doses compared to control (Table 2). However, withdrawal of treatment for two months resulted in almost complete recovery (Table 2).

**Sperm Count:** The cauda epididymal sperm count of treated mice had decreased in comparison to the control (Table 2). After withdrawal of treatment, recovery was noted to have increased after two months (Table 2). In the uteri of females mated with treated males, the implantation sites were absent compared to 12-14 in the control, so that fertility was nil in treated mice (Table 2). Withdrawal of treatment resulted in significant recovery of fertility (Table 2).

Scanning Electron Microscopy (SEM) of normal cauda epididymal spermatozoa had scimitar shaped head (Figure 1). In the treated mice spermatozoa from cauda epididymis, head, midpiece and tail showed abnormalities compared to the control. Deflagellated spermatozoa were also observed (Figures 2 and 3).

Silver nitrate staining of cauda epididymal sperm of the control mouse revealed a clear differentiation of the acrosomal, post acrosomal and midpiece regions (Figure 4). However in NaF treated animals, the staining was diffuse with no proper demarcation (Figures 5 and 6).

## Discussion

Treatment induced a loss in fertility rate when normal cycling female mice were mated with treated males. Hall and Howell (11) observed that infertility is a relatively common manifestation of deficiency in trace elements, including deficiencies of copper, zinc, manganese, iodine and



selenium. Messer *et al* (3) demonstrated that low fluoride intake over two generations showed a progressive decline in litter production in female mice and addition of fluoride to the diet of females restored their reproductive capacity, even though earlier they were demonstrated as infertile. Kour and Singh (12) reported that in fluoride ingested mice testis showed a lack of maturation and differentiation of spermatocytes, cessation of spermatogenesis and necrotic seminiferous tubules. Moreover, a clear relationship between fluorosis and testis damage was observed by these authors in mice administered 500 and 1000 ppm NaF. Similar results have been obtained in mice given 10 and 20 mg NaF/kg body weight for 30 days (5). The testis, epididymis and vas deferens were affected more than seminal vesicles and prostate. As a consequence of the alterations in histology of these organs by NaF, particularly epididymis and vas deferens, their internal milieu is rendered

Table 1

S1 No.	Group	Treatment	Duration (Days)	Day of Autopsy
1.	Control	—	—	Along with Treated
2.	NaF Treated	10 mg/kg Body Weight Equivalent to 230 ppm/animal/day	30	31st
3.	NaF treated	20 mg/kg Body Weight Equivalent to 400 ppm/animal/day	30	31st
4.	NaF treated	Withdrawal of Treatment for one month	30	31st
5.	NaF treated	Withdrawal of treatment for two months	60	61st

Table 2

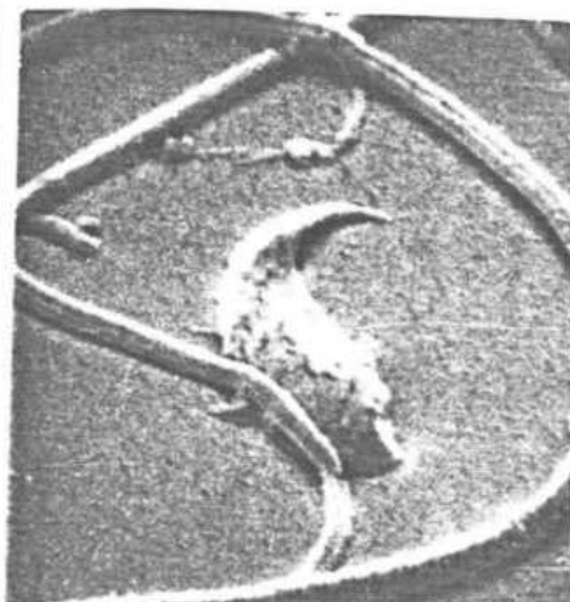
Sperm Motility, Count and Fertility Rate in Control, NaF-Treated and NaF-Withdrawal Group of Mice

Parameter	Control	NaF-Treated		NaF-Withdrawal	
		10 mg/kg body weight	20 mg/kg body weight	1 month	2 months
Cauda Epididymal Sperm motility (%)	80.0 $\pm$ 0.28	38 $\pm$ 2	24 $\pm$ 1	44 $\pm$ 0.3	74.6 $\pm$ 1.7
Cauda Epididymal Sperm Count ( $10^6$ /mL)	45.0 $\pm$ 0.58	34 $\pm$ 3	32.8 $\pm$ 0.5	37.7 $\pm$ 0.6	43.4 $\pm$ 1.6
Fertility Rate	95-100% +ve	nil	nil	nil	75% +ve

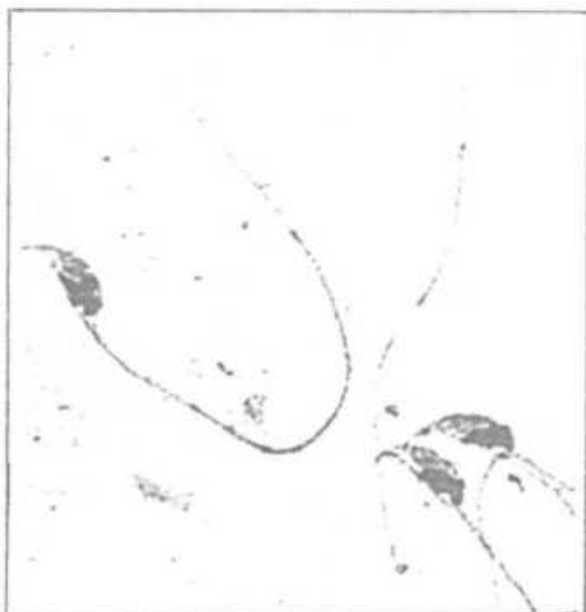
Figure 1 (right)  
Normal Spermatozoa



Figures 2 and 3 (below)  
Abnormal Spermatozoa



**Figure 4 (right)**  
Clear Demarcation (Control)



**Figures 5 and 6 (below)**  
Poor Demarcation (Treated)



hostile for the sperm motility, metabolism and survival (5), which was probably responsible for loss of fertility. Another factor leading to reduction of fertility might be due to the large number of deflagellated spermatozoa, as well as their acrosomal, midpiece and tail abnormalities caused by scanning electron microscopy and the modified silver nitrate staining technique (8).

The fluoride may interfere with spermatozoa maturation at the epididymal level or with the secretion of accessory glands. Since the histology of the reproductive organs was affected, their metabolism would be altered. The present study therefore elucidates certain important features of fluoride effects, namely (1) induction of infertility in male mice by altering the sperm structure and function; (2) the changes were transient and reversible since sperm density and motility were restored and significant fertility regained.

### Conclusion

NaF does not cause permanent damage to reproductive organs, since normalcy in histoarchitecture and fertilizability of spermatozoa was restored within 2 to 3 months after withdrawal of treatment.

### Acknowledgement

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## STUDIES ON ALTERATIONS IN BRAIN LIPID METABOLISM FOLLOWING EXPERIMENTAL FLUOROSIS

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**SUMMARY:** The neurotoxic effect of fluoride on lipid content of brain was assessed in rabbits during experimental fluorosis. Sodium fluoride at 5, 10, 20 and 50 mg/kg body weight/day was injected subcutaneously for 100 days into 60 rabbits of both sexes. The control animals were given 1 cc distilled water/kg body weight/day for the same period. Biochemical studies showed hyperlipidemia, hyperphospholipidemia and hypertriglyceridemia in the brain of treated animals of both sexes. The maximum increase in total lipids, phospholipids and triglycerides of brain occurred in animals treated with 50 mg NaF/kg. In male rabbits, the cholesterol content of brain rose suddenly ( $p < 0.001$ ) in the 5 mg fluoride group, followed by gradual decline in 10, 20 and 50 mg fluoride groups. In females, the cholesterol level rose ( $p < 0.001$ ) in animals of the 5, 10 and 20 mg fluoride groups and fell suddenly in the 50 mg fluoride group. Fluoride exerts an inhibitory effect on the free fatty acids in brain of both sexes. The relevance of these results in experimental fluorosis is discussed.

**Key words:** Brain; Cholesterol; Fluoride; Free fatty acids; Phospholipids; Rabbit; Total lipids; Triglycerides.

### Introduction

The manifestations of the initial phase of fluorosis indicate injury to the central nervous system and the spinal cord. In humans, the neurological complications in advanced fluorosis in the form of partial and complete paralysis of arms and legs, headache, vertigo, spasticity in the extremities, visual disturbances and impaired mental acuity have been reported (1).

Fluoride is known to enter the brain and the blood brain barrier fails to exclude it from the nervous tissue (2). Accumulation of fluoride may induce a wide variety of changes in physiological and biochemical parameters. But due to lack of precise experimental data it is difficult to draw conclusions concerning the effect of fluoride on the nervous system.

The present investigation is aimed at elucidating alterations in lipid metabolism in brain of rabbit in experimental fluorosis.

### Materials and Methods

**Experimental Design:** Sixty albino rabbits of both sexes weighing 400-650 gm were divided into 5 groups of 12. They were given fluoride subcutaneously at 5, 10, 20 and 50 mg/kg body weight/day for 100 days. The control animals were given 1 cc distilled water/kg body weight/day for the same period.

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All the animals were maintained on standard laboratory chow and water was supplied *ad libitum*. After 100 days, the control and  $F^-$  treated animals were sacrificed and the brains immediately removed for various biochemical studies. Extraction of total lipids was done by the method of Folch *et al* (3). Total lipids were estimated gravimetrically.

*Separation of neutral lipids:* Silica gel G thin layer plates (20x20 cm) were prepared for thin layer chromatography (4). Dried plates activated at 100°C for 90 minutes, were developed in a solvent system consisting of n-hexane:diethyl ether: glacial acetic acid (90:10:1 v/v). The chromatograms were air dried and stained with iodine vapours in sealed chambers. The resultant yellow spots are identified, marked and taken into extracting solvent: n-hexane:diethyl ether, 1:1 v/v. Pooled extracts evaporated to dryness were used for spectrophotometric analysis.

*Determination of various lipid constituents:* Triglycerides were determined by the method of VanHandle and Zilversmit (5). Estimation of phospholipids was done as described by Ames (6). Quantitative analysis of free fatty acids was done by the method of Chakrabarty *et al* (7) and cholesterol was assessed by the method of Stadtman (8).

Significance of the resulting data was determined by Student's t-test.

## Results

Changes in the levels of total lipids and their various components are shown in Tables 1 and 2.

Differences in the level of total lipids in the brain of fluoridated and control rabbits of both sexes were significant ( $p < 0.05 - 0.001$ ). The female rabbits showed a higher percent increase in total lipid content of brain as compared to the males (Figure 1).

The concentration of phospholipids in the brain showed a significant ( $p < 0.001$ ) increase in the experimental animals of both sexes compared with the controls (Figure 2).

The level of neutral lipids in the brain showed a 16% increase in male rabbits treated with 5 mg fluoride. The level was slightly decreased (5%) in animals treated with 10 mg fluoride. Again 16% decrease in neutral lipid content of brain was observed in animals of the 20 mg fluoride group. The level of neutral lipids returned to the control values in animals treated with 50 mg fluoride. In females the amount of neutral lipids in the brain showed a slight to moderate elevation in all  $F^-$  treated groups compared with the control (Figure 3).

Brain triglyceride levels were highly elevated in fluorotic animals of both sexes (Figure 4). The females showed a greater increase in all of the  $F^-$  treated groups compared with the control. The highest percent increase was seen in animals of the 50 mg fluoride group (150.7% in males vs 265.5% in females).

**Table 1.** Effect of fluoride on total lipids, phospholipids and neutral lipids in the brain of rabbits (Data are means  $\pm$  SD)

Constituent	Treatment F mg/kg b.w.	Male	Percentage of control	Female	Percentage of control
Total lipids (mg/g w.w.)	0(control)	69.81 $\pm$ 3.372		60.76 $\pm$ 9.418	
	5	78.28 $\pm$ 7.187*	+12.1	90.61 $\pm$ 9.164***	+49.1
	10	81.82 $\pm$ 9.200*	+17.2	95.07 $\pm$ 8.058***	+56.4
	20	79.28 $\pm$ 5.610**	+13.5	102.22 $\pm$ 7.952***	+68.2
	50	253.36 $\pm$ 21.346***	+262.9	245.91 $\pm$ 10.539***	+304.7
Phospho- lipids (mg/g w.w.)	0(control)	30.36 $\pm$ 0.550		28.36 $\pm$ 0.130	
	5	38.99 $\pm$ 0.250***	+28.4	40.84 $\pm$ 0.350***	+44.0
	10	36.44 $\pm$ 0.240***	+20.0	46.44 $\pm$ 0.210***	+63.7
	20	41.04 $\pm$ 0.510***	+35.1	44.31 $\pm$ 0.325***	+56.2
	50	39.86 $\pm$ 0.260***	+31.2	42.87 $\pm$ 0.296***	+51.1
Neutral lipids (mg/g w.w.)	0(control)	41.83		42.60	
	5	48.55	+16.0	56.28	+33.8
	10	39.35	-5.92	50.16	+19.2
	20	35.02	-16.2	43.77	+4.0
	50	40.83	-2.3	50.06	+19.0

P values as compared with control: \*  $p < 0.05$  \*\*  $p < 0.01$  \*\*\*  $p < 0.001$

**Table 2.** Effect of fluoride on triglycerides, free fatty acids and cholesterol in the brain of rabbits (Data are means  $\pm$  SD)

Constituent	Treatment F mg/kg b.w.	Male	Percentage of control	Female	Percentage of control
Triglycerides (mg/g w.w.)	0(control)	12.17 $\pm$ 1.091		11.12 $\pm$ 0.015	
	5	19.12 $\pm$ 0.550**	+57.1	21.44 $\pm$ 0.220**	+92.8
	10	19.23 $\pm$ 0.150**	+58.0	31.53 $\pm$ 0.180**	+183.5
	20	17.66 $\pm$ 0.160**	+45.1	29.19 $\pm$ 0.190**	+162.5
	50	30.52 $\pm$ 0.166**	+150.7	40.65 $\pm$ 0.226**	+265.5
Free fatty acids (mg/g w.w.)	0(control)	22.94 $\pm$ 0.230		25.15 $\pm$ 0.110	
	5	9.96 $\pm$ 0.109**	-56.5	20.57 $\pm$ 0.140**	-18.2
	10	14.34 $\pm$ 2.842**	-37.4	8.92 $\pm$ 0.169**	-64.5
	20	12.93 $\pm$ 0.120**	-43.6	7.52 $\pm$ 0.220**	-70.0
	50	6.62 $\pm$ 0.155**	-71.1	5.46 $\pm$ 0.190**	-78.2
Cholesterol (mg/g w.w.)	0(control)	6.72 $\pm$ 0.306		5.79 $\pm$ 0.061	
	5	19.47 $\pm$ 0.310**	+18.0	14.27 $\pm$ 0.440**	+146.4
	10	5.78 $\pm$ 0.336*	-13.9	9.71 $\pm$ 0.169**	+67.7
	20	4.43 $\pm$ 0.046**	-34.0	7.06 $\pm$ 0.094	+21.9
	50	3.69 $\pm$ 0.052**	-45.0	3.95 $\pm$ 0.370**	-31.7

P values as compared with control: \*  $p < 0.05$  \*\*  $p < 0.001$

Figure 1. Total Lipids in Brain during Experimental Fluorosis

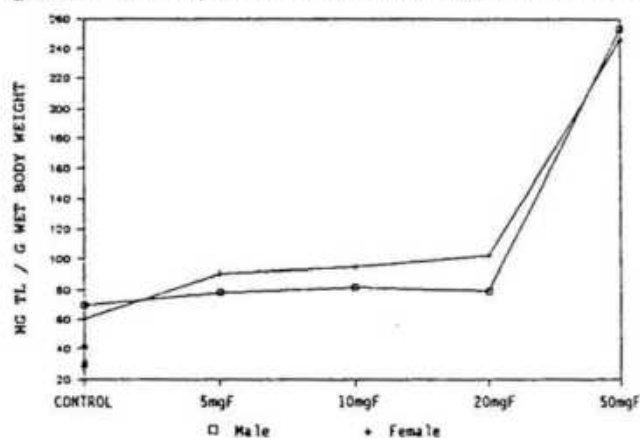


Figure 2. Phospholipid Content of Brain in Experimental Fluorosis

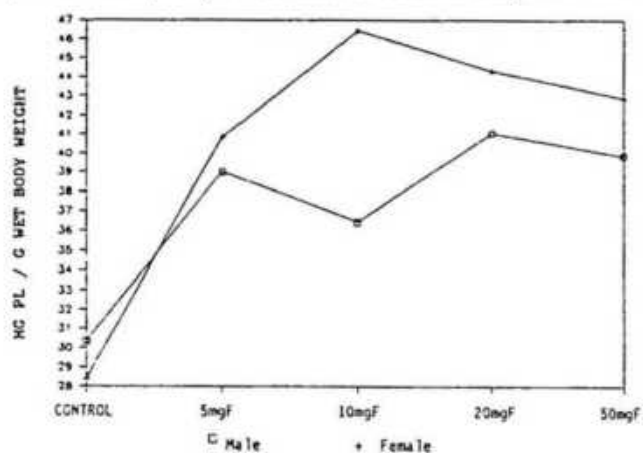


Figure 3. Neutral Lipids in Brain in Experimental Fluorosis

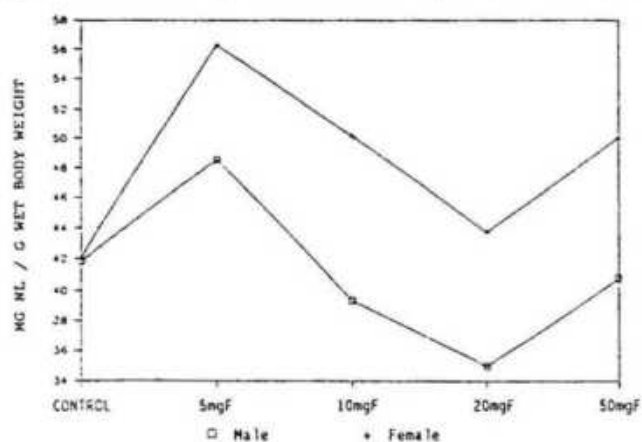




Figure 4. Triglyceride Levels in Brain of Rabbit in Fluoride Intoxication

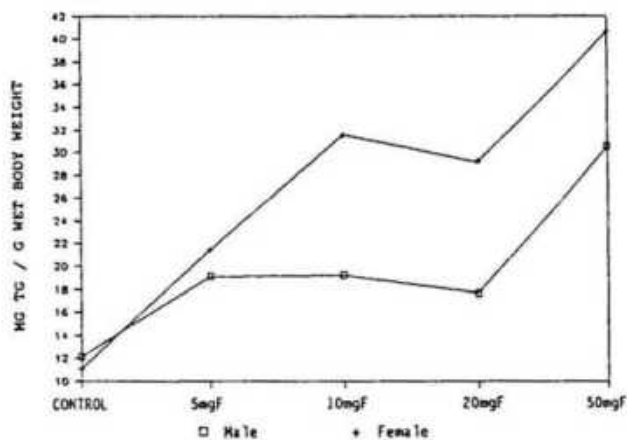


Figure 5. Free Fatty Acid Levels in Brain in Experimental Fluorosis

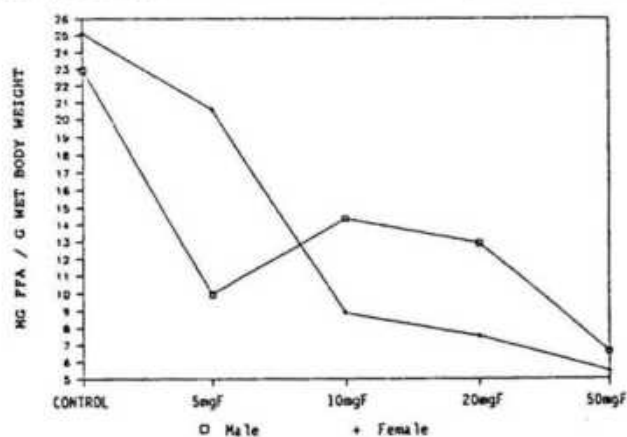
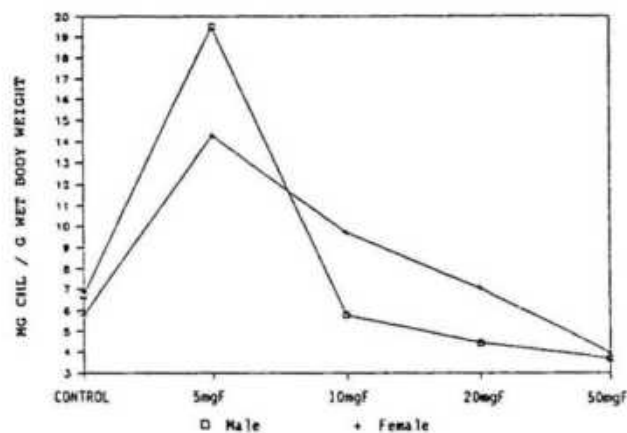


Figure 6. Cholesterol Levels in Brain during Experimental Fluorosis



Fluoride caused a decrease in brain free fatty acids in the experimental animals of both sexes (Figure 5).

The cholesterol content of the brain showed a sudden rise in male animals treated with 5 mg fluoride followed by rapid decline ( $p < 0.001$ ) in subsequent groups. In females, the brain cholesterol showed a significant increase ( $p < 0.001$ ) in animals treated with 5, 10 and 20 mg fluoride, whereas it significantly declined ( $p < 0.001$ ) in animals treated with 50 mg fluoride (Figure 6).

### Discussion

In this study on rabbits, there are appreciable changes in the brain lipid metabolism induced by fluoride. They are similar to the disorders known as "lipid storage diseases". Lipidosis is a disorder of lipid metabolism leading to abnormal fat accumulation in body tissues particularly in the liver and brain (9). The present data indicate hyperlipidemia in the brain of rabbits of both sexes resulting from fluoride intoxication. Hyperlipidemia may occur due to enzymatic defect, the inability of brain to degrade the lipid in the body.

Since lipids are transported in association with carrier proteins, it is possible that hyperlipidemia may result from a defect in lipoprotein metabolism. Several possible mechanisms are suggested:

- a) an inhibition of the production of plasma lipoproteins;
- b) a block in lipoprotein apoprotein synthesis;
- c) a block in the synthesis of lipoprotein from lipid and apoprotein;
- d) a failure to provide the phospholipids found in lipoproteins;
- e) a deficiency in lipotropic factors.

The deficiency of a lipotropic agent causes triglycerides to accumulate. Elevation of triglycerides may lead to a decrease in the synthesis of free fatty acids during fluoride intoxication (10). The decrease in free fatty acids synthesis in soft tissues during experimental fluorosis has been reported earlier (11-13). The hyperlipidemia, hypertriglyceridemia and hyperphospholipidemia represent excessive mobilization of fat (14).

Fluoride inhibits many enzymes involved in lipid metabolism - *e.g.* lipases, phospholipases which are capable of hydrolyzing the fatty acids from phospholipids (15). The inhibition of these enzymes could result in elevated levels of phospholipids and decrease in free fatty acids.

In this study, the brain cholesterol was significantly elevated in the early phase of intoxication (5 mg fluoride group), but declined in subsequent fluoridated groups of male animals, whereas in females, hypercholesterolemia in the brain was found in animals treated with 5, 10 and 20 mg/kg fluoride. The cholesterol content of the brain was highly elevated in female animals of the 5 mg fluoride group (146%) and the 10 mg group (67%). In animals of the 20 mg fluoride group the cholesterol levels in the brain were moderately elevated (21%). The levels were significantly decreased (31%) in animals receiving the highest dose of fluoride (50 mg/kg body weight). Hypercholesterolemia may be due to the deficiency of liposomal lipase which hydrolyzes cholesterol esters taken up by the cell.

Several studies involving the effects of fluoride on serum lipids have been reported. Townsend and Singer (16) observed decrease in serum cholesterol in guinea pigs. On the other hand, Vatassery *et al* (17) reported an increase in serum cholesterol in guinea pigs which received deionized water containing 25 ppm fluoride for 13 weeks. According to Singer and Armstrong (18) an increase in fluoride intake did not influence serum cholesterol.

As a result of an imbalance in the synthesis and breakdown of the lipids in the brain due to fluoride intoxication, the neurons of the cerebellar cortex showed degeneration. In addition to this, there was retarded development, paraplegia and quadriplegia.

### Conclusions

1. Fluoride interferes with lipid metabolism in the brain of experimental rabbits. The abnormal accumulation of lipid in rabbit brain may be due to deficiency of a lipotropic agent.

2. Hyperlipidemia, hyperphospholipidemia and hypertriglyceridemia in the brain may result from a defect in lipoprotein metabolism.

3. Depletion of cholesterol and free fatty acid in the brain of experimental rabbits may be the result of decreased lipolysis due to inhibition of lipase.

4. Hypercholesterolemia in rabbit brain may be due to the deficiency of liposomal lipase which is necessary to hydrolyze the cholesterol esters.

Whether similar changes take place in humans after excessive ingestion of sodium fluoride is yet to be ascertained.

### Acknowledgement

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## FLUORIDE BOUND TO PLASMA CONSTITUENTS IN CATTLE

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**SUMMARY:** The technique of dialysis commonly used to measure the extent of drug binding to plasma constituents was used to determine the percentage of fluoride bound to the plasma constituents in cattle. Determinations were carried out on the plasma of 22 cows, from 4 different herds, immediately after blood sampling and, for 8 cows, on a plasma that had been stored at  $-30^{\circ}\text{C}$  for 7, 21, or 60 days. The percentage of fluoride bound to plasma constituents was  $20.7 \pm 19.2$ . In one of the herds, the mean value was notably lower ( $4.8 \pm 2.8$ ). In the same herd, individual differences were marked. The binding to plasma constituents did not correlate with the ionic fluoride concentration in the plasma. The 7-day or 21-day deep freezing did not change the results.

**Key words:** Cattle; Fluoride binding; Plasma constituents.

### Introduction

For several years now, as a result of improvements in analytical methods, it has been relatively easy to determine accurately the ionic (free) fluoride in the plasma. This determination is carried out in most experimental studies of fluorosis, and sometimes, notably in pharmacokinetic studies, may even be the principal parameter examined.

Ionic fluoride, however, represents only a portion of the plasma fluoride. Information concerning ionic, ionisable, and total fluoride may be found in the literature. The portion of total fluoride that is not in a free ionic state is bound to the plasma constituents. This situation is analogous to that of drugs, and it is for this reason that we tried to apply to this problem methods used to measure the extent of drug binding to plasma constituents. We adopted the same kinetic method of dialysis used by Zini (1), taking into consideration the suggestions made by Kurz *et al* (2).

### Materials and Methods

The dialyses were carried out in racks each containing five troughs. Each trough consisted of two 2-mL compartments separated by a VISKING regenerated cellulose membrane 25  $\mu\text{m}$  in thickness, 11.34  $\text{cm}^2$  in area, with a pore diameter of 150 to 200 nm. One hour before each dialysis the membranes were left to soak in distilled water to eliminate impurities and facilitate ion passage. The troughs were filled through two lateral apertures which were then carefully sealed.

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It was decided, as a result of preliminary tests described by Diagbouga (3) to fill the five troughs of each rack in the following way:

troughs 1, 2, and 3: 2 mL of the same plasma + fluoride against phosphate buffer pH 7.4;

trough 4: Plasma against phosphate buffer at pH 7.4;

trough 5: phosphate buffer at pH 7.4 + fluoride against phosphate buffer at pH 7.4.

Three hours before the initiation of dialysis, a given amount of fluoride (0.95  $\mu\text{g/mL}$ ) was added to the plasma and to the phosphate buffer. The filled racks were placed in a Dianorm® apparatus with the thermostat set at 39°C (body temperature of healthy cattle), and the troughs were rotated for 2 hr at a rate of 20 revs/min in a water-bath.

At the end of dialysis the phosphate buffer was collected and the fluoride content determined by a fluoride ion-specific electrode using the known addition method (Tacussel *et al* (4)). The plasma fluoride concentration had been determined in the same way, prior to dialysis.

The percentage of fluoride bound to the plasma constituents ( $\beta$ ) was calculated from the following formula, proposed by Kurz *et al* (2):

$$\beta = \left( 1 - \frac{C_1 - C_2}{C_3 - (C_1 - C_2)} \right) \times 100$$

in which:

$C_1$  is the fluoride concentration in the phosphate buffer in troughs 1, 2, or 3;

$C_2$  is the fluoride concentration in the phosphate buffer in trough 4;

$C_3$  is the fluoride concentration in the non-supplemented phosphate buffer in the troughs.

The plasma samples originated from blood samples (Table 1) taken from 5 cows that had been acquired for teaching purposes by the E.N.V.A. (National Veterinary School, Alfort) several weeks earlier, from 5 cows (breeding unit 1) in the herd belonging to the National Agronomy Institute at Paris-Grignon (I.N.A.P.G.), and from 12 cows in 2 breeding units (No. 2 and 3) near an aluminium plant. The blood was collected on sodium heparine in Vacutainer tubes with blue stoppers (Aubry & Co., France) and centrifuged within 24 hours at 3500 revs/min for 20 min.

In all cases, the percentage of fluoride bound to the plasma constituents was examined within 24 hours of plasma preparation ( $t_0$ ).

The determination was repeated for 8 animals from breeding units 2 and 3, using plasma that had been stored at -30°C for 7, 21, or 60 days.

The different results were compared by variance analysis and Student's test.

Table 1. Ionic plasma fluoride concentrations and percentage of fixation on plasma constituents at day 0, in different herds

Origin	Number of the animal	Sex	Race	Age (years)	Ionic fluoride concentration (mg/l)	Percentage of fixation on plasma constituents (mean of 3 measures)	Fixation of plasma constituents (mean percentage and standard deviation) in the breeding unit
E.N.V.A.	236	Female	Priesian	7-8	0.03	69.5 $\pm$ 4.4	27.3 $\pm$ 23.8
	246	"	"	"	0.03	15.2 $\pm$ 1.5	
	561	"	"	"	0.04	19.3 $\pm$ 1.1	
	252	"	"	"	0.03	19.3 $\pm$ 2.5	
	618	"	"	"	0.03	13.0 $\pm$ 4.6	
Breeding unit number 1	418	Female	Priesian	4	0.19	1.0 $\pm$ 0.0	4.8 $\pm$ 2.8
	268	"	"	8	0.19	4.6 $\pm$ 2.1	
	451	"	"	6	0.24	8.0 $\pm$ 5.2	
	414	"	"	6.5	0.27	3.3 $\pm$ 1.1	
	136	"	"	9	0.29	7.0 $\pm$ 4.3	
Breeding unit number 2	648	Male	Tarine	2.5	0.29	61.3 $\pm$ 1.5	28.4 $\pm$ 20.6
	476	Female	"	6.5	0.28	59.6 $\pm$ 1.5	
	050	"	"	7.5	0.29	18.0 $\pm$ 5.0	
	078	"	"	4	0.25	27.0 $\pm$ 3.0	
	767	"	Charolaise	3	0.29	17.6 $\pm$ 2.9	
	976	"	"	"	0.30	16.0 $\pm$ 8.6	
	391	"	"	"	0.31	21.6 $\pm$ 2.9	
	355	"	"	6	0.30	6.0 $\pm$ 2.6	
Breeding unit number 3	996	Female	Tarine	6.5	0.30	14.0 $\pm$ 2.6	16.8 $\pm$ 12.3
	304	"	"	10.5	0.25	6.6 $\pm$ 5.1	
	666	"	"	4.5	0.43	12.0 $\pm$ 11.5	
	101	"	"	2	0.15	34.6 $\pm$ 2.5	

Table 2  
Influence of deep-freezing on the percentage  
of fluoride bound to the plasma constituents  
(Mean of three measures)

Percentage of fluoride bound to the plasma constituents at different times				
Number of the animal	t0*	t7**	t21**	t60**
767	17,6 ± 2,9	10,6 ± 9,2	31,0 ± 1,0	14,3 ± 4,9
976	16,0 ± 8,6	15,6 ± 3,2	20,3 ± 6,6	8,6 ± 4,2
391	21,6 ± 2,9	22,0 ± 4,0	16,0 ± 6,5	8,0 ± 0,0
355	6,0 ± 2,6	22,6 ± 2,1	22,3 ± 0,6	1,6 ± 2,9
996	14,0 ± 2,6	N.D.	15,3 ± 0,6	6,3 ± 0,6
304	6,6 ± 5,1	N.D.	9,6 ± 4,0	11,0 ± 1,7
666	12,0 ± 11,5	N.D.	16,6 ± 4,0	2,3 ± 2,1
101	34,6 ± 2,5	N.D.	16,0 ± 5,0	16,6 ± 1,1
Mean ± SD	16,1 ± 9,2	17,7 ± 5,7	18,4 ± 6,3	8,6 ± 5,3

\* t0 = dosage 24 hours after the plasma preparation.

\*\* t7, t21 or t60 = dosage after storage at -30°C for 7, 21 or 60 days.



## Results

The animals from E.N.V.A., which were fed mainly on forage, exhibited a very low ionic fluoride concentration in the plasma ( $0.032 \text{ mg F}^-/\text{L} \pm 0.004$ ) (Table 1). The animals from the experimental herd at I.N.A.P.G. (unit 1), which received a mineral-rich ration, showed a much higher fluoride content ( $0.24 \text{ mg F}^-/\text{L} \pm 0.05$ ) that was significantly different from the former ( $p < 0.001$ ). The plasma fluoride contents of the animals in unit 2 were very homogeneous ( $0.29 \text{ mg F}^-/\text{L} \pm 0.02$ ) and were only a little different from those of unit 1 ( $p < 0.05$ ).

The plasma fluoride contents of the animals from unit 3, which were dairy cows of different ages and production levels that did not receive exactly the same food ration, were less homogeneous ( $0.28 \text{ F}^-/\text{L} \pm 0.12$ ).

For the animals as a whole, the percentage of fluoride bound to plasma constituents was  $20.7 \pm 19.2$  (the standard deviation was very high). Although the majority of values were between 10 and 30 percent, extreme values ranging from 1 to 69.6 percent were observed.

The mean values of animals from E.N.V.A. and from unit 2 were very similar, whereas the plasma fluoride contents were very different ( $p < 0.001$ ).

The animals in unit 1, which had very similar fluoride contents to those in unit 2, showed very different binding percentages ( $p < 0.05$ ). There did not therefore seem to be any correlation between plasma fluoride content and the percentage of fluoride bound to plasma constituents.

The influence of deep-freezing on the percentage of fluoride bound to the plasma constituents is shown, in Table 2.

At  $t_7$  and  $t_{21}$  the results did not differ significantly from  $t_0$ , whereas they were significantly lower ( $p < 0.001$ ) at  $t_{60}$ . The concentrations of ionic fluoride were measured in plasma that had been thawed after different periods. These concentrations were identical to those obtained at  $t_0$ .

## Discussion

For the low values of about 10 to 20 percent, determination of the percentage of fluoride bound to the plasma constituents was not very accurate due to the method of calculation. For example, a difference of 3.6 percent in the determination of  $C_i$  led to a difference of 40 percent in the calculation of percentage binding. This explains the often considerable dispersal of the three results obtained from the same sample of plasma.

It is difficult to compare the obtained results with those published in the literature because the latter are highly disparate.

In 1968, Taves (5) was the first to establish the presence of ionic fluoride and bound fluoride in human serum. The values were very low:

0.01 to 0.02  $\text{mg F}^-/\text{L}$  for ionic fluoride and

0.08 to 0.10  $\text{mg F}^-/\text{L}$  for the total fluoride determined after mineralization and diffusion. Taves (6) concluded, in light of electrophoretic

studies, that 80% of the total fluoride was bound to the albumin in human serum.

Venkateswarlu (7) in 1975 obtained analogous results, whereas other authors such as Singer and Armstrong (8), Singer and Ophaug (9), and Belisle and Hagen (10) obtained lower binding percentages (45 to 75 percent) in serum samples with low fluoride concentrations.

Other authors such as Venkateswarlu *et al* (11), Paez *et al* (12), Yamamoto *et al* (13) also measured the ionizable fluoride (i.e. the fluoride measurable with an electrode after deproteinization with perchloric acid for example). In man this ranged from 12 percent (13) to 20 percent (12).

Few assays have been carried out in animals. Milhaud *et al* (14) found the percentage of bound fluoride in sheep to be 53 to 72 percent, whereas in the calf, according to Bessho *et al* (15), the ionizable fluoride represents 64 to 85 percent of the total fluoride.

### Conclusion

In the diagnosis of fluorosis, the determination of ionic fluoride — the diffusible form of fluoride — is of great interest, since it is easy to carry out and is well-codified. Bound fluoride is more difficult to measure; the various determination techniques are based on very different principles and give disparate results. The percentages obtained in man appear to be higher than in animals.

### Acknowledgement

The authors are very grateful to Professor Tillement, Faculté de Médecine de Créteil (94000), for providing the facilities required to undertake the work. We also highly appreciate the competence and efficient help provided by Professor Zini and his colleagues. We also thank Professor Toutain (INRA-Toulouse) for his advice.

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## FLUORIDE TREATMENT IN OSTEOPOROSIS

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Abstracted from leading article in *Postgraduate Medical Journal* 67 323-6 1991

This article commences by stating: "The use of fluoride for the treatment of established vertebral osteoporosis in adults with the symptomatic crush fracture syndrome is approved for use in 8 European countries. This treatment was suggested by the low prevalence of osteoporosis in some areas where the drinking water contained moderately high concentrations of fluoride and by the enormously increased bone density characteristic of fluorosis."

The authors then review the conflicting clinical studies, drawing attention to the high doses used in the recent double-blind ones which suggested a greater risk of fracture from the use of fluoride. They conclude:

"Despite recent reports concentrating on the problems associated with high-dose fluoride regimens, fluoride therapy for established vertebral crush fracture syndrome has been shown to be of value and associated with an increase in bone density and a reduced incidence of vertebral fracture. Patients should be monitored carefully, preferably using fluoride concentrations and a measurement of osteoblastic response such as osteocalcin or the more recently developed quantitation of type 1 collagen synthesis available from measurement of procollagen peptide. Only low doses are recommended for use in patients who are vitamin D replete and receiving calcium supplementation. Recently interest in the intermittent use of the bisphosphonate, etidronate, has been stimulated by several studies showing its efficacy in preventing bone loss in postmenopausal women. It is an inhibitor of bone resorption and in these patients small changes were noted in bone density. Fluoride therapy, however, remains the only known anabolic treatment for osteoporosis apart from anabolic steroids with their well-documented side effects of liver toxicity. Despite concern from a few centres about a possible increased incidence of hip fracture in patients on high-dose treatment, this form of therapy should not be abandoned."

Key words: Fluoride therapy; Hip fractures; Osteoporosis; Vertebral fractures.  
Reprints: Dr H Berry, King's College Hospital, Denmark Hill, London SE5 9RS, UK.

PERIOSTITIS DUE TO LOW-DOSE FLUORIDE INTOXICATION  
DEMONSTRATED BY BONE SCANNING

T R Weingrad, M J Eymontt, J H Martin and M D Steltz  
Bryn Mawr PA, USA

Abstract from *Clinical Nuclear Medicine* 16 59-61 1991

In a patient with severe upper and lower extremity pain, bone scanning showed markedly increased activity along long bones. After low-dose sodium fluoride therapy for the treatment of osteoporosis was discontinued, the patient promptly became asymptomatic, and the bone scanning improved at 10 weeks.

Key words: Bone scanning; Fluoride therapy; Osteoporosis.

Reprints: Dr Tina R Weingrad, Nuclear Medicine Bryn Mawr Hospital,  
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### ADVERSE EFFECTS OF OSTEOPOROSIS TREATMENT WITH FLUORIDE

K J Münzenberg, F Möller and W Koch  
Bonn, Germany

Abstract from *Munchener Medizinische Wochenschrift* 133 (5) 56-8 1991  
(in German, Abstract in English)

Apart from gastrointestinal complaints, pain in the lower extremities is the most common adverse effect of fluoride therapy. Complaints in the extremities are the result of stress fractures and of calcium-phosphate deposition in the joint capsule or in paraarticular tissue. The fractures may be clearly demonstrated by Magnetic Resonance imaging. Where osteoblasts do not react to fluoride with new bone formation and where mainly fluorapatite production by circumvention of brushite and octacalcium-phosphate formation occurs, calcium homeostasis is deranged. This was demonstrated in one of our patients with a lethal outcome on fluoride therapy.

Key words: Fluoride therapy; Stress fractures; Osteoporosis.

Reprints: Prof Dr med K J Münzenberg, Orthopädische Universitätsklinik,  
Sigmund-Freud-Str 25, 5300 Bonn 1, Germany.

### BONE FRAGILITY OF THE PERIPHERAL SKELETON DURING FLUORIDE THERAPY FOR OSTEOPOROSIS

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Johannesburg, South Africa

Abstract from *Clinical Orthopaedics and Related Research* (261) 268-71 1990

Bone fragility during fluoride therapy for osteoporosis was observed in 24 (37.5%) of 64 patients treated with sodium fluoride, calcium, and vitamin D for 2.5 years who developed episodes of lower-limb pain during treatment. Eighteen (28%) of these patients had clinical and roentgenographic features of 41 stress fractures and 12 new spinal fractures. There were 26 periarticular, six femoral neck, three pubic rami, three tibia and fibula, one great trochanter, and two subtrochanteric fractures. Vertebral fractures appeared first, then periarticular, then femoral neck, and lastly long-bone shaft fractures. All fractures were spontaneous in onset. The peripheral fracture rate during treatment was three times that in untreated osteoporosis. Roentgenograms must be repeated at intervals of three to four weeks before the pathognomic callus becomes visible, and the diagnosis can be made. Trabecular stress fractures tend to occur in the first 18 months of treatment, and cortical stress fractures occur after 30 months of therapy.

Key words: Bone fractures; Bone fragility; Fluoride therapy; Osteoporosis.

Reprints: C M Schnitzler, Medical School, York Road, Parktown,  
Johannesburg 2193, South Africa.

# ENAMEL DEFECTS IN A FLUORIDATED SOUTH-EAST ASIAN COMMUNITY

N K Chellappah, H Vignejsa and G L Lo  
Singapore

Abstract from *Australian Dental Journal* 35 530-5 1990

The prevalence and distribution patterns of enamel defects in maxillary incisors was assessed in 194 Singaporean children aged 11-15 years and belonging to three different ethnic groups. All were born and continuously resident in Singapore, which has a tropical climate. The water supply was fluoridated in 1957 at a level of 0.7 ppm. The mouth prevalence of defects was 71.5 per cent and the tooth prevalence was 55.9 per cent; 82 per cent of all affected teeth demonstrated white lesions of various forms. Although there was no sex difference in the prevalence and distribution pattern of defects, some racial differences were observed. The results were compared with data from other studies where the same classification of defects was used.

Key words: Enamel defects; Fluoride; Singapore.

Reprints: Department of Operative Dentistry, University of Singapore, Singapore.

# EFFECTS OF FLUORIDE AND CHLORHEXIDENE ON THE MICROFLORA OF DENTAL ROOT SURFACES AND PROGRESSION OF ROOT-SURFACE CARIES

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Abstract from *Journal of Dental Research* 70 150-3 1991

The effects of fluoride and chlorhexidine varnishes on the microflora of dental root surfaces and on the progression of root-surface caries were studied. Forty-four patients, surgically treated for advanced periodontal disease, were distributed at random among three groups. All patients received a standardized preventive treatment. Furthermore, the dentition of the patients in the two experimental groups was treated, at three-monthly intervals, with chlorhexidine and fluoride and fluoride varnish, respectively. Patients in the control group received no additional treatment.

In the experimental groups, plaque samples were collected from selected sound and carious root surfaces at baseline and at three, six, and nine months after the onset of the study. The presence of root-surface caries was scored at baseline and after one year. In addition, the texture, depth, and color of the root-surface lesions were monitored.

Mutans streptococci on root surfaces were suppressed significantly ( $p < 0.05$ ) during the whole experimental period in the chlorhexidine varnish group, but not in the fluoride varnish group. A non-significant increase in the number of *Actinomyces viscosus/naeslundii* was noted after treatment with chlorhexidine and fluoride varnish.



The increase in the number of decayed and filled root surfaces after one year was significantly lower in the experimental groups than in the control group. After treatment with chlorhexidine varnish, significantly more initial root-surface lesions had hardened than in the other groups.

Key words: Chlorhexidine varnish; Fluoride varnish; Root-surface caries; Root-surface microflora.

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## FLUORIDE INTAKE OF INFANTS IN NEW ZEALAND

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Abstract from *Journal of Dental Research* 69 1828-33 1991

Since the fluoride ( $F^-$ ) intake of New Zealand infants and young children is not known, a study was designed to determine and compare the  $F^-$  intake of infants, aged 11 to 13 months, residing in fluoridated (F) and non-fluoridated (NF) areas. Parents of 60 infants duplicated quantitatively and qualitatively all food and drink that the infants ingested during a three-day period. The acid-diffusible  $F^-$  content in the liquid homogenate was isolated by the HMDS-HCl diffusion technique (Taves, 1968) and measured by a fluoride electrode. The ionic  $F^-$  in samples of breast milk was measured directly by the electrode. In the F area, the  $F^-$  content of the food and drinks of 31 subjects ranged from 0.130 to 0.679 mg/kg (mean, 0.320; SD, 0.168); in the NF areas, the  $F^-$  content of the food and drinks of 29 subjects ranged from 0.036 to 0.281 mg/kg (mean, 0.095; SD, 0.053). The dietary intake ranged from 0.089 to 0.549 mg F/day (0.009 to 0.056 mg F/kg bw) in the F area, and from 0.038 to 0.314 mg F/day (0.004-0.038 mg F/kg bw) in the NF area. Including  $F^-$  from tablets and toothpastes, total intake ranged from 0.093 to 1.299 mg F/day (0.009-0.150 mg F/kg bw) and from 0.039 to 0.720 mg F/day (0.004-0.061 mg F/kg bw) in F and NF areas, respectively.

The mean dietary intake of infants in the F area was about half the recommended "optimal" range; in the NF areas, the dietary intake was five to seven times less than the optimal. Sources of high fluoride intake such as soy-milk formulae and tea, however, raised the fluoride content of the diet to near optimal levels. The use of F toothpastes and tablets raised the intake to near optimal levels. The optimal intake was exceeded by one child in the F area as a result of excessive toothpaste use. It was calculated that, if a child on soy formula in the non-fluoridated areas were to ingest fluoride supplements and large amounts of toothpaste as well, the total fluoride intake of this infant could exceed optimal levels. This finding stresses the need for identification of potential sources of high fluoride intake in a child's diet before any form of fluoride supplementation is recommended.

Key words: Fluoridation; Infant fluoride intake; New Zealand.

Reprints: Department of Community Dental Health, University of Otago, PO Box 647, Dunedin, New Zealand.

DIMINUTIONS OF FLUORINE CONTENTS IN LICHENS DUE TO  
A REGRESSION OF POLLUTION IN AN ALPINE VALLEY  
(MAURIENNE, SAVOIE, FRANCE) FROM 1975 TO 1985

Gladys Belandria, Juliette Asta and Jean-Pierre Garrec  
Grenoble and Nancy, France

Abstract from *Revue d'Ecologie Alpine, Grenoble* 1 45-58 1991

Fluorine analyses were made for different species of lichens collected in the course of 11 years (from 1975 to 1985) in the Arc valley (Maurienne, Savoie, France). A general decrease of fluorine contents in lichens was observed for instance from 54 to 21 ppm for *Parmeliaceae*, from 106 to 12 ppm for *Peltigera canina* and from 124 to 44 ppm for *Umbilicaria cylindrica*. This decrease of the fluorine contents for lichens reveals perfectly well the variations of the level of fluorine pollution for the atmosphere from 1975 to 1985. It is confirmed here that the fluorine contents of *Parmeliaceae* and of *Peltigera canina* are respectively higher than those of fruticulous filamentous lichens and those of *Cladonia* of the *pyxidata* group, that the fluorine contents of lichens are lower in places distant from the sources of pollution, but depend also very much on the air currents in the valley. Fluorine contents of substrates (bark and ground) are always higher than those of the lichens. For *Usnea muricata*, *Peltigera canina* and *Cladonia* gr. *pyxidata*, the fluorine contents measured in 1985 in that valley are not higher than those found for lichens of the same kinds living in places where there is no fluoride contamination.

Key words: Alpine valley; Bioaccumulator; Fluoride pollution; Lichens; Maurienne.

Reprints: J Asta, Université Joseph Fourier Grenoble I. Laboratoire de Biologie Alpine, BP 53X - 38041 Grenoble cédex, France.

ADSORPTION AND DESORPTION OF FLUORIDE IN NAILS

Anna Machoy-Makrzynska  
Szczecin, Poland

Abstract from *Environmental Sciences* 1 137-41 1992

It is shown that fluoride adsorption constitutes an important process in the accumulation of this element in human nails. Adsorption in nails is a reversible process. The elements that bind fluoride in the nails are most probably bivalent metals (Ca, Mg, Fe), as well as keratin, which display considerable affinity with regard to fluoride. A small amount of fluoride in nails, of the order of a few ppm, should be looked upon as a permanent component of nails, regardless of the person's age.

Key words: Environment; Fluoride accumulation; Fluoride adsorption; Fluoride in nails.

Reprints: Institute of Pharmacology, Pomeranian Medical Academy, Szczecin, Poland.



### FLUORIDE: AN ADJUVANT FOR MUCOSAL AND SYSTEMIC IMMUNITY

J E Butler, M Satam and J Ekstrand  
Iowa City IA, USA

Abstract from *Immunology Letters* 26 217-20 1990

Fluoride, the agent responsible for reduction of dental caries worldwide, and a recognized proliferative agent, is a potent adjuvant when given intragastrically to rats. Intragastric fluoride causes increases in the size and cellularity of the Peyer's patches and mesenteric lymph nodes as well as the number of plasma cells secreting IgG and IgA antibodies to ovalbumin given in their drinking water. Rats ingesting NaF and fed OA showed a significant increase in surface immunoglobulin expression on lymphocytes from the Peyer's patches and mesenteric lymph nodes. The frequency of CD4<sup>+</sup> T cells in these lymphoid tissues was elevated while that of CD8<sup>+</sup> T cells was significantly decreased. In separate experiments, rats parenterally immunized with myelin basic protein (MBP) and fed NaF twice weekly, had significantly elevated serum IgG antibody activity to MBP compared to similarly immunized rats not receiving NaF. The supplemental fluoride prescribed for infants and especially that which is inadvertently ingested by children and adults given fluoride gels, is within the concentration range of that which produced the effects we observed in rats. The adjuvant effect we describe thus has relevance for fluoride therapy worldwide.

Key words: Adjuvant; Antibody; Fluoride; Peyer's patches; Proliferation.  
Reprints: J E Butler, Department of Microbiology, University of Iowa,  
Iowa City IA 52242, USA.

### METAL SHIFT IN RATS EXPOSED TO FLUORIDE

Y Yoshida, K Kono, M Watanabe, H Watanabe, S Inoue, Y Tanioka,  
T Dote, Y Orita, K Umebayashi and H Nagaie  
Osaka, Japan

Abstract from *Environmental Sciences* 1 1-9 1991

The influence of fluoride (F) on the distribution of metals in rats exposed to low concentrations of F for one year was studied. Results showed that F exposure did not influence the body-weight growth curve of experimental animals. F concentrations in the serum, urine, lung, liver, kidney, thigh bone and upper incisors of the experimental group were higher than those of the control group. Administration of F caused a shift in the concentration of metals. In particular, the increase of Zn in the serum and the decrease of Se and increase of Al in whiskers were notable. The experimental data suggest that, in addition to serum and urine, whiskers may serve as a possible indicator of the biological body burden of F in rats.

Key words: Biological index; Fluoride administration; Metal shift.  
Reprints: Department of Hygiene and Public Health, Osaka Medical College,  
2-7, Daigakumachi, Takatsuki City, Osaka, Japan.

## MORE NEWS ON THE CONFERENCE

The following is the timetable for the 19th Conference of ISFR, which will be held in Kyoto, Japan, September 8-11 1992.

- September 8 1pm Registration at Renaissance Hall.  
6pm Welcome Reception at Hotel RANTEI of Arashiyama.
- September 9 9am Opening Ceremony at Renaissance Hall.  
10am to 12 Special lectures by Professors Yu, Miller and Bély  
(see page 54)  
12 to 1.30pm Business Meeting of ISFR.  
2 to 5pm Presentation of Papers.
- September 10 9am to 12 Presentation of Papers.  
1pm to 5pm Poster Session.  
6pm Banquet at Kyoto Century Hotel.
- September 11 9am to 12 Presentation of Papers.  
12.30pm Sundry Distillery and Matsushita Electronics.

The registration fee for the Conference is ¥30,000 and ¥10,000 for each accompanying person. Limited accommodation is available at Kyoto Century Hotel. Conference rate: ¥10,000 single, ¥20,000 double. For further information write to:

ISFR 92 Scientific Secretariat  
c/o Dr Koichi Kono  
Department of Hygiene and Public Health  
Osaka Medical College  
2-7 Daigakumachi, Takatsuki City  
Osaka, 569 Japan  
(TeleFAX 0726-84-6519)

The Secretariat is happy to issue letters of invitation to assist applicants in obtaining visas and travel funds.

## INSTRUCTIONS TO AUTHORS

Papers should present original investigations. Review papers are also accepted.

1. **General.** The submitted paper, with a copy, should be written concisely in English. Either American or British spelling will be accepted. Double space with generous margins. Measures should be in metric system.

2. **Title.** A concise but informative title should be followed by the name(s) of the Author(s). The address where the research was carried out should appear at the bottom of the first page.

3. **Summary.** Begin with a brief factual summary.

4. **Key words.** List the major themes or subjects.

5. **Introduction.** State the reason for the work with a brief review of previous work on the subject.

6. **Materials and Methods.** Condense. However, if the methodology is new or developed by the author(s) it can be more detailed.

7. **Results.** List the direct conclusions of the work.

8. **Discussion.** Deal with general conclusions, referring to other work on the subject. In short papers Results and Discussion may be combined.

9. **Abbreviations or Acronyms.** Define, either in brackets or in footnotes, when they first appear.

10. **Acknowledgements.** Keep brief. They may include funding source, technical assistance, text editing and useful comments.

11. **References.** Identify in the text by bracketed numerals. Number references consecutively in the order in which they first occur. For repeated (identical) references, re-use the original reference number. Arrange the list of references by number, not alphabetically. Give all authors up to four. When more than four, add *et al* after the third. Italicize (or underline) name of journal and volume number, book titles and Latin or non-English words like *et al*. For examples of reference style, see current issues of journal.

Points to note are:

For journal references follow the order:

Author(s). Title. *Journal (spelled out in full) volume number* page number(s) year.

For books the order is:

Author(s) or Editor(s). *Book Title*. Publisher, Place and year of publication followed if appropriate by relevant page number(s).

For article or chapter in a book:

Author(s). Title of article/chapter. In: Book reference as above.