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# FLUORIDE

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FLUORIDE, official journal of the International Society for Fluoride Research, publishes quarterly reports on biological, chemical, ecological, industrial, toxicological and clinical aspects of inorganic and organic fluoride compounds.

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tween fluoridated water and tooth decay is actually weaker and less significant than the positive correlation now reported between fluoridated water and hip fractures. The claimed lower tooth decay rates in fluoridated areas rest on studies using the imprecise "DMF" measure of decay (that is, number of decayed, missing and filled teeth or tooth surfaces). It is a very subjective measure, easily influenced by the bias of the examiner (20). The reductions in tooth decay claimed to be due to fluoridation (21, 22) sound impressive when expressed as a percentage reduction in such "DMF". In fact, they are usually less than half a tooth per child - sometimes less than half a tooth surface (23) - which is not even statistically significant, let alone clinically significant, given the highly subjective nature of caries diagnosis. The diagnosis of hip fractures, of course, is much less subjective.

However, there is much other evidence pointing to a causal connection between fluoridation and fractures. Recent reviews in the *New England Journal of Medicine* (24) and the *Journal of Bone and Mineral Research* (25) and recent rigorous well-designed clinical studies of the side effects of fluoride treatment for osteoporosis (10-12) suggest that fluoride, far from strengthening bone as expected, actually increases bone fragility and fractures. The authors conclude that fluoride should no longer be recommended for such clinical use. An earlier Swiss case study of bilateral hip fractures following fluoride therapy had concluded "these data suggest a causal link between fractures and fluoride in patients with renal failure" (26). Earlier studies of endemic fluorosis in India had also reported reduced tensile strength of fluoride-affected bones (27,28).

The claim that these studies are irrelevant to the fluoridation issue (because of the high doses involved) is discounted by studies in Finland (29,30). Some old people who had drunk fluoridated water for 10 years or more, especially those with impaired kidney function, had bone fluoride levels (30) similar to those which have been reported following fluoride therapy for osteoporosis (31). An earlier study reported: "The highest fluoride content in bone ash was observed in women with severe osteoporosis" (29). Fluoridation has now ceased in Finland (Arnala, personal communication 1991). In Australia and New Zealand, as in most English speaking countries, such monitoring of old people's bones has not occurred, and fluoridation is still vigorously promoted.

Clearly the evidence now strongly suggests that low fluoride doses over long periods as well as high doses for short periods can damage bones, making them more liable to fracture. Our belief that the amount accumulated from fluoridated water would be insignificant is now discounted by the new evidence. The situation may well worsen as people reach old age after spending greater proportions of their lives ingesting low fluoride doses.

We have long known that about a half the fluoride we ingest is excreted by our kidneys - the rest accumulates in our bones (32,33). It is released very slowly (about a half in 20 years) only if fluoride exposure ends. People with impaired kidney function, being less able to excrete fluoride, accumulate more. The gradual

accumulation causes a disturbance of structure - an increase in bone mass (sclerosis). In contrast osteoporosis (literally "porous bone") is a decrease in bone mass. Hence the attempts to reverse the decrease with large doses of fluoride. But the bone sclerosed by fluoride, though denser, is more fragile than normal bone. Unlike the effect on teeth, which occurs only during the period of tooth formation in the first few years of life, the disturbance to bone can continue throughout life.

Children excrete fluoride less efficiently than adults, so retain even more in their bones (2,32-34). Fluoridation since the 1950s has increased the fluoride intake of millions of today's old people for up to half their adult lives. An unanswered question is: what will have been the effect on the bones of today's children by the time they reach old age?

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John Colquhoun

FLUORIDE INGESTION AND ITS CORRELATION WITH  
GASTROINTESTINAL DISCOMFORT

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**SUMMARY:** This study was carried out to assess the effect on the human gastroduodenal mucosa of drinking naturally fluoridated water and treating patients with 30 mg sodium fluoride for otosclerosis. Ten cases each of skeletal fluorosis and otosclerosis and twenty cases of non-ulcer dyspepsia (NUD) were investigated through routine clinical investigations, chemical investigations of body fluids and drinking water for fluoride, radiographs, stool examination for ova, cysts and worms, abdominal sonography, upper gastrointestinal endoscopy, jejunal aspirates for *Giardia lamblia*, histopathology of biopsies of intestinal and gastric mucosa and scanning electron microscopy of the mucosa. Patients of all three groups, compared with a control group of normal healthy volunteers, presented gastrointestinal problems and discomfort. Four patients with non-ulcer dyspepsia also presented radiological evidence of skeletal fluorosis. Analysis of ingested drinking water revealed fluoride concentrations of 0.49 - 11.36 ppm. Histopathological studies revealed non-specific lesions. Stool examination revealed ova of *Ascaris lumbricoides* in two NUD patients, while the rest had normal stool on examination. Jejunal aspirates were negative for *Giardia lamblia* in all the subjects. Scanning electron microscopic studies revealed widespread damage to the mucosa, viz. (a) mucus droplets were not visible, (b) loss of microvilli, (c) cracked-clay appearance of the duodenal mucosa and (d) desquamated epithelium of gastric mucosa. It is concluded: 1) Ingested fluoride damages gastroduodenal mucosa. 2) Gastrointestinal discomfort can be an early warning sign of fluorosis. 3) Fluoride toxicity should be considered a possible reason for non-ulcer dyspepsia, especially in fluorosis endemic areas. 4) Gastrointestinal discomfort during sodium fluoride therapy calls for extreme caution and close monitoring. 5) Gastrointestinal discomfort in the form of dyspeptic symptoms should be an important diagnostic feature when identifying fluorosis patients and should not be dismissed as non-specific.

**Key words:** Fluoride ingestion; Gastrointestinal discomfort; Non-ulcer dyspepsia; Osteofluorosis; Otosclerosis.

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## Introduction

Gastrointestinal symptoms such as severe gastric pain, nausea, repeated vomiting and diarrhoea are common in acute fluoride toxicity suggesting gastrointestinal intolerance to fluoride (1-3). Some reports also suggest gastrointestinal involvement in chronic fluoride toxicity. Roholm (1937) observed for the first time gastrointestinal symptoms in 80-90% of cryolite workers with skeletal fluorosis (4). Waldbott (1956) reported dyspeptic symptoms in the form of anorexia, nausea, vomiting, abdominal pain and diarrhoea with intermittent constipation in chronic fluoride toxicity (5). Gastric ulcer and haemorrhages have also been reported in chronic fluoride toxicity (6,7).

However, because of the lack of specific knowledge of the pathogenesis of these gastrointestinal problems, gastrointestinal symptoms are not yet generally accepted as an associated manifestation of chronic fluoride toxicity. The purpose of the present study was to investigate the effect of chronic fluoride poisoning on gastrointestinal mucosa.

## Materials and Methods

### A. Patients

Cases were selected from outpatient clinics of the All India Institute of Medical Sciences, New Delhi.

(i) Skeletal fluorosis: Ten confirmed cases of skeletal fluorosis (all male) with age ranging from 19 to 50 years (mean  $34.6 \pm 9.3$ ) were selected from orthopaedic, neurology and endocrinology clinics. Gastrointestinal symptoms were not taken into consideration while selecting the patients.

(ii) Otosclerosis patients on sodium fluoride therapy: Ten patients with otosclerosis (6 male and 4 female) in the age range of 13-49 years (mean  $31.4 \pm 15.7$ ) were selected from the otorhinolaryngology clinic. These patients were on oral sodium fluoride therapy of 30 mg daily for 3 months to 12 months. None of the otosclerosis patients had any symptoms referable to the upper gastrointestinal tract prior to sodium fluoride therapy.

(iii) Non-ulcer dyspepsia (NUD) patients: Twenty patients with non-ulcer dyspepsia (14 male and 6 female) ranging in age from 19 to 62 years (mean  $32.90 \pm 11.20$ ) diagnosed according to Johnson's criteria (8), were selected from the gastroenterology clinic. Patients with documented peptic ulcer, gallstones or a previous history of abdominal surgery were excluded from the study. The purpose of including NUD patients in the study was to decide whether fluoride could be a causal factor for dyspeptic symptoms, as the exact etiopathogenesis of NUD is not known.

(iv) Control group: Ten subjects from the surgical clinic, with



non-gastrointestinal problems, volunteered to serve as controls.

## B. Methods

All subjects were evaluated clinically for dyspeptic symptoms (9). A dietary history was obtained from each subject including their source of drinking water to assess all possible means of fluoride intake, as some food items are known to have high fluoride contents (10,11). All patients and control subjects underwent the following investigations.

(i) Standard liver and renal function tests were done to evaluate the hepatic and renal function profiles (12).

(ii) Stool examination for cysts and ova for evidence of parasitic infection.

(iii) Ionic fluoride levels in serum, urine and drinking water were determined for each of the patients and control subjects by ION85 ION ANALYSER (Radiometer, Copenhagen) (13,14).

(iv) Radiological evaluation of fractures, bone density and calcification of ligaments was performed for evidence of fluorosis (15). The evaluating radiologist was blinded to the clinical symptoms and the group to which the subject belonged.

(v) Ultrasound examination: Abdominal sonography was done to rule out any involvement of liver, gall-bladder, portal and splenic veins, spleen or pancreas.

(vi) Gastrointestinal examination: All patients underwent upper gastrointestinal endoscopic examination using an Olympus GIF-Q10 fiberoptic endoscope. The mucosal findings on endoscopy were classified as erythema, erosions or petechiae (16). Antral and duodenal biopsies were obtained using punch biopsy forceps for histopathological as well as scanning electron microscopic studies. Jejunal aspirates were collected in each subject to examine for presence of *Giardia lamblia*.

(vii) Histopathological examination: Haematoxylin and eosin stained sections were examined for abnormalities.

(viii) Scanning electron microscopic examination: The tissues obtained from the antrum and duodenum were initially washed gently in normal saline solution to remove debris and mucus. A second rinse was in 0.1 M phosphate buffer, followed by fixation in Karnovsky's fluid (17) for 6 hours. The specimens were again washed in 0.1 M phosphate buffer and fixed in 0.5% aqueous OsO<sub>4</sub> for an hour. After further washes in 0.1 M phosphate buffer the specimens were dehydrated through a graded series of acetone. The tissues were then critical point dried followed by sputter coating with gold and examined under a scanning electron microscope (Philips 501B) at 15 KV.

## Results

(i) Gastrointestinal complaints: Dyspeptic symptoms were observed in 80% of the otosclerosis patients on sodium fluoride therapy, whereas all the fluorosis patients in the present study had mild to severe gastrointestinal problems (Table 1). The clinical symptoms of NUD patients are also shown in Table 1. The most frequent symptom was abdominal pain followed by vomiting, nausea and anorexia. None of the control subjects had any upper gastrointestinal tract symptoms (Table 1).

(ii) Fluoride determination: All the drinking water samples of the fluorosis patients had fluoride levels above the permissible limit (1.0 ppm). Consequently fluoride levels in the serum and urine were also above normal levels in all the fluorosis patients except for one. Similarly, in the otosclerosis group, serum and urine fluoride levels were higher than normal in every subject, although only one out of the ten otosclerosis patients had drunk water with fluoride level above the normal limit, indicating that sodium fluoride therapy is the main source of fluoride intake. In the NUD group 11 out of 20 drinking water samples had abnormal levels of fluoride (>1.0 ppm). However, none of the NUD patients had normal levels of fluoride in serum and urine. In the control group 3 subjects had serum fluoride levels slightly higher than normal value, whereas all the drinking water and urine samples were within the normal limits.

(iii) Liver and renal function test: No abnormalities were noted in any of the patients and control subjects.

(iv) Stool examination: Two NUD patients were found to have the ova of *Ascaris lumbricoides* in their stool. Others were normal.

(v) Ultrasound examination: The abdomens were normal in all the patients and control subjects.

(vi) Radiological examination: All the fluorosis patients presented with classical features of osteofluorosis, thereby confirming their diagnosis. However, no fluorotic changes were observed in the X-rays of otosclerosis patients on sodium fluoride therapy. In the NUD group, four patients had radiological features of fluorosis, such as generalized increase in bone density and calcification of interosseous membrane, which are suggestive of chronic fluoride toxicity. X-rays of the control group did not show any abnormality (Table 2).

(vii) Upper gastrointestinal endoscopy: Diffused erythema with petechiae were observed in the antrum in 5 (25%) and in the duodenum in 4 (20%) patients of the NUD group. In the otosclerosis group, petechiae, erosions and erythema in the antrum and/or duodenum were observed in 9 (90%) patients. In the osteofluorosis group, the endoscopic appearance of the gastric and the duodenal mucosa was normal in 3 patients, while gastric

Table 1  
Fluoride levels in drinking water, serum and urine with  
gastrointestinal symptoms in the four study groups

Condition	Age in years (Mean $\pm$ SD)	Fluoride levels in ppm (Mean $\pm$ SD)			Gastrointestinal complaints					
		Water (A/N)*	Serum (A/N)*	Urine (A/N)*	Pain	Vomit- ing	Diarrhoea	Constipation	Nausea	Anorexia
Control (N = 10)	25.60 $\pm$ 7.26	0.36 $\pm$ 0.19 (0/10)	0.04 $\pm$ 0.04 (3/7)	0.065 $\pm$ 0.02 (0/10)	0	0	0	0	0	0
Osteo- fluorosis (N = 10)	34.60 $\pm$ 9.29	4.86 $\pm$ 4.66 (10/0)	0.12 $\pm$ 0.08 (9/1)	2.09 $\pm$ 2.64 (10/0)	10	1	2	7	3	5
Oto- sclerosis# (N = 10)	31.40 $\pm$ 15.67	0.34 $\pm$ 0.10 (1/9)	0.47 $\pm$ 0.28 (10/0)	2.21 $\pm$ 1.04 (10/0)	3	1	2	1	3	3
Non-ulcer dyspepsia (N = 20)	32.95 $\pm$ 11.20	1.20 $\pm$ 0.75 (11/9)	0.103 $\pm$ 0.08 (20/0)	1.34 $\pm$ 0.95 (20/0)	19	16	4	8	11	9

\* Number of samples with abnormal (A) fluoride levels/number of samples with fluoride levels within the normal (N) range.  
(Normal range: Water  $\leq$  1.0 ppm; Serum  $\leq$  0.02 ppm; Urine  $\leq$  0.10 ppm)

# Otosclerosis patients were on NaF therapy 30 mg/day for 3-12 months

Table 2  
Results of different investigations

	RADIOLOGY		ENDOSCOPY (ANTRUM + DUODENUM)			HISTOPATHOLOGY				
	Generalized increase in bone density	Interosseous membrane calcification	NAD*	Erythema	Petechiae	Erosion	Non-specific gastritis	Non-specific duodenitis	Gastric ulcer	NAD*
Control (N = 10)	0	0	10	0	0	0	10	1	0	9
Osteo-fluorosis (N = 10)	10	9	0	1	0	6	3	7	0	3
Oto-sclerosis# (N = 10)	0	0	10	2	4	3	1	10	3	0
Non-ulcer dyspepsia (N = 20)	4	3	16	5	4	0	11	10	5	7

\* NAD = No abnormalities detected

# Otosclerosis patients were on NaF therapy 30 mg/day for 3-12 months

erosions were observed in 6 (60%) patients and one had gastric erythema with petechiae. All in the control group had normal endoscopic appearance (Table 2).

(viii) Tests for *Giardia lamblia*: Jejunal aspirates were negative in all the patients and control subjects.

(ix) Histopathology: Histological evaluation of the gastric and duodenal mucosa revealed non-specific gastritis and duodenitis in all the ten otosclerosis patients. In the osteofluorosis group chronic atrophic gastritis was observed in 7 patients (70%), while in the NUD group, non-specific antral gastritis and duodenitis were found in 13 patients (65%) and gastric ulcer in one patient (5%). In the control group one subject (10%) had non-specific chronic mild gastritis (Table 2).

(x) Scanning electron microscopy: On scanning the surface morphology of the mucosal layer of antrum and duodenum, significant cytomorphologic abnormalities were observed in all the otosclerosis and osteofluorosis patients. The epithelium of the gastric antrum and duodenal mucosa showed loss of microvilli (Fig. 1). Duodenal mucosa of some patients revealed a "cracked-clay" like appearance (Fig. 2). In addition to the above abnormalities, the micrographs of 3 patients in the otosclerosis group and 4 patients in the osteofluorosis group revealed abrasion and desquamation of the gastric epithelium (Fig. 3,4). In the NUD group the above noted abnormalities were observed in 17 patients (85%) in both the antral and duodenal mucosa. Among the remaining 3 patients, one had normal surface morphology of both the antral and duodenal mucosa, while two had normal duodenum but their antral mucosa showed loss of microvilli and surface abrasion. These structural abnormalities were not observed in normal healthy control subjects (5-7) (Table 3).

Table 3  
Cytomorphological Abnormalities Noted in Antrum (A) and Duodenum (D) in Scanning Electron Microscopic Examination

	Scanty microvilli		Cracked clay appearance		Surface abrasions		Desquamated epithelium		NAD*
	A	D	A	D	A	D	A	D	
Control (N=10)	0	0	0	0	0	0	0	0	10
Osteofluorosis (N=10)	7	2	0	7	4	0	4	0	0
Otosclerosis † (N=10)	3	3	2	4	3	0	3	0	0
Non-ulcer dyspepsia (N=20)	8	4	1	12	2	0	8	0	1

\* NAD = No abnormalities detected

† Otosclerosis patients on NaF therapy (30mg/day) for 3-12 months



Figure 1. Epithelium of the gastric mucosa showing loss of microvilli, giving a bald appearance to the cells (X 7000)



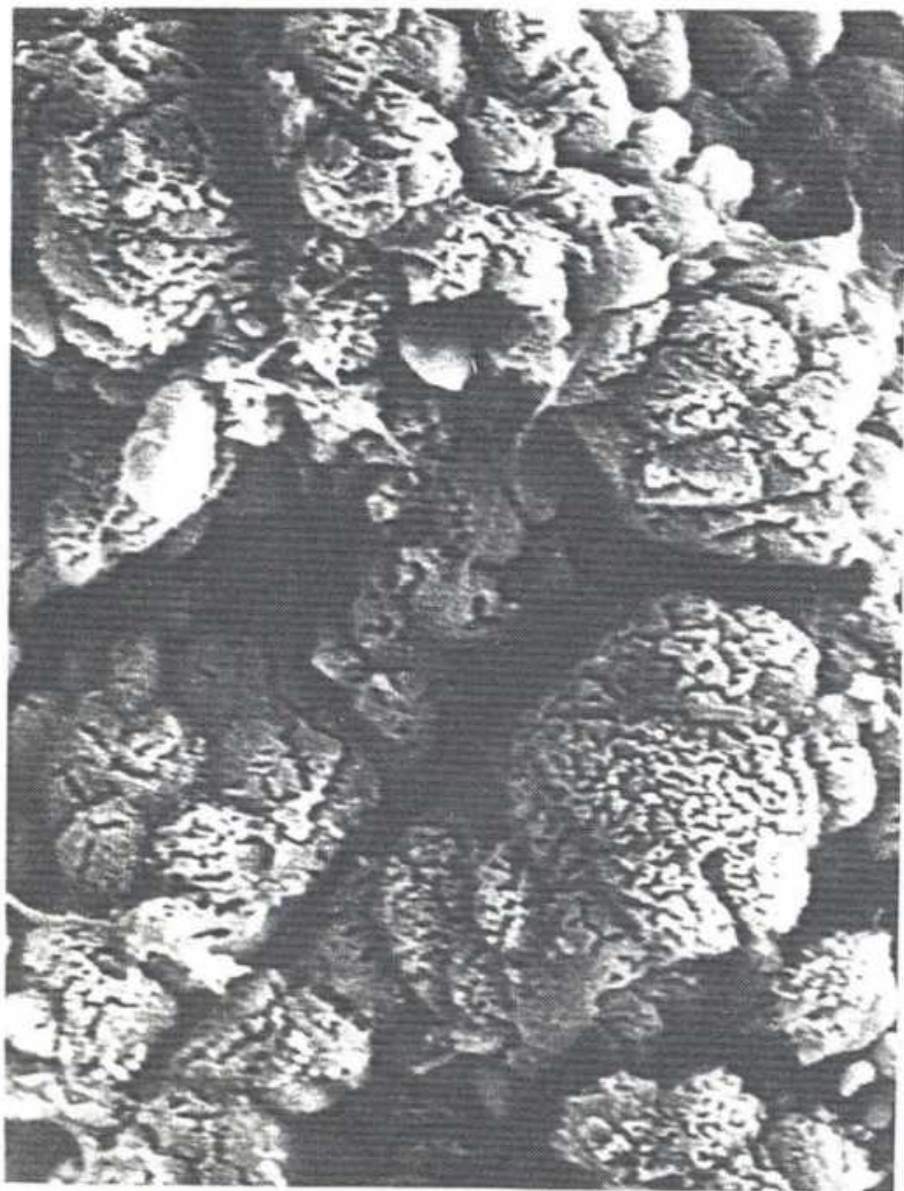


Figure 2. Scanning electron micrograph of duodenal mucosa showing "cracked-clay" appearance (X 1750)

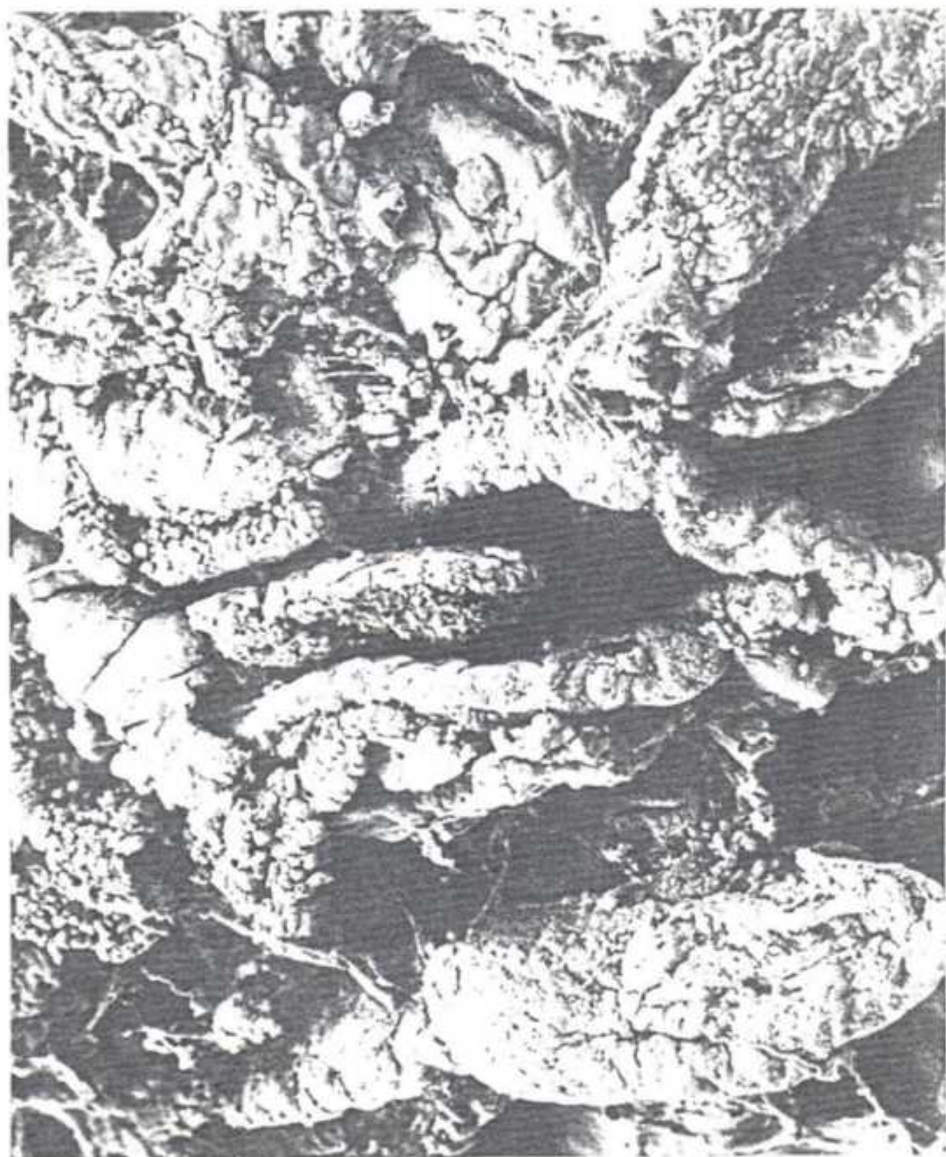


Figure 3. Low magnification scanning electron micrograph of the duodenal villi showing surface abrasions (X 224)



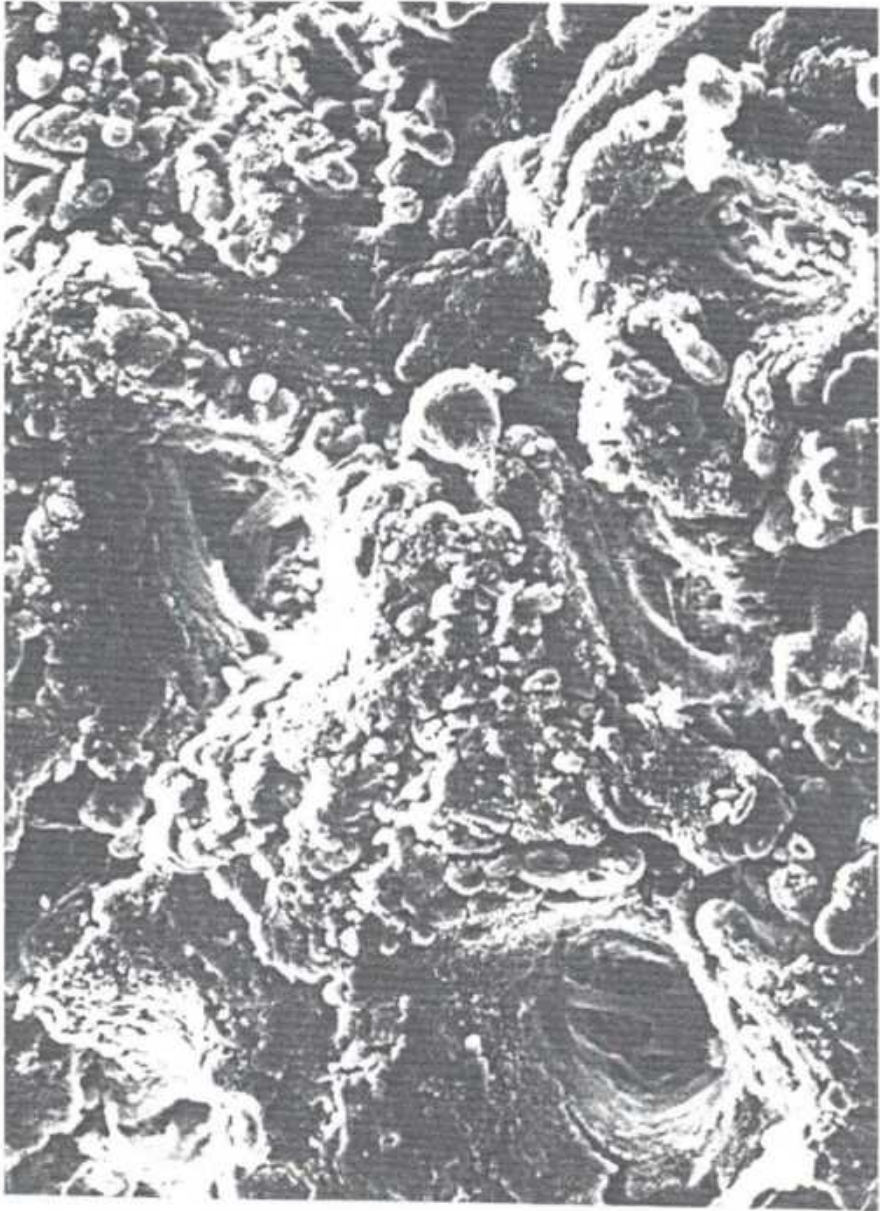


Figure 4. Gastric mucosa showing severe surface abrasions and desquamated epithelium (X 896)



Figure 5. Scanning electron micrograph of duodenal villi of a control subject showing smooth leaf-like structure with surface kinks (X 280)

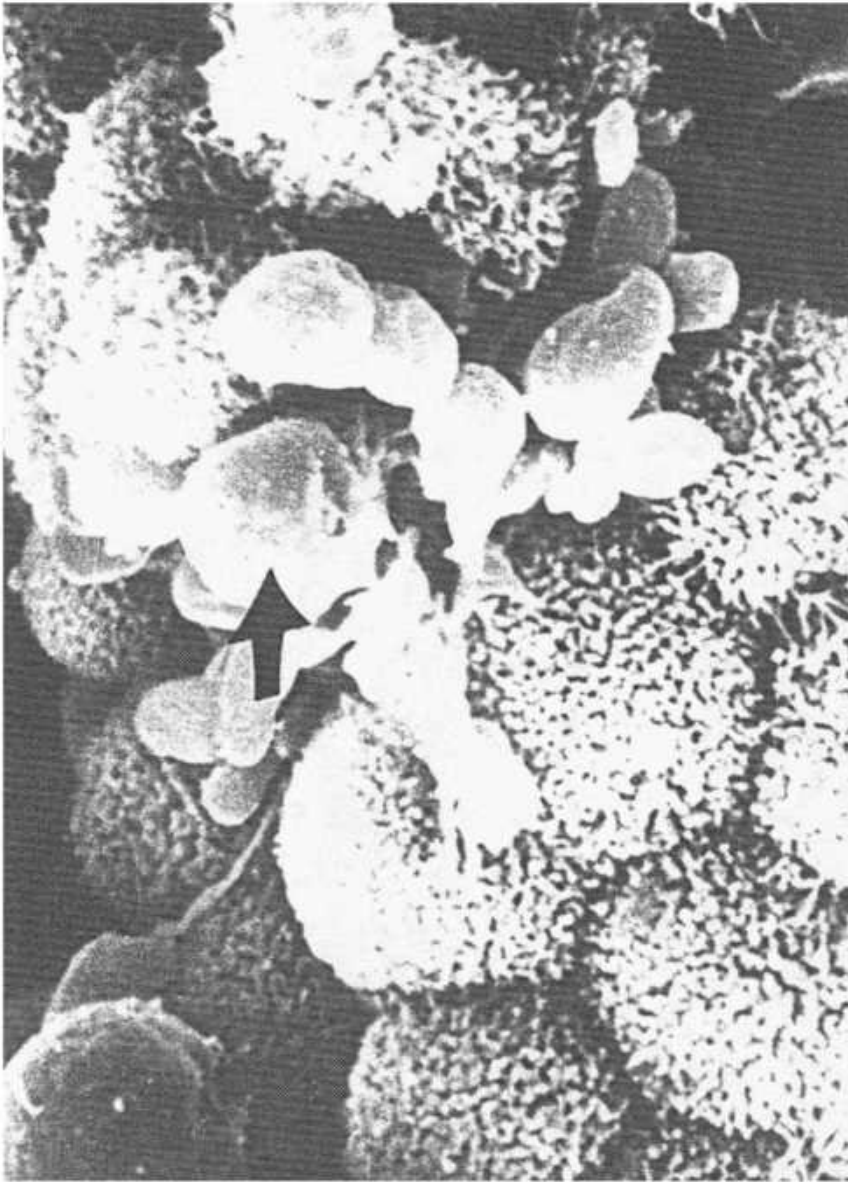


Figure 6. Gastric mucosa showing normal epithelium with microvilli.  
Mucus secretion can also be seen (arrow) (X 7000)

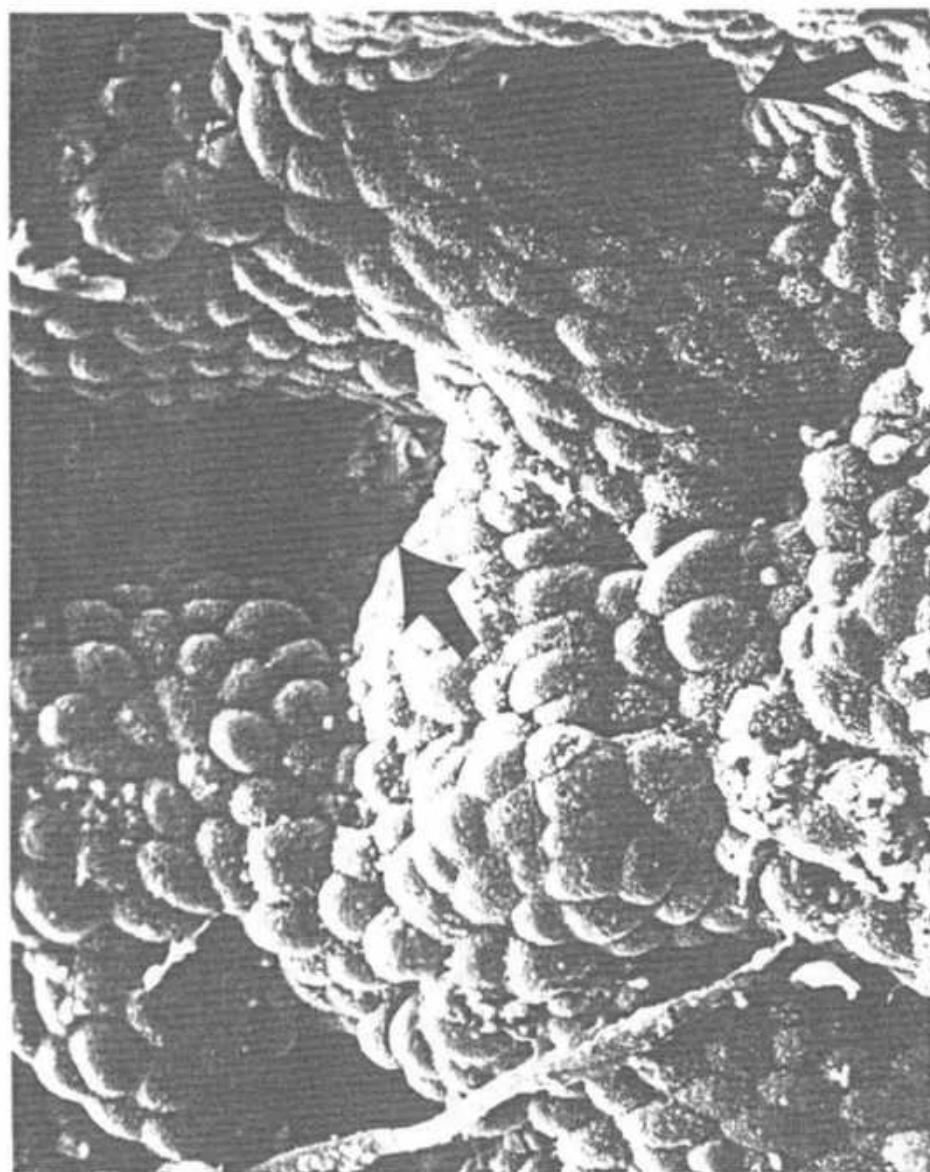


Figure 7. Gastric mucosa showing normal epithelium with gastric pits (arrow) (X 896)



### Discussion

The response of the gastrointestinal mucosa to the ingestion of fluoride has not before been adequately evaluated. The present study demonstrates in detail different pathological and biochemical abnormalities which help in understanding the etio-pathogenesis of gastrointestinal problems in chronic fluoride toxicity.

In patients on fluoride therapy, among the commonest side-effects are gastrointestinal symptoms. Jowsey *et al* (1972) reported epigastric dyspepsia in 54.6% of the osteoporosis patients on fluoride therapy (18). In another study on fluoride therapy Riggs *et al* (1990) observed gastrointestinal symptoms in 44% of osteoporosis patients (19). In the present study 80% of the otosclerosis patients on sodium fluoride therapy had dyspeptic symptoms. The high incidence of dyspeptic symptoms in the present study could be due to the special emphasis given to gastrointestinal problems leading to an increased awareness. In the osteofluorosis group all the patients were found to have mild to severe gastrointestinal problems. Roholm (1937) also observed gastrointestinal symptoms in 80-90% of cryolite workers with skeletal fluorosis (4). The high incidence of dyspeptic symptoms and above normal levels of fluoride in serum and urine of the otosclerosis patients on sodium fluoride therapy and osteofluorosis patients suggest an etiological association between chronic fluoride toxicity and gastrointestinal symptoms.

Non-ulcer dyspepsia (NUD) is a very common clinical problem but it still remains an ill-defined entity. Although a number of causal factors have been suggested (20-23), none has consistently been shown to result in NUD. The purpose of including NUD patients in the present study was to determine whether fluoride toxicity could be an etiological factor for NUD, as most of the patients coming to the hospital of the All India Institute of Medical Sciences come from Delhi and the neighbouring States, which are known endemic areas for fluorosis.

The presence of high serum and urine fluoride levels in all the NUD patients is striking and contrasts sharply with the normal fluoride levels in the control subjects, who belonged to the same geographical areas as the patients. The main source of fluoride toxicity in NUD patients is drinking water, as 11 out of the 20 NUD patients had fluoride levels in their drinking water above the permissible limit, whereas all the control subjects had fluoride levels within the permissible limits. The NUD patients with high serum and urine fluoride levels, but consuming water with fluoride within the permissible limit, could have ingested fluoride through some typical food habits, as some food items are known to have very high fluoride content (10,11). Three control subjects also had slightly raised serum fluoride values, in spite of normal fluoride levels in urine and drinking water. That could be due to fluoride ingestion from

some food items or drinking water infrequently used by control subjects. The presence of high fluoride levels in serum and urine of the NUD patients clearly suggest that chronic fluoride toxicity is one of the causal factors for non-ulcer dyspepsia. Further, it was observed on radiological investigation that 4 non-ulcer dyspepsia patients had classical features of osteofluorosis, although they reported to the hospital only with non-ulcer dyspeptic complaints and had no clinical manifestation whatsoever of osteofluorosis or any other bone disorder. These four NUD patients were therefore considered to be in the initial stages of osteofluorosis. Thus it appears that in chronic fluoride toxicity the stomach and proximal small intestine are the first to show toxic manifestations in the form of dyspeptic symptoms and the skeletal involvement leading to osteofluorosis is a later phenomenon of chronic fluoride toxicity. For these reasons dyspeptic symptoms can be considered an early warning sign of chronic fluoride toxicity.

Upper gastrointestinal endoscopic findings revealed mucosal injury in the form of erythema, petechiae and erosions in 90% of otosclerosis, 70% of osteofluorosis and 45% of NUD patients. Spak *et al* (1989) also observed similar endoscopic findings in 20 healthy volunteers after a single dose of 20 mg sodium fluoride (24). The similarity in endoscopic findings in the two studies indicate that fluoride is the causal factor.

The histopathological examination revealed nonspecific gastritis and duodenitis in 100% of otosclerosis, 70% of osteofluorosis and 70% of NUD patients. These findings are in agreement with an earlier study by Pashley *et al* (1984), on rats subjected to 10 mg sodium fluoride for one hour (25). Both the endoscopic and histopathological findings demonstrated that the otosclerosis group on sodium fluoride therapy of 30 mg/day for 3 months or more are most adversely affected, followed by the osteofluorosis and NUD patients.

The scanning electron microscopic examination revealed more details of the deleterious effects of fluoride on gastroduodenal mucosa. These ultrastructural findings are in accord with other observations in the present study. In an earlier electron microscopic study, Susheela and Das (1988) reported severe cytomorphological abnormalities, viz. "cracked-clay" appearance, surface abrasion and cell degeneration in the duodenal mucosa of rabbits subjected to chronic fluoride toxicity (26). Pashley *et al* (1984) observed desquamated surface epithelium and widening of the junctions between adjacent epithelial cells in the stomach of rats subjected to 10 mg sodium fluoride for one hour (25). The observations emerging from these experimental studies are in conformity with the ultrastructural findings of the present investigation on human subjects, thereby clearly establishing that the gastroduodenal mucosa is severely damaged by the toxic effects of fluoride, resulting in dyspeptic symptoms.

The mechanism of action of fluoride on the gut mucosa may be as follows: Fluoride by chemically reacting with gastric hydrochloric acid forms hydrofluoric acid (HF) in the stomach. Unlike other mineral acids, hydrofluoric acid has high penetrating and corrosive properties. The corrosive nature of the hydrofluoric acid possibly leads to inflammation, petechiae, ulceration and other mucosal abnormalities in the stomach and proximal small intestine. On the other hand, due to its penetrating properties, hydrofluoric acid may easily enter the epithelial cell and get dissociated to hydrogen and fluoride ions. As fluoride ions are known inhibitors of many enzyme systems (27), the pathogenesis of mucosal damage could be due to this indirect effect of enzyme system inhibition.

### Conclusions

1. Ingested fluoride damages gastroduodenal mucosa.
2. Gastrointestinal discomfort can be an early warning sign of fluorosis.
3. Fluoride toxicity should be considered a possible reason for non-ulcer dyspepsia, especially in fluorosis endemic areas.
4. Gastrointestinal discomfort during sodium fluoride therapy calls for extreme caution and close monitoring.
5. Gastrointestinal discomfort in the form of dyspeptic symptoms should be an important diagnostic feature when identifying fluorosis patients and should not be dismissed as non-specific.

### Acknowledgement

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## EFFECT OF INDUSTRIAL FLUOROSIS ON HAEMOGRAM OF CAMELS

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**SUMMARY:** Fluoride pollution may have ill effects on livestock and vegetation as well as on humans. The Manquabad Super-Phosphate Factory, Assiut Province, Egypt, emits hydrofluoric acid (HF) gas. According to the present study, fluoride affected the haemograms of 114 camels at various distances from the factory. Blood sera and whole blood, analyzed to determine fluoride levels and haematological parameters, showed that elevated mean corpuscular volume (MCV) values were correlated with levels of serum fluoride. Decreases in total erythrocytes (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) levels were associated with the elevated MCV values. Lymphocytosis, neutropenia and monocytopenia were present in animals from affected areas. This study showed that anaemia may be associated with chronic fluorosis in camels, and that use of the haemogram in diagnosis of fluoride toxicity is not valid. Diagnosis should be confirmed by dental lesions, bone changes and chemical analysis.

**Key words:** Anaemia; Camels; Haemogram; Industrial fluorosis.

### Introduction

Variations in the constituents of blood cells are useful in the diagnosis of ailments affecting the liver, pancreas, bone and other organs or systems. Few data are available concerning the effects of industrial fluorosis on blood constituents of ruminants. In cattle, high eosinophils may be an early manifestation of fluoride toxicity (1), as is anaemia (2). The present study concerns the number of red blood cells per unit volume of blood, haemoglobin, haematocrit and mean corpuscular volume in herds exposed to varying levels of fluoride.

In Assiut Province, Egypt, the Manquabad Super-Phosphate Factory emits fluorine and other waste by-products, which have noticeably damaged the environment around the factory.

### Materials and Methods

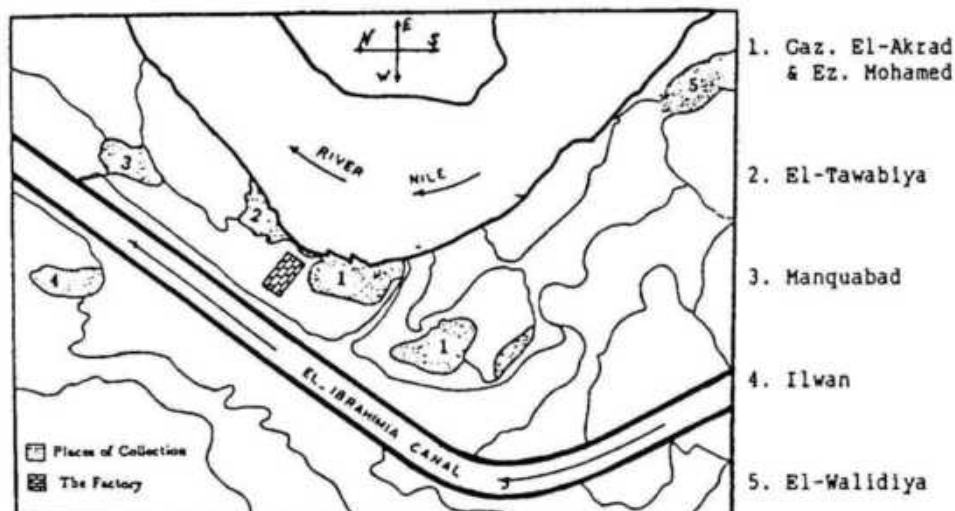
A total of 77 camels (*Camelus dromedarius*) were examined from different areas where fluoride intoxication was evident in the vicinity of the Manquabad Super-Phosphate Factory, Assiut

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Province, Egypt (Figure 1). An additional 37 camels from the Manfalut area 25 km due north of the factory served as low-fluoride controls.

Figure 1. Map of the Factory and Places of Sample Collection



According to routine clinical examination and laboratory investigations conducted for each camel, 55 camels showed clinical signs of intoxication. Age, sex, number of camels, location and angle of direction are shown in Table 1. Camels were selected from six localities (Gazert El-Akrad, Ezabet Mohamed, El-Tawabiya, Manquabad, Ilwan and El-Walidiya) around the Super-Phosphate Factory; the control group was from an area about 25 kilometers from the factory (Manfalut). Two blood samples were taken from each animal. The first sample of anti-coagulated blood was collected to determine total erythrocytes (TRBCs), haemoglobin (Hb), packed cell volume (PCV), total leukocytes (TWBCs) and differential leucocyte count (DLC). The second sample (10ml) was used to produce serum to estimate fluoride levels. Fluoride ions were determined by an expandable ions analyzer EA (920, Orion Research, "Model-94-90-00")(3).

TRBCs, T/L\*, TWBCs\*\* and Hb concentration (g/L) were determined by an Electronic Blood Cell Counter, Dyne 300, Sequol-turner (4). The apparatus was calibrated for direct measurement of RBCs, WBCs and Hb. PCV was determined by the micro-haematocrit method, with a micro-haematocrit centrifuge (5). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemo-

\* T/L = Terra/Liter =  $10^{12}/L$

\*\* G/L = Giga/Liter =  $10^9/L$

globin concentration (MCHC) were estimated (4). DLC was recorded (6). Two hundred white blood cells were counted to determine the percentage of each type of leukocytic cell. Data were analysed to determine the group means and standard error and the T test was applied to determine significant differences between group means (7).

Table 1. Location and Characteristics of Camels in Vicinity of Factory

Localities	Distance (Km) from factory	Location from factory	O	No. of Animals		age (years)
				Males	Females	
Gaz. El-Akrad	0 - 0.75	South	0°	10	12	7-11
Ez. Mohamed	1.5 - 2.5	Southwest	15°	7	4	7-12
Gaz. El-Tawabiya	0.5 - 1	Northeast	17°	3	6	8-12
Manquabad	1 - 2	Northwest	22°	10	4	7-10
Ilwan	2	Northwest	40°	5	4	7-12
El-Walidiya	4	Southeast	18°	7	5	8-12
Manfalut	25	North	0°	15	22	7-12

O = Angle of direction



Figure 2. Abnormal slight staining of teeth

## Results

Fifty-five of the 77 animals examined, located as far as 2.5 km from the factory, exposed signs of chronic fluorosis: loss of appetite, mottled teeth, brownish discoloration, pitting, fast wearing of permanent teeth.

Blood serum levels of fluoride are presented in Table 2.

Haematological characteristics including TRBCs, Hb concentration, PCV, MCV and MCHC are shown in Tables 3 and 4. Total and differential leukocyte counts are shown in Tables 5 and 6.

Bone fluoride levels and teeth lesions in camels were previously studied by the author. The former are presented in Table 7 and the latter in Figures 2-7.

Table 2  
Serum Fluoride Levels (ppm)

Localities	No. of animals examined	Distance (Km) from factory	Males	Females
Gaz. El-Akrad	22	0 - 0.75	1.90 $\pm$ 0.30*** 0.70-3.50	1.25 $\pm$ 0.07*** 0.70-1.80
Ez. Mohamed	11	1.5 - 2.5	1.20 $\pm$ 0.09*** 0.90-1.60	0.89 $\pm$ 0.16*** 0.80-1.30
El-Tawabiya	9	0.5 - 1	0.44 $\pm$ 0.03*** 0.39-0.50	0.45 $\pm$ 0.12** 0.12-0.74
Manquabad	14	1 - 2	0.58 $\pm$ 0.05*** 0.35-0.76	0.59 $\pm$ 0.03*** 0.50-0.65
Ilwan	9	2	0.38 $\pm$ 0.11* 0.37-0.66	0.26 $\pm$ 0.01*** 0.11-0.51
El-Walidiya	12	4	0.25 $\pm$ 0.07 0.12-0.63	0.28 $\pm$ 0.06** 0.21-0.50
Manfalut (control)	37	25	0.11 $\pm$ 0.02 0.10-0.32	0.10 $\pm$ 0.02 0.10-0.23

\*\*\* P < 0.001

\*\* P < 0.01

\* P < 0.05

Table 3  
Total RBCs Count (T/L) Haemoglobin Concentration (G/L)  
In Examined Camels at the Studied Areas

Localities	No.	RBCs (T/L)		Haemoglobin (G/L)		PCV (%)	
		Males	Females	Males	Females	Males	Females
Gaz. El-Akrad	22	8.1 $\pm$ 0.3*** 6.4 - 10.7	7.6 $\pm$ 1.4 5.1 - 9.1	109.0 $\pm$ 5.5*** 74 - 127	114.0 $\pm$ 5.0*** 92 - 142	24.3 $\pm$ 0.8* 21 - 28	24.3 $\pm$ 0.9* 9.7 - 28
Ez. Mohamed	11	8.4 $\pm$ 1.0 5.5 - 13.4	7.4 $\pm$ 0.7** 5.5 - 8.7	128.0 $\pm$ 7.0 103 - 152	125.0 $\pm$ 4.3*** 115 - 135	25.7 $\pm$ 1.2 22 - 31	28.5 $\pm$ 2.1 24 - 34
El-Tawabiya	9	8.7 $\pm$ 0.6* 7.7 - 9.9	7.9 $\pm$ 0.5** 6.7 - 9.8	126.0 $\pm$ 7.4 118 - 141	125.0 $\pm$ 18.7 119 - 130	28.0 $\pm$ 0.6 27 - 29	26.8 $\pm$ 2.0 23 - 28
Manquabad	14	7.8 $\pm$ 0.5*** 6.8 - 9.1	6.0 $\pm$ 0.2 5.5 - 6.3	101.2 $\pm$ 5.0*** 91 - 118	104.0 $\pm$ 5.0*** 95 - 115	27.0 $\pm$ 1.0 23 - 34	21.3 $\pm$ 0.6** 20 - 23
Ilwan	9	9.5 $\pm$ 0.7 8.5 - 10.8	9.6 $\pm$ 1.2 7.5 - 11.7	123.0 $\pm$ 11.0 103 - 139.5	126.0 $\pm$ 36.0 101 - 198	30.3 $\pm$ 0.9** 29 - 32	30.0 $\pm$ 1.20** 28 - 32
El-Walidiya	12	9.3 $\pm$ 0.6 7.1 - 11.7	9.3 $\pm$ 0.9 7.4 - 11.1	150.2 $\pm$ 4.2 137 - 168	146.5 $\pm$ 3.1 139 - 163	25.1 $\pm$ 3.1 24 - 29	26.3 $\pm$ 1.3 25 - 30
Manfalut (control)	37	10.5 $\pm$ 0.5 8.1 - 12.3	10.1 $\pm$ 0.6 7.4 - 14.7	146.2 $\pm$ 7.3 107 - 190	153.0 $\pm$ 4.0 120 - 190	26.8 $\pm$ 0.7 22 - 30	27.1 $\pm$ 0.7 26 - 30
*** P<0.001		** P<0.01	* P<0.05				

Table 4  
MCV (fl), MCH (Pq) and MCHC (%)  
In Examined Camels at the Studied Areas

Localities	No.	MCV (fl)		MCH (Pq)		MCHC (%)	
		Males	Females	Males	Females	Males	Females
Gaz. El-Akrad	22	30.3 $\pm$ 1.2 25.9 - 37.6	32.2 $\pm$ 0.9* 26.3 - 37.2	13.4 $\pm$ 1.7 9.3 - 20.1	13.9 $\pm$ 0.5 8.9 - 19.7	43.9 $\pm$ 2.5** 30.8 - 56.6	47.2 $\pm$ 1.9** 36.8 - 57.7
Ez. Mohamed	11	32.6 $\pm$ 2.6 19.0 - 37.7	39.2 $\pm$ 3.4** 33.3 - 46.6	15.9 $\pm$ 0.9 11.3 - 18.6	9.9 $\pm$ 1.8* 6.3 - 11.8	49.8 $\pm$ 2.1 44.8 - 66.8	44.7 $\pm$ 2.0*** 40.0 - 46.8
El-Tawabiya	9	35.7 $\pm$ 1.3*** 33.2 - 37.5	35.0 $\pm$ 2.5* 23.4 - 38.1	15.7 $\pm$ 2.2 12.1 - 16.8	16.2 $\pm$ 0.9 12.1 - 18.3	45.1 $\pm$ 1.9** 42.0 - 48.6	46.7 $\pm$ 1.1*** 43.2 - 51.7
Manquabad	14	34.9 $\pm$ 1.2** 29.9 - 38.5	35.4 $\pm$ 2.1** 32.2 - 41.7	13.7 $\pm$ 1.1 14.9 - 19.6	17.3 $\pm$ 1.3 15.2 - 20.8	37.8 $\pm$ 1.9*** 30.0 - 48.3	48.9 $\pm$ 1.3** 48.2 - 51.4
Ilwan	9	32.1 $\pm$ 1.3* 29.6 - 34.03	32.2 $\pm$ 3.9 27.3 - 40.0	15.8 $\pm$ 0.4 13.8 - 16.8	16.2 $\pm$ 1.3 15.1 - 20.8	40.4 $\pm$ 2.5*** 35.6 - 43.5	41.3 $\pm$ 6.6* 36.1 - 61.9
El-Walidiya	12	27.7 $\pm$ 2.3 22.0 - 41.0	27.65 $\pm$ 2.3 23.0 - 37.0	16.5 $\pm$ 1.3 14.0 - 23.6	16.5 $\pm$ 1.3 12.9 - 18.7	59.7 $\pm$ 2.1* 57.0 - 65.0	59.7 $\pm$ 1.1* 50.0 - 61.0
Manfalut (control)	37	26.9 $\pm$ 1.6 20.3 - 37.0	28.6 $\pm$ 1.1 25.0 - 35.1	16.2 $\pm$ 1.5 12.8 - 26.8	15.2 $\pm$ 0.8 11.4 - 22.6	53.7 $\pm$ 1.5 43.0 - 61.5	55.4 $\pm$ 1.7 40.7 - 65.5

\*\*\* P&lt;0.001

\*\* P&lt;0.01

\* P&lt;0.05

Table 5  
Total WBC count (G/L), Lymphocytes and Band Neutrophils Percentage  
In Examined Camels at the Studied Areas

Localities	No.	WBC (G/L)		Lymphocytes Percentage		Band Neutrophils Percentage	
		Males	Females	Males	Females	Males	Females
Gaz. El-Akrad	22	12.1 $\pm$ 0.9** 7.5 - 16.9	15.2 $\pm$ 2.3 8.2 - 20.5	30.9 $\pm$ 1.5 22 - 37	39.5 $\pm$ 3.5 28 - 61	4.5 $\pm$ 0.9 3 - 10	4.4 $\pm$ 0.9 2 - 10
Ez. Mohamed	11	16.1 $\pm$ 2.9* 12.3 - 23.8	16.8 $\pm$ 3.1 9.0 - 21.8	44.1 $\pm$ 5.1** 30 - 68	48.5 $\pm$ 4.7 37 - 59	3.1 $\pm$ 1.9 0 - 14	2.0 $\pm$ 1.1 0 - 5
El-Tawabyia	9	9.5 $\pm$ 1.0 7.8 - 11.4	13.8 $\pm$ 3.8 7.5 - 15.3	41.6 $\pm$ 2.4*** 37 - 45	44.3 $\pm$ 3.9** 34 - 62	2.3 $\pm$ 0.9 1 - 4	7.0 $\pm$ 0.7** 5 - 9
Manquabad	14	14.4 $\pm$ 1.3*** 10.0 - 15.7	10.7 $\pm$ 0.4 9.5 - 11.4	36.1 $\pm$ 3.3* 25 - 50	22.8 $\pm$ 0.9*** 20 - 24	4.3 $\pm$ 1.3 1 - 14	5.0 $\pm$ 1.3 4 - 6
Ilwan	9	14.6 $\pm$ 1.9** 11.2 - 17.7	10.4 $\pm$ 0.5 9.6 - 11.2	28.7 $\pm$ 2.3 25 - 33	32.3 $\pm$ 2.3 28 - 36	2.3 $\pm$ 1.2 0 - 4	5.0 $\pm$ 1.7 2 - 8
El-Walidyia	12	14.6 $\pm$ 0.9*** 12.0 - 18.5	13.6 $\pm$ 2.2 9.3 - 19.6	45.1 $\pm$ 2.2*** 41 - 66	44.8 $\pm$ 3.8** 38 - 55	3.6 $\pm$ 0.5 1 - 5	4.0 $\pm$ 0.4 3 - 5
Manfalut (control)	37	8.7 $\pm$ 0.7 5.3 - 12.9	12.3 $\pm$ 1.2 5.6 - 18.7	28.30 $\pm$ 1.5 20 - 36	31.7 $\pm$ 2.3 22 - 40	4.9 $\pm$ 0.5 3 - 8	4.30 $\pm$ 0.5 3 - 7

\*\*\* P&lt;0.001

\*\* P&lt;0.01

\* P&lt;0.05

Table 6  
Segmented Neutrophils, Eosinophils and Monocytes in the Blood  
of Examined Camels at the Studied Areas

Localities	No.	Segmented Neutrophils		Eosinophils Percentage		Monocytes Percentage	
		Males	Females	Males	Females	Males	Females
Gaz. El-Akrad	22	51.5 $\pm$ 2.8 38 - 71	43.6 $\pm$ 3.3 31 - 61	6.8 $\pm$ 1.2 2 - 12	6.3 $\pm$ 0.8 3 - 12	4.3 $\pm$ 1.1 1 - 12	5.7 $\pm$ 0.8 2 - 10
Ez. Mohamed	11	39.0 $\pm$ 3.2*** 26 - 52	40.3 $\pm$ 3.2** 34 - 49	10.1 $\pm$ 2.2 2 - 19	6.8 $\pm$ 2.1 3 - 12	4.0 $\pm$ 0.7 2 - 6	2.5 $\pm$ 0.9** 1 - 4
El-Tawabiya	9	41.5 $\pm$ 1.5*** 39 - 44	37.2 $\pm$ 0.8*** 26 - 40	7.7 $\pm$ 1.7 6 - 11	7.0 $\pm$ 2.3 1 - 17	6.3 $\pm$ 1.2 4 - 8	5.30 $\pm$ 1.3 3 - 11
Manquabad	14	48.5 $\pm$ 2.7 42 - 66	59.8 $\pm$ 1.7*** 55 - 62	6.9 $\pm$ 1.5 1 - 13	8.3 $\pm$ 1.3 6 - 12	2.3 $\pm$ 0.5*** 1 - 7	4.3 $\pm$ 1.1 3 - 7
Ilwan	9	49.0 $\pm$ 1.2 47 - 51	46.7 $\pm$ 2.9 41 - 51	15.0 $\pm$ 2.1* 11 - 18	9.3 $\pm$ 0.33 8 - 10	4.3 $\pm$ 0.9 3 - 6	6.7 $\pm$ 0.33 6 - 7
El-Walidiya	12	45.1 $\pm$ 4.2 33 - 50	44.0 $\pm$ 3.5 35 - 51	3.6 $\pm$ 0.57* 2 - 6	5.5 $\pm$ 1.3 3 - 9	2.4 $\pm$ 0.5*** 1 - 5	2.5 $\pm$ 0.6*** 1 - 4
Manfalut (control)	37	52.3 $\pm$ 1.5 45 - 58	50.8 $\pm$ 1.7 41 - 58	8.0 $\pm$ 1.9 2 - 21	7.6 $\pm$ 1.0 3 - 12	5.3 $\pm$ 0.4 3 - 7	5.6 $\pm$ 0.6 3 - 8

\*\*\*  $P < 0.001$ \*\*  $P < 0.01$ \*  $P < 0.05$



Table 7

Overall Means of Fluoride Levels (ppm) in Bone Ash of Examined Bones from Clinically Healthy and Fluorosed Camels (previous study)

	Clinically Healthy Camels (37)			Camels showing signs of fluorosis (53)		
	Metacarpal	Metatarsal	Ribs	Metacarpal	Metatarsal	Ribs
Proximal extremities of the bones	824 $\pm$ 42 590-1240	902 $\pm$ 78 620-1612	717 $\pm$ 33.4 620-868	4046 $\pm$ 360* 2945-6200	5215 $\pm$ 645* 3100-8060	3513 $\pm$ 246* 2790-4340
Shaft of the bone	775 $\pm$ 485 590-1240	757 $\pm$ 53.3 590-1302	828 $\pm$ 70 682-1550	3695 $\pm$ 446* 2015-6200	4167 $\pm$ 433* 2950-6510	3996 $\pm$ 588* 2170-7750

\*  $P < 0.001$



Figure 3. Moderate course mottling of enamel



Figure 4. Definite mottling and staining



Figure 5. More advanced lesion (definite mottling and abrasion)

Figure 6 (next page, above) Pitting and slight wearing

Figure 7 (next page, below) Fast wearing



### Discussion

A significant decrease ( $P<0.001$ ) in the total erythrocytic count (Erythropenia) in affected camels from Manquabad (both males and females) and for males only at Gazert El-Akrad was observed. Only females were affected from Ezabet Mohamed. The decrease ( $P<0.05$ ) in male and female camels at El-Tawabiya was significant.

There was a significant decrease ( $P<0.001$ ) in haemoglobin in males and females from Gazert El-Akrad and Manquabad.

Decrease in PCV was significant in camels at Gazert El-Akrad and Ilwan for both sexes. The lack of significant differences may reflect the normally wide variations in PCV in camels' blood (8).

MCV was significantly higher ( $P<0.001$ ) in male camels from El-Tawabiya, and in camels from Manquabad. In female camels from Ezabet Mohamed MCV was significantly higher ( $P<0.01$ ), as were MCV values from El-Tawabiya and Gazert El-Akrad ( $P<0.05$ ).

Decreases ( $P<0.05$ ) of MCH in female camels were significant from only Ezabet Mohamed. MCHC values decreased ( $P<0.01$ ) in male camels from Gazert El-Akrad, Manquabad and El-Tawabiya, and in female camels from Ezabet Mohamed ( $P<0.001$ ). MCHC values decreased ( $P<0.01$ ) in all female camels from all areas except Ilwan.

Most of the animals were afflicted by anaemia, the macrocytic hypochromic type in areas of Ezabet Mohamed, El-Tawabiya and Manquabad and the normocytic hypochromic type in the areas of Gazert El-Akrad. Animals in Ilwan also showed signs of anaemia characterized by decreases in MCHC and haemoglobin concentrations.

Macrocytic anaemia was associated with increased values of MCV whereas hypochromic anaemia was characterized by decreased Hb, MCH and MCHC values. The increased size of RBCs could reflect the effects of chronic fluoride intoxication on bone marrow. Fluoride intoxication can depress bone marrow activity in cattle (9).

Fluoride concentration of bones and teeth is the most important biochemical indication of chronic fluoride intoxication (10,12). The bone fluoride study (Table 7) revealed significant increase ( $P<0.001$ ) in fluoride content of bones from affected animals, which reached about five times the level of the control group. Proximal extremities of affected metacarpal metatarsal bones retained higher levels of fluoride than their shafts, similar to those obtained by Shupe and Olson (10) in bovine fluorosis. Although bone fluoride was high, bony exostoses were not observed in any bones of affected camels.

The highly significant elevation in fluoride levels recorded in our results in Gazert El-Akrad and Ezabet-Mohamed in comparison with the other areas of investigation may be due to their proxi-

mity to the plant as well as to the fact that the wind direction is the same for both; the closer to the plant the greater was the effect (11).

Anaemia was related to elevated fluoride levels in the serum and bones, one of the clinical signs of fluorosis in cattle (12). Anaemia in fluorotic cattle is attributed to lower levels of blood folic acid (1) RBC counts and Hb concentration. PCV and MCV were low in fluorotic camel herds (13,2) and in goats reared in polluted areas near the Manquabad Super-Phosphate Factory (14).

Total leukocyte count in male camels from all investigated areas was significantly higher, except in El-Tawabiya. Camels from areas near the factory with high fluoride levels were afflicted with lymphocytosis. There was a significant decrease in the percentage of neutrophils (Neutropenia) and monocytes (Monocytopenia), results similar to previous investigations involving cattle and goats (1,15).

Our results do not seem to coincide with the concepts of Shupe et al (16) because none of their various fluoride diets had any detectable harmful effect on the animals' blood morphology or the haemopoietic system (16).

#### Conclusion

The results of this present study showed that anaemia was associated with chronic fluorosis in camels. Use of the haemogram is not considered valid in the diagnosis of chronic fluoride toxicity which must be confirmed by tooth lesions and bone fluoride determination.

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FLUORIDE CONCENTRATION IN TILAPIA FISH (*Oreochromis leucostictus*) FROM LAKE NAIVASHA, KENYA

Joseph K. Gikunju \*

**SUMMARY:** Fluoride concentration ( $\mu\text{g/g}$ ) in *Oreochromis leucostictus* (Tilapia Fish) living in water containing 24 mg F/L were: (Mean  $\pm$  SEM) muscles  $1.97 \pm 0.16$ , skin  $4.96 \pm 0.27$ , gills  $143.1 \pm 8.89$  and bones  $210.6 \pm 24.7$  (all the values on wet weight basis). For fillet, skin, and gill tissues, fluoride levels were not related to the weight of the fish. The relationship between fish weight and fluoride concentration in bone, however, was significant ( $p < 0.05$ ). Hence *Oreochromis leucostictus* possibly has a saturation level of fluoride for fillet, skin, and and gill tissues, or perhaps a fluoride excretion mechanism exists in the Tilapia fish. The fluoride content of fish muscle may contribute to the total daily intake of fluoride and hence predispose to dental fluorosis. However, water and other high fluoride foods also have to be considered in order to reach a definite conclusion.

**Key words:** Diet; Fish; Fluoride accumulation; Fluorosis; Kenya; Lake Naivasha; *Oreochromis leucostictus* (Tilapia fish).

## Introduction

Endemic dental fluorosis is widespread in Kenya. On the whole, 30 to 50% of the population is affected, but there are regional differences in prevalence and severity (1). In several countries fish and marine products have been identified as a major source of dietary fluoride (2-4). Aquatic organisms living in high fluoride environments accumulate fluoride, especially in osseous tissues. However, there are reports of fluoride accumulation in muscles and skin of some temperate fish (5). Fish is an important source of food in Kenya; however, little information is available regarding fluoride levels in fish including *Oreochromis leucostictus* (Tilapia fish), which is consumed locally around Kenya's Lake Naivasha.

The main objective of this work was to determine the fluoride concentration in muscles, skin, gills, and bones of *Oreochromis leucostictus* and whether such fluoride levels might be contributing to a high daily intake of fluoride and a consequent health hazard.

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## Materials and Methods

### *Sampling:*

A total of 90 Tilapia fish (*Oreochromis leucostictus*) with weight ranging from 33 to 250g (mean weight 95g) were caught from Lake Naivasha with a fine net. The fish were kept in a cooler with ice before they were transported to the laboratory where fillet, skin, gills, and bones were dissected. Fillet was simply peeled from the bone to avoid scrapping of bone chips onto the fillet. Water samples were collected in clean 500ml polyethylene bottles. The samples were transported to the laboratory in a cool box and then stored at -20 C before analysis.

### *Fluoride analyses:*

The pH and F levels were determined with a digital pH meter (3020 Orion®) and a F specific ion electrode (Orion® 96-09) respectively. Calibration of the F electrode was repeatedly checked with appropriate standards during the measurements. To determine fluoride concentration in water standards and samples, the solutions were adjusted to pH 5.0 and 5.5 before being buffered with TISAB reagent.

### *Reagents:*

Fluoride standard solution (100ppm, 94-09-07, Orion®). TISAB III (Total ionic adjustment buffer), (940911, Orion®). TISAB II (94-09-09, Orion®).

### *Fluoride standard-calibration curve:*

Fluoride standard solutions (0.1, 1.0 and 10.0ppm) were prepared by diluting the 100ppm standard solution with deionised water. Two parallel tubes were filled with 3.0ml standard fluoride solution and 0.3ml buffer added to each tube before analysis. A calibration curve was prepared from these standards. The average relative millivolt value for each standard was plotted against the fluoride concentration on a 4-cycle semilogarithmic paper. The difference between a ten-fold increase in fluoride concentration was between 54 and 60 relative millivolts (Figure 1).

### *Fish:*

The total F levels of the tissues were assessed after preparation according to Birkeland (6). After homogenization and drying for 24 hours at 105°C, about 45mg of the dry sample was dissolved in acid (equal parts of 11.6 M perchloric acid and 14.3 M nitric acid) and then neutralized and buffered to pH 5.2 - 5.5 with an alkaline mixture of 7.8 M sodium hydroxide and 1.0 M trisodium citrate. The dissolution and buffering took place in a closed compartment (Figure 2). The accuracy of the method was checked by spiking and recovery experiments. Recoveries of added quantities of fluoride at various levels were as follows (Mean  $\pm$  SEM%): Blank, 99.6  $\pm$  2, fillet 87.4  $\pm$  4, skin 101  $\pm$  16, gills 105  $\pm$  6, bones 109  $\pm$  3.



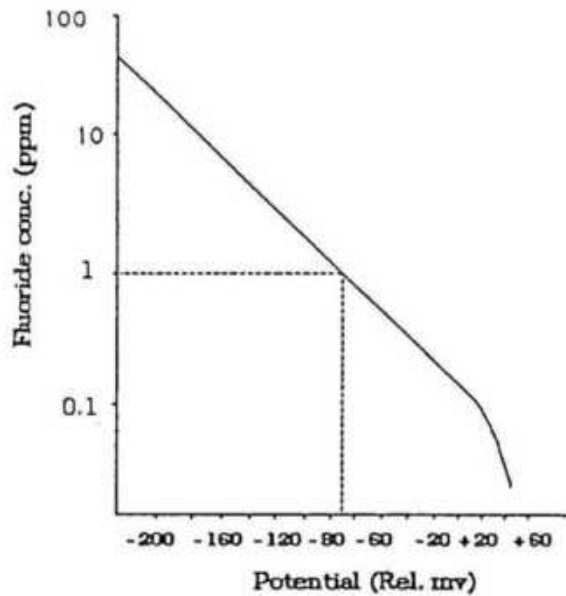


Figure 1. Fluoride concentration calibration curve

Digestion tube for fluoride samples

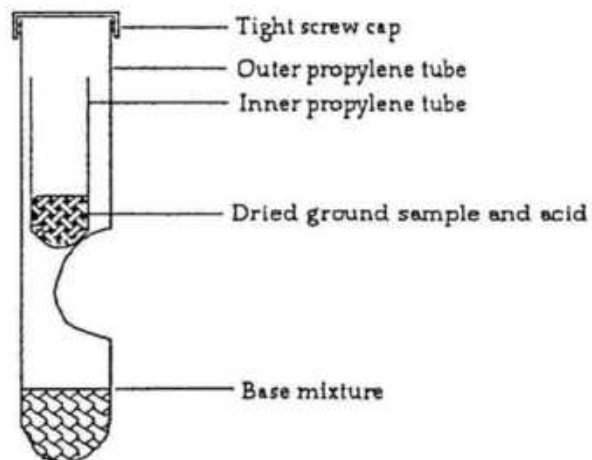


Figure 2. Closed double tube arrangement used for dissolution and buffering of fluoride analysis samples

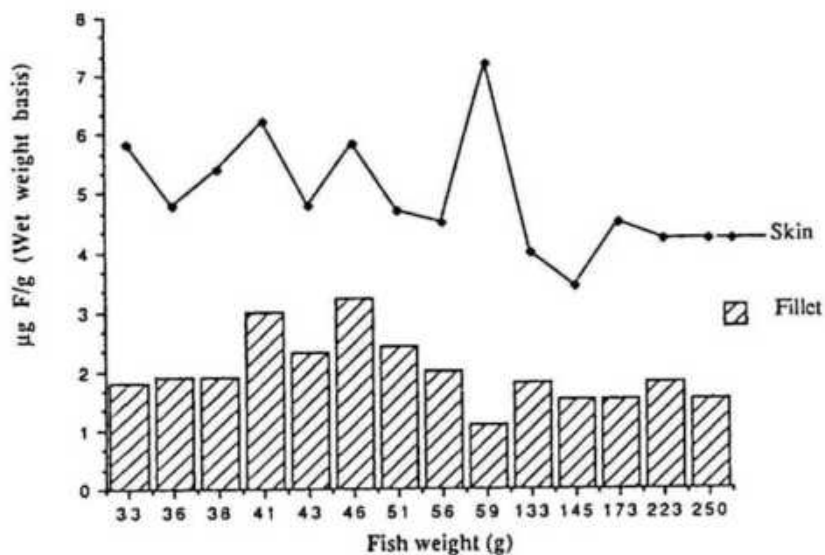


Figure 3. Relationship between fluoride concentration in fillet and skin of *Oreochromis leucostictus* and the fish weight.

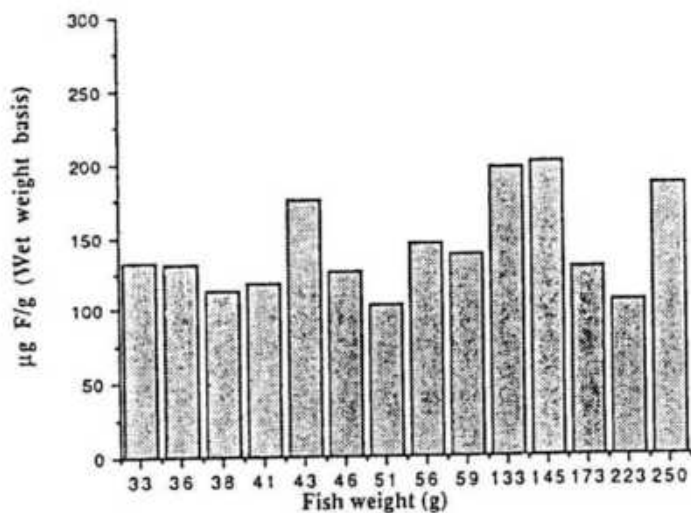


Figure 4. Fluoride concentration in gills of *Oreochromis leucostictus* of varying fish weight.

## Results

The fluoride concentration of water samples from Lake Naivasha was 2.4mg F/L (mean) and 2.4-2.6 (range). Fluoride concentrations in the various body parts of *Oreochromis leucostictus* in relation to fish weight are shown in Figures 3-5.

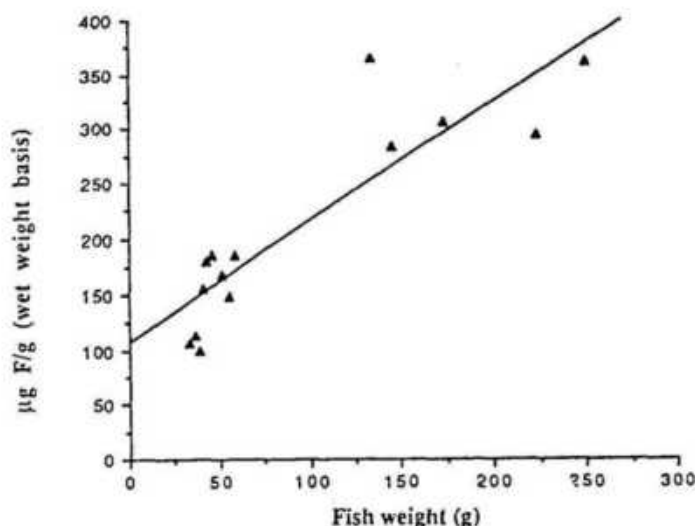


Figure 5. Fluoride levels in bone tissue of *Oreochromis leucostictus* of different fish weights.

## Discussion

The recorded F concentration of 2.4-2.6mg F/L in Lake Naivasha agree fairly well with the values of 1.3-1.5mg F/L reported by Kariuki *et al* (7). The much higher values reported by earlier investigators (8,9) may depend partly on seasonal variations and different sites of collection, but another possibility is analytical errors in the F determinations caused by the high pH and salinity of the water.

The fluoride concentrations in bone (Fig.5) were consistent with the reports of a direct correlation between the F levels in fish tissues and the fish size (10). It is known that considerable accumulation of F occurs in the skeletons of marine animals, and to varying degrees in the fish skin (11). This was also the case in *Oreochromis leucostictus*. Fish weight and fluoride concentration were significantly related at the 0.05 level ( $p = 0.045$ ).

On a wet weight basis, the fluoride levels in the fillet of *Oreochromis leucostictus* (Fig.3) were 2µg F/g. Reported values of F levels in fish fillet show considerable variation (11-14). Oelschleger *et al* (12) reported fluoride levels of 1.2-0.3µg F/g in the fillet of six species of fresh-water fish and four species of sea-water fish, and the same range was reported by Demmel *et al* (13) in studies on fresh and sea water fish. Wright and Davison (11) reported fillet of three species of sea water fish to contain 1.0-1.8 µg F/g, while Manthey (14) found 8-14µg F/g in fillet of four species of Antarctic sea water fish. Sea water normally contains 1.2-1.5mg F/L. Uptake of F may not only take place from water, but also from food. The high F levels in Antarctic fish are attributed to consumption of krill which concentrate and accumulate F by several orders of magnitude. The bones of the krill-consuming fish contained 258-1,841µg F/g on wet weight basis. The bone fluoride concentrations of the fish from Lake Naivasha were therefore relatively low compared with the levels reported in Antarctic fish.

The fillet is often the only part of the fish consumed by humans. Intake of 200g fillet with 2µg F/g would provide 0.4mg F, which is well below the 1.5-4.0mg F regarded as a safe daily F intake in adults (15). In children the recommended maximum daily intake is about 0.06mg F/kg body weight, but an increase to 0.1mg F/kg may cause dental fluorosis (16,17). Nevertheless, consumption of fillet with 2µg F/g by children will only make a moderate contribution to the amount capable of causing fluorosis.

*Oreochromis leucostictus* from Lake Naivasha are often used to make soup which is prepared by cooking fish with skin and bones. This may increase the F intake considerably. On the other hand, the reported values give the total fluoride (both ionic and bound F) in the tissues. Similar to the mechanism in higher vertebrates, the deposition of fluoride in fish bone is assumed to depend on anion-exchange (usually with OH<sup>-</sup>) in the hydroxyapatite complex (11). There is great uncertainty regarding the bioavailability of fluoride in most foods (18). Probably only a fraction of the F in the bones will be extracted into the soup, and of this only a part will be absorbed into the gastrointestinal tract. Accordingly, it appears that the F content of fish is not a major contributor to the prevalent and severe fluorosis in the Rift Valley area (1).

#### Acknowledgement

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## 1992 CONFERENCE

The 19th Conference of ISFR will be held in Kyoto, Japan, on September 8-11 1992. Kyoto, "Capital of Peace", was for about 1000 years the centre of Japanese politics and culture. Today it is still considered the "Cultural Heart of Japan" and is one of the Japanese people's most beloved cities. It is where Buddhist architecture, the art of landscape gardening and other fine arts and crafts developed remarkably around 1580AD. Those historic attractions, as well as the city's excellent shopping facilities and friendly open-minded citizenry, make Kyoto a most promising venue for an enjoyable and interesting conference.

The conference will be held at the Renaissance Hall. The banquets will be at the Kyoto Century Hotel. Both venues are close to Kyoto Railway Station. The language of the conference will be English.

The scientific programme of keynote addresses, special lectures and workshops is still in the planning stage as the submission of papers for presentation continues until April 30. Workshop topics will be:

1. Analytical methods for fluoride
2. Environmental fluoride pollution
3. Biological effects of fluoride
4. Effects of fluoride on humans

More news will be in the next issue of Fluoride. ISFR members and other researchers have received an Announcement and Call for Abstracts. The registration fee 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. Early booking is desirable. 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 will be happy to issue letters of invitation to assist applicants in obtaining visas and travel funds.



EFFECT OF FLUORIDE TREATMENT ON THE FRACTURE RATE  
IN POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS

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Edmund YS Chao, Heinz W Wahner, Joan M Muhs,  
Sandra L Cedel, L Joseph Melton III  
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Abstract from *New England Journal of Medicine* 322 802-9 1990

Although fluoride increases bone mass, the newly formed bone may have reduced strength. To assess the effect of fluoride treatment on the fracture rate in osteoporosis, we conducted a four-year prospective clinical trial in 202 postmenopausal women with osteoporosis and vertebral fractures who were randomly assigned to receive sodium fluoride (75 mg per day) or placebo. All received a calcium supplement (1500 mg per day). Sixty-six women in the fluoride group and 69 women in the placebo group completed the trial.

As compared with the placebo group, the treatment group had increases in median bone mineral density of 35 percent ( $P < 0.0001$ ) in the lumbar spine (predominantly cancellous bone), 12 percent ( $P < 0.0001$ ) in the femoral neck, and 10 percent ( $P < 0.0001$ ) in the femoral trochanter (sites of mixed cortical and cancellous bone), but the bone mineral density decreased by 4% ( $P < 0.02$ ) in the shaft of the radius (predominantly cortical bone). The number of new vertebral fractures was similar in the treatment and placebo groups (163 and 136, respectively;  $P$  not significant), but the number of nonvertebral fractures was higher in the treatment group (72 vs. 24;  $P < 0.01$ ). Fifty-four women in the fluoride group and 24 in the placebo group had side effects sufficiently severe to warrant dose reduction; the major side effects were gastrointestinal symptoms and lower-extremity pain.

We conclude that fluoride therapy increases cancellous but decreases cortical bone mineral density and increases skeletal fragility. Thus, under the conditions of this study, the fluoride-calcium regimen was not effective treatment for postmenopausal osteoporosis.

Key words: Fluoride therapy; Hip fracture; Osteoporosis.  
Reprints: Dr Riggs, Mayo Clinic, 200 First Street SW,  
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## FLUORIDE AND BONE - QUANTITY VERSUS QUALITY

Robert Lindsay  
New York, USA

Abstracted from *New England Journal  
of Medicine* 322 845-6 1990

"Postmenopausal osteoporosis is a major public health problem, whose impact is expected to reach epidemic proportions during the early part of the next century as the population ages." After that observation, this article reviews recent findings on the treatment of osteoporosis with doses of sodium fluoride - an agent which had raised great hopes because it was known to increase bone density. "Unfortunately, the use of fluoride is often associated with side effects, including abnormalities of bone structure. Fluoride is incorporated into hydroxyapatite, altering the size and structure of the crystals and perhaps thereby decreasing the mechanical competence of the bone. When calcium intake is inadequate, the administration of fluoride also results in an impairment of mineralization. The bone formed in response to fluoride use may be somewhat disorganized, at least before remodeling, resembling immature woven bone rather than adult lamellar bone. Therefore, although the risk of fractures normally rises as the bone mass declines, increases in bone mass with fluoride treatment may not reduce fracture rates."

The article reviews and discusses the recent clinical trials by Riggs *et al* (see abstract above) and Hedlund and Gallagher (abstract in *Fluoride* 23 No.3). Commenting on the disturbing suggestion that fluoride fails to reduce vertebral fractures and increases the risk of hip fractures, Lindsay concludes "until studies designed as rigorously as that of Riggs *et al* are performed with other preparations, formulations, or dosages, it is difficult to recommend use of fluoride in clinical practice."

Pointing out that "we do not yet have an ideal therapy for postmenopausal osteoporosis" Lindsay stresses the urgent need for targeted research into age-related bone changes, concluding finally: "The alternative is a not-too-distant future in which the incidence of osteoporotic fractures will reach epidemic proportions, and costs may escalate beyond our capacity to pay."

Key words: Fluoride therapy; Hip fracture; Osteoporosis.  
Reprints: Dr Robert Lindsay, Helen Hayes Hospital,  
West Haverstraw NY 10993, USA.

## FLUORIDE IN THE PREVENTION OF OSTEOPOROSIS AND FRACTURES

L Joseph Melton III  
Minnesota USA

Abstract from *Journal of Bone and Mineral  
Research* 5 Suppl 1 163-7 1990

Age-related fractures, especially hip fractures, produce sufficient morbidity and mortality to make osteoporosis a disease worth preventing, and accurate techniques exist to identify groups at high risk of fracture by virtue of low bone mass. While the need for prevention is evident, no specific program of fluoride use for this purpose has been devised, and epidemiologic data provide little support for the notion that exposure to fluoride reduces hip fracture incidence. At present, fluoride cannot be recommended as a prophylactic agent for the fractures that are the primary adverse health outcome of osteoporosis.

Key words: Fluoride therapy; Hip fracture; Osteoporosis.  
Reprints: Section of Clinical Epidemiology, Department of  
Health Sciences Research, Mayo Clinic and Foundation,  
Rochester MN, USA.

## REGIONAL VARIATION IN THE INCIDENCE OF HIP FRACTURE US WHITE WOMEN AGED 65 YEARS AND OLDER

Steven J Jacobsen, Jack Goldberg, Toni P Miles,  
Jacob A Brody, William Stiers, Alfred A Rimm  
Milwaukee WI, USA

Abstracted from *Journal of the American  
Medical Association* 264 6-8 1990

This study examined the geographic distribution in the United States of hip fractures among white women aged 65 years and over. It presented an ecological analysis using county-level data on such fractures, totalling 541,985, from all hospital discharges during 1984 through 1987. Weighted least-squares regression methods were used to examine various associations. Unadjusted and multi-factor adjusted results were presented. The risk factors examined included the percentage of the population served with fluoridated water. A weak positive association be-

tween that factor and hip fracture incidence was strengthened after adjustment for other risk factors. The study's Figure, a county map of hip fracture incidence rates, displayed a distinct north to south geographic pattern, with higher rates in the south where there is a cluster of high risk counties - a pattern confirmed by the ecological analysis. The authors commented: "The results from the ecological regression analysis suggest that soft and fluoridated water, poverty, reduced sunlight exposure and rural location all increase the risk of hip fracture. The strong positive association of latitude with hip fracture incidence among women is undiminished after adjustment for the confounding effects of these risk factors." Discussing possible reasons for the latitude association, they observed: "it is of interest that the ecological analyses suggest factors that are strongly associated with diet (*i.e.* poverty and rural location). Further, the observed cluster of high risk for hip fractures in the southeastern United States corresponds closely with those areas that suffered epidemic levels of diseases of nutritional deficiency, such as pellagra, early in this century." However, they concluded "No presently recognized factor or factors adequately explain this observed geographic variation."

**Key words:** Ecological analysis; Fluoridation; Hip fracture; Latitude; Poverty; Rural location; Sunlight; Water hardness.  
**Reprints:** Dr Jacobsen, Division of Biostatistics/Clinical Epidemiology, Medical College of Wisconsin, PO Box 26509, Milwaukee WI 53226, USA.

#### WATER FLUORIDE CONCENTRATION AND FRACTURE OF THE PROXIMAL FEMUR

C Cooper, C Wickham, R F Lacey, D J P Barker  
 Southampton, England

*Abstract from Journal of Epidemiology  
 and Community Health 44 17-9 1990*

**Study objective** - The aim of the study was to examine the relationship between water fluoride concentration and the incidence of hip fracture, since evidence on this is at present inconsistent.

**Design** - Numbers of hospital admissions for fractures of proximal femur were obtained from hospital activity analysis data for the years 1978-1982. The fracture rates were compared with water fluoride concentrations in 39 county districts of England and Wales (fluoride concentrations had been measured in these districts between 1969 and 1973 as part of the British Regional Heart study).

**Patients** - During the study period, 4121 men and 16272 women aged 45 years and over were discharged from hospital after hip fracture.

**Results** - Poor correlations were found between discharge rates and both total ( $r=0.16$ ,  $p=0.34$ ) and natural ( $r=0.01$ ,  $p=0.95$ ) water fluoride concentrations.

**Conclusions** - Water fluoridation to levels of around 1 mg/litre is unlikely to reduce hip fracture incidence markedly in this country.

**Key words:** Fluoridation; Hip fracture; Osteoporosis.

Reprints: Dr Cooper, MRC Environmental Epidemiology Unit,  
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#### WATER FLUORIDATION AND HIP FRACTURE

Cyrus Cooper, Carol A C Wickham,  
David J R Barker, Steven J Jacobsen  
Southampton, England

Abstracted from *Journal of the American  
Medical Association* 266 513-4 1991

In a letter to the editor, the authors of two recent large-scale ecological studies of hip fractures (Jacobson *et al* 1990, Cooper *et al* 1990 - see preceding abstracts) wrote, concerning the original English finding of lack of association between hip fractures water fluoride concentration: "the results from the recent US study prompted us to reexamine our data. Our original statistical methods did not adequately account for differences in precision of the county-specific rate estimate. We reanalyzed the data using a weighted least-squares technique (weighting each county by the size of the population aged 45 years and older) to allow for these differences. We found a significant positive correlation between fluoride levels and discharge rates for hip fracture . . . We present these data for two purposes. First, given the widespread use of fluoridated water in public water supplies for the prevention of dental caries, any risk or benefit associated with this practice will affect extremely large numbers of persons. . . Of course, this approach remains hampered by the problems common to all ecologic studies. The relationship observed may be spurious due to the confounding of some other factor that has not been accounted for in our analysis. Furthermore, an adverse impact of such low levels of fluoride appears biologically implausible, despite the recent trials suggesting such a consequence at much higher doses than in our study. Nevertheless, this positive association demands further investigation at the individual level. Our second purpose is to stress the methodologic issue of weighting in this type of analysis. The precision with which each county-specific rate is estimated is directly related to the size of the population. Analyses that fail to adequately account for this variation in precision give inappropriate emphasis to counties in which there is greater error in measurement of the rate. These data

provide a striking example of such a bias obscuring the detection of potentially important associations." The letter's accompanying Figure depicts graphically the rise in hip fracture discharge rates as water fluoride levels rise from zero to one part per million.

Key words: Ecologic studies; Fluoridation; Hip fracture.  
Reprints: Dr Cooper, Southampton General Hospital MRC  
Environmental Epidemiology Unit, Southampton, England.

# A PROSPECTIVE STUDY OF BONE MINERAL CONTENT AND FRACTURE IN COMMUNITIES WITH DIFFERENTIAL FLUORIDE EXPOSURE

MaryFran R Sowers, M Kathleen Clark,  
Mary L Jannausch, Robert B Wallace  
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Abstract from *American Journal  
of Epidemiology* 133 649-60 1991

In 1983/1984, a study of bone mass and fractures was begun in 827 women aged 20-80 years in three rural Iowa communities selected for the fluoride and calcium content of their community water supplies. The control community's water had a calcium content of 67 mg/liter and a fluoride content of 1 mg/liter. The higher-calcium community had water with a calcium content of 375 mg/liter and a fluoride content of 1 mg/liter. The higher fluoride community's water had 15 mg/liter of calcium and 4 mg/liter of fluoride naturally occurring. In 1988/1989, a follow-up study characterized the 684 women still living and available for study. Residence in the higher fluoride community was associated with a significantly lower radial bone mass in premenopausal and postmenopausal women, an increased rate of radial bone mass loss in premenopausal women, and significantly more fractures among postmenopausal women. There was no difference in the 5-year relative risk of any fracture in the higher-calcium community versus the control community; however, the relative risk was 2.1 (95% confidence interval (CI) 1.0-4.4) in women in the higher-fluoride community compared with women in the control community. There was no difference in the 5-year risk of wrist, spine or hip fracture in the higher-calcium community versus the control community; however, the 5-year relative risk for women in the higher-fluoride community, compared with women in the control community, was 2.2 (95% CI 1.1-4.7). Estimates of risks were adjusted for age and body size.

Key words: Bone and bones; Calcium; Fluoridation; Fluorides;  
Fractures.  
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SCIENTIFIC KNOWLEDGE IN CONTROVERSY: THE  
SOCIAL DYNAMICS OF THE FLUORIDATION DEBATE

Brian Martin

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Reviewed by Neville Hicks \*

Well over \$100 million of taxpayer's money is spent on medical research in Australia every year but taxpayers can rest easy. All of the money is allocated by the peers of those who get the money, and "only scientifically rigorous proposals" are funded. Judgements about scientific rigour are always based on "objective knowledge" and the funding process is not influenced by mateship, deals, networks or hope of reciprocal favours.

Brian Martin from the University of Wollongong thinks that life among the scientists is always more controversial than that point of view suggests. In his latest book he sets out to show how scientists in dispute behave like non-scientists, attacking their opponents rather than the "facts" and choosing facts to suit prior commitments. The dispute in this case is about the fluoridation of water supplies, a long-running debate in which Australian and New Zealand scientists have been particularly active. Martin argues that "it is impossible to separate the scientific and power dimensions of the fluoridation issue". The proponents of fluoridation have always implied that scientific considerations came before ethical and political concerns: Martin shows that both the pros and the antis mix up science, ethics and politics.

The argument for fluoridation rests on the claim that it will dramatically reduce tooth decay. This view was challenged over thirty years ago, when in 1959 a Melbourne University dentist, Phillip Sutton, pointed out the inadequacy of base-line statistics prior to fluoridation, the sampling methods of surveys after fluoridation, and the tests for tooth decay. Proponents of fluoridation began to argue that the history of the trials was irrelevant since various "natural experiments" had shown that fluoridation worked. Then another Australian, Mark Diesendorf, pointed to the "significant declines in tooth decay in un-fluoridated regions", and to the fact that the declines in decay have continued "long after the maximum effects" of fluoridation should have been obtained.

The technical argument is hardly the stuff of gripping public debate. Instead, the fight has been over more emotive questions about risks, individual rights and forms of decision making. The antis think that trials should be set so that the safety of fluoride has to be proved, whereas the proponents believe that the benefits must be demolished conclusively before fluoridation is withdrawn.

The appeal to individual rights has been particularly powerful in the United States and relies both on American individualism and on the concept of the purity of water, which many people see as something which should not be "adulterated". (The pros argue that fluoridation simply restores the "natural" mineralisation to urban water supplies.) Related to this is a set of arguments about whether fluoridation decisions should be made by experts directly, by experts advising officials, by commissions of inquiry, by officials acting without advice or by popular referendum. Proponents tend to favour a specialist approach, antis to favour a popular method.

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## THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH

In October 1962 the first international scientific conference on fluoride research was held in Bern, Switzerland. The sixty researchers who attended had recognized the need for such meetings and discussions and expressed great satisfaction with the resulting exchange of information and views. The papers and discussions were published in 1964. In 1966 the American Society for Fluoride Research held a meeting in Detroit that included participants from Europe and Asia as well as North America. The success of those conferences led to the founding of the International Society for Fluoride Research (ISFR), which has met regularly since 1968. Biochemists, botanists, physicists, chemists, physicians, dentists, veterinarians, agriculturists and engineers from many parts of the globe have participated in its meetings and published their work in its journal. Thus through its conferences and its journal the Society has contributed to the advancement of fluoride research. Society members hold widely differing views on the role of fluoride in air, water and soil pollution, but the Society does not become involved in political issues. The Society's journal, *Fluoride*, covers all aspects of fluoride research and is listed in *Science Citation Index*, *Excerpta Medica*, *Biological Abstracts*, *Chemical Abstracts*, *Pollution Abstracts*, *Oceanic Abstracts* and *Current Contents*. Professors S S Jolly and A K Susheela of India, and Professor J Franke of Germany were Presidents of the Society in recent years. The current President is Professor H Tsunoda of Iwate Medical University, Japan. The current Secretary is Professor G W Miller (address: Utah State University, Logan, Utah, USA). Dr George Waldbott, a Michigan physician and Fellow of the American Academy of Allergy, was founding Secretary of the Society and edited *Fluoride* until his death in 1982. His widow, Edith Waldbott, carried on as Interim Editor, with the assistance of Acting Editor Professor A W Burgstahler of the University of Kansas. Her age and health caused her to discontinue during 1991. The Editorial Office was transferred to Auckland, New Zealand, in January 1992.

Researchers are invited to apply to the Secretary for ISFR membership. The annual membership fee (US\$25 - please pay to the New Zealand Office) includes a reduced annual journal subscription.

## 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 issue 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.