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INTERACTIONS BETWEEN FLUORINE AND PHOSPHORUS

A Machoy-Mokrzynska and Z Machoy

Orthophosphate is known to appear in all living cells. Phosphate esters act as mediators in numerous metabolic processes. How can the dominant part of phosphate in biological processes of the cell be substantiated? One fact is that the pK_1 of phosphoric acid is about 2.0, which allows phosphate compounds to ionize at physiological pH. They are unable to diffuse freely across the cell membrane, and therefore they remain inside the cell. On the other hand, the chemically active fluoride ion penetrates cell membranes with ease. Does a mutual interaction take place between the two anions within the cell?

Until now a large number of interactions occurring between fluoride and many metal cations have been described (1). Here we note several examples of possible fluoride interactions with phosphate. One of the first reports by Warburg and Christian in 1942 deals with fluoride inhibition of the enzyme enolase (2). The inhibitory action of fluoride was thought to arise from a magnesium-fluorine-phosphate complex. However, some years later Larner (3), studying the inhibition of enolase by fluoride, discovered a decreased glycolysis in the cells, with enzyme inhibition occurring only in the presence of phosphate and arsenate. Further investigations by Peters *et al* were performed on enolase in animals and plants (4). These workers showed that enolase was not inhibited by fluorine-phosphate, but by the bivalent ion of FPO_3^{-2}, which is able to form a complex with magnesium, a known activator of this enzyme.

In 1952 Slater and Bonner (5) found that succinic dehydrogenase inhibition by fluoride increased in the presence of phosphates. In the absence of fluoride, phosphate itself was a weak competitive inhibitor. Similarly in the absence of phosphate, the activity of this enzyme was inhibited by fluoride only slightly. The studies we carried out on succinic dehydrogenase confirmed that fluoride and phosphate intensified the inhibition of this enzyme.

Describing different mechanisms of inhibitory action exerted by fluoride on the activity of enzymes, Cimasoni (6) mentions the possibility for fluoride to bind with phosphate at the catalytic site of the enzyme. Among enzymes inhibited by fluoride are numerous phosphatases, kinases as well as ATP-ases which are associated with energetic cell processes (7). The fall in ATP levels in cells induced by fluoride has a detrimental effect on many living processes.

Fluorine compounds do not always act as inhibitors of enzyme activity. For instance, the activity of adenylate cyclase increases in the presence of fluorine compounds (7). The mechanism of this phenomenon is not yet fully understood. However, it was noted that in the presence of added fluoride, phosphates start liberating themselves from the membranes, which may be evidence that adenylate cyclase undergoes dephosphorylation (8). The cited enzyme intensifies its activity in the presence of NaF and Na_2PO_3F, although Na_2PO_3F practically fails to release fluoride, since fluoride is covalently bound to phosphorus (9). Phosphate also gets separated during interaction of fluoride with another enzyme - phosphoglucomutase (10).

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In enzymology there are studies dealing with the binding of anions to enzymes. For example, Reiman (11) determined that phosphate anions link with cytochrome oxidase, a significant respiratory enzyme in plants and animals. It is believed that active sites may be competitively occupied by fluoride.

It should be noted that enzymes were not the only objectives of these studies. Great affinity of fluoride for phosphate was observed in determining the mineral composition of urinary calculi. The presence of ammonium ion, calcium, magnesium and phosphorus in urinary calculi is a decisive factor with regard to the amount of bound fluorine.

For many years it has also been evident that there is considerable affinity of fluorine for hydroxyapatite, which is a compound of calcium with phosphate. Fluoroapatite existing in bones exerts a decisive influence on physiological and chemical properties of bones. Therefore, bones exposed to mechanical pressure, *e.g.* heel bone or ankle bone, contain as a rule larger amounts of fluorine (12).

The above examples suggest important interactions of fluorine with phosphorus. Further studies are needed to elucidate the mechanisms of these interactions.

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STUDIES ON EFFECTS OF FLUORIDE IN 36 VILLAGES OF MEHSANA DISTRICT, NORTH GUJARAT

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SUMMARY: A survey was carried out in 36 fluoride endemic villages of Mehsana District of North Gujarat. Urine and blood samples of fluoride-afflicted human population and their drinking water were analysed for fluoride content and compared with samples from different parts of Ahmedabad city (control). The fluoride content in water samples of Ahmedabad city was within the permissible limits, but was high in endemic villages. The urine and serum of individuals from these villages also showed a higher concentration of fluoride than in the control population. The enhanced Na^+ and K^+ levels in the urine of the fluorotic populations indicates a probable electrolyte imbalance and altered kidney functions. Similarly, higher activities of serum transaminases (SGOT and SGPT) might be due to altered liver function, since both of these enzymes are known markers (of liver function). Normal steroidogenesis in fluorotic subjects was evident by the unaffected serum testosterone levels. Serum cholesterol was also in the normal range which indicates that fluorotic subjects were not suffering from hypercholesterolemia. Serum sialic acid, a known marker for detection of fluorosis, was reduced in cases from endemic villages. This might be due to escalated concentration of glycosaminoglycans, which hinder hormone-receptor interaction. Thus, the above data reveal altered liver and kidney function in fluorosis-afflicted individuals with high urine and serum fluoride but low sialic acid levels compared to normal controls.

Key words: Fluoride; Human population; Mehsana; Survey study.

Introduction

Fluoride is ubiquitously distributed in the soil, water, food and air. Water is ordinarily the principal medium of fluoride intake by the human population. Excessive concentrations of fluoride in drinking water lead to crippling fluorosis in endemic areas. More than a million people in India are afflicted with skeletal and dental fluorosis. The fluoride absorbed through the gastrointestinal tract is rapidly distributed to all the tissues by simple diffusion. Fluorine, the most electronegative element, can rapidly cross the cell membrane, skeletal and cardiac muscle, liver, skin (1) and the erythrocytes (2). Even placental transfer of fluoride by diffusion is known which can impose deleterious effects on foetal development (3). Under certain conditions, the absorbed fluoride can affect virtually every phase of human metabolism.

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Endemic fluorosis is prevalent in 15 states in India where, due to the hot climate, large quantities of water containing comparatively high levels of fluoride are drunk (4). Gujarat is one of the 15 states where fluorosis occurs in five districts. A large faction of population, especially of economically backward classes, were found highly susceptible to the disease. A survey was therefore carried out in 36 villages of Mehsana district of Gujarat state. Drinking water samples, along with blood and urine, were collected to study the alterations brought about by fluoride ingestion in these individuals.

Materials and Methods

Thirty six villages located in the Mehsana district of North Gujarat were suveyed. The inhabitants of these villages, prior to the collection of urine and blood samples, were checked for apparent mottled teeth, back pain, stiffness of back and joints and other abnormalities including skeletal problems. Most of these individuals were unable to bend due to a stiff back, which indicated that they were afflicted with fluorosis. The details of each case and the source of drinking water were recorded in the proforma sheet. A total of 210 samples of urine and 68 samples of blood were obtained. The blood was transferred to culture tubes after collection by using hypodermic syringe and allowed to clot fully, and the serum was separated by centrifugation. The urine samples were collected in clean, dry plastic bottles. Immediately after collection, 2 to 3 drops of toluene were added to prevent fungal growth. Drinking water samples from all these villages were also collected. Blood, urine and water samples from inhabitants of Ahmedabad city and its vicinity were collected and used as controls for various biochemical parameters.

Fluoride content in water, urine and serum

Fluoride concentrations (expressed in ppm) in water, urine and serum samples were determined with an Ion Selective Electrode Orion Model 701A.

SGOT and SGPT

The photometric determinations of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were carried out by the method of Reitman and Frankel (5). A 0.2 ml sample of diluted serum (1:2) was incubated at 37° C with 1 ml of buffered solution (pH 7.4) (α-ketoglutaric acid and aspartic acid for SGOT; α-ketoglutaric acid and alanine for SGPT). One ml of colouring reagent was added and kept at room temperature for 20 minutes. To these, 10 ml of 0.4 N NaOH was added. The respective quantities of oxaloacetate and pyruvate formed were measured in a Bausch and Lomb Spectronic 88 Colorimeter at 546 nm and expressed as mU/ml.

Cholesterol

Cholesterol concentration in serum was determined by the method of Pearson *et al* (6). To 5 ml of colouring reagent (para-toluenesulphonic acid in glacial acetic acid and acetic anhydride 40:60 respectively), 0.2 ml of serum and 1 ml of concentrated sulphuric acid (H_2SO_4) was added. Cholesterol reacts at room temperature with acetic anhydride and H_2SO_4 gives an intense brown-red complex, which was measured in Spectronic Colorimeter 106 at 620 nm and expressed as mg/100 ml serum.

Testosterone

The levels of testosterone in serum were assayed by using radioimmunoassay (Double antibody technique) of Peterson and Swerdloff (7) with reagents standardised in RIA kit supplied by M/s Serono Laboratories, Italy. An unlabelled hormone of unknown concentration in the standard (sample) competes with a known concentration of radiolabelled hormone for the limited binding sites of the specific antibodies. At the end of the incubation, the antibody-bound and free hormone were separated by the addition of second antibody, after which precipitation occured. The pellet was then counted by placing each tube for one minute in a Beckman Automatic Gamma Counter (Model 5500). The hormone concentrations of the samples were quantitated by measuring the radioactivity associated with the bound particles of the samples or standards and expressed as ng/ml.

Sialic acid

Sialic acid concentrations in serum were determined by the method of Jourdian *et al* (8). To 0.5 ml of diluted serum (1:10), 0.1 ml of periodic acid (0.04 M) was added and kept in an icebath for 20 minutes. To these was added 1.25 ml of resorcinol (0.6%) and the solutions boiled at 100° C for 15 minutes, cooled and then 1.25 ml of t-butynol alcohol was added. The periodate oxidation of glucosidically bound sialic acid gives a chromogen, which reacts with resorcinol. The colour intensity was measured in a Spectronic 106 Colorimeter at 630 nm and expressed as μg/ml serum.

Urinary Na^+, K^+

The sodium and potassium levels of urine were estimated on a Systronics Flame Photometer, Digital Unit Type 125, by the method of Dean (9) and expressed as ppm. Solutions of NaCl and KCl (1 to 9 ppm) were used as standards. Diluted urine for analysis was sprayed as a fine mist into a non-luminous flame, which becomes coloured according to the characteristic emission of the metal. A narrow band of wavelength corresponding to the element being analysed was selected by a light filter and allowed to fall on a photodetector, which was the concentration of the element measured in the digital display.

Statistics

A minimum of 20 replicates were taken for each parameter and the data were statistically analysed using Student's 't' test.

Results

Fluoride in water

Fluoride content in water was significantly higher ($p < 0.001$) in Mehsana district, a fluoride endemic area, compared to the non-endemic areas selected from Ahmedabad city and its vicinity (Table 1).

Fluoride in urine

Fluoride level was enhanced significantly ($p < 0.001$) in the urine of fluorotic individuals compared to controls (Table 1).

Fluoride in serum

Serum fluoride concentration was also increased significantly ($p < 0.001$) in the fluorotic human population of Mehsana district in comparison to controls (Table 2).

Table 1
Fluoride concentration in water and urine of control and fluoride endemic population

Area	Water Fluoride (ppm)	Urine Fluoride (ppm)
Control (Ahmedabad)	0.62 ± 0.02	0.67 ± 0.01
Range	0.6 - 1.04	0.1 - 1.5
n	24	50
Endemic Region (Mehsana District)	2.2 ± 0.05	4.2 ± 0.5
Range	1.5 - 3.9	1.4 - 8.9
n	36	210

Values are mean ±S.E.
n = number of samples

Table 2
Fluoride concentration in serum in control and endemic population

Area	Fluoride (ppm)
Control (Ahmedabad)	0.04 ± 0.002
Range	0.03 - 0.05
n	25
Endemic Region (Mehsana District)	0.32 ± 0.04
Range	0.18 - 0.79
n	68

Values are mean ±S.E.
n = number of samples

Table 3
Activities of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGOT) in control and endemic population

Area	SGOT (mU/ml)	SGPT (mU/ml)
Control (Ahmedabad)	15 ± 0.80	10 ± 0.84
Range	12.5 - 20	6.5 - 13.5
n	25	25
Endemic Region (Mehsana District)	58 ± 4	56 ± 6
Range	14 - 86	16 -120
n	68	68

Values are mean ±S.E.
n = number of samples

Table 4
Serum cholesterol and testosterone levels in control and endemic populations

Area	Cholesterol (mg/100ml)	Testosterone (ng/ml)
Control (Ahmedabad)	184 ± 5.0	6.4 ± 0.42
Range	150 - 195	3 - 9
n	25	25
Endemic Region (Mehsana District)	190 ± 5	6.38 ± 0.45
Range	153 - 220	2.85 - 8.8
n	68	68

Values are mean ±S.E.
n = number of samples

Table 5
Serum sialic acid concentration in control and endemic population

Area	Sialic acid (μg/ml)
Control (Ahmedabad)	200 ± 0.88
Range	180 - 236
n	25
Endemic Region (Mehsana District)	168 ± 4
Range	114 - 223
n	68

Values are mean ±S.E.
n = number of samples

Table 6
Na^+, K^+ levels in urine of control and endemic populations

Area	Na^+ (ppm)	K^+ (ppm)
Control (Ahmedabad)	2850 ± 314	1670 ± 265
Range	1300 - 4000	600 - 3000
n	50	50
Endemic Region (Mehsana District)	3567 ± 272	2541 ± 227
Range	1200 - 7900	750 - 5500
n	210	210

Values are mean ±S.E.
n = number of samples

Serum SGOT and SGPT

The activities of SGOT and SGPT in the endemic populations increased significantly ($p < 0.001$) compared to controls (Table 3).

Serum cholesterol

Cholesterol in serum showed no alterations in fluoride-afflicted individuals in comparison to controls (Table 4).

Serum testosterone

Serum testosterone levels were also unaffected in fluorotic individuals compared to controls (Table 4).

Serum sialic acid

The concentration of sialic acid was decreased significantly ($p < 0.01$) in the fluorotic populations in comparison to the control population (Table 5).

Urinary Na^+, K^+

The Na^+ and K^+ levels in urine of fluoride afflicted individuals showed a significant increase ($p < 0.001$) compared to controls (Table 6).

Discussion

The fluoride content in drinking water of the endemic villages was higher than the permissible level of 1 ppm, according to WHO (10). Serum and urinary fluoride concentrations were also significantly higher in the fluorotic subjects of these areas. Extensive literature shows that fluoride, even at very low concentrations, inhibits several enzymes involved in various metabolic processes of the body (11,12). In the present study the high fluoride content in urine and serum due to greater intake through water probably adversely affects the general body metabolism in the fluorotic cases.

Renal tissues are highly sensitive to fluoride, which thereby causes damage to the kidneys. As the damage increases, clearance of fluoride decreases (13). The toxic effects of fluoride are aggravated by the altered clearance of electrolytes. Thus, the rise in Na^+ and K^+ levels of urine could be attributed to changes in electrolyte balances in intercellular and intracellular fluids, which in turn may influence the movement of water in and out of the cellular matrix. The differential distribution of these two cations is essential in many membrane systems, where energy requiring active transport is functional. Fluoride in excess in the intracellular region results in Na^+ influx and K^+ efflux (14). The altered ionic concentrations might result in dysfunction of aldosterone action at selective resorption sites in kidney, which can cause the decrease in body weight due to loss of water along with the salts. Suketa and Terui (15) also reported that altered Na^+ and K^+ levels in urine and serum of fluoride-intoxicated rats might be due to a disturbance of adrenal function. Therefore, changes in Na^+ and K^+ levels in the urine of fluorotic subjects might cause altered adrenal function in these cases.

The activities of SGOT and SGPT are known markers of liver function. The activities of both these transaminases were significantly increased in the fluorotic human beings, which would indicate the hepatocellular death or damage and changes in liver function (16) in the fluorotic human population. Triggered activity of these transaminases and liver damage following the ingestion of fluoride in different species of animals have also been reported (17,18).

In the present study, serum cholesterol and testosterone levels of fluoride afflicted human subjects were found to be unaffected. This observation suggests unaltered steroidogenesis in these individuals. Similar findings were also obtained in rodents (mice, rabbits and rats) by fluoride ingestion. The Leydig cell morphology was also unchanged (19,20). Despite unaltered cholesterol and testosterone levels in fluoride ingested rodent models, various androgen-dependant parameters in different target organs were adversely affected, especially cauda epididymis leading to dysfunction of sperm and thus contributing towards low fertility. In corroboration of these results, recently Neelam *et al* (21) reported prevalence of infertility among residents in high fluoride endemic areas in Andhra Pradesh, India. This might be due to alterations in the conversion of testosterone into its potent metabolite, 5α-dihydrotestosterone and the enzyme 5α-reductase; or to impaired hormone-receptor interaction and consequent impaired target organ response due to increase in prostaglandins E2 and PGF2 by fluoride (22). Both these PGs are known to manifest antiandrogen effects on the male reproductive system (23,24). Hence, further studies in this direction are under way at present.

In the serum of fluorotic human subjects, sialic acid concentration decreased significantly. Susheela and Jha (25) and Jha *et al* (26) also reported decreased sialic acid and increased glycosaminoglycans in the serum of fluorotic subjects and have suggested that sialic acid concentration is a marker for detection of fluorosis. Sialic acid, a sialomucoprotein, maintains equilibrium with its derivatives, the glycosaminoglycans, in the serum. The latter are known to attach themselves to exogenous proteins of the plasma membrane by glycosidic and glucosidic bonds and thus play an important role in the interaction of hormone-membrane bound receptor molecules. Hence, the above mentioned alterations in sialic acid might cause disturbance in hormone action at the target cell in fluorotic cases.

From the above data, it is essential to investigate a therapeutic agent that could effectively ameliorate the effects of fluoride in the endemic population, and is easily available and cheap. The role of ascorbic acid and calcium individually and in combination in the mitigation of fluoride effects has been reported elsewhere (27). The role of vitamin C in the mitigation of fluoride effects has been investigated by Venkateswarlu and Narayana Rao (28). Therefore, in view of our animal data, studies on the ameliorative role of ascorbic acid and calcium individually and/or in combination are called for to help relieve the suffering of millions afflicted with fluorosis.

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FLUORIDE LEVELS IN BOREHOLE WATER AROUND NAIROBI

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Nairobi, Kenya

SUMMARY: Endemic dental fluorosis is widespread in Kenya. Overall, 30 to 50% of the population are affected, but with regional differences in prevalence and severity. High fluoride levels in drinking water have often been associated with dental fluorosis. The present work was therefore designed to determine the fluoride concentrations in borehole water and the possible public health hazard resulting from drinking water with high fluoride levels in some areas around Nairobi. Water samples from 50 boreholes were collected and fluoride levels determined with a fluoride ion selective electrode. All samples were analysed in duplicate. The mean fluoride concentration was 4.1 ppm with a standard error (SE) of 0.5 ppm. The maximum concentration of fluoride recorded was 32 ppm while the lowest value was 0.13 ppm. Of all the samples 84% had fluoride concentrations above 0.7 ppm. The results indicate that borehole water may present a potential health hazard to consumers and therefore should be analyzed for fluoride levels before being recommended for domestic or industrial use.

Key words: Boreholes; Fluoride water; Nairobi.

Introduction

Fluorine is widely distributed in the environment and ranks 13th among the elements in order of abundance in the earth's crust. High levels of fluoride in drinking water are found in some countries, such as India, China, Japan and parts of the middle East and Africa (1). In these areas cases of osteosclerosis and crippling fluorosis have been observed. The geology of Kenya makes it one of the places in the world where fluorides occur in the highest concentrations, not only in rocks and soil but also in surface and ground waters (2). Accordingly, the amount of fluorides to which part of the Kenyan population are exposed rank among the highest in the world (3).

The water-soluble fluoride is of greatest interest since it may affect plant and animal life. In saline soils the dominance of sodium and the resultant greater solubility usually leads to fluoride concentrations of several ppm. In river water most of the fluoride exists as free fluoride ions, although salinity increases the complexed fraction. Ground water in Kenya has very high fluoride concentrations in general (4,5). The highest concentration of fluoride in Kenyan water occurs in water from some springs, boreholes, and in some of the lakes in the Rift valley (2).

Fluoride enters the water cycle by leaching from soils and minerals into ground water and surface water. Fluoride concentration in water is affected by factors such as availability and solubility of fluoride-containing minerals, porosity of the rocks or soils through which the water passes, temperature, pH, and the presence of other elements which may complex with fluoride (6).

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The population within the city of Nairobi has increased enormously over the last few years, forcing people to recede to its suburbs where ground water is becoming an important alternative to piped water. As the population in towns and cities continues to increase, more and more difficulties are being encountered in finding sufficient water supply for domestic and industrial use. Therefore ground water is increasingly being used to augment the surface water supply. Ground water is generally assumed to be of good quality and hence in most cases is used directly from the borehole. Water in its natural state is one of the purest compounds known. However, today it is not easy to find a source of fresh water that has not been disturbed by man (7).

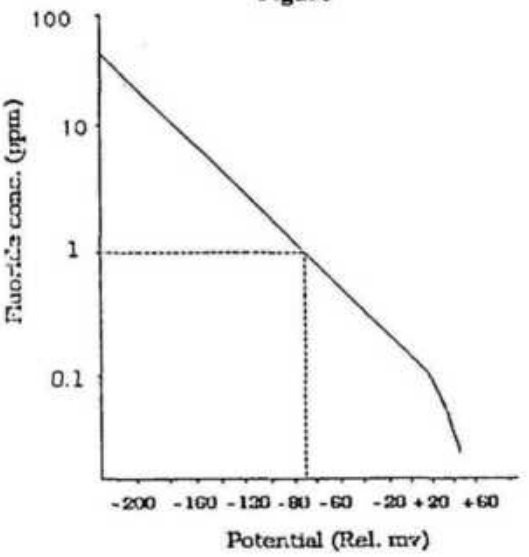
Materials and Methods

Water samples were obtained from boreholes in Ongata Rongai, Ngong, Kabete, Athi River and Ruiru areas. The samples were collected in clean 500 ml polyethylene bottles, transported to the laboratory in a cool box and then stored at -20°C before analysis. The pH and F levels were determined with a digital pH meter (3020 Orion) and a combination fluoride electrode (Orion 96-09), respectively.

Prior to analysis the solutions were adjusted to pH 5.0-5.5 before being treated with TISAB III (total ionic strength adjustment buffer Orion 940911).

Fluoride standard solutions (0.1, 1.0 and 10.0 ppm) were prepared by diluting a 100 ppm standard solution (Orion 94-09-0) with deionised water. Two parallel tubes were filled with 3.0 ml of standard fluoride solution, and 0.3 ml of TISAB III buffer was added to each tube before analysis. A calibration curve was prepared from these standards (See Figure). The average relative millivolt value for each standard was plotted against the fluoride concentration on a 3 or 4 cycle semi-logarithmic paper. The difference between a ten-fold increase in fluoride concentration was between 54 and 60 relative millivolts.

Figure



Results and Discussion

Table

Fluoride levels in boreholes around Nairobi

Range of F concentration (ppm)	% Total No. of Boreholes
< 0.7 ppm	16
> 1 ppm	80
> 1.5 ppm	72
> 3 ppm	48
> 5 ppm	24
> 10 ppm	3

Fluoride is a major problem in ground water as it can cause dental fluorosis, especially in children. The use of drinking water containing 1-1.2 or more ppm (8) may cause dental fluorosis. Waterborne fluoride may also cause skeletal fluorosis with debilitating symptoms of chronic fluorosis.

A previous investigation of water samples from 1286 boreholes in various parts of Kenya revealed fluoride concentration above 1 ppm in 61% of the boreholes and above 5 ppm in 20% (10). In our study 80% of the borehole water samples had fluoride levels above 1 ppm while 24% had fluoride concentrations above 5 ppm. A parallel study on the microbiological quality of water from some areas around Nairobi (11) revealed contamination of borehole water with coliforms in up to 42% of all the samples collected. Therefore the assumption that borehole water is safe for consumption is not valid according to bacteriological evaluation. Similarly, borehole water should not be assumed to be fit for human consumption as most of the boreholes samples may present a potential health hazard to consumers and therefore should be analyzed for fluoride levels before being recommended for domestic as well as for industrial use.

Acknowledgement

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PHYTOTOXIC EFFECTS OF GASEOUS FLUORIDES ON GRAIN CROPS IN THE SOUTHEAST UKRAINE

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SUMMARY: A study was carried out to determine the effects of airborne fluorides on grain crops grown in the south-east part of the Ukraine. In zones polluted by fluorine-containing industrial emissions, accumulation rates of fluoride in straw of winter wheat and spring barley were 10-200 times higher than unpolluted ones. In the immediate vicinity of the emission source the yield of wheat and barley dropped by 60% and their quality deteriorated. Fumigation experiments in chambers demonstrated the following effects of hydrogen fluoride on 10-day seedlings of wheat and barley: decreased apparent photosynthesis, increased rate of respiration, decreased respiration ratio, increased dry mass, and length and biomass of the above-ground parts. Fluoride accumulation was linear with dose, and coefficients of uptake for both crops were determined under both natural and experimental conditions.

Key words: Fluorine-containing industrial emissions; Grain crops; Phytotoxic effects; Pollution; Ukraine.

Introduction

It is well known that gaseous fluorides are the most phytotoxic among industrial pollutants (1-4). Fluorine emissions in some industrial regions present a great problem since their concentrations in the atmosphere can have a very adverse affect on vegetation and crops. The area selected for our study is in the south-east part of the Ukraine (Dniepropetrovsk region) in which are located many industrial sources of fluorine-containing emissions: metallurgical, chemical and building material plants. At the same time this area is also a region of intensive agriculture. More than 95% of it is agricultural land.

In the present study the direct effects of airborne fluoride on the appearance, metabolism, yield and fluorine accumulation by grain plants have been investigated. Effects on crop growth and yield under these conditions are difficult to determine and little is known about them.

To gain such information we undertook a study of:

1) The relationship between concentration of fluoride in the atmosphere near the fluoride-emitting source and its accumulation in plants grown in this area.
2) The accumulation coefficients of fluoride accumulation for wheat end barley.
3) The effects of the access of F^- on the quality and quantity of crop yield.
4) The damage to agriculture generally.
5) The effects of experimental fumigation by different HF concentrations on wheat and barley seedlings.

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Materials and Methods

Two species of grain crops were chosen for this study: Odesskaja semi-dwarf winter wheat and Zernogradskii 73 spring barley. The crops were sampled from the vicinity of a big fluoride-emitting non-ferrous metallurgy plant. The crops were grown downwind of the fluoride source at distances 0.3 to 5 km from it. The control and study plants were grown under irrigation conditions. The soils were ordinary chernozyoms, which represent the more important soil type of this region. The soils of the control and study areas were treated with nitrogen-phosphorus-potassium fertilizers: NPK (12-8-3).

Because the effects of air pollutants on crops depend largely upon the pollutant dose, ecologic, climatic, and agrotechnical factors, the control area was chosen to be similar to the study area. The control area was situated 60 km from the plant and was free of industrial pollution of any type. The control and study areas differed only in their degree of pollution.

Starch, cellulose, protein, gluten, and ash in grain were determined by the methods of Pleshkov (5).

In laboratory experiments seedlings of wheat and barley were grown in water culture comprising a ¼ strength Khnop's solution. After the seedlings were cultured for 10 days, HF fumigation was conducted for 1 hour every day in polyethylene chambers (0.15 m^3). HF concentration was 0.2 mg m^{-3} (high concentration) or 0.02 mg m^{-3} (low concentration). After harvesting, the rate of apparent photosynthesis and of respiration were determined by the Photo-Warburg method (6). The biomass, dry mass, and length of the above-ground part were also determined.

The total fluorine content in grain crops and in seedlings was determined with an ion-selective electrode according to the method described by Garcia-Ciudad *et al* (7).

Results and Discussion

Fluoride has a very important characteristic - it accumulates in the plant. The "normal" fluorine content in plants generally ranges from 2 to 20 ppm on a dry-weight basis (1-4,7). Accumulation of fluoride in crops has a significance that transcends the effect of fluoride on plant growth and quality and quantity of crop yield.

The fluoride content in grain and straw of grain crops in zones of year-round pollution with fluoride-containing industrial emissions is shown in Table 1. The crops accumulated fluorides produced from a non-ferrous metallurgy plant in high concentrations, with a maximum of 439.0 mg kg^{-1} compared with a background control level of 2.4 mg kg^{-1}. Accumulation also decreased in straw with increasing distance from the plant.

We suggest that the concentration of fluoride in wheat and barley is proportional to the dose, *i.e.* to the product of the concentration of gaseous fluoride (HF) and the duration of exposure. This relationship can be expressed by the dose-rate equation (2): $\Delta F = KCT$, where ΔF represents the fluoride concentration in the tissue after correction for the background concentration, C is the mean concentration of atmospheric fluoride, T is the exposure time in days, and K is the accumulation coefficient (ppm F $mg^{-1}m^3day^{-1}$). Thus, it was found that winter wheat and spring barley exposed to atmospheric fluoride in the field had mean accumulation coefficients f 5.2 and 12.8, respectively.

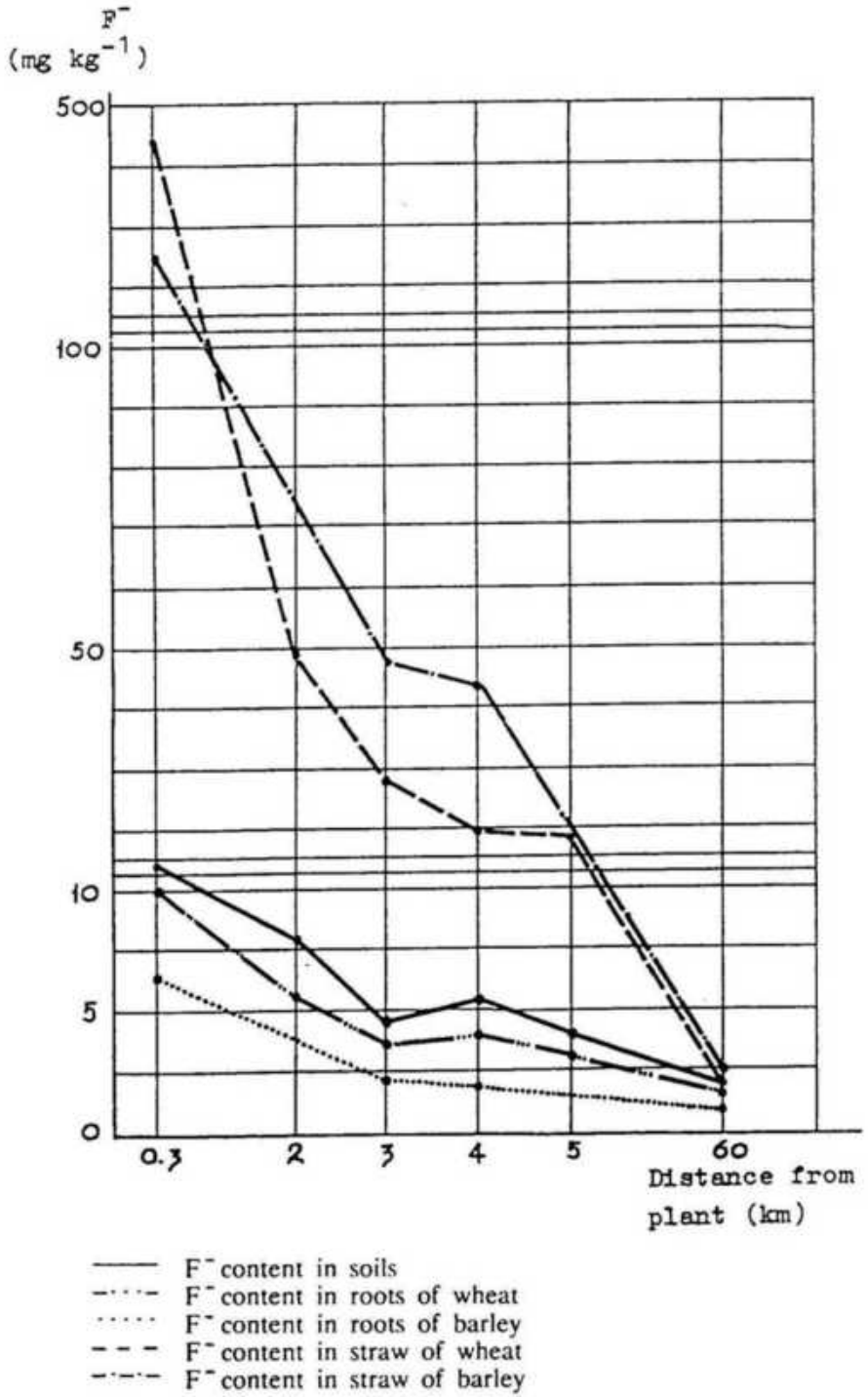
Table 1
Fluoride accumulation in straw and grain of wheat and barley (mg kg^{-1} dry weight)

DISTANCE FROM PLANT (km)	GRAIN	STRAW
	WINTER WHEAT	
0.3	2.2 ±0.02	439.0 ±0.15
2	1.1 ±0.04	49.1 ±0.10
3	0.0	28.5 ±0.07
4	0.0	19.6 ±0.08
5	0.0	18.1 ±0.06
60 (Control)	0.0	2.4 ±0.08
	SPRING BARLEY	
0.3	5.2 ±0.02	245.0 ±0.09
3	0.6 ±0.03	48.5 ±0.10
4	0.6 ±0.01	44.9 ±0.08
60 (Control)	0.0	2.4 ±0.06

Table 2
Quality of grain yield of wheat and barley (% dry weight)

DISTANCE FROM PLANT (km)	TOTAL NITROGEN	PROTEIN	GLUTEN	STARCH	CELLULOSE	ASH
			WINTER WHEAT			
0.3	2.0 ±0.04	11.4	25.5 ±0.26	74.5 ±0.53	3.5 ±0.10	2.8 ±0.15
2	3.1 ±0.06	17.7	34.6 ±0.27	69.4 ±0.23	3.1 ±0.12	2.3 ±0.09
3	3.3 ±0.02	18.8	39.5 ±0.26	62.8 ±0.38	3.0 ±0.13	2.1 ±0.12
4	3.1 ±0.07	17.8	40.3 ±0.31	62.4 ±0.23	3.1 ±0.14	2.0 ±0.09
5	3.5 ±0.04	20.0	40.9 ±0.55	61.3 ±0.46	2.4 ±0.08	1.9 ±0.03
60 (Control)	4.4 ±0.04	21.7	45.5 ±0.47	60.2 ±0.19	2.7 ±0.08	1.7 ±0.09
			SPRING BARLEY			
0.3	1.8 ±0.05	10.3	20.2 ±0.25	68.2 ±0.33	3.7 ±0.03	3.2 ±0.14
3	2.6 ±0.04	14.8	27.0 ±0.21	60.4 ±0.47	3.1 ±0.15	2.9 ±0.07
4	2.7 ±0.04	15.4	27.4 ±0.20	60.7 ±0.36	2.9 ±0.03	3.0 ±0.10
60 (Control)	2.9 ±0.08	16.5	29.6 ±0.28	52.3 ±0.45	2.7 ±0.06	2.5 ±0.12

Figure


F⁻
(mg kg⁻¹)
500
100
50
10
5
0
0.3
2
3
4
5
60
Distance from plant (km)
F⁻ content in soils
F⁻ content in roots of wheat
F⁻ content in roots of barley
F⁻ content in straw of wheat
F⁻ content in straw of barley

Experimental evidence is fairly conclusive that the amount of fluoride normally accumulated from the soil is small and that there is little relationship between the concentration of fluoride in the soil and that of the plant. The deposition of air-borne fluorides on soil usually has little or no effect on the fluoride content of the plant. It was shown here that fluoride uptake and accumulation by vegetation over a relatively long period of time is related to the atmospheric concentrations to which it is exposed (See Figure).

It was further established that fluoride injury to the grain crops depends upon its accumulation level. As a result, significant changes in the concentrations of starch, cellulose, protein, gluten and ash were found in plants near the fluoride source (see Table 2). Thus, the amounts of total nitrogen and gluten in grain of wheat and barley decreased by 7.9-47.4 and 6.9-37.9%, and by 10.1-44.0 and 7.4-31.7%, respectively. At the same time, the amounts in starch, cellulose and ash in grain of wheat and barley increased by 1.8-23.8 and 16.6-30.4%, and 11.1-29.6 and 7.4-37.0%, and 11.8-64.7 and 20.0-28.0%, respectively. The coefficients of correlation between every index of grain quality (content of total nitrogen, gluten, ash, starch and cellulose) and the content of fluoride in crops are shown in Table 3. On the basis of these results it can be concluded that the quality of yield decreased with the increase of F^- accumulation in crops.

It was also possible to determine the rate of change in every index of grain quality during the growing season for both crops. The winter wheat has a 7-month growing period (not counting 2 winter months) and spring barley has a 3-month growth period. Our calculations, for example, indicate that the F^- content of starch in the grain of barley increased about three times more rapidly than in the grain of wheat. These results suggest that winter wheat is less susceptible to fluoride injury than spring barley.

Finally, in the immedate vicinity of the emission source grain yield of wheat and barley was reduced by about 60%. It is evident therefore that a number of these changes are associated with extended pollution from fluorine-containing industrial emissions.

Biochemical effects induced by fluoride that are not associated with chlorosis or necrosis are diverse and suggest that many different areas of metabolism can be affected. Often there is a degree of confusion and uncertainty in the evaluation of fluoride-induced injury in the field. This is due to the fact that most vegetation will display symptoms of one form or another as a result of a number of environmental and pathologic conditions. Foremost among the agents or factors that mimic fluoride-induced injury are other air pollutants. That is why the laboratory experiments under controlled condition were considered necessary.

Results of the laboratory experiments are shown in Table 4. It was found that the fluoride concentration in the above-ground parts of wheat and barley seedlings increased in the range of 14.9 and 25.3 mg kg^{-1} respectively, during the 10-day exposure period. Fluoride accumulation was linear with dose. Accumulation coefficients were 6.97 for wheat seedlings and 13.07 for barley seedlings. It is evident that barley accumulated fluoride twice as intensively as wheat.

Among the best documented effects of fluoride on metabolic pathways of the plant are decreasing rate of apparent photosynthesis and increasing rate of respiration (2-4). Many different possibilities exist as to the locus of fluoride action. The pathways of respiratory activity are likely primary sites of fluoride toxicity.

The sensitivity of the pathway of respiratory activity to fluoride fumigation was determined for wheat and barley seedlings in the absence of foliar lesions. Thus, the oxygen uptake by wheat and barley seedlings increased 6 and 5 times, respectively, after exposure to high concentrations of HF, and release of CO_2 increased threefold for both species. The respiration ratio decreased from 0.78 to 0.43 and from 0.71 to 0.40, respectively, for wheat and barley.

Photosynthesis is more sensitive to HF fumigation than respiration. Thus, O_2 from high-fluoride treated seedlings of wheat and barley was reduced by about 30 and 24%, respectively. Fluoride-induced inhibition of apparent photosynthesis depends directly upon the ambient concentration of HF and the amount of fluoride in the seedlings. Elevated levels of fluorine caused significant disturbances in plant metabolism with major changes in the rate of apparent photosynthesis and respiration.

Also recorded in Table 4 are fluoride-induced changes in length, biomass, and dry weight of seedlings.

In summary, atmospheric concentrations of fluoride, duration and frequency of exposure, age or stage of development of the plant, and plant species are the major factors which determine plant susceptibility to pollutants. The application of results from experimental fumigations to field problems is also limited. It is important to combine the two approaches.

Table 3
Coefficients of correlation between the fluoride content in plants and indices of grain yield of wheat and barley

	TOTAL NITROGEN	GLUTEN	STARCH	CELLULOSE	ASH
		Winter Wheat			
F^- content in grain	-0.78	-0.98	+0.96	+0.68	+0.92
F^- content in straw	-0.74	-0.92	+0.92	+0.70	+0.90
		Spring Barley			
F^- content in grain	-0.88	-0.93	+0.84	+0.67	+0.88
F^- content in straw	-0.92	-0.83	+0.80	+0.65	+0.92

Table 4
Effect of fluoride fumigation on wheat and barley seedlings

INDEX	HF CONCENTRATION (mg m^{-3})				CONTROL	
	0.2		0.02			
	WHEAT	BARLEY	WHEAT	BARLEY	WHEAT	BARLEY
RATE OF RESPIRATION: O_2 UPTAKE (mg $g^{-1}h^{-1}$)	1.19±0.084	0.72±0.091	0.76±0.047	0.41±0.006	0.19±0.012	0.15±0.012
CO_2 EXHALATION (mg $g^{-1}h^{-1}$)	0.52±0.029	0.29±0.006	0.42±0.028	0.20±0.009	0.15±0.006	0.10±0.009
RESPIRATIONM RATIO	0.43±0.009	0.40±0.003	0.55±0.007	0.51±0.009	0.78±0.006	0.71±0.006
RATE OF APPARENT PHOTOSYNTHESIS, O_2 EXHALATION (mg $g^{-1}h^{-1}$)	13.67±0.259	8.76±0.176	16.2±0.315	9.39±0.198	19.42±0.21	11.58±0.035
BIOMASS OF ABOVE-GROUND PART (mg)	58±0.06	160±0.06	61±0.06	152±0.09	55±0.06	149±0.06
DRY WEIGHT(%)	8.8±0.09	11.7±0.12	8.0±0.04	11.1±0.04	7.7±0.1	10.4±0.07
LENGTH OF ABOVE-GROUND PART (cm)	11.4±0.08	19.3±0.1	11.0±0.1	18.1±0.08	9.0±0.09	16.0±0.11
F^- ACCUMULATION (mg kg^{-1} DRY WEIGHT)	14.9±0.23	25.3±1.11	1.3±0.08	2.7±0.08	0.0	0.0

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A STUDY OF DAMAGE TO HARD TISSUE OF GOATS DUE TO INDUSTRIAL FLUORIDE POLLUTION

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SUMMARY: In 40 native goats, 6-10 months old, pastured for 18 months in an area severely polluted by industrial fluoride, bone fluoride was about 10 times higher than in controls. Serum calcium decreased significantly whereas alkaline phosphatase (ALP) increased. Mandibles, ribs and teeth, examined by radiography scanning electron microscopy (SEM), showed thinning of compact bone, decrease in bone density, loose and porotic surface of bone, differences in height and mineralization of teeth and abnormal structures of enamel and dentine. Excessive grass fluoride with negative balance of calcium during the dry grass season is the main cause for osteopenia. Moreover, variation of grass fluoride levels and of calcium availability during the green grass and dry grass seasons are key factors which cause significant differences in abrasion of teeth and shortening of life-span.

Key words: Bone; Goats; Industrial fluorosis; Teeth.

Introduction

Numerous goats in Baotou in Inner Mongolia are impaired due to industrial fluoride pollution. In an area of severe pollution, poor production performance and shortened life-span of goats cause enormous economic loss. Sawteeth are clinical features of goats due to industrial fluoride (1). When the changes are severe enough to affect food intake and mastication, goats die at a young age from hunger and cachexia. Recently Bao (2) reported that osteoporosis is the main skeletal change of goats in industrial fluorosis. The following experiment was carried out to study industrial fluoride damage to goats and its causes.

Materials and Methods

Forty native goats, 6-10 months old, were pastured in an extremely polluted area of Baotou for 18 months. Twenty goats of the same breed and age, in a fluoride free zone, were used as controls. During the experiment ribs of fluorotic and control goats were obtained by operation. Monthly bone samples were ashed and fluoride content determined with a fluoride ion-selective electrode. Monthly grass samples were collected from November 1987 to October 1988 (0.5 g of grass powder was put into a 100 ml beaker; 20 ml 0.05 N HNO3 was added; 4 hours later 20 ml 0.1 N KOH was added; the solution was boiled for 15 minutes; after cooling, pH was adjusted to 5.5 with acid). Blood samples were collected from 16 fluorotic and 20 control goats, and calcemia and serum alkaline phosphatase determined during the second dry grass spell. Mandibles, metacarpal bones, ribs and teeth samples, obtained at slaughter from 6 fluorotic and 4 control goats, were examined by radiography and scanning electron microscope (SEM: Japan EMASIC-40) following Zhan Chongwan's method (3).

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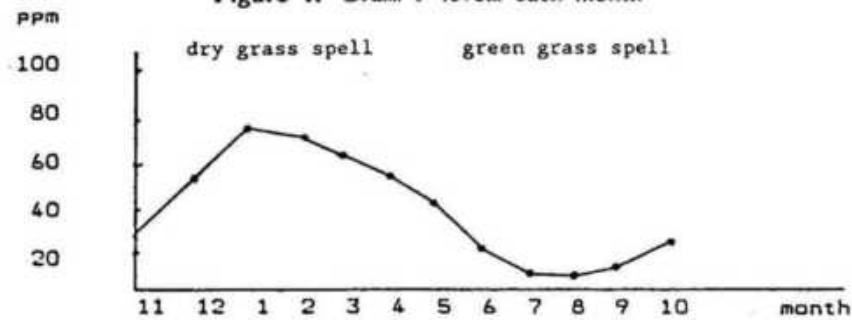
Results

Chemical Studies: Figure 1 shows that the grass fluoride level each month was not constant. It decreased during the green grass spell (May to October), and increased during the dry grass spell (November to April).

Figure 1. Grass F levels each month



Data in Table 1 illustrate that bone F rose during the first dry grass spell (November to April, 1988), and fell during the following green spell (May to October, 1988). It rose again during the second dry grass spell (May to April, 1989). Table 2 shows calcemia and serum ALP levels.

Table 1

F Level of Control and F Group at Different Times

Group	Time	N	Bone F (ppm/D.W.)
Control	April, 1989	10	613 ±64
Fluoride	December, 1987	15	2168 ±425[a]
Fluoride	January, 1988	15	5808 ±193[b]
Fluoride	April, 1988	8	6825 ±247[b]
Fluoride	October, 1988	16	4709 ±153[b]
Fluoride	January, 1989	12	6371 ±171[b]
Fluoride	April, 1989	10	7786 ±528[b]

a. significantly different from control, $p < 0.001$
b. significantly different from a, $p < 0.001$

Table 2

Calcemia and Serum Alkaline Phosphatase

	N	Ca (mmol/L)	μALP (/L)
Control	20	2.5 ±0.2	20.32 ±14.31
Fluoride	16	2.1 ±0.3[a]	40.76 ±20.13[a]

a. significantly different from control, $p < 0.01$

Bone Morphological Observation: Widened marrow cavity, thinned compact bone and decreased bone density of mandibles in F Group (Figure 3) compared with control (Figure 2). In the place with bone outgrowths, compact bone was thickened, but its structure by radiography remained low in density. Surface of mandibles and ribs was smooth in controls, and porotic in the fluoride group under SEM.

Morphological Observation of Teeth: The rate at which teeth wore down became increasingly significant due to varied speeds of wear. The first pair of incisors, the height of which was 35 percent of the control was much shorter than the second pair of incisors. The height of these was 60 percent of the control (Figure 4). The rear half of the first upper molar became long and sharp. The central part of the second lower molar was severely worn (Figures 3, 5).

According to the radiographs, control molars were well-mineralized (Figure 2). Those of the F group, however, were generally inadequate in calcification, especially the upper part of second lower molar as Figure 3 shows. The enamel section of normal incisors appeared semi-transparent, whereas that of the abnormal incisors was chalky. Moreover enamel rods of the former were of regular size and width and heavily calcified (Figure 6). On the other hand enamel rods of irregular size and width showed low density in some area of the F group (Figure 7) under SEM. In comparison with the control (Figure 8), dentinal apertures had irregular shape and non-smooth edges (Figure 9).

Discussion

Relationship of Grass F and Climate: Grass fluoride dissolved in acid-base solution only accounted for 6.8 ±2.8 percent of total grass F (Baotou Environmental Monitoring Station). Accordingly, total grass F in the experimental area was comparatively low from July to September and markedly high from January to February. Changes in grass F during the one year are related to the climate in this region. Baotou is located at 41°N and 110°E; it belongs to the dry, cold area. There are about 115 days of no frost, 250-300 mm of annual rainfall during July to September; less snow in winter results in low grass F in the green grass spell and high grass F during the dry grass spell. Continual rainfall could wash the fluoride dust off the grass, in addition to a comparative decrease in pollution with grass growing in the green spell. In winter due to less snow fluoride dust collects on the dry grass.

Relationship of Bone F and Grass F: Table 1 shows that bone F increased in the first dry grass spell; it decreased in the following green spell and increased again in the second dry grass spell. That the change in bone F accompanied that of grass F is one characteristic of fluorosis in Baotou.

Effect of Fluoride on Calcemia and Serum ALP: In this study, the calcemia level in the F group is significantly lower than that in the control ($p < 0.01$), which agrees with previous results which show that rabbits (4) and sheep (5) in chronic fluorosis are hypocalcemic. Serum ALP is twice as high in the F group as in the control. The high level of ALP agrees with the increase of ALP in fluorosis of sheep (5,6) and cattle (7). Previous experimentation by the authors indicated that ALP levels in rabbits rose markedly with doses of NaF. Serum samples, heated at 56°C for 15 minutes, revealed that alkaline bone phosphatase was mainly responsible for the rise of serum ALP (8). Thus an increase of serum ALP is an important parameter of animals in chronic fluorosis.

Figure 2. Mandible of control goat

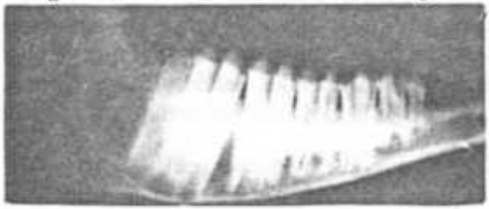


Figure 3. Fluorotic mandible

Figure 4. Fluorotic & control teeth

Figure 5. Fluorotic cheek teeth

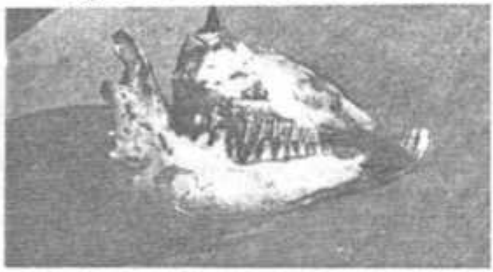


Figure 6. Enamel rods of control

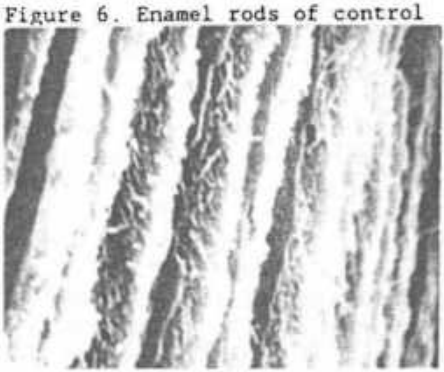


Figure 7. Fluorotic enamel rods

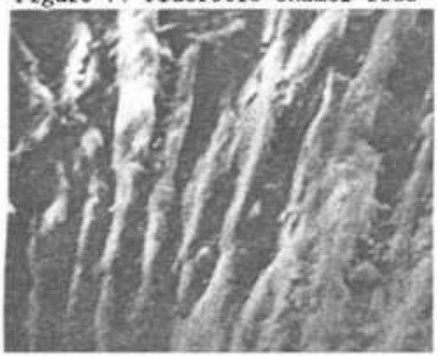
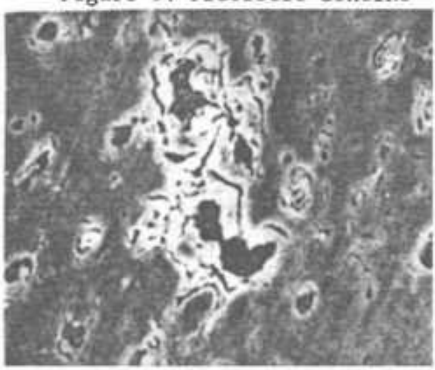


Figure 8. Dentine of control

Figure 9. Fluorotic dentine



Analysis of Causes of Osteopenia in Goats in an Industrially Fluoride-Polluted Area: Bao (2) observed that, of 94 goats with industrial skeletal fluorosis, decreased bone density and thinned compact bone were apparent in all 74 of those 2 years old, in 15 to 20 of those 3 to 5 years old, respectively. However, Wang (9) observed that osteosclerosis was the principal change of industrial skeletal fluorosis in 100 humans (99 males, 1 female) in contact with fluoride (average 15.9 years). Franke (10) concluded that the changes caused by excessive fluoride are associated with species differences, duration of intake and individual sensitivity. In particular, the level and age of F intake is important: in small doses, fluoride causes osteosclerosis. Large doses, on the other hand, cause osteopenia and osteomalacia due to decreased availability of calcium especially in animals with a high calcium demand. Comparison of conditions of workers in factories, where fluorides are emitted, with conditions of animals in industrially fluoride-polluted areas revealed the following differences: **1.]** doses of F intake every day are great, especially during the dry grass period, **2]** animals came into contact with fluoride when very young (about 2 months old) and **3]** the ewes account for the majority in a flock of goats, because wethers (emasculated rams) are killed for mutton at ages 2 to 3 years. Moreover, ewes at and above 2 years of age are pregnant for 5 months every year, which requires calcium in growing animals as well as when ewes are pregnant and lactating. Absorption of calcium by the intestine can be greatly affected by fluoride. Both pregnancy and lactation result in hypocalcemia and inadequate calcification of bone. Low calcemia further causes dissolution of bone salts by parathyroid action. The series of pathological changes lead to decreased density of bone. In particular, bone changes are more likely to occur in pregnant and lactating ewes, because pregnancy occurs during the dry grass period (from November to March) at which time a negative balance of calcium is obvious.

Exploration of Causes Which Lead to Sawteeth:

Incisors: The fact that the crowns of the first pair of incisors are formed during the first dry spell, and the second pair of incisors later during the second green grass spell, causes the first incisors to be less hard than the second incisors. Thus the first pair is more easily worn. In addition, the first pair has a longer period of abrasion. Hence the first incisors are shorter than the second incisors.

Molars: The front half of the upper first molar opposes the lower first molar, and the rear half opposes the lower second molar. The upper and lower first molars develop in the first 6 months of age - *i.e.* during the first green grass spell. Thus their calcification is better because of calcium supplementation from milk and reduced effect of grass fluoride. In contrast, the second molars develop later, during the first dry spell, so their calcification is inadequate (Figures 3, 5). Thus, as the front half of the upper first molar wears against the lower first molar, the rear half becomes comparatively longer because the lower second molar did not at first grow out enough to meet and contact the upper first molar. Then, when the surfaces did make contact and begin to wear against each other, they gradually assumed opposite shapes due to the marked difference in their hardness. The premolar teeth develop during the 12th to 18th month of life - *i.e.* during the second green spell - so abraded normally.

Conclusion

In the area polluted by industrial fluoride in Baotou in Inner Mongolia, China, the dry climate is an essential condition which results in excessive grass fluoride during the dry seasons. Excessive grass fluoride and negative balance of calcium during the dry seasons are the main causes of osteopenia. Differences in the grass fluoride levels and of calcium availability during the green grass and dry grass seasons are key factors affecting hardness of teeth.

Acknowledgement

The authors thank Mr Qing Xiao-su who helped in taking some of the X-ray photographs.

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THE INFLUENCE OF SODIUM HYDROGEN CARBONATE ON THE ELIMINATION OF FLUORIDE IN RATS

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SUMMARY: The impact of sodium hydrogen carbonate ($NaHCO_3$) on fluoride absorption and elimination in rats was investigated. Fluoride levels in urine, bones, teeth and hair were determined in rats receiving sodium fluoride (NaF) in drinking water, alone or in combination with $NaHCO_3$, for 9 weeks. Another group of animals received sodium hydrogen carbonate in drinking water for 5 weeks after termination of the treatment with sodium fluoride.

Key words: Bones; Drinking water; Fluoride in urine; Sodium fluoride; Sodium hydrogen carbonate; Teeth; Rats.

Introduction

Elevated environmental and occupational exposure to fluorine compounds involves an increase in fluoride absorbtion in humans and animals. Excessive intake of fluoride over a long period of time is harmful; in extreme cases it may even result in crippling skeletal fluorosis.

To prevent extensive fluoride uptake into the organism different courses of action were undertaken. On the one hand, various methods for reduction of fluoride emissions from environmental and industrial sources were applied; on the other, new methods in prevention and therapy of fluoride poisoning were explored. In the latter case, a medium to effectively reduce the whole body fluoride burden would be of great value. Some authors indicate that metabolic alkalosis protects against acute fluoride poisoning as a result of enhanced renal fluoride clearance rate (1-4).

The aim of this study was to investigate the influence of sodium hydrogen carbonate ($NaHCO_3$) on fluoride absorption and desorption from body deposits. It was hoped that this treatment might have preventive or therapeutic value in subchronic fluoride intoxication.

In this experiment fluoride levels in urine, hair and calcified tissues (bones, teeth) were determined in rats that received sodium fluoride (NaF) in drinking water and, simultaneously or subsequently, were treated with $NaHCO_3$.

Materials and Methods

Experimental Design

Male Wistar rats, six weeks old, weighing 150 ±20g were divided into four groups and given, as drinking water, the following solutions (Table 1): Group.I, tap water; Group II, 0.2% $NaHCO_3$; Group III, 20 ppm NaF; Group IV, 20 ppm NaF and 0.2% $NaHCO_3$.

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Table 1
Experimental Design
(first part)

Group	n	solution	n_1
I	8	tap water	4
II	8	0.2% $NaHCO_3$	4
III	15	20 ppm NaF	6
IV	8	20 ppm NaF and 0.2% $NaHCO_3$	8

n - number of animals treated
n_1 - number of animals sacrificed after 9 weeks of treatment

Table 2
Experimental Design
(second part)

Group	n	solution
I	4	tap water
II	4	0.5% $NaHCO_3$
IIIa	5	0.5% $NaHCO_3$
IIIb	4	tap water

n - number of animals treated

After 9 weeks of treatment some animals were sacrificed. Bones and teeth were collected for fluoride determination. The remaining animals received, for the next 5 weeks as drinking water, the following solutions (Table 2): Group I, tap water; Group II, 0.5% $NaHCO_3$; Group IIIa, 0.5% $NaHCO_3$; Group IIIb, tap water.

Subsequently the animals were sacrificed and the material collected for fluoride determination. During the entire experiment the fluid intake was measured. No significant differences in fluid intake were noted between various groups of animals. The tap water contained 0.3 mg F^-/L. The animals were fed a standard pellet diet.

Preparation of samples

Fluoride content was determined in urine, bones, teeth and hair. Animals were placed in metabolic cages every week and 24-hour portions of urine were collected. Samples of urine (4 ml) were diluted with an equal volume of Tisab Buffer (pH 5.2) (5).

Femur distal epiphyses were used as bone samples, incisors as tooth samples. Specimens of bones and teeth (0.1 g weight) were cleaned, dried, crushed and extracted with 6 ml of 2 M perchloric acid for 1 hour in room temperature. Then 2.4 ml of 0.5 M sodium citrate solution was added and the samples were centrifuged.

Samples of hair were collected after 4, 9 and 14 weeks of the experiment. Specimens of hair taken from the dorsal part of the rat were rinsed on a fritted glass filter with acetone, detergent, and redistillated water. After drying, 100-mg aliquots were treated with 67% sodium hydroxide solution and heated in boiling water until both were completely dissolved (about 1 hour). Cooled and neutralized (with diluted hydrochloric acid) samples made up with water to 4 ml, were diluted with equal volumes of Tisab Buffer. All analytical procedures were performed in polyethylene or teflon tubes.

Determination of fluoride

Fluoride concentrations were measured by a fluoride-specific electrode and Ag/AgCl reference electrode with a double jacket. Calculations were based on a response factor from a standard curve prepared daily. Recovery of F from analyzed materials amounted to 100 ±8%.The coefficient of variation in different kinds of material was 5 to 12%. Significance was determined by Student's t-test.

Results and Discussion

The extent of fluoride excretion depends upon the acidity of the urine. In a pharmacokinetic study, according to Ekstrand *et al* (1), the apparent plasma half-life of fluoride was longer when urine was acid (4.3 ±0.6 hr) than when it was alkaline (2.4 ±0.4 hr). Using this information, Reynold *et al* (2), and Whiteford *et al* (3), in an attempt to decrease the fluoride body burden and facilitate its renal excretion in experimental sodium fluoride intoxication, proved the protective influence of alkalosis in acute fluoride toxicity. To load the rats with fluorides they received a 20 mg/l solution of NaF in drinking water for 9 weeks, a concentration similar to levels encountered in areas with endemic fluorosis (6). Their mean daily water intake was about 30 mL; approximately 0.6 mg of fluoride was taken up by the animals.

Table 3

Fluoride content in bones and teeth of rats (mg F^-/g)

Tissue		9 week exposure period				5 week convalescence			
	Group:	I	II	III	IV	I	II	IIIa	IIIb
Bones	$\bar{x}$	0.50	0.58	1.06*	0.94*	0.61	0.56	0.93*	0.95*
	±SD	0.11	0.12	0.38	0.19	0.03	0.08	0.11	0.14
Teeth	$\bar{x}$	0.15	0.18	0.52*	0.48*	0.16	0.14	0.32*	0.28*
	±SD	0.04	0.05	0.10	0.09	0.02	0.02	0.07	0.04

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

Table 4

Fluoride content in hair of rats (μg F^-/0.1 g)

		Group:	I	II	III	IV	IIIa	IIIb
Exposure period	4 weeks	$\bar{x}$	0.23	0.20	0.21	0.28	-	-
		±SD	0.23	0.11	0.07	0.12	-	-
	9 weeks	$\bar{x}$	0.23	0.24	0.33	0.31	-	-
		±SD	0.12	0.33	0.26	0.26	-	-
Convalescence period (5 weeks)		$\bar{x}$	0.20	0.14	-	-	0.20	0.11*
		±SD	0.04	0.04	-	-	0.07	0.03

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

Simultaniously one group of animals (Group IV) received a 0.2% solution of $NaHCO_3$. The authors assumed that this treatment might have some preventive effect by decreasing fluoride uptake and increasing its renal excretion.

Another group of animals (Group IIIa) received a 0.5% solution of $NaHCO_3$ for 5 weeks after the exposure to NaF was terminated. This alkaline solution was thought likely to facilitate mobilization of fluoride deposits from the calcified tissues and have some therapeutic value.

The NaF and $NaHCO_3$ solutions apparently did not influence the palatability of the drinking water since there were no differences in daily fluid intake between intoxicated and control groups.

The mean urinary pH of rats drinking tap water was about 6.50, whereas in animals receiving $NaHCO_3$ it was 7.01. The increase of $NHaCO_3$ concentration in the second part of the experiment failed to change the urinary pH significantly.

In this experiment, as anticipated in animals dosed with NaF, the urinary fluid level was about twice as high as in controls (Figure 1). No significant differences in urinary fluoride were found between the control groups, namely between rats drinking tap water (Group I) and those receiving 0.2% $NaHCO_3$ solution (Group II).

In animals dosed with NaF, on the average a small increase of urinary fluoride was noticed, which was also in rats receiving $NaHCO_3$ (Group IIIa) when compared to those given NaF alone Group IIIb). However, this increase was not statistically significant.

Urinary fluoride and urinary pH under the present experimental conditions were not correlated. The correlation coefficient between these parameters in all animals treated with NaF was $r = 0.34$; in rats given $NaHCO_3$ it was $r = 0.38$.

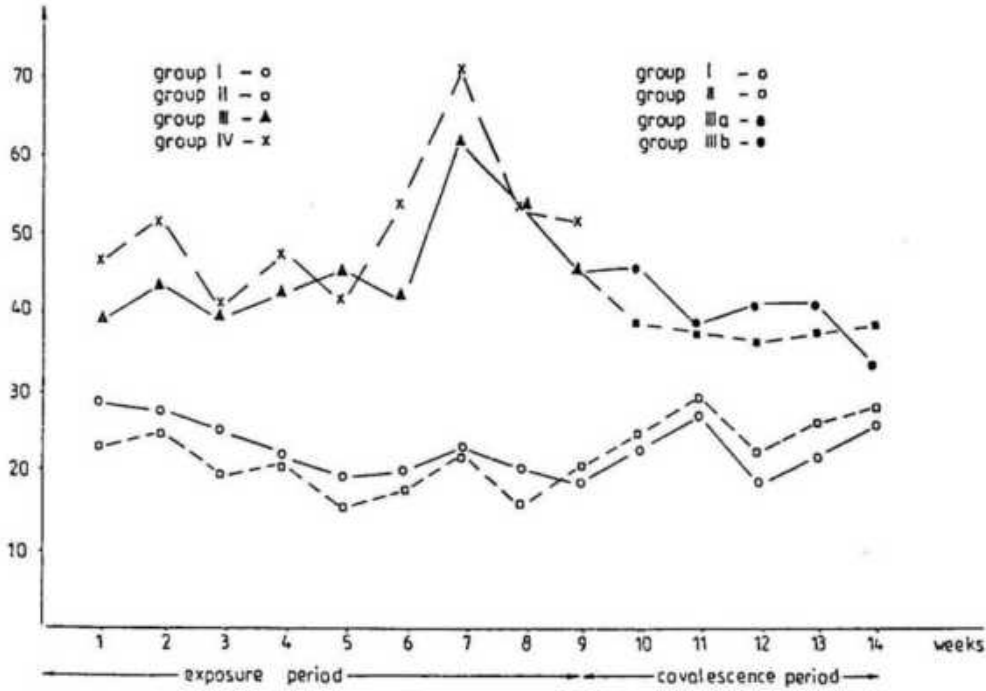
Fluoride content in bones and teeth of animals after 9 weeks of treatment with NaF was significantly higher than in controls (Table 3). In rats receiving NaF in combination with $NaHCO_3$ the mean fluoride levels were slightly lower, but the difference was not significant.

The fluoride levels in urine and calcified tissues also remained higher after the 5-week convalescence (when NaF was no longer being administered) in intoxicated animals (Group IIIa and Group IIIb) compared to controls (Groups I and II). In intoxicated animals, however, differences between rats receiving $NaHCO_3$ Ggroup IIIa) and tap water (Group IIIb) were not significant.

A 4-week exposure to NaF failed to influence the hair fluoride (Table 4). After 9 weeks of dosing, however, this parameter had increased. Because of the large dispersion of results, the increase was not significant. During the convalescence period the fluoride level in hair decreased in all groups of animals, with a significant difference ($p < 0.01$) between animals receiving sodium fluoride alone (Group III) and controls (Group I).

This experiment indicated that, under the present experimental conditions, the fluoride load of bones and teeth, but not of hair, was partial. Treatment with $NaHCO_3$ was not sufficiently effective to exert preventive or therapeutic effects. To reduce amounts of fluoride retained in the organism, a treatment causing distinct alkalosis should be applied.

Figure 1
Urinary fluoride in rats



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EVALUATION OF SPATIAL VARIATION IN WATER SOLUBLE FLUORINE CONTENT OF THE SOILS OF DIFFERENT AGRO-CLIMATIC ZONES OF HARYANA, INDIA

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SUMMARY: Data on water soluble fluorine (F) content of 470 soil samples (collected at a grid of 10 x 10 km) of the different agro-climatic zones of the Haryana State, India, were statistically analyzed for their spatial variability. The frequency distribution of the data was found to be normal. The mean values of the water soluble F were 4.42, 4.60, 4.23, 2.47 and 2.12 mg/kg for the hot and arid, hot and dry, hot and semi-dry, hot and sub-humid, and hot and humid zones of the State, respectively, with a mean value of 4.19 mg/kg for the entire State. This distribution of the F levels was mainly attributed to the soil pH as a linear correlation between the two parameters was statistically significant. The highest variation of F was observed in the hot and semi-dry zone (coefficient of variation, CV = 54.6%). In other zones the variation was low to medium (CV = 24.1 to 38.4%). Finally, a relationship between standard error and number of observations has been worked out so as to use it to estimate the number of samples to be collected in future F monitoring in the soils of the study area for a given precision and probability level.

Key words: Agro-climatic zones; Alluvial plains; Haryana, India; Spatial variability; Water soluble soil fluorine.

Introduction

Fluorine (F) may cause tooth enamel fluorosis in human beings. Amongst other sources, soil water soluble F is an important one for human beings via animals and plants. In nature fluorine is widely distributed as a constituent of most soils and rocks. It is the 13th most abundant element having an average value of 650 mg/kg in the earth's crust (1).

Several studies have been conducted to assess the F status of soils from different parts of the world (2-9). In most cases, spatial variability of F levels was not taken into consideration. This study is an attempt to obtain data on water soluble F levels in soils of the different agro-climatic zones of Haryana State in the Indo-Gangetic Alluvial plains of India and to analyze the data for spatial variability.

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2 Chaudhary Charan Singh Haryana Agricultural University Research Station, Krishi Gyan Kendra, Rohtak 124001, India.

Materials and Methods

The study area consisted of Haryana State in the north-west of India (Figure 1). Its area is 44222 km^2. It lies between the Thar desert in the south and the Himayas in the north. The landscape is a part of the Indo-Gangetic Alluvial plains. The thickness the alluvium varies from a few meters to 1200 m. The State is mostly flat with a saucer shaped depression in the middle which runs east to west. It has a subtropical, semi-arid, continental monsoonal climate with average rainfall of 300 to 1000 mm. It has been divided into 5 agro-climatic zones: Hot and arid, hot and dry, hot and semi-dry, hot and sub-humid and hot and humid with < 300, 300-500, 500-750, 750-1000 and > 100 mm annual rainfall, respectively (Figure 1). The soils are mainly light to medium textured and dominated by illite and kaolinte clay minerals, have mainly alkaline reaction, and are low in organic carbon and nitrogen, low to medium in phosphorus and mostly high in potassium. (10).

A grid map of the State was prepared for soil sampling at a grid of 10 x 10 km. Five soil samples were collected randomly for each grid location, were composited, and a sub-sample of each retained. The sampling depth was 50 cm covering the most important part of the root zone. Water soluble F content of the samples was determined colorimetrically (11) - this method is spectrophotometric using Zirconium-Eriochrome Cyanine-R Lake.

Statistical analysis

The usual statistical concepts used for analysis of a given variable assume that the variable is normally distributed, *i.e.* the central limit theorem applies to the distribution of the given variable. To see whether the F data were normally distributed or not, the cumulative probabilities of F values were plotted on the cumulative probability paper as described by Dahiya *et al* (12). The parameter is normally distributed when such a plot is a straight line. To test the goodness of fit of a normal distribution, Chi-square test (13) was used.

When F data were found to confirm normal distribution, mean, m; standard deviation, s; and coefficient of variation, CV = (s/m)x 100, were estimated using the usual statistical formulae (12), which are based on normal distribution of the variable (F). With CV < 25, between 25-50 and >50, the variation was taken to be low, medium and high, respectively (12, 14)

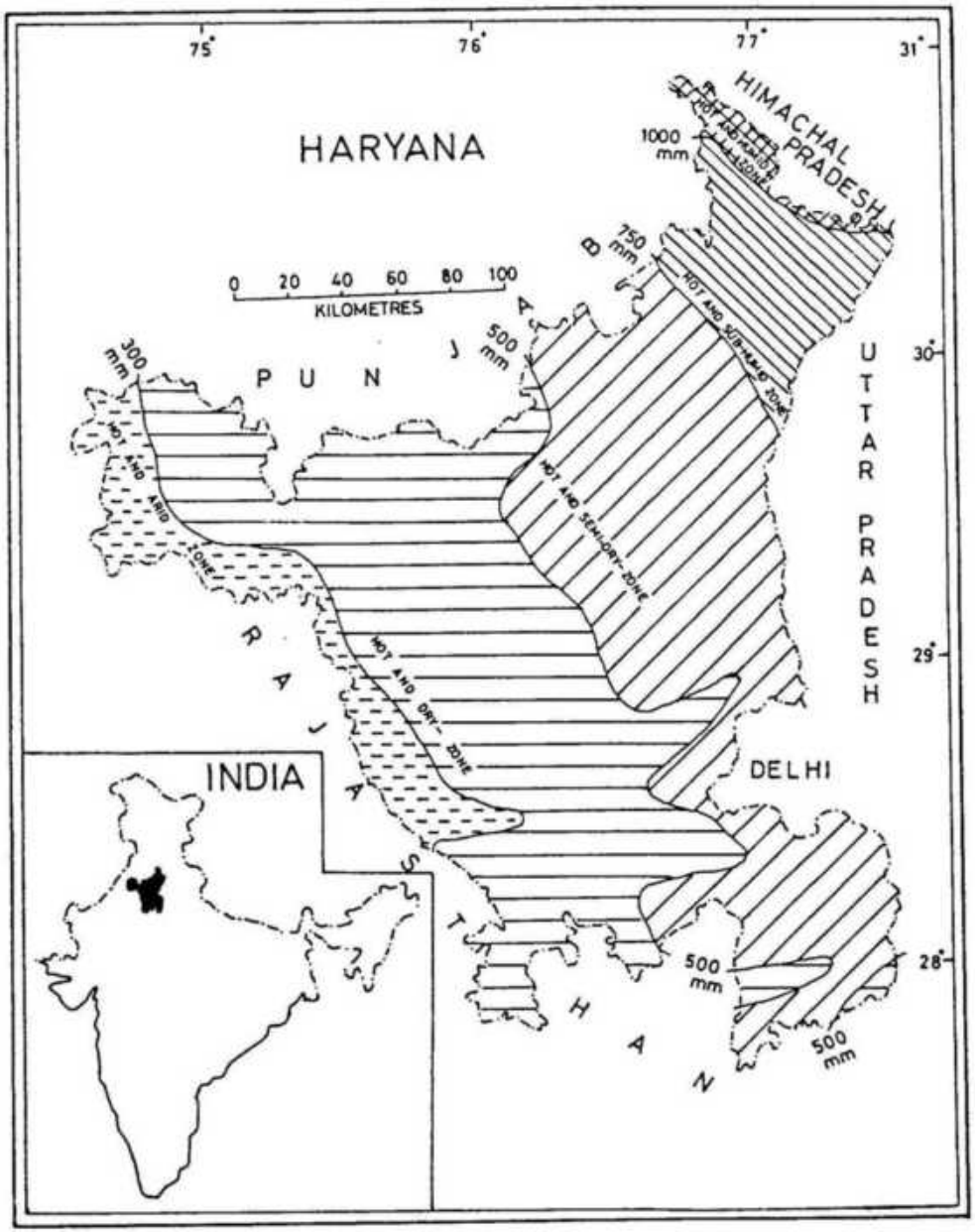
A relationship between standard error and the number of observations was worked out so as to use the relationship to determine the optimum sample size (number of observations) required to estimate the mean F value with a given degree of precision and significance level.

Results and Discussion

Figure 2 shows the plot of the cumulative probability versus F content values of the soil samples. It is seen that the plot is nearly linear. Hence, the water soluble F content of the soils of Haryana is normally distributed. Furthermore, the Chi-square test indicated that deviation from normal distribution was not statistically significant. The data from individual agro-climatic zones were also found to be normally distributed.

Figure 1

Index map of Haryana showing the agro-climatic zones



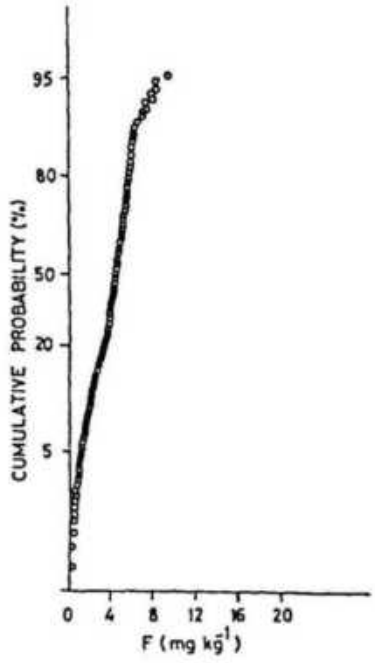


Figure 2

Cumulative frequency plot of water soluble F content of Haryana soils

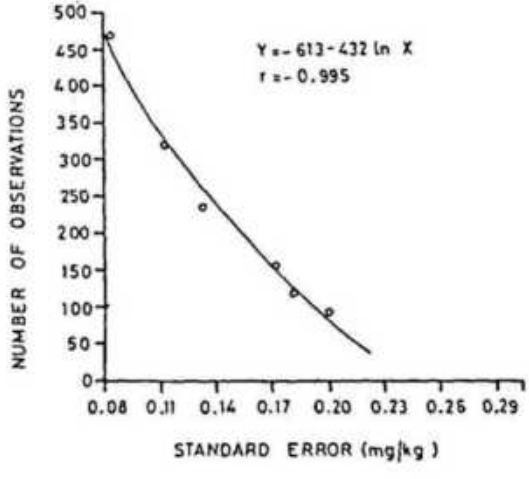


Figure 3

Relationship between number of observations and standard error

The mean, standard deviation and CV values for different agro-climatic zones and for the whole State are given in Table 1. Since F was normally distributed, these statistical parameters, estimated using formulae based on normal distribution, can be used to characterize the soils of the study area for their water soluble F content.

It is seen from Table 1 that the hot and dry zone has the highest mean value of F content, 4.62 mg/kg, with a range of 1.55 to 8.45 mg/kg. It was closely followed by hot and arid zone (m = 4.42 mg/kg, range = 1.60-11.50 mg/kg) and the hot and semi-dry zone (m = 4.23 mg/kg, range = 0.50-19.13 mg/kg). The mean values of the hot and sub-humid and hot and humid zones were nearly half of the mean values of the other three zones: m = 2.47 and 2.12 mg/kg, range = 0.25-4.65 and 1.00-3.60 mg/kg, respectively. It was statistically estimated (15) that for 95% confidence level, the real means would be expected to be within 10.6% (4.42±0.47 mg/kg), 3.3 (4.62±0.15), 8.3 (4.23±0.35), 12.1 (2.47±0.30), 20.2 (2.12±0.43) and 3.8% (4.19±0.16 mg/kg) of the measured means, respectively, in the hot and arid zone, hot and dry zone, hot and semi-dry zone, hot and sub-humid zone, hot and humid zone and the whole state.

In general, the mean F content value decreased (Table 1) as we moved from hot and dry zone (annual rainfall < 300 mm), situated in the south-western part of the state, to the hot and humid zone (annual rainfall > 1000 mm), situated in the north-eastern part. This may mainly be attributed to the soil pH, the mean values of which also behaved in a similar fashion (Table 1). Thus, the mean F values in the hot and arid, hot and dry and hot and semi-dry zones varied from 4.23 to 4.62 mg/kg and pH values varied from 7.80 to 7.82. The mean F content values dropped rapidly to 2.47 and 2.12 mg/kg with a sharp drop of pH to 7.05 and 5.62, respectively, in the hot and semi-humid and hot and humid zones.

To confirm the fact that F content levels in the soil varied directly with soil pH, soil F values were correlated with soil pH. It was observed that soil F was significantly correlated with soil pH ($r = 0.907^{**}$). The following equation was thus obtained:

$$F = -4.38 + 1.10\,pH$$

The CV values given in Table 1 indicate low variation (CV = 24.1) in the hot and dry zone, high (CV = 54.6) in hot and semi-dry zone and medium variation (CV = 37.7-43.4) in the rest of the zones. However, medium variation (CV = 43.4) was observed in the whole of the state. That was so because the soils of the Haryana State are mostly formed from alluvium which itself is not very heterogeneous (10). The normal distribution of the F data (Figure 2) may be attributed to the overall medium variation in the parameter under study. Warrick and Nielson (14) and Dahiya *et al* (12) concluded from a literature survey that the properties having low and moderate variations tend to be normally distributed.

The F data were further analyzed by grouping them into three classes: < 2.5, 2.5-5.0 and > 5.0 mg/kg. The percentage of the total number of observations falling in each class is given in Table 2. It is seen from Tables 1 and 2 that the highest number of F observations from different zones (52.1-72.5%) fell in the frequency classes in which their means were included, with the exception of hot and humid zone in which the mean fell in the frequency class < 2.5 mg/kg, but the highest number of samples

Table 1

STATISTICAL PARAMETERS OF WATER SOLUBLE F IN SOIL SAMPLES OF DIFFERENT AGRO-CLIMATIC ZONES

AGRO-CLIMATIC ZONES	N	RANGE	m	s	CV	SOIL pH
		(mg/kg)				
HOT AND ARID	51	1.60-11.50	4.42	1.70	38.4	7.86
HOT AND DRY	200	1.55 - 8.45	4.62	1.12	24.1	7.89
HOT AND SEMI-DRY	169	0.50-19.13	4.23	2.31	54.6	7.82
HOT AND SEMI-HUMID	37	0.25 - 4.65	2.47	0.94	38.1	7.05
HOT AND HUMID	13	1.00 - 3.60	2.12	0.80	37.7	5.62
THE WHOLE STATE	470	0.25-19.13	4.19	1.82	43.4	7.75

N = number of observations. m = mean. s = standard deviation.
CV = coefficient of variation.

Table 2

PERCENTAGE OF F OBSERVATIONS IN DIFFERENT FREQUENCY CLASSES

AGRO-CLIMATIC ZONES	% SAMPLES IN CLASSES		
	< 2.5	2.5-5.0	> 5.0
	(mg/kg)		
HOT AND ARID	2.0	72.5	25.5
HOT AND DRY	2.5	60.5	37.0
HOT AND SEMI-DRY	21.3	52.1	26.6
HOT AND SEMI-HUMID	70.3	29.7	0.0
HOT AND HUMID	46.2	53.8	0.0
THE WHOLE STATE	28.5	53.7	17.8

were contained in the class 2.5-5.0 mg/kg. It was further noticed that although the mean values were 4.42, 4.62 and 4.23 mg/kg, respectively, in the first three zones (Table 1), the highest percentage of their total number of samples falling in the class 2.5-5.0 mg/kg were not in this order (Table 2). They were in the order of 72.5, 60.5 and 52.1% for the three zones, respectively. This indicates that to reach a reasonable conclusion about the water soluble F levels, or any other soil variable, of any area, primary consideration should be given to the frequency distribution of the data. An intensive litrature survey by Warrick and Nielsen (14) and Dahiya *et al* (12) also supports this conclusion.

Figure 3 gives the relationship between standard error, estimated by using different sets of F observations, and the number of observations in different sets. The optimum number of samples to be collected in future F monitoring in the soils of the study area for a given precision and probability level can be estimated from this relationship. Suppose that one wishes to determine the water soluble F content of the soils of the study area for mapping with confidence interval m ±0.1m, *i.e.* one wishes one's estimate to be within 10% of the true mean at the 95% confidence level. Here m is the estimated mean which is 4.19 mg F/kg for the Haryana soils (Table 1). The specified limits are, therefore, 4.19±0.419 mg/kg and are equivalent to requiring a standard error of approximately 0.21 mg/kg (*i.e.* 0.419/1.96, where 1.96 is the Student's t value at 95% confidence level with infinite degree of freedom). Figure 3 shows that 61 observations are sufficient to give this precision.

Conclusions

1 The water soluble soil F data followed a normal distribution.
2 The mean soluble F values of the soils of different agroclimatic zones varied from 2.12 to 4.62 mg/kg.
3 The variation in the water soluble F data was low in one zone, medium in three zones and high in one zone.
4 The soluble F data was significantly correlated with soil pH.
5 In four zones, 52.1 to 72.5% of the total number of observations fell in the frequency class 2.5 to 5.0 mg F/kg and in one zone, 70.3% in the class < 2.5 mg F/kg.

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UNDERGROUND POTABLE WATER FLUORIDE LEVELS OF THE TOWN OF HISAR AND TOTAL FLUORIDE INTAKE OF SELECTED FAMILIES

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SUMMARY: The study was conducted to determine fluoride distribution in underground potable water and total fluoride intake of 15 selected families of the town of Hisar. One hundred and ninety nine underground water samples were collected from five zones of the town and their fluoride levels determined. Three groups of five families were selected on the basis of fluoride levels in drinking water (Group 1, <1 ppm, Group 2, 2.5 to 3.0 ppm, Group 3 >4 ppm). Three days weighment of cooked food and record of food and water intake were done. Food and water fluoride content were analyzed. The mean water fluoride of the town ranged from 2.26 to 5.55 ppm. The coefficient of variation of the zonal fluoride levels was very high thus making the means unreliable as representative zonal values. The fluoride intake of adolescents and adults of Group 3 was significantly ($P<0.01$) more than the two other groups. In 4 to 7 year olds, intake of fluoride in Group 3 was significantly ($P<0.01$) higher than that of Group 1.

Key words: Fluoride intake; Hisar; Potable water fluoride; Underground water; Water fluoride level.

Introduction

Surveys within Punjab and Haryana have revealed that water fluoride levels in Sangrur and Hisar range from 0.11 to 13.64 ppm (1). No scientific data on the potable water fluoride levels and the total fluoride intake of residents of the town of Hisar is available. Because fluorosis is a health problem this study was planned to estimate the fluoride intake of representative families residing in Hisar. An initial survey of the habits regarding the water used for cooking and drinking revealed that the majority of the families preferred and used underground water instead of municipality (canal) tap water for this purpose. It was also deemed important to identify the major contributor (food or drinking water or both) to the total intakes of fluoride by those consuming drinking water with different fluoride levels.

Materials and Methods

Selection of areas and families

A preliminary survey was conducted of all localities of Hisar in which underground water was used for drinking and cooking purposes.One hundred and ninety nine samples from the various localities of the town were collected and analysed statistically for mean, standard deviation, coefficient of variation (CV) and safety levels on the basis of different zones. Selection of families could not be made randomly from different zones on the basis of zonal means due to the very high zonal CV values.

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Thus, families were selected on the basis of individual water fluoride levels, irrespective of residential zones. Three groups of five families each were selected. The range of fluoride in water for these three groups was: Group 1, <1 ppm; Group 2, 2.5 to 3 ppm; Group 3, >4 ppm. Only families which had inhabited the specific house for three years were selected.

Measurment of fluoride intake

The actual three day weighing of cooked food method (2) was conducted for all the members of the fifteen families. Forms were prepared and a daily 24 hour record of the total food eaten by each person was maintained by measuring food and fluid intake and plate waste. One twentieth of the sample of the total amount consumed by the subjects was collected in polyethelene containers and tightly covered with plastic lids. Drinking water intake was similarly recorded. Collected food was homogenised, dried and powdered for analysis.

Fluoride estimations

Fluoride in food samples was determined by the method of Ville (3) using an ion selective electrode. The fluoride levels in water were analyzed by the spectrophotometric method (4) using Zirconium-Eriochrome Cyanine-R Lake.

The data obtained were statistically analysed to differentiate between inter-group fluoride intake levels.

Results and Discussion

Water fluoride levels

Hisar town was divided into five zones (north, east, west, south and central). Each zone covered from about 6 to 15 residential localities (Table 1). Altogether 49 residential localities were covered. One hundred and ninety nine underground water samples were collected from these 49 residential localities. Table 2 indicates the means, standard deviations and coefficient of variation in the water fluoride levels in the various zones. The extent of safe, marginally toxic and toxic samples within each zone has also been calculated. Data presented in Table 2 indicate that the south zone has the highest mean value (5.5 ppm) of fluoride. In other zones means were much less than in the south zone. The mean values of these zones give the impression of an almost uniform water fluoride level of 2.26 to 2.91 ppm. However, the fluoride content of water samples collected from the different localities projected considerable difference within the localities. The data, when observed under classification for so-called fluoride safety limits - namely safe (< 1 ppm), marginally safe (1-2 ppm) and toxic (>2 ppm) - indicated that the highest percentage (27%) of safe samples were from the north zone.

South zone failed to have a single sample, the fluoride value of which was within the safe limit. Sixty one to 93 per cent of samples from the five zones separately contained more than toxic limits of fluoride in water. Within a locality the variation in fluoride was compared through coefficient of variation (CV). The CV values were moderately high except in the central zone where the CV was moderate (42%). In other zones the variability was 56.1 to 75.3%. Maximum intrazonal variability occurred in the south zone. The north and west zones also had quite high variability values of 60

Table 1

Residential localities covered by different zones

ZONES	LOCALITIES
NORTH	Textile Mill, Vinod Nagar, Padav Bazar, Cloth Market New Model Mandi, Jain Gali, Tibba Danasher
EAST	Shanti Nagar, Housing Board Colony, Labour Colony, Bank Colony, Model Town, Urban Estate II, Industrial Area, Dhakka Basti, DC Colony
WEST	Talaki Gate, Rishi Nagar, Nagori Gate, Devi Bhavan, Aggarwal Colony, Double Phatak, Balsamand Road, Adarsh Colony, Prem Nagar
SOUTH	Cahal Colony, Camp Chowk, Green Park, Lajpat Nagar, Patel Nagar, Jawahar Nagar, Krishna Nagar
CENTRAL	Parijat Chowk, Rajguru Market, Railway Station, Rampura Mohalla, Saini Mohalla, Thandi Sarak, New Urban Estate, Moti Nagar, Kamla Nagar, Jahaz Pul, Jyoti Pura, Dogra Mohalla, Professor Colony, New Subji Mandi, Adarsh Nagar

Table 2

Zonal water fluoride distribution in Hisar town

ZONE	NO. OF SAMPLES	WATER F^- mean mg/L	S.D.	C.V.(%)	WATER F^- LEVELS <1mg/L	1-2mg/L	2mg/L
					% of sample		
NORTH	26	2.26	1.36	60.31	27	12	61
EAST	30	2.92	1.64	56.10	17	7	76
WEST	36	2.92	1.84	64.60	8	22	70
SOUTH	29	5.55	4.18	75.32	0	7	93
CENTRAL	78	2.37	0.99	11.99	9	21	70
WHOLE TOWN	199	3.00	2.30	76.66	14	15	71

S.D. - standard deviation C.V. - coefficient of variation

Table 3

Total fluoride intake of families consuming potable underground water of differing fluoride levels

	AGE GROUP (yrs)		WATER F⁻ ppm < 1(Group 1)	2.5-3.0(Group 2)	> 4(Group 3)
CHILDREN	1-3		0.56(1)	-	1.63(1)
	4-6		0.76(1)	2.75(1)	3.84(1)
	7-12	RANGE	0.82-1.57	2.46-3.38	4.9-11.33
		MEAN ±SE	1.15 ±0.33(4)	2.92 ±0.46(2)	8.02 ±1.85(3)
ADOLESCENT	13-18	MALE RANGE	0.88-2.35	2.57-4.6	13.54-14.75
		MEAN ±SE	1.43 ±0.27(5)	3.53 ±0.57(3)	14.14 ±0.61(2)
		FEMALE RANGE	1.20-2.06	2.68-4.56	6.75-9.1
		MEAN ±SE	1.66 ±0.43(3)	3.62 ±0.39(4)	7.77 ±0.57(4)
ADULT		MALE RANGE	1.08-2.52	3.51-4.96	9.00-14.9
		MEAN ±SE	1.75 ±0.46(10)	4.17 ±0.23(6)	11.59 ±1.33(5)
		FEMALE RA	.97-2.15	3.22-4.48	7.6-14.75
		ME	49 ±0.19(7)	3.97 ±0.16(7)	11.33 ±1.03(8)

Values in parentheses indicate numbers of subjects

Table 4

Estimated daily fluoride intake from foods and drinking water

AGE	DAILY FLUORINE INTAKE FROM DRINKING WATER (mg) Group 1	Group 2	Group 3	FROM FOOD (mg) Gp1	Gp2	Gp3	TOTAL Gp1	Gp2	Gp3
CHILDREN (yrs)									
1-3	0.21(37.5)	-	1.40(86)	0.35	-	0.23	0.56	-	1.63
4-6	0.51(67)	2.38(86)	3.62(94)	0.25	0.37	0.22	0.76	2.75	3.84
7-12	0.76(61)	2.50(85)	7.49(93)	0.39	0.42	0.53	1.25	2.92	8.02
ADOLESCENT									
MALE	0.96(67)	2.99(85)	13.10(93)	0.47	0.54	1.05	1.43	3.53	14.14
FEMALE	1.11(96)	2.94(81)	7.19(93)	0.55	0.68	0.58	1.16	3.62	7.77
ADULT									
MALE	1.14(65)	3.33(80)	8.30(92)	0.61	0.84	0.70	1.75	4.17	9.00
FEMALE	0.92(62)	3.28(83)	10.52(93)	0.57	0.69	0.81	1.49	3.97	11.31

Figures in parentheses indicate the percentage of the total intake

and 64 per cent respectively, which indicates the need of a more extensive sampling of the zones with very high CV values. The variation in the water fluoride levels amongst and within the zones can be attributed to the irregular distribution of fluoride bearing minerals in the soil, the main source of fluoride in water. The depth of the pump may or may not be the determining factor because of the lack of information on the exact depth of the pumps in this study and the controversial reports of the effect of well depth on the water fluoride levels (1,5). These observations suggest that mean zonal values could not be used as zonal representatives due to high CV levels of water fluoride levels.

Fluoride intake

Regarding fluoride intake data of subjects from three groups of families (Table 3), of the fifteen families studied, there were 14 children between 1 to 12 years, 21 adolescents and 43 adults including both sexes. The fluoride intake of children (1-3 years) from Groups 1 and 2 was 0.56 and 1.63 mg/d respectively; of 4 to 6 year olds it was 0.76, 2.75 and 3.84 mg/d in Groups 1, 2 and 3, respectively.

In 7 to 12 year olds, fluoride intake was 1.15, 2,92 and 8.02 mg/d for the threegroups. When these values were compared with each other the fluoride intake of Group 2 was more than Group 1 and that of Group 3 was more than Group 2 but not significantly. However, the fluoride intake of Group 3 was significantly ($P<0.01$) more than that of Group 1.

Male adolescents consumed 1.43, 3.55 and 14.14 mg/d of fluoride in Groups 1, 2 and 3, respectively. In comparison to each other fluoride intake of Group 1 and Group 2 was similar whereas it was significantly ($P<0.01$) more than in Group 3 compared to the other two groups. The intake of female adolescents was 1.66, 3.62 and 7.77 mg/d from Groups 1,2 and 3, respectively. The trend of comparison amongst the groups was similar to that in adolescent males.

Adult males consumed 1.75, 4.17 and 11.59 mg of fluoride/day in Groups 1, 2 and 3, respectively. In females the intake was 1.49, 3.97 and 11.33 mg fluoride from the three groups respectively. The adult males and females from Group 3 consumed significantly ($P<0.01$) more fluoride compared to Groups 1 and 2, which had similar intake values. Thus it is evident that the intake levels varied for families consuming different levels of fluoride in drinking water.

The data of relative contribution from food and water to the total intake of fluoride are presented in Table 4. Drinking water contributes considerably more to the daily intake compared to food. The quantity of fluoride in cooked food consumed by the subjects increased with the increase in concentration of fluoride in the drinking water of the families. Even in Group 1, with least water fluoride levels, the fluoride intake from water was found to be more than 61% in different age groups, except for 1-3 year olds. Nanda (6) found that the main reason for the high fluoride content in the cooked food was the high fluoride content of cooking water, salt and spices used.

According to another report by Lakdwala and Panekar in 1973 (7) where fluoride levels of drinking water are low, the contribution to the total fluoride intake in food items is significant. Kramer *et al* in 1974 (8) reported that when the fluoride intakes are between 3 and 4 mg/d, 50 percent of the intake is derived from diets.

Conclusion

It is evident that underground drinking water fluoride levels are positively associated with the total fluoride intake of subjects. From the observation of Hisar town the picture is indicative of drinking water being the main contributor to total fluoride consumption in families that habitually and preferentially use underground water for cooking and drinking.

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BIOCHEMICAL EFFECTS OF FLUORIDE ON LIPID METABOLISM IN THE REPRODUCTIVE ORGANS OF MALE RABBITS

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SUMMARY: The effect of fluoride on testicular lipid metabolism was assessed in male albino rabbits in experimental fluorosis. Fifty male albino rabbits were administered sodium fluoride (5, 10, 20, and 50 mg/kg body weight/day) subcutaneously for 100 days. The control animals were given 1 cc distilled water/kg body weight over the same period. Compared with controls, the experimental animals, especially those given 50 mg NaF/day/kg of body weight, showed abnormal accumulation of lipids in testes. Hyperphospholipidemia, hypertriglyceridemia, and hypercholesterolemia in testes indicate enhanced lipid biosynthesis in response to fluoride toxicosis. A progressive significant ($p<0.001$) increase in amount of free fatty acids was observed in testes of fluoridated animals. The increase of concentration of all lipid classes except free fatty acids in testes was directly correlated with the increase in dosage of fluoride administered.

Keywords: Albino rabbits; Cholesterol; Experimental fluorosis; Free fatty acids; Phospholipids; Testes; Total lipids; Triglycerides; Sodium fluoride.

Introduction

Fluoride induces toxicological effects in reproductive organs in experimental animals. In mice, alterations in the reproductive organ structure and metabolism as well as reduction in fertility have been reported (1). Tokar (2) found an association between fluorosis and hypogonadism. In human beings, Tarinsky (3) recorded a 2-3 fold increase in symptoms of oligospermia and azoospermia in male workers suffering from industrial fluorosis. Several reports in the literature suggest a definite correlation between infertility and fluorosis (4-7). The present investigation is an attempt to elucidate the testicular lipid metabolism in experimental fluorosis.

Materials and Methods

Animals and treatment

Fifty male albino rabbits weighing 400-650 gm were divided into five groups of ten animals each. They were administered fluoride (as NaF) subcutaneously in the dosage of 5, 10, 20, and 50 mg NaF/kg body weight/day for 100 days. The control animals were injected with 1 cc distilled water/kg body weight/day for the same period. All animals were maintained on standard laboratory chow; water was supplied *ad libitum*. After 100 days, the control and treated animals were sacrificed under ether anaesthesia, and testes were immediately removed, weighed, and kept in chloroform: methanol (2:1 v/v) for the extraction of lipids (8).

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Separation of neutral lipids

The silica-gel G thin-layer plates (20 x 20 cm) were prepared for thin-layer chromatography by employing the method of Freeman and West (9) with slight modifications. The dried plates, activated at 110° C for 90 minutes, were developed in *n*-hexane:diethyl ether:acetic acid glacial (90:10:1 v/v). The chromatograms were air dried and stained with iodine vapour in sealed chambers to provide yellow spots. These spots were identified, marked, and taken into extracting solvent (*n*-hexane:diethyl ether: 1:1 v/v). Pooled extracts evaporated to dryness under reduced pressure were taken up in a known volume of chloroform:methanol (1:1 v/v). Extracts containing different lipids fractions were used for spectrophotometric analysis.

Triglycerides

Triglycerides in the testes of control and treated rabbits were determined by the method of Van Handel and Zilversmit (10).

Phospholipids

Quantitative analysis of phospholipids was done according to the method of Ames (11).

Cholesterol

The estimation of cholesterol in testes of control and treated rabbits was carried out by the method of Stadtman (12).

Free fatty acids

The free fatty acids were assayed by the method of Chakrabarty *et al* (13).

Statistical analysis

Results are shown as mean ±SD. Significance was determined by Student's t-test.

Results

The Table clearly shows that administration of fluoride to rabbits profoundly enhanced the synthesis of lipids in the testes.

Figure 1 shows the mean total lipid and triglyceride concentration in the testes of fluoridated and control animals. Total lipid content of the testes in all treated groups of animals was significantly elevated ($p < 0.001$) over the controls. Similarly, triglyceride levels in testes of treated animals were profoundly enhanced compared to the controls. The differences were statistically significant ($p < 0.001$). Values for treated groups reached a maximum 168.1 ±5.73 mg/g of tissue vs 7.7 ±0.18 in the controls.

Differences in the level of phospholipids in testes of fluorotic groups of animals and control were highly significant (Figure 2). The levels of significance were between $p < 0.001$ and 0.02.

The concentration of cholesterol in testes registered a moderate elevation (33.3%) in animals treated with 5 mg of NaF. In subsequent experimental groups of animals, the amount of cholesterol was highly elevated, the maximum being in animals treated with 50 mg of NaF (Figure 2).

TABLE

Lipid profile of rabbit testis during experimental fluorosis (Data are mean ± SD)

Treatment NaF mg/kg body weight	Total lipids	Phospho-lipids	Triglycerides	Cholesterol	Free fatty acids
1 cc distilled water (control)	25.1 ±0.75	9.5 ±3.04	7.7 ±0.18	2.1 ±0.08	5.1 ±0.09
5	44.9 ±1.75[a] (+79)	14.1 ±0.40[a] (+48)	15.4 ±0.20[a] (+104)	2.8 ±0.73[c] (+33)	13.6 ±0.07[a] (+167)
10	113.0 ±9.78[a,d] (+350)	29.6 ±1.60[a,d] (+212)	27.7 ±2.46[a,d] (+260)	4.4 ±0.3[a,d] (+110)	14.1 ±0.13[a,d] (176)
20	151.7 ±16.08[a,e] (+504)	37.5 ±1.28[a,d] (+295)	32.3 ±0.41[a,e] (+319)	4.5 ±0.29[a,NS] (+114)	15.6 ±0.12[a,d] (+206)
50	247.2 ±19.67[a,d] (+884)	48.5 ±0.62[a,d] (+410)	168.1 ±5.73[a,d] (+2083)	7.4 ±1.02[a,d] (+252)	9.1 ±0.51[a,d] (+78)

Results are expressed as mg/g w.w. of tissues.
P values compared to the control: [a] $p < 0.001$; [b] $p < 0.02$; [c] $p < 0.05$.
Significant values in 5 mg vs 10 mg F^- group, 10 mg vs 20 mg F^- group and 20 mg vs 50 mg F^- group are: [d] $p < 0.001$; [e] $p < 0.01$; [NS] non-significant.
Figures in parentheses indicate percent change.

Figure 1
Testicular total lipid and triglyceride
in experimental fluorosis

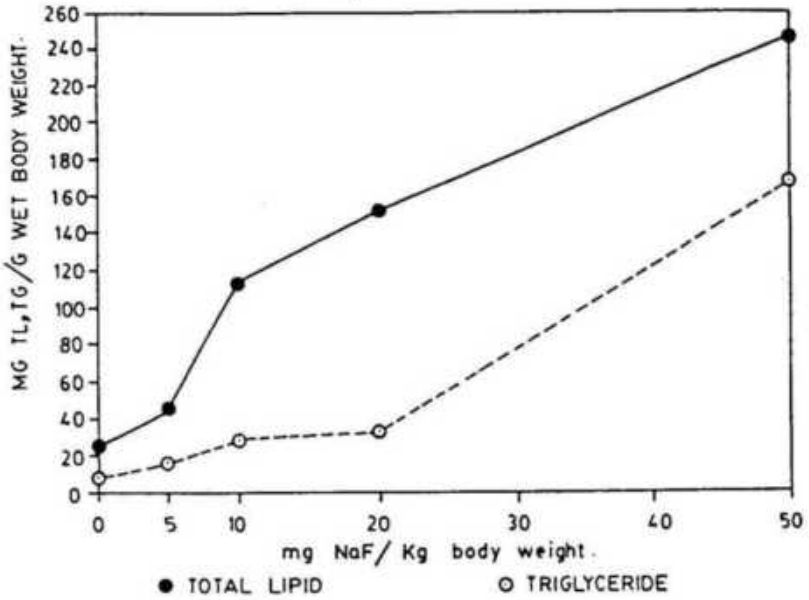
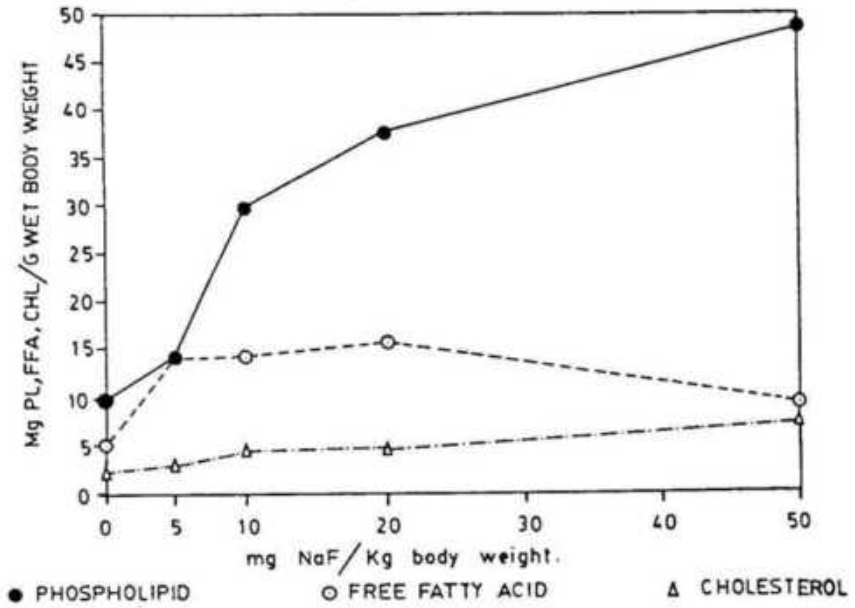


Figure 2
Testicular phospholipid, free fatty acid and cholesterol
in experimental fluorosis



The experimental group of rabbits showed significant ($p < 0.001$) increase in free fatty acid content of testes (Figure 2). There was significant elevation ($p < 0.001$) in the amount of free fatty acids in the 5 mg vs 10 mg NaF group and the 10 mg vs 20 mg NaF group and a decrease ($p < 0.001$) in the 20 mg vs 50 mg NaF group.

Discussion

In the fluoridated animals abnormal quantities of lipids, phospholipids, triglycerides, cholesterol and free fatty acids accumulated in the testes. As shown in Table 1, the amount of these lipids deposited in the testes increased in direct relation to the increase in the dosage of fluoride administered. The maximum increase in lipids level was seen in animals of the highest fluoride group (50 mg NaF).

The results obtained from this study, which confirm our earlier reports, suggest a strong association between fluorosis and alteration in lipid metabolism in rabbits (14-16).

The high levels of lipids in the testes of experimental animals in response to fluoride toxicosis strongly indicate an imbalance between the synthesis and breakdown of the lipid in the testes. Fluoride is known to inhibit hormone sensitive lipase, thus not only reducing the release of free fatty acids but of glycerol as well and results in enhanced lipogenesis (17). The increased fatty acid levels suggest increased triglyceride synthesis, decreased fatty acid oxidation, and increased cholesterol synthesis. Similar hypercholesterolemic effects in the serum of experimental animals after exposure to fluoride have been reported (18,19). However, Chinoy and Sequeira (1) found no significant changes in testes cholesterol in fluoride-treated mice.

Hyperlipidemia, hyperphospholipidemia, hypertriglyceridemia also indicate excessive mobilization of fat (20). The noted degenerative changes in spermatocytes, Leydig cells, and sertoli cells (7) reflect disturbances in the synthetic processes in the testes of rabbits in experimental fluorosis. Since the various stages of spermatogenesis are controlled by different hormones, it appears likely that testosterone, the male sex hormone required for the maintenance of spermatogenesis (21), is lowered in fluoride intoxication (1), reflecting hormonal imbalance in the body (22).

Conclusions

1. This investigation demonstrates a significant elevation in all lipid classes, thereby indicating enhanced lipogenesis in testes of rabbits in response to fluoride toxicosis.
2. The quantity of testicular lipids in different fluoridated groups of animals is influenced by the dosage of fluoride administered.

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PROTEIN DEGRADATION IN SKELETAL MUSCLE OF RABBIT DURING EXPERIMENTAL FLUOROSIS

A Shashi,[1] J P Singh [2] and S P Thapar [3]
Patiala and Ludiana, India

SUMMARY: Sixty albino rabbits, ranging in weight from 400 to 650 g, were segregrated into five groups of twelve each. One group of twelve animals served as control. The remaining four groups were given NaF in the concentration of 5, 10, 20, and 50 mg/kg body weight/day, respectively. After 100 days, all the animals were sacrificed and their skeletal muscle was analyzed for proteins and amino acids. Compared with controls the experimental animals, especially those administered 20 and 50 mg/kg of NaF, showed higher degradation of proteins and amino acids. The amount of proteins and amino acids decreased in intoxicated animals as the dose of fluoride was increased.

Key words: Amino acids; Experimental animals; Fluorosis; Proteins; Rabbits.

Introduction

Fluorosis is a slow progressive degenerative disorder known to affect the structure and function of the skeletal system and teeth (1,2). The tendinous attachment of muscle gets calcified, and calcification extends into its fasciculi (3). The fluoride ion has great affinity for calcium and high calcium content of the skeletal muscle makes it more susceptible to the toxic effects of fluoride (4). Muscles turn pale due to reduction in myoglobin content (5). Fluoride also enhances the permeability of sarcolemma, and creatinine phosphokinase levels are raised in the blood stream due to the highly permeable membrane of the muscle cell (6). In the rabbit, electron microscope studies reveal that all components of muscle fibres including the actin and myosin are affected by fluoride (7). In view of the significant role of skeletal muscle in work performance and endurance, investigations were carried out on muscle proteins of male and female rabbits administered heavy doses of fluoride experimentally.

Materials and Methods

Experiment

Albino rabbits of both sexes weighing 400-650 gm were kept under normal laboratory conditions. The animals were fed a standard rabbit pellet diet, supplied *ad libitum*. The animals were divided into five groups of twelve animals each. Subcutaneous injections of 1 cc of distilled water/kg/day were given to the animals of the control group. The remaining groups of animals were administered 5, 10, 20 and 50 mg of sodium fluoride injections per kg/body weight every day. The experiments were continued up to three and a half months. At the end of the experimental period, all animals were sacrificed under ether anaesthesia. Owing to paralysis the animals of group IV given 50 mg fluoride/kg body weight did not complete the total duration of experiment (their maximum survival was 70 days). From all animals the skeletal muscle from the thigh was removed and weighed on an electronic balance.

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Extraction of proteins

The acidic and basic proteins were extracted separately. The tissue was homogenized in ice cold 10% TCA to precipitate the proteins. The homogenates were incubated at 70°C for 20 minutes, cooled, and centrifuged. The supernatant was discarded and the residue gave the total proteins in a sample. The lipids and nucleic acids were removed by washing with ethanol. The residue was then treated with a known volume of 0.2 *N* HCL and incubated at 100°C for 30 minutes. The resulting supernatant was taken as extract for basic proteins. The residue was treated with 0.1 *N* NaOH and kept overnight at room temperature. The supernatant served as an extract for acidic proteins.

Estimation

Determination of both acidic and basic proteins was done colorimetrically by the method of Lowry *et al* (8) using bovine serum albumin as standard. The quantitative determination of free amino acids was done on a spectronic-2 colorimeter by the method of Troll and Cannon (9). All data were subjected to statistical analysis.

Results and Discussion

The treated animals showed considerable decrease in weight, and retarded growth, compared with the controls. The muscle was pale in colour in fluoridated rabbits. There was significant reduction in acidic and basic protein content in skeletal muscle of the experimental groups of rabbits as compared to those of the controls (Table). The free amino acids also registered a significant ($p < 0.001$) decrease in skeletal muscle of fluorotic animals. A greater decrease in proteins and amino acids was recorded in animals treated with 20 and 50 mg fluoride/kg body weight.

The present study demonstrates that fluoride has significant effects on muscle proteins and amino acids. Since fluoride is known to affect the rate of cellular protein synthesis, which is mainly due to impairment of peptide chain initiation (10,11), the present findings suggest that inhibition of protein synthesis in skeletal muscle may be due either to increased proteolysis or to decreased protein synthesis. Ravel *et al* (12) have observed that sodium fluoride acts as a specific inhibitor of protein synthesis by interfering with new peptide chains on ribosomes. The depletion in free amino acids appeared to be due to decreased transamination, in agreement with evidence that fluoride inhibits the oxidative decarboxylation of branched-chain amino acids and simultaneously promotes protein breakdown (13).

The disturbance of protein synthesizing systems in fluorosis has been attributed to a decrease in activity of a group of enzymes catalysing the key processes of cellular metabolism .The enzymes were glutamine synthetase catalysing certain stages of amino acid biosynthesis and methionine activating enzyme of the liver (14). The present findings stand in close agreement with those of Kathpalia and Susheela (15). They observed that large doses of fluoride given orally to rabbits induced a reduction in protein content ranging from 10 to 46 percent in most body tissues including cardiac muscle, liver, lung, pancreas, skeletal muscle and spleen. During the present investigation, fragmentation and degeneration of muscle fibres and, in some cases, complete necrosis was observed, leading to muscle wasting and impairment of energy metabolism (16). Similar changes have also been encountered in patients with skeletal fluorosis (7).

TABLE

Proteins and free amino acid levels in skeletal muscle of albino rabbit during fluoride intoxication (Values are mean ± SD)

Component	Sex of animal	Control	Group I (NaF 5mg)	Group II (NaF 10 mg)	Group III (NaF 20 mg)	Group IV (NaF 50 mg)
Acidic protein	Male	22.4 ±0.32	18.0 ±0.29[a] (-20)	5.3 ±0.14[a] (-76)	3.2 ±0.08[a] (-86)	0.2 ±0.25[a] (-99)
	Female	19.0 ±1.29	8.1 ±0.12[a] (-57)	6.7 ±0.08[a] (-65)	5.1 ±0.05[a] (-73)	0.7 ±0.06[a] (-102)
Basic protein	Male	3.3 ±0.06	2.3 ±0.04[a] (-30)	1.8 ±0.09[a] (-45)	0.7 ±0.05[a] (-79)	0.6 ±0.04[a] (-82)
	Female	4.9 ±0.11	2.7 ±0.21[a] (-45)	2.0 ±0.18 (-59)	1.0 ±0.07 (-80)	0.5 ±0.04[a] (-90)
Total protein	Male	25.7	20.4 (-21)	7.2 (-72)	3.9 (-85)	0.8 (-97)
	Female	32.9	10.9 (-66)	8.7 (-74)	6.2 (-81)	1.2 (-96)
Free amino acids	Male	3.7 ±0.21	0.8 ±0.09[a] (-78)	0.6 ±0.02[a] (-84)	0.4 ±0.25[a] (-89)	0.1 ±0.41[a] (-97)
	Female	4.6 ±0.03	1.7 ±0.01[a] (-63)	1.4 ±0.09[a] (-70)	0.6 ±0.01[a] (-87)	0.3 ±0.02[a] (-93)

Protein and free amino acid values are expressed in mg/g wet weight of tissue. Values in parentheses represent percent change.

[a] $p < 0.001$ indicate comparison of experimental groups with control.

Conclusion

The present experimental investigations provide ample evidence that muscle protein is a major target element for fluoride action. Sodium fluoride retards protein synthesis in direct proportion to the amount of fluoride administered.

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IS INGESTION OF FLUORIDE AN IMMUNOSUPPRESSIVE PRACTICE ?

P R N Sutton
Melbourne, Australia

Abstracted from *Medical Hypotheses 35* 1-3 1991

This paper records several observations which suggest that the habitual ingestion of small doses of fluoride, even as small as the 1 mg/L contained in fluoridated water, may decrease the function of the immune system.

In 1987, Sutton advanced the hypothesis that, as a result of the normal resorption of bone containing a high concentration of fluoride (which forms in those who habitually drink fluoridated water), the released fluoride could pass through the Haversian canals into the marrow and damage, even destroy, some of the developing cells of the immune system.

There is laboratory evidence that fluoride can affect the efficacy of the cells of the immune system in several ways: for instance, Gibson reported in 1981 that a 6h exposure of white cells to 0.1 ppm fluoride reduced their unrestricted migration rate by 21% (and 0.5 ppm fluoride reduced it by 74%).

Wilkinson studied the effect of NaF on the locomotion and chemotaxis of human neutrophils and monocytes, using a microphore filter assay and a time-lapse photographic assay, and found that there was total inhibition of neutrophil locomotion when the cells were exposed to NaF at 10^{-2} M. The dose-response curve of monocytes was similar to that of neutrophils. At concentrations greater than 10^{-4} M, NaF inhibited locomotion of both types of cell, but this was not seen if lower concentrations of NaF were used. He concluded that: "these experiments give no reason to believe that fluoride at levels used in drinking water supplies, or at levels likely to be found in the body fluids of individuals drinking fluoridated water, has any deleterious effect on the locomotor properties of the leucocytes involved in defense against infectious decease". But that statement does not take into account the release of fluoride during the resorption of high-fluoride bone developed as a result of habitually drinking fluoridated water (1 mg/L). This practice greatly enhances the annual rate of increase of the fluoride concentration in "total" bone from approximately 5 ppm to 26 ppm in women, and from 3 ppm to 18 ppm in men, resulting in a considerable accumulation of fluoride in their bones.

Alhava *et al*, in 1980, found that the mean fluoride concentration in cancellous bone was 2070 ppm in 24 fluoridated women of average age of 69 years who had been drinking fluoridated water for approximately 20 years. However, in 23 women of average age 64 years who lived in a non-fluoridated area, the mean fluoride concentrationwas only 622 ppm. The mean fluoride concentrations in men were lower - 1360 ppm in the fluoridated area and 447 ppm in the control one, as in the women less than a third of the mean bone fluoride concentration found in the fluoridated area.

That concentration of fluoride in cancellous bone in women (2070 ppm) is more than 200 times (and in men more than 150 times) the "high" concentration of fluoride (NaF greater or equal to 10^{-3} M) which Wilkinson stated "...inhibited locomotion of both neutrophils and monocytes" in laboratory experiments. Furthermore, Rich and Feist stated that fluoride deposited in bone is located mainly in the walls of the canaliculi and of the lacunae containing the osteocytes. Therefore, the fluoride concentration in those places is likely to be much higher than that found in total cortical bone (2070 ppm in females and 1360 ppm in men).

It is not known in what concentration of fluoride is attained in human canaliculi and lacunae when this high-fluoride bone surrounding them is resorbed and its fluoride content released into the small volume of fluid contained in them. However, in cattle, it is sufficiently high to inactivate or kill osteocytes.

It is postulated that, as a result of the resorption of this high-fluoride bone, the fluid in the canaliculi will contain a high concentration of fluoride, some of which will stream into the marrow, producing a prolonged exposure of some developing immune system cells to concentrations of fluoride which could considerably exceed the level and exposure time (NaF 10^{-3} M for 30 min) which Wilkinson found caused inhibition of the locomotor action of neutrophils and monocytes in vitro. In addition, Gabler *et al* observed the effect of F^- (0.0-5.0 mmol/L) pre-treatment on the kinetics of O_2 generation by human neutrophils, and stated: F^- inhibits the activation and activity of neutrophils."

Allman *et al* found that if "fluoridated water (NaF at 1 ppm)" was fed to rats for 6 weeks, their 3', 5' cyclic AMP levels in the six tissues tested were increased significantly - in liver, tibia and heart by more than 100%. They stated: "It is clear that low levels of NaF are able to cause an elevation of tissue cAMP."

Curnutte *et al* found that "20 mM F^- is a potent stimulus for O_2 production by neutrophils" and that it "abolishes phagocytosis."

The above mentioned observations suggest that fluoride released in high concentrations during the normal absorbtion of high-fluoride bone, formed as a result of the habitual ingestion of fluoridated drinking-water for a period of years, may damage some immune system cells and reduce the efficacy of others. Following on the recent string of about 40 in vitro studies which have found that fluoride is a mutagen even when in low concentration, this prospect raises further doubts about the safety of compelling whole populations to ingest daily, for the whole of their lives, uncontrollable and cumulative doses of fluoride through their drinking-water.

This evidence that the ingestion of fluoride may damage the cells of the imune system certainly raises the question whether HIV+ patients should be permitted to drink fluoridated water.

Key words: Bone marrow; Fluoridated water; Immunosuppressive.
Reprints: Dr P R N Sutton, 163A New Street, Melbourne, Australia 3106.

THE SEVERITY OF DENTAL FLUOROSIS IN CHILDREN EXPOSED TO WATER WITH A HIGH FLUORIDE CONTENT FOR VARIOUS PERIODS OF TIME

T Ishii and G Suckling
Tokyo, Japan and Wellington, New Zealand

Abstract from *Journal of Dental Research* 70 (6) 952-6 1991

Inhabitants of the Ikeno district of Japan were accidentally exposed to drinking water containing 7.8 ppm fluoride (F) for 12 years, after which water with 0.2 ppm was substituted. Dental examinations of local inhabitants revealed that only children aged seven years or less at the introduction or aged 11 months or more at the removal of the high-F water had fluorosis.

Regular inspections were made of the 86 children between those age limits. The severity of fluorosis in three tooth types (first permanent molars, upper central incisors, and first premolars) was assessed and related to the period of use of the high-F water. Continuous exposure throughout tooth development resulted in severe changes in all three tooth types. With limited exposure, the age at the beginning and at the end was an important factor in determining the severity of the fluorosis. The pattern of change from normal to severe fluorosis differed in the three tooth types, influenced by their respective times of formation. Two "at risk" periods for the production of moderate or severe fluorosis were evident. One started at birth and ended early in tooth development, while the other started later and ended at eruption.

The duration of F exposure, although determining the initial degree of fluorosis, did not influence the rate of post-eruptive enamel loss.

Key words: Dental fluorosis; Japan.
Reprints: Ministry of Health and Welfare, 1-2-2, Kasumigaseki, Chiyoda-Ku, Tokyo 100, Japan.

HIP FRACTURES AND FLUORIDATION IN UTAH'S ELDERLY POPULATION

Christa Danielson, Joseph L Lyon, Marlene Egger and Gerald K Goodenough
Utah, USA

Abstract from *Journal of the American Medical Association 286* 746-8 1992

Objective.- To test the effect of water fluoridated to 1 ppm on the incidence of hip fractures in the elderly.

Design.- Ecological cohort.

Setting.- The incidence of femoral neck fractures in patients 65 years of age or older was compared in three communities in Utah, one with and two without water fluoridated to 1 ppm.

Patients.- All patients with hip fractures who were 65 years of age and older over a 7-year period in the three communities, excluding (1) those with revisions of hip fractures, (2) those in whom the hip fracture was anything but a first diagnosis, (3) those in whom metastatic disease was present, or (4) those in whom the fracture was a second fracture (n=246).

Outcome Measure.- Rate of hospital discharge for hip fracture.

Results.- The relative risk for hip fracture for women in the fluoridated area was 1.27 (95% confidence interval [CI] = 1.08 1.46) and for men was 1.41 (95% CI = 1.00 to 1.81) relative to the nonfluoridated areas.

Conclusions.- We found a small but significant increase in the risk of hip fracture in both men and women exposed to artificial fluoridation at 1 ppm, suggesting that low levels of fluoride may increase the risk of hip fracture in the elderly.

Key words: Fluoridation; Hip fractures; Utah.
Reprints: Dr Danielson, Department of Family and Preventive Medicine, University of Utah, 50N Medical Dr, Salt Lake City, UT 84132, USA.

FLUORIDATION AND OSTEOPOROSIS '92

The recent *Journal of the American Medical Association* report by Danielson *et al* (1) documents again the positive correlation between fluoride and increased risk of hip fracture. In typical knee-jerk fashion, some public health service spokesmen are quick to make unsubstantiated claims that the study may somehow be in error or that the picture is clouded by supposed conflicting studies. The purpose of this letter is to clarify the picture for those who might be confused by biased and misinformed rebuttals that have arisen with the publication of this latest study.

Fluoride "therapy" for osteoporosis

It has been proposed in the past that, since fluoride is "calcium seeking", it might be a good treatment for those with osteoporosis. This has resulted in a number of "therapeutic" fluoride trials. The results of these trials are now available and the conclusion is that fluoride has no place in the treatment of osteoporosis. These trials include those of Riggs (2), of Kleerekoper (3), and of Hedlund and Gallagher (4), all of which reported significantly increased hip fracture incidence as well as an unacceptable rate of gastrointestinal and osteoarticular side effects in the treated group compared to the controls. Professor Avioli of the Washington University School of Medicine concluded in 1987 that "sodium fluoride therapy is accompanied by so many medical complications and side effects that it is hardly worth exploring in depth as a therapeutic mode for postmenopausal osteoporosis" (5). Riggs, after several years of touting fluoride for osteoporosis, finally conceded in 1989 that fluoride had no place in osteoporosis treatment (6). In late 1989, the chairman of the FDA advisory commitee reviewing fluoride's effect on fracture incidence was quoted as saying the FDA "should quietly forget" about fluoride (7).

Fluoridation and osteoporosis prevention

The hypothesis that fluoridation might somehow help prevent osteoporosis and bone fracture received its impetus in 1966 when Bernstein *et al* reported a difference in osteoporosis prevalence between high- and low-fluoride areas in North Dakota (8). What appears to have been forgotten is that Bernstein went on to study the metabolic effects of fluoride on bone and concluded that fluoride was toxic to bone and not likely to be of any bone benefit (9). Similar studies abroad (10-13) reached the same conclusion: fluoride is toxic to bones.

In a 1986 retrospective study (14) and a prospective study concluded in 1991 (15), Sowers *et al* reported a definite correlation of fluoridation status and an *increased* susceptibility to fracture when comparing communities with fluoride at 1 ppm and 4 ppm. Kleerekoper, in his *JAMA* editorial concerning the recent Danielson study, mistakenly suggests that Sowers ignored other differences in the water, such as calcium content. In fact, Sowers deliberately chose communities with differing levels of calcium in the drinking water so that this factor could also be tested and it was found not to be of any importance to fracture incidence.

In 1990, Jacobsen *et al* reported on an assortment of ecologic variants relative to over 500.000 hip fractures in white women in all counties of the 48 contiguous US states; he found a definite positive correlation between fluoridation status and hip fracture incidence. After correcting for the other possible variants, this positive correlation became even stronger (16).

In 1991, Jacobsen joined with Cooper of Great Britain to correct an error in applying statistical methods to Cooper's earlier paper concerning hip fracture incidence and fluoridation status in 38 districts in England. When properly addressed, the correct results showed a positive correlation of hip fracture with fluoridation status even though the difference in water fluoride levels ranged only from <0.1 to 0.9 ppm (17).

Fluoride promoters, however, claim that there exists one study that found fewer hip fractures in a fluoridated community compared to an unfluoridated one. This is the Finnish study by Simonen and Laitinen (18), a two page report that appeared in *Lancet* in 1985. Apparently, the authors had collected a computer-generated readout of hip fracture diagnoses from Kuopio and another community that had recently started using computer data in their respective hospitals. They did not notice that their Kuopio data produced results indicating approximately equal numbers of hip fractures for both men and women. Since the common ratio of hip fractures between men and women is 1:4, this result means that their Kuopio case finding was faulty; it was missing 3/4 of the female hip fractures. The control community data exhibited the usual sex ratio of fractures and thus was more likely to be correct. The authors also were unaware that researchers at the Kuopio University had already published several papers (10-13) on the bone damaging effect of fluoride. A further defect of the Simonen and Laitinen study is that the residence of the fracture patients was never established; it is likely that a university hospital will attract patients from outside the city itself and thus from a population not drinking the fluoridated city water.

Though the defects in the study were obvious to all knowledgeable researchers, *Lancet* never published an admission of this error. The city fathers, however, were less obtuse; in comparing the work of their own university researchers with the obviously faulty *Lancet* article, they realized that continuing fluoridation was a mistake and shortly thereafter, voted to discontinue the practice. Thus, the only community in Finland that was fluoridating their water in those years, is now fluoride free. One wonders at the mental competence of those that still insist this one report, faulty as it is, is sufficient reason to continue to place the rest of us at the greater risk of fracture induced by fluoridation.

The Danielson JAMA report (1)

Simply put, Danielson found that the risk of hip fracture was approximately 30% higher for women and 40% higher for men exposed to fluoridated (1 ppm) drinking water when compared to those with unfluoridated water. The effect was particularly strong in those women who were exposed to fluoridated water during the time of their menopause, a time of active bone remodeling. In older women, when bone remodeling is less and the incorporation of fluoride in bone is less, the effect was less strong. Further, the confounding factors of smoking and alcohol use which might dilute the fluoride significance of other studies, are not present in the populations of this study; thus the effect of fluoride is all the more clear to see.

Conclusion

Fluoride is toxic to bones and increases risk of fracture at all levels of exposure including fluoridation at 1 ppm. Regardless of any other consideration, this is reason enough to discontinue fluoridation immediately.

John R Lee, M.D.

15 August 1992.

(References next page)

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CORRECTION OF ABSTRACT

John C. - I understand why you chose to omit the phrase about treatment savings, but it would have been less noticeable had you reconstructed the lines to make English sense. As it is, the omission draws attention to itself.

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[The above communication accompanied a photocopy of page 130 of *Fluoride* Vol. 24 No. 4 1991, drawing attention to some missing words from the Abstract from *Journal of Dental Education*. We thank Mr Small for drawing our attention to the error. The passage affected should have read: "In general, the articles failed to incorporate the declining prevalence of dental caries into their analyses and to fully document costs associated with water fluoridation. Treatment savings from dental care averted secondary to water fluoridation were not appropriately incorporated into the cost-effective analyses, thereby overestimating the marginal cost associated with fluoridation."]

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