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THE KYOTO CONFERENCE

The XIXth Conference of the International Society for Fluoride Research (ISFR) was held September 8-11 1992 in Kyoto, Japan. Chairman and host of the Conference was Professor Yasuhisa Yoshida, Department of Hygiene and Public Health, Osaka Medical College. He was assisted by his very able Secretary General, Professor Koichi Kono, and the Executive Committee from the same Department and College. Professor Humio Tsunoda, Department of Hygiene and Public Health, Iwate Medical University, Morioka, presided over the Conference as President of ISFR.

Special lectures were presented by Professor M H Yu, Western Washington University, USA, Professor Miklos Bély, National Institute of Rheumatology, Hungary, and Professor Gene W Miller, Utah State University, USA. Professor Yu presented an overview of the biological aspects of fluoride on plants with a special emphasis on germination and enzymatic reactions involved. Professor Bély emphasized the structure and function of bone tissue and articular cartilage in osteofluorosis. He detailed morphology of bone tissue and articular cartilage and presented polarization optical methods in ultrastructural research to study them. Professor Miller discussed the effects of fluoride on higher plants and emphasized early physiological and biochemical disorders. Fluoride effects at physiological levels on many sensitive enzymes were presented. Of particular interest were membrane ATPases, which were especially fluoride sensitive.

About 70 papers and posters were presented on topics including: analytical methods for fluoride, environmental fluoride pollution, biological effects of fluoride and effects of fluoride on humans. Topics involved studies on humans, animals, plants and microorganisms from over 170 participants frm Japan, Canada, USA, Hungary, China, Switzerland, India, Britain, France, Norway, New Zealand, Denmark and Germany. Summaries of a few of them follow:

Professor Sakurai and others from the University of Shizuoka, Japan, presented research on how hyperglycemia and hyperglucose urine could be induced in rats by a single dose of fluoride. *In vitro* effects of fluoride on glucose transport were examined in renal brush border membranes and basolateral membranes. The renal brush border membrane was found to be resistant to fluoride, whereas the basolateral membrane was very sensitive. Na^+, K^+ and ATPase activity was almost completely suppressed by 10 mM fluoride.

Professor Milhaud, Ecole Nationale Vétérinaire, France, studied effects of fluoride on sheep molars and found they were influenced by the stage of mineralization. If molars were in the process of mineralization during fluoride administration, the enamel was thin, a marked decrease in hardness occurred and there was a low resistance to wear. To a lesser extent the same phenomenon occurred in molar teeth that had mineralized before fluoride intake. Deep changes occurred in the structure and direction of crystals in enamel formed with fluoride administration as shown by scanning electron microscopy.

Dr J Colquhoun, University of Auckland, New Zealand, presented results showing the decline in primary tooth decay in New Zealand had started before fluoridation was introduced. The decline over a 40 year period was discussed. A recent steep decline is correlated with a change in diagnostic criteria within the School Dental Service.

Suketa and others, University of Shizuoka, discussed osteoporosis and mottled teeth in animal fluorosis in terms of metabolic disorders of calcium. They found that kidney calcification was caused by a large fluoride dose to rats. They concluded that the stimulation of calcium incorporation by fluoride was due to the opening of a calcium channel gate, rather than by direct damage of brush border membranes by fluoride. The calcium level elevation in organelles of rat kidney after fluoride administration was discussed in terms of changes in protein kinase activity.

Nagaie, Yoshida and others, Osaka Medical College, Japan, presented a poster on the effects of food intake on serum and urinary fluoride concentrations as an indicator of occupational fluoride exposure. The intake of tea and marine products containing fluoride markedly influenced concentrations in the biological fluids. It is important to closely monitor and eliminate the effects of fluoride-containing foodstuffs on the serum and urine before using serum and urinary fluoride concentrations as an indicator of occupational fluoride exposure.

Research presented was new and original and provided valuable information for those present. Many presentations will be published shortly in *Fluoride*. Thanks were given to the hosts for a very successful Conference and all participants were pleased with the presentations and discussions. The next Conference will be held in Beijing, China, in 1994, followed by Budapest, Hungary, in 1996.

Gene W Miller

PUBLICATIONS IN 1993

In this issue we commence publication of the Conference proceedings, opening with Professor Miller's important review. Further papers and abstracts from the Conference will be in later 1993 numbers. Other research in this issue includes the Chlebna-Sokól and Czerwinski paper, mentioned in our last issue in the report on the Symposium in Poland. Their finding of early bone-structure disturbances in young males with dental fluorosis is significant, in view of the recent USA reports of much higher incidences of osteosarcoma among young males in fluoridated areas. The history of reports on the fluoride relationship to osteosarcoma is not unlike that for hip fractures described by Dr John Lee in our No.3 1992 issue. Earlier suggestions of no relationship, or even a benefit, based on small-scale surveys (see abstracts on page 68), are now refuted by more comprehensive and thorough ecologic studies (see abstracts on pages 66 and 67).

John Colquhoun

THE EFFECT OF FLUORIDE ON HIGHER PLANTS

WITH SPECIAL EMPHASIS ON EARLY PHYSIOLOGICAL AND BIOCHEMICAL DISORDERS

Gene W Miller *
Logan, Utah, USA

SUMMARY: One of the earliest manifestations of fluoride toxicity in plants is a change in respiratory rates. Either stimulation or inhibition occurs depending on a number of factors like plant species, concentration of fluoride, age of tissue, length of exposure, pH of culture medium and interaction between various mineral elements and fluoride. Fluoride-inhibited tissue respiration may be due in large part to inhibition of respiratory enzymes. Succinic dehydrogenase and malic dehydrogenase have been shown in *in vivo* and *in vitro* experiments to be inhibited by physiological concentrations of fluoride.

The reasons for fluoride-stimulated respiration are less obvious. Several species of plants have shown an increased use of the pentose phosphate pathway when exposed to fluoride. The activities of glucose-6-phosphate dehydrogenase, cytochrome oxidase, catalase and peroxides were enhanced in fluoride injured tissues. The rise in respiration may be linked to fluoride as a phosphorylative uncoupler. When tissue respiration was enhanced in fluoride tissue (HF fumigation) ATPase activity of mitochondria was increased. This higher ATPase activity could result in increased ADP pools within the cell. Examination of the respiratory parameters of mitochondria from corn that were exposed to fluoride exhibited increased oxygen consumption, reductions in respiratory control and ADP/O ratios. ATPase was inhibited using 30 mM NaF. This is consistent with the reduced phosphorus/oxygen observed in mitochondria fluoride studies. Fluoride treatment of isolated mitochondria reduced the energized membrane potential and, since simultaneously increased respiration was observed, fluoride may have been acting as a weak classic uncoupler.

We have reported the disruption of the tonoplast membrane and indicated it is the most fluoride-sensitive membrane as shown by electron microscopy. Tonoplast ATPase at 10 mM fluoride is inhibited. The maintenance of the transmembrane pH gradient is disrupted. Initially the chloride antiport movement inwardly during extrusion is blocked by fluoride competitively in the transport channel. Later ATPase was inhibited directly by fluoride. The ATPase in the plasmalemma membrane in sugarbeet was inhibited by fluoride as low as 5 mM. There was apparently formation of a MgF_2 complex at the active site of the enzyme preventing binding of the normal Mg-ATP substrate.

There appear to be major differences in the concentration of fluoride required to elicit the various responses between *in vivo* and *in vitro* experiments. This can be explained on the basis of subcellular fluoride partitioning. The basis for this model resides in the weak acid characteristics of HF (pKa 3.45), which would be in 2 forms, HF and F^-, and directly dependent on the pH of the immediate microenvironment. This can be calculated mathematically by the Henderson-Hasselbalch equation:

$$pH = pKa - \log(F^-)/(HF)$$

The two fluoride forms differ by 6 magnitudes in their ability to pass through lipid membranes. Thus concentration differences of fluoride between the apoplast (outside cell wall) and the cytoplasm, mitochondria and chloroplast would be great. If 1.9 ppm (parts per million) fluoride were in the apoplast (ph 5.8) then 47, 298 and 190 ppm would be found in the cytoplasm (pH 7.2), chloroplast (pH 8.0) and mitochondria (pH 7.8), respectively.

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Presented to the XIXth Conference of ISFR, Kyoto, Japan, September 8-11 1992.

Such a model indicates that low fluoride levels in the apoplast can be concentrated to high levels in organelles which could induce physiological damage. Such build-up of fluoride in the cytoplasm and organelles compared to the fluoride absorbed into the apoplasm may also explain cytogenetic effects of fluoride. A close relationship exists between calcium deficiency and chromosome abnormality. Treatment of chromosomes with EDTA causes dispersion of chromosomes through the dissolution of ionic bridges by the removal of calcium. Fluoride may also bind with calcium and destroy such ionic bridges.

Levels of ambient fluoride near an industrial plant were 200 ppb (parts per billion) (0.2 mg/m³). At 0.2 kilometer from the plant wheat leaf levels were over 500 ppm (dry weight basis) or about 3 mM in undried leaf. A concentration in the cytoplasm could be in excess of 75 mM. The percentage of mutations under these conditions was 2-6 times higher than the control and the spectrum of chromosome aberrations showed changes. Grain seedlings are highly sensitive to the mutagens in gaseous form such as HF.

Key words: Chromosome abnormality; Enzymes; Fluoride; Higher plants; Mutagenic; Mutations; Respiration; Toxicity.

INTRODUCTION

Fluoride is found in man's natural environment and, under normal conditions, is present in our food, water, soil, air, vegetation, and body. Concentrations of fluoride found in various components are shown in Table 1. Much fluoride in our environment comes from industrial sources and the natural fluoride chain is supplemented in fluoride by a man-made chain as illustrated in Figure 1. Fluoride may be significantly increased in air, water, food, soil, vegetation, and mammals by industrial sources.

Fluoride and its effects on the physiology and metabolism of plants have been the subjects of various reviews (1-7). Widely distributed in nature, fluoride is found in varying amounts in mineral rocks, soils, gases from volcanoes, and water, and it also occurs naturally in plant and animal tissue. The natural fluoride content of plant foliage ranges from 1 ppm in hay to 30 ppm in potato leaves on a dry weight basis depending on factors such as soil and water characteristics and plant species (8). Despite a few reports of beneficial effects of fluoride on plants (9), it is not considered an essential mineral nutrient. The perceived benefits may be related to a "sparing action" on the requirement for the halogen, chloride.

Although the detrimental effects of fluoride on plants and animals have been known for some time, it has been considered a serious toxicant to vegetation only since the industrial expansion during and following World War II. By-products emitted from specific industrial operations such as those producing steel, aluminum, ceramics and phosphate fertilizers are major sources of atmospheric fluoride contamination. Plants exposed to fluoride in the atmosphere accumulate it in their foliage, which may affect the growth and development of the plants themselves or of animals ingesting the foliage. Fluoride therefore has attracted attention as a major problem in air pollution.

In regions of the Western United States, water from deep wells are used for sprinkle-irrigation crops. Waters from 300 of these wells were tested and 70 percent were found to have fluoride concentrations from 2-30 ppm. These waters may be consumed directly by animals or the animals may eat forage which was sprinkle-irrigated with the water. Vegetation may accumulate high levels of fluoride under these conditions.

Fluoride from industrial sources may elevate concentrations in the air far above ambient levels (less than 0.1 ppb). A survey of US cities (industrial areas) in 1960 found levels of 2-13 ppb with high values of 50 ppb on particular days. Such high levels may induce an accumulation in plants far in excess of 30 ppm. This vegetation, when continuously ingested by livestock, may result in skeletal and dental damage. Problems may manifest particularly in the legs (Figure 2). The bones of animals which ingested different fluoride levels exhibit different degrees of injury (Figure 3). Animals ingesting water containing 2.5 ppm fluoride over a prolonged period would show similar injuries.

TABLE 1. FLUORIDE IN THE NATURAL ENVIRONMENT

Component	Description of fluoride
SOIL	76 ppm in sandy soil. 2640 ppm in heavy clay. High F soils generally have high $CaCO_3$ (90% F bound)
AIR	Average fluoride concentration 0.04 - 1.2 ppb. Less than 8% urban areas and 0.2% rural areas have over 0.1 ppb F.
WATER	Average levels 0.1 to 1 ppm. India has waters with high fluoride (low calcium) up to 25 ppm. America has waters (high calcium) up to 25 ppm.
VEGETATION TERRESTIAL MAMMALS OCEAN	Normal levels 1 - 15 ppm. Tea may have 400 ppm. Bone less than 1000 ppm, soft organs less than 5 ppm. Ionic fluoride 0.4 - 0.7 ppm (a similar amount bound as magnesium).
SEAFOOD	High in fluoride: Mackerel 27 ppm (fresh weight), fish protein 761 ppm (dogfish).
ENTIRE FOOD CHAIN, AMOUNT CONSUMED PER DAY BY HUMANS	0.4 - 0.8 mg/day (USA fluoride-free areas), 1.0 mg/day (Czechoslovakia) 1.0 mg/day (world average)

ppm - parts per million ppb - parts per billion mg - milligram

FIGURE 1. FLUORIDE IN THE ENVIRONMENT THROUGH INDUSTRIAL AND OTHER MAN-MADE SOURCES

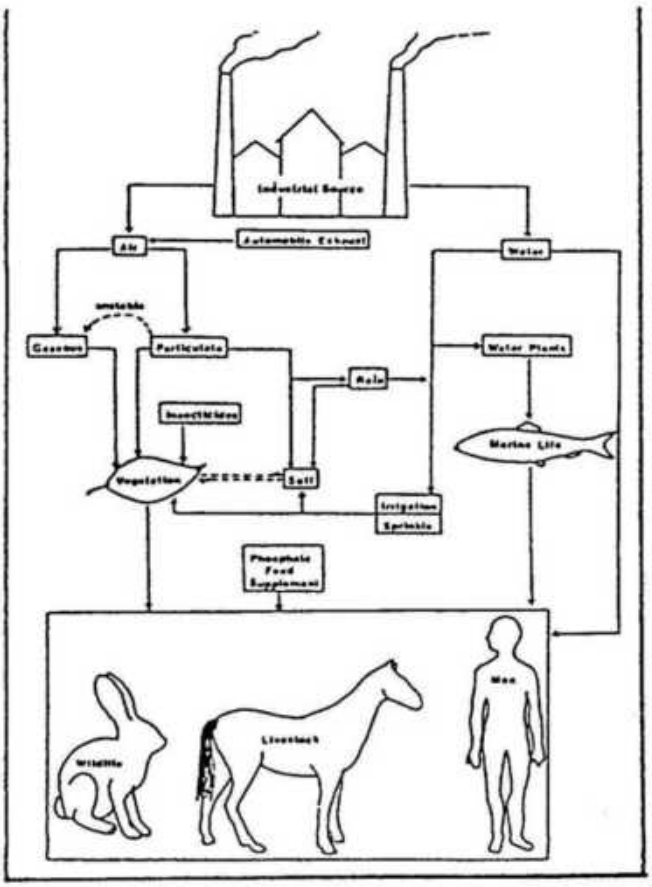


FIGURE 2. Fluoride toxicosis from vegetation
Photo courtesy of Dr J L Shupe, Utah State University

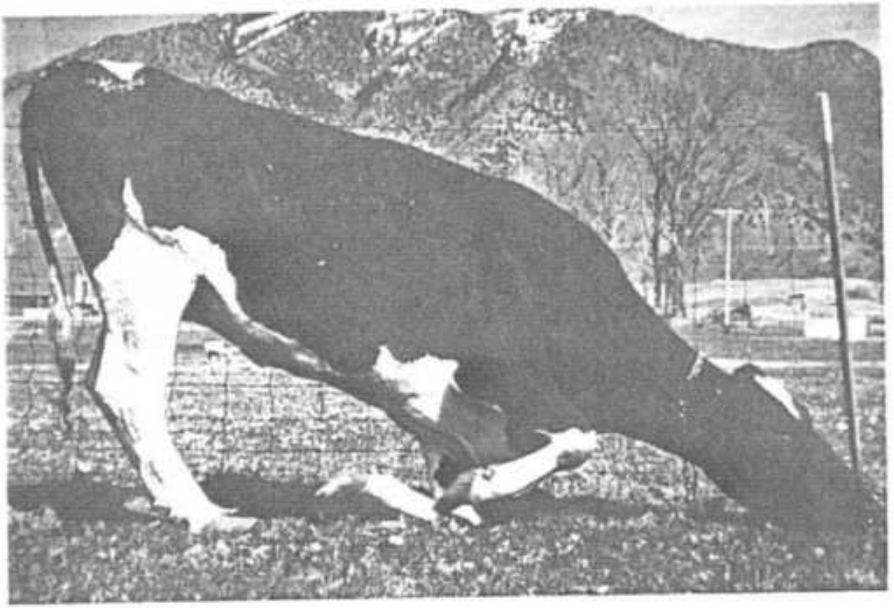
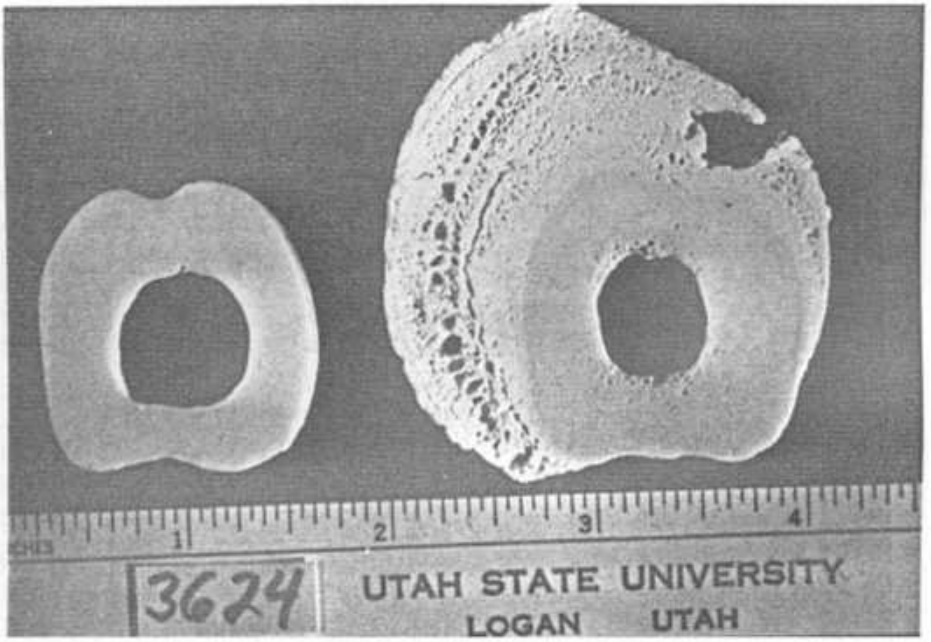


FIGURE 3. Cross section of Bovine metatarsal bone (hind leg)
Left: normal Right: abnormal osteoporosis
Photo courtesy of Dr J L Shupe, Utah State University



PLANTS

Gaseous fluoride is taken up primarily through the stomata of transpiring plants, since considerably less fluoride accumulates under conditions when stomata are closed. There is negligible contribution to leaf fluoride content by uptake through the roots. Once in the leaf, hydrogen fluoride will dissolve within the aqueous phase of the substomal cavity, where the ionic form will move through the apoplasmic space of the cell walls of the mesophyll cells with the transpirational stream to the leaf tips and margins. Leaf tips and margins accumulate the highest concentration of fluoride and are the site of initial visible injury (Figure 4). Some of the dissolved fluoride does not reach the leaf margins but rather crosses the plasmalemma, entering the cell where it accumulates within sub-cellular organelles (*e.g.* mitochondria, chloroplasts and vacuoles). Fluoride concentrations increase in the leaf tissue at almost a linear rate when the leaf is exposed to a constant fluoride level over time (Figure 5).

EFFECTS AT DIFFERENT LEVELS OF BIOLOGICAL ORGANIZATION

Plants are affected at different levels of biologic organization through the chemical interactions that occur once fluoride is taken into the tissue. Effects on the cell or biochemical mechanisms in the cell are manifested by alterations in this tissue or organism that ultimately affect the entire ecosystem. The fluoride effects are complex because they are involved in many biochemical reactions, processes and sites at different levels of organization and time of occurrence. To understand the effects of fluoride on plant growth and development in terms of physiological, cytological and biochemical manifestations, the integrated and controlled reactions of the entire cell, tissue and organism must be considered.

CELLULAR AND STRUCTURAL DAMAGE

Histochemical studies of fluoride-injured plants have indicated that the damage to leaves first occurs in the spongy mesophyll and lower epidermis, followed by distortion and disruption of chloroplasts in the palisade cells. The upper epidermis is last to exhibit any distortion or collapse (20).

Wei (10) descibed a slight disorganization of mesophyll cells in HF-fumigated soybean (*Glycine max*, Merr) after 3 days of fumigation at 40 ppb. Chlorosis developed later and, after five days, a few spongy mesophyll cells and lower epidermal cells collapsed. After six days, necrosis was evident, cellular organization was disrupted, and no chloroplasts were recognizable.

The first noticeable cellular change consisted of increased and aggregated endoplasmic reticulum (Figure 6). Subsequently (second day of fumigation), small vacuoles were formed in the cytoplasm, and phytoferretin accumulated in chloroplasts. With further fumigation, lipid droplets accumulated in the cytoplasm; mitochondrial membranes showed a slight swelling, and a few mitochondria had lost the electron density of the matrix (Figure 7). At this time the tonoplast appeared to break up into vesicles and many multi vesicular bodies appeared in the cytoplasm. Mitochondria later began to degenerate, but the chloroplasts remained unaffected until after five days of fumigation.

Tonoplast breakdown releases phenols and organic substances, which induce osmotic changes in the cytoplasm as well as exerting toxic effects on it, thereby hastening degeneration of other cell organelles.

ENZYMES

Fluoride has an effect on enzymes associated with glycolysis, respiration, photosynthesis and other reaction systems (Table 2). Enolase (11) is particularly sensitive to fluoride and is inhibited *in vitro* at concentrations as low as 10^{-4}M. Membrane ATPases are also quite sensitive to low fluoride concentrations. Some enzymes such as glucose-6-phosphate dehydrogenase, catalase and peroxidase are enhanced *in vitro* by fluoride.

FIGURE 4. Fluoride damage in apricot leaves showing necrosis in tips and around leaf margins

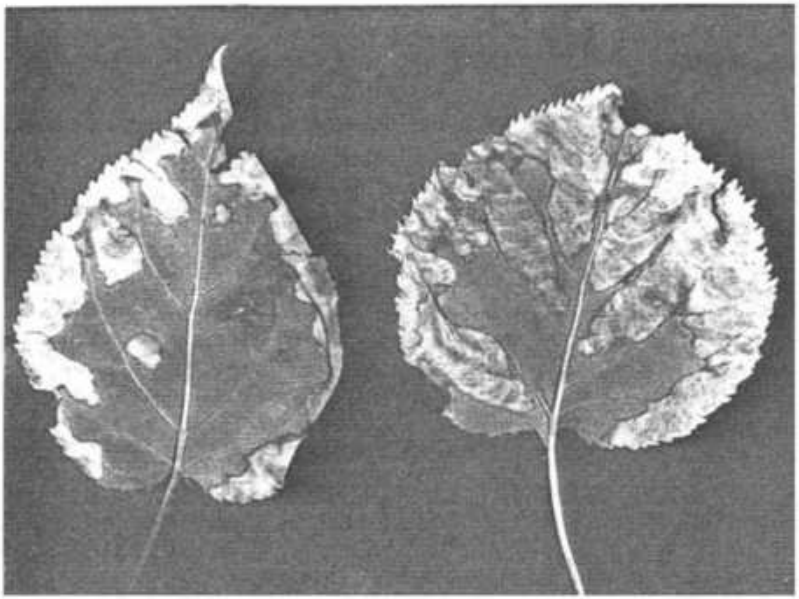
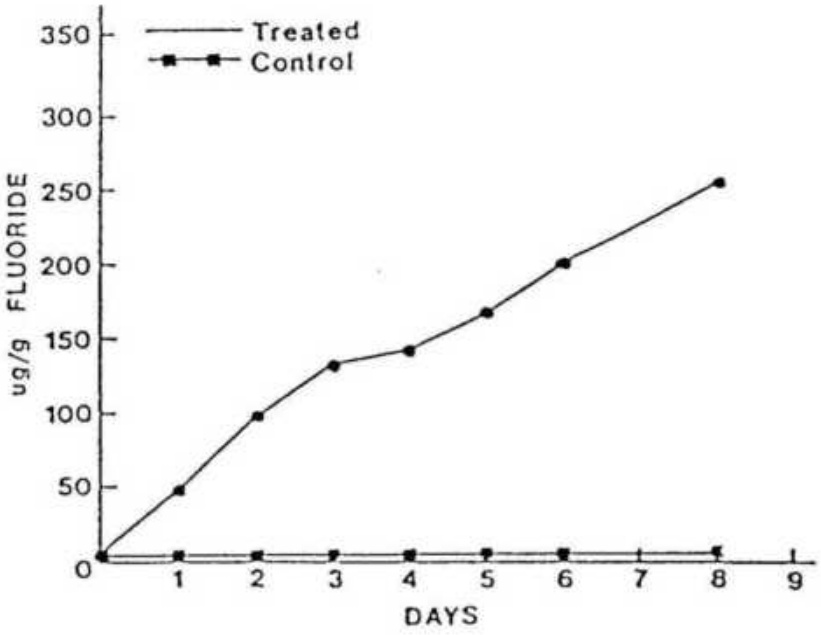


FIGURE 5. Accumulation of fluoride in the leaves of corn plants fumigated with 40 ppb F over an 8-day period



Physiological data of whole plant response to elevated levels of fluoride would seem inconsistent with concentrations required to affect biochemical mechanisms *in vitro*, as shown in Table 2. Reconciliation of the apparently higher *in vitro* concentrations and the anticipated lower *in vivo* observations has been obtained by steady-state modeling of the subcellular partitioning of fluorides into cellular fractions driven by a non-ionic diffusion gradient. This model is based on the physical properties of fluoride in solution: HF is a weak acid and exists in two forms at a physiological pH: F^- and HF. The two forms exhibit extremely different permeability coefficients through lipid bilayer membranes (46). This is illustrated in Figures 8 and 9. The HF species is much more permeable and would diffuse and concentrate where a pH gradient exists. Comparable fluoride concentrations that one could find in the various cell fractions are shown in Table 3.

RESPIRATION RATE

One of the earliest manifestations of fluoride toxicity in plants is a change in respiration rates. Alterations in oxygen consumption and carbon dioxide evolution of plants exposed to fluoride result from changes in glucose catabolism via the respiratory pathways. Either stimulation or inhibition occurs depending on a number of factors such as plant species, concentration of fluoride, age of tissues, length of exposure, pH of culture medium, and interactions between various mineral elements and fluoride.

Just as general plant susceptibility to fluoride varies with the species, so does the effect of fluoride on respiration. Thus, *Chenopodium murale*, which is very sensitive to hydrogen fluoride fumigation, may be injured when treated with less than 1 ppb F for a few days, whereas soybean (*Glycine max*) may be injured only by prolonged fumigation at above 20 ppb F (47).

Fluoride at low concentrations has enhanced oxygen uptake in *Chlorella pyrenoidosa* (48), bush bean plants *Phaseolus vulgaris* (49) and soybean plants *Glycine max* (47). Oxygen uptake in plants is accelerated at fluoride concentrations below those that induce visible foliar symptoms of fluorosis (47) (Figure 10). Increased oxygen consumption as a result of fluoride treatment has been related to light exposure. According to Suketa *et al* (50), the oxygen uptake by gladiolus leaves in the light was increased by HF, but in darkness HF slightly decreased oxygen uptake.

Fluoride inhibition of respiration has been reported in a number of plant species, including *Chenopodium, Glycine max, Chlorella, Triticum vulgare* and *Rubus hispidus*. Fluoride at both high and low concentrations resulted in an initial stimulation followed by inhibition of respiration in soybean leaf tissue (Figure 10).

While fluoride-induced respiratory effects have been studied extensively, the mechanism involved in the observed changes is not well understood. Decreased tissue respiration following exposure to fluoride may be attributed in large part to an inhibition of respiratory enzymes (Table 2).

On the other hand, the reasons for fluoride-induced respiratory stimulation in plant tissues are less obvious. *Polygonum*, after exposure to fluoride, exhibited an increased use of the pentose phosphate pathway. This was evident with fluoride-stimulated respiration in *Polygonum* and fluoride-inhibited respiration in *Chenopodium*. Subsequently, our laboratory showed that the activities of cytochrome oxidase, peroxidase, catalase, and glucose-6-phosphate dehydrogenase were enhanced in fluoride-injured tissues. Fluoride may disrupt basic cellular energetics in some manner and thus increase oxygen consumption by increasing amounts of phosphate acceptor (ADP) or donor (ATP). We have proposed that increases in ADP levels might be partially responsible for fluoride-stimulated respiration in necrotic tissues.

We attempted to establish whether respiration changes induced by fluoride were correlated with ADP concentrations by using 2,4-dinitrophenol (DNP), an uncoupler of oxidative phosphorylation. The rise in respiration may be due solely to the action of an uncoupler. All classic uncouplers allow respiration to proceed near maximal rates while

phosphorylation does not occur. Results show a close similarity between the effects of DNP and of fluoride (*i.e.* at low concentrations DNP stimulated respiration while at high concentrations it was inhibitory). Further experiments indicated that a significant DNP-induced respiratory response occurred only in older leaves (47).

Miller and Miller (21) subsequently investigated the respiration of tissue and the ATPase activity of isolated mitochondria from soybean leaves treated with fluoride. A correlation was found among tissue respiration, mitochondrial respiration and mitochondrial ATPase activity. When tissue respiration was enhanced by fluoride treatment, the respiration and ATPase activity of mitochondria were increased. When respiration decreased in fluoride-inhibited tissue, the respiratory rate and ATPase activity were reduced (Table 4).

MITOCHONDRIA AND MEMBRANE EFFECTS

Green soybean leaf tissue that had been fumigated for 48 hours with 9-12 ppb hydrogen fluoride displayed increased oxygen consumption and an apparently higher than normal ATPase activity, which might result in higher than average ADP pools within the cell (Table 4). Since fluoride effects on respiration remain unresolved by these observations, it is useful to examine the organelle (the mitochondrion) responsible for respiration when it is free of cytoplasmic interference.

The mitochondrion, the site of cellular respiration, is a specialized organelle that couples oxygen consumption with oxidative phosphorylation. The organelle consists of two membrane envelopes surrounding a central space referred to as the matrix. The outer membrane is freely permeable to molecules of less than 5,000-10,000 molecular weight. The inner membrane functions as a permeability barrier to most molecules, thus separating mitochondrial components (*e.g.* the tricarboxylic acid cycle enzymes found in the matrix) from the cytosol. The inner membrane also has the electron transport system embedded vectorially across it, as well as a coupled ATPase located on the matrix side. Coordination between cytoplasmic and mitochondrial reactions requires the movement of metabolic intermediates across the inner membrane.

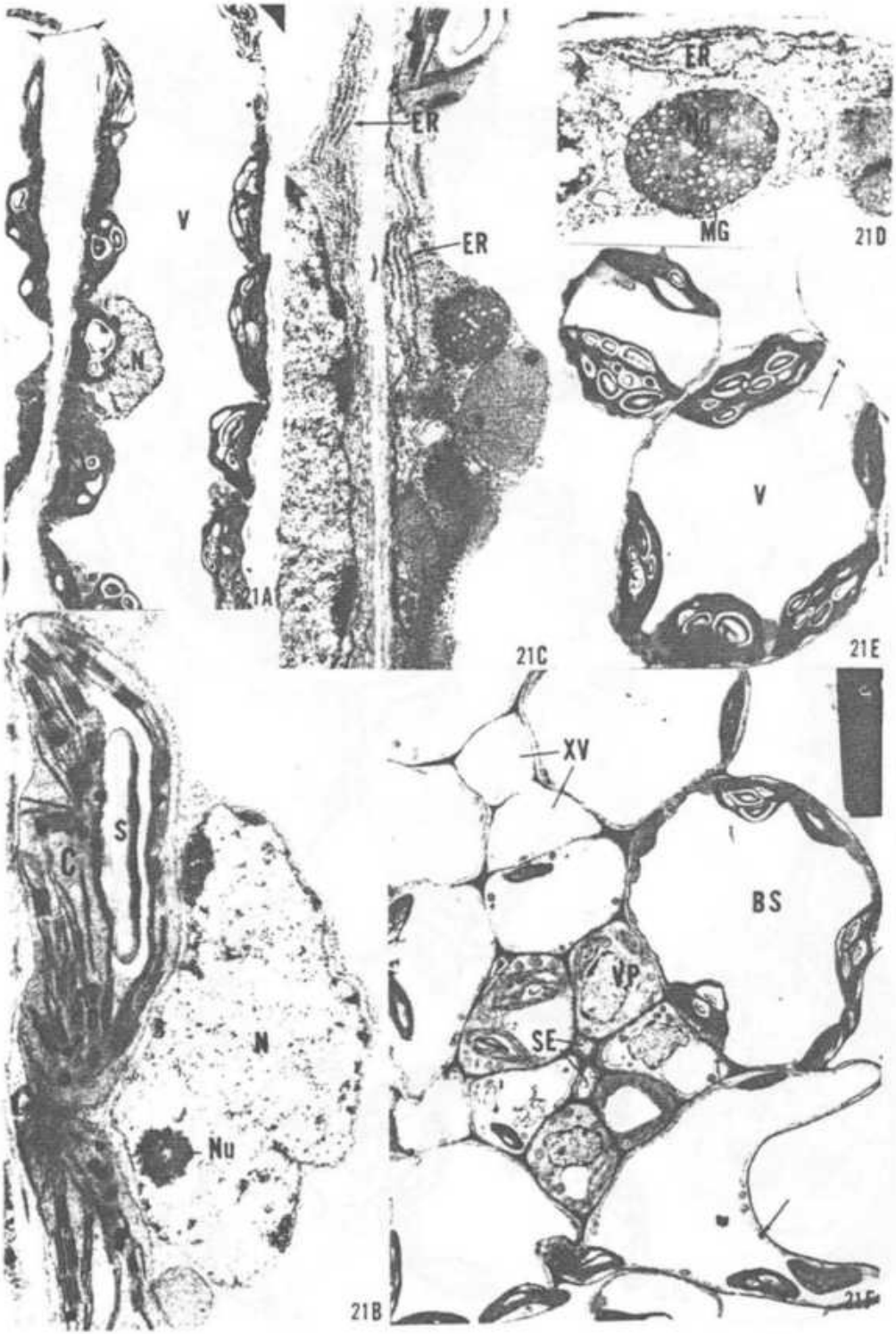
Mitochondria isolated from young green soybean plants (*Glycine max*) fumigated with 9-12 ppb hydrogen fluoride showed a stimulation of respiration after two days of treatment (21). When exposure was continued for an additional two days, oxygen consumption was inhibited.

Miller and Miller (21) proposed that a possible explanation for these fluoride-induced ATPase phenomena might be a disruption of membrane integrity. It had been previously established that mechanical disruption of pea mitochondria resulted in an increased ATPase activity (51). Other lines of evidence point to the membrane as a possible site of fluoride damage (52).

Examination of the respiratory parameters of mitochondria isolated from etiolated 5-day-old corn shoots and exposed to 30 mM sodium fluoride *in vitro* displayed increased rates of oxygen consumption compared with the control (22). In addition to increased rates of oxygen consumption, reductions in respiratory control and ADP/O ratios were

FIGURE 6 (opposite page) Cells from leaves fumigated with 40 - 50 ppb of hydrogen fluoride for 1 day

21A. Palisade mesophyll cells showing normal peripheral location of cell components (x3,000).
21B. Portion of palisade mesophyll cell showing normal structure of chloroplast and nucleus with nucleolus (x13,000).
21C. Arrows show stacks of parallelly aggregated rough ER in the palisade cytoplasm (x13,000).
21D. A mitochondrion with DNA-containing nucleoid and several mitochondrial granules also showing stack of many ER (x16,000).
21E. Normally organized spongy mesophyll cells with arrow showing the presence of a few small lipid-droplet-like globules in the cytoplasm (x3,000).
21F. Survey micrograph showing normal vascularization except a low lipid-droplet-like globules are observed (arrow) in bundle sheaf parenchyma cells (x3,000).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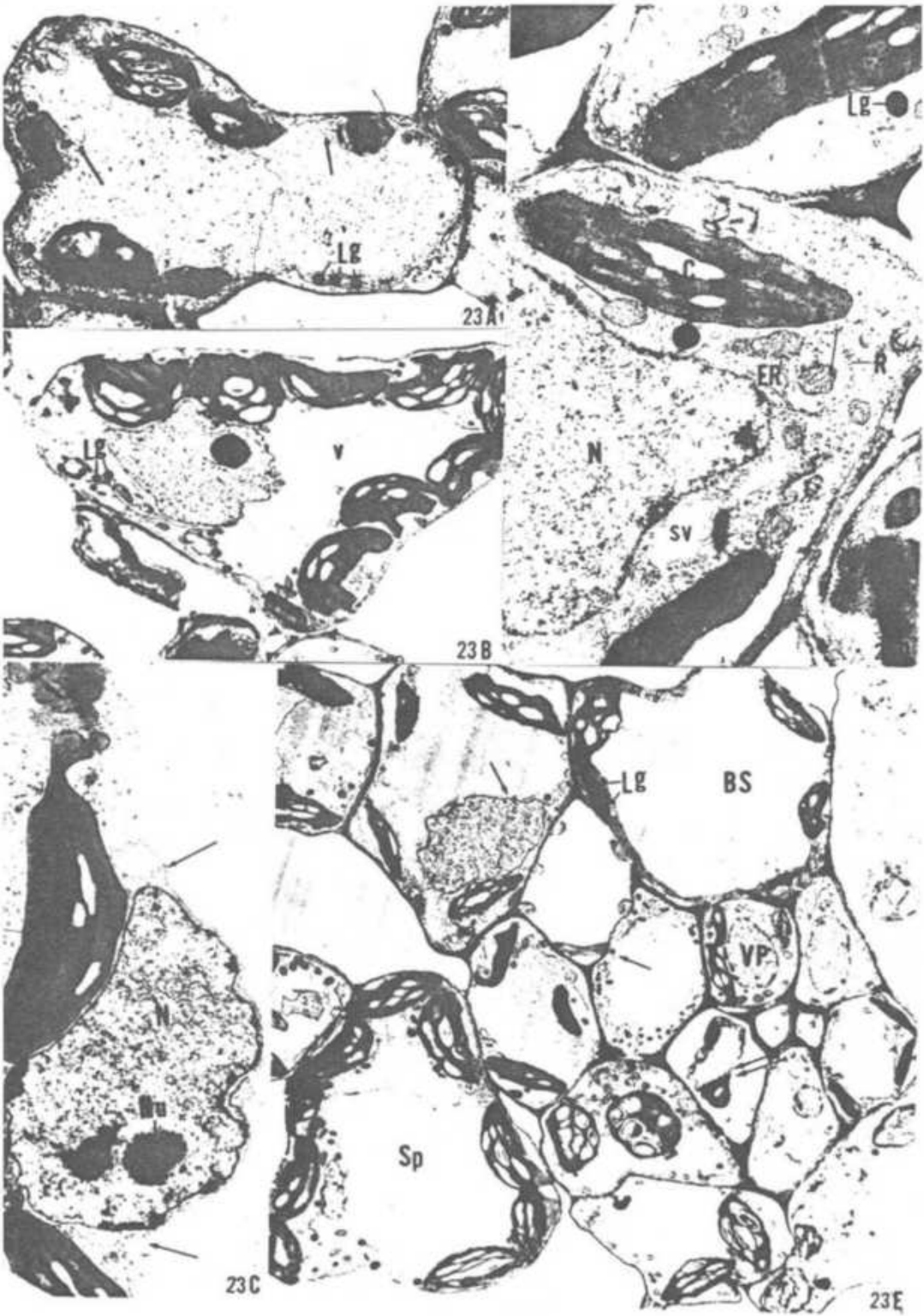
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FIGURE 7 (opposite page) Various injured cells from slightly chlorotic leaves exposed to 40 - 50 ppb for 3 days

23A. Palisade mesophyll cell showing increased size and number of lipid globules in the peripheral cytoplasm. Arrows indicate the breaking of tonoplast (x3,600).
23B. Spongy mesophyll cell also containing many lipid-droplets-like globules (x3,600).
23C. Portion of a mesophyll cell showing normal nucleus and chloroplast. Arrows indicate the disruption of tonoplast (x9,200).
23D. Bundle sheath cells showing the presence of small vacuoles, ribosomes and lipid-droplets-like globules in the cytoplasm. Slightly dilated mitochondrial cristae is indicated by an arrow (x13,800).
23E. A survey micrograph showing the vascular bundle and adjacent spongy mesophyll cells. Arrows show the vesiculated tonoplast. Double arrows show the clumped cell mass within a vascular parenchyma cell (x3,000).

FIGURE 8. Hypothetical model of fluoride accumulation by a nonionic diffusion gradient. Increase of protons at the exterior of the membrane induces HF formation enhancing fluoride transport across the membrane

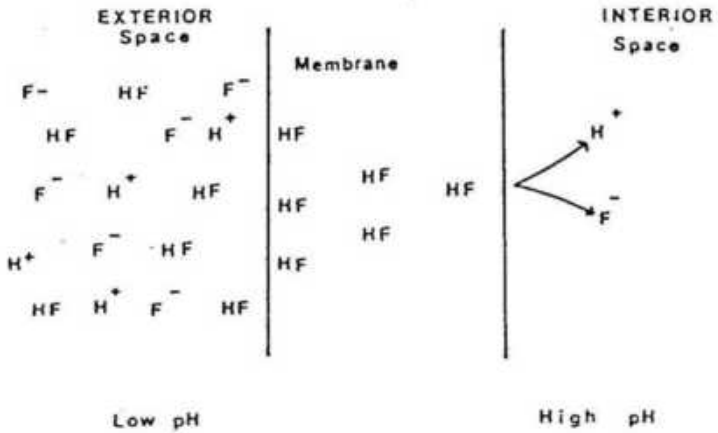


FIGURE 9. The diffusion of fluoride (HF) across the plasma membrane and accumulation in various organelles of the cell

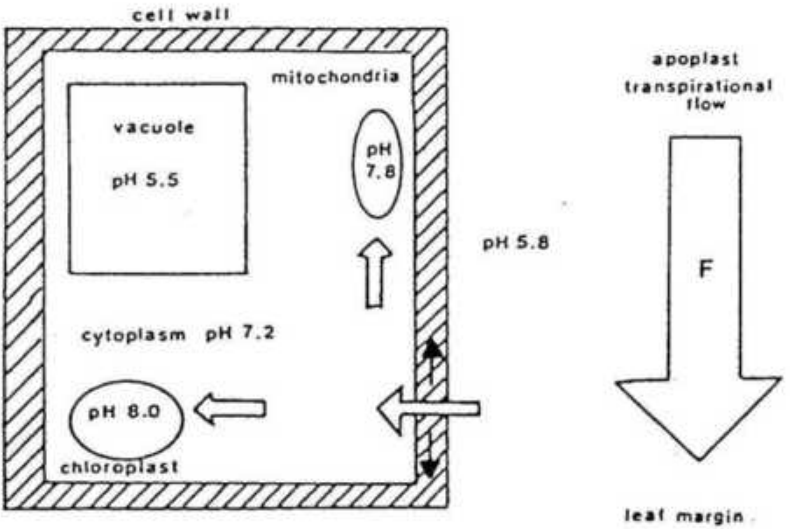


TABLE 2. Enzymes or systems affected by fluoride in higher plants

Enzymes or systems	Fluoride concentration mM	Effect*	Reference
Glycolysis			
Pyruvate kinase		[3]	McCune *et al* 1964 (12)
Phosphoglucomutase	1	[1]	Yang & Miller 1963a (13)
UDP glucose fructose transglucosylase	10	[2]	Yang & Miller 1963a (13)
Hexokinase		[2]	Melchior and Melchior 1956 (14)
Glucose 6 phosphate dehydrogenase		[3]	Lee *et al* 1966 (15)
Pentose phosphate shunt		[3]	Ross *et al* 1962 (16)
Enolase	5	[1]	Miller 1957 (11)
PEP carboxylase		[3]	Yang & Miller 1963b (17)
Respiration			
Cytochrome oxidase	10	[3]	Poovaiah & Wiebe 1971 (18) Lee *et al* 1966 (15)
Peroxidase	10	[2,3]	Lee *et al* 1966 (15), Poovaiah& Wiebe 1971 (18)
Catalase	10	[3]	Lee *et al* 1966 (15)
Polyphenol oxidase	10	[2,4]	Lee *et al* 1966 (15)
Ascorbic acid oxidase	10	[2,3,4]	Lee *et al* 1966 (15)
Succinic dehydrogenase	10	[2,4]	Lovelace & Miller 1967a (19) 1967b (20)
Mitochondrial ATPase	30	[1,3]	Miller & Miller 1974 (21) Pushnik & Miller 1983 (22)
Malic dehydrogenase	10	[4]	Lovelace & Miller 1967b (20) Psenak *et al* 1974 (23)
Photosynthesis			
Photophosphorylation	10	[2]	Giannini *et al* 1985 (24)
Uroporphyrinogen decarboxylase		[2]	Chen & Miller 1974 (25)
Hill reaction		[2]	Ballantyne 1972 (26)
Chlorophyll biosynthesis		[2]	Newman 1960 (27) Wallis *et al* 1974 (28)
Chloroplast ATPase	30	[2]	Giannini *et al* 1988 (29)
Membrane			
Ultra structure		[1]	Wei 1972 (10)
Plasmamembrane ATPase	5	[1]	Giannini *et al* 1987a (30)
Tonoplast ATPase	30	[1]	Giannini *et al* 1987b (31)
Others			
Phytase		[2]	Hauskrecht 1972 (32)
Pyrophosphatase		[2]	El-Badry & Bassham 1970 (33)
Amylase		[2]	Rockwood 1919 (34)
Phosphatase		[2]	Massart & Dufait 1942 (35) Lorenc-Kubis & Morawiecka 1978 (36)
Esterase		[2,3]	Mendoza *et al* 1969 (37) Yee-Meiler 1975 (38)
Nitrate reductase		[2]	Kadam *et al* 1980 (39)
Urease		[2]	Dixon *et al* 1980 (40)
Cellulose synthesis		[2]	Ordin & Skoe 1963 (41)
Protein synthesis		[2]	Kalinnikov & Tolokonnikov 1971 (42)
Lipid synthesis		[2,4]	Harwood & Stumpf 1971 (43) Simola & Koskimies-Soininev 1980 (44)
Nuclear aberrations and mutations		[1,3]	Gritsan 1993 (45)

* [1] Very sensitive *in vitro* [3] Enhanced *in vivo*
[2] Inhibited *in vitro* [4] Inhibited *in vivo*

TABLE 3. Henderson-Hasselbalch distribution of subcellular fluoride

		Fluoride Concentration	
Cellular Fraction	pH	(mM)	(ppm)
Apoplast	5.8	0.1	1.9
Cytoplasm	7.2	2.5	47.5
Chloroplast	8.0	15.7	298.3
Mitochondria	7.8	10.0	190.0

Calculations determined using the Henderson-Hasselbalch equation assuming a pKa of 3.45.

FIGURE 10. Effect of fluoride concentration on the respiratory rate of soybean leaf discs. O, 10^{-3} M KF; Δ, $5x10^{-3}$ M KF; □, 10^{-2} M KF. KCl supplemented at the same concentration as KF was used as control. A slight injury was observed for $5x10^{-3}$ M KF starting from 2 days of treatment; severe injury was shown on leaves treated with 10^{-2} M KF after 2 day treatment. No visible injury was obvious with the others.

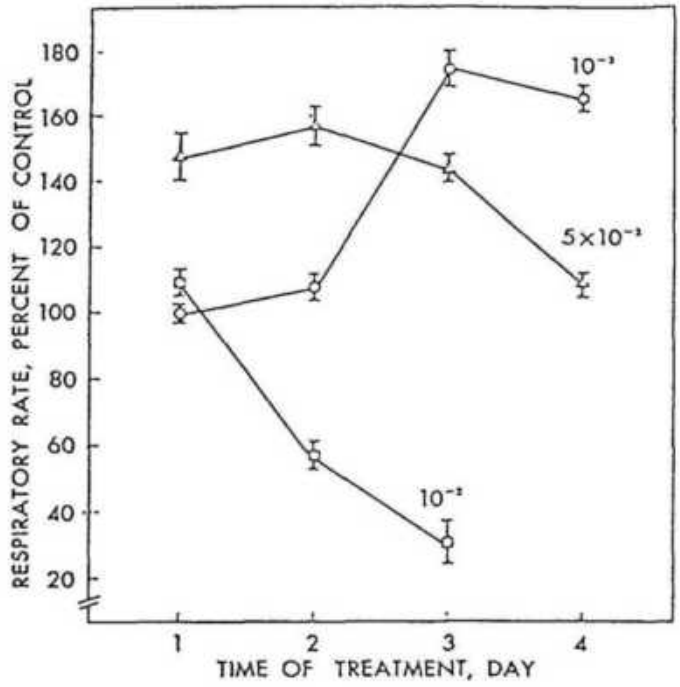


TABLE 4. Influence of HF-fumigation on respiration of soybean leaf tissue and ATPase activity of extracted mitochondria

Treatment	Extracted mitochondrial protein mg/30 g tissue	Respiration rate O_2 nmol/min/10 discs	ATPase activity P_i nmol/min/mg protein
48h			
Control	0.25	24 ± 2.3	37 ± 0.8
Fluoride	0.28	34 ± 5.2	44 ± 1.3
96h			
Control	0.24	18 ± 2.3	39 ± 4.0
Fluoride	0.29	10 ± 2.7	23 ± 4.8

From Miller and Miller, *Physiologia Plantarum 32* 115-121 1974

observed. Investigating these observations, we used an externally coupled ATP-regenerating reaction to determine fluoride effects on mitochondrial localized ATPase activity. It was determined that a 30 percent reduction in the externally coupled enzyme activity occurred in intact mitochondria. This suggested a 30 percent reduction in the availability of the rate-limiting ADP that was generated by the mitochondrial ATPase, in response to externally supplied ATP. To eliminate the possibility of interference in the reaction rate by a reduced activity of membrane-bound transporters, submitochondrial vesicles and trypsin-solubilized ATPase (F_1) were used. ATPase still exhibited an approximate 30 percent reduction in activity when 30 mM sodium fluoride was included in the reaction mixture.

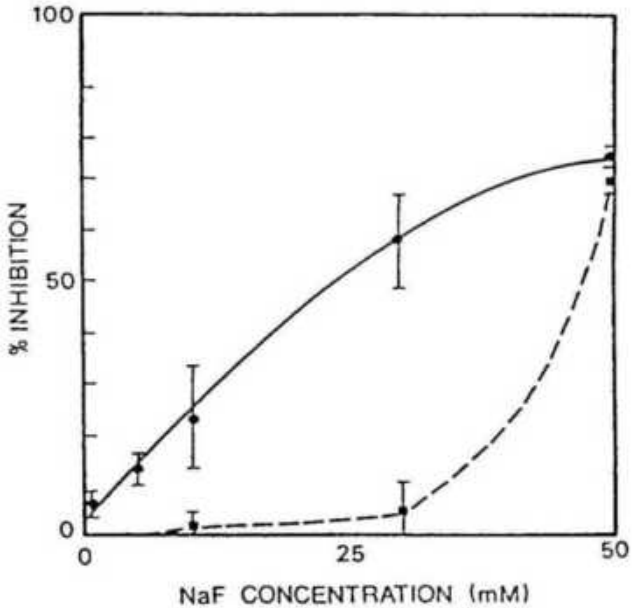
It was concluded from these experiments that exposure to 30 mM sodium fluoride inhibited mitochondrial ATPase activity. Further ATPase inhibition with fluoride exposure is consistent with the reduced phosphorus/oxygen observed in mitochondrial fluoride studies (19,21,22,53). From those observations it was concluded that fluoride did not inhibit the electron transfer process but impacted directly on the mitochondrial F_1 ATPase. The results were less clear concerning the cause of the observed respiratory stimulation seen in the same studies.

Explanation of the increase in respiratory activity required an alternative interpretation. It had been previously demonstrated that dinitrophenol (DNP), a classic uncoupler of oxidative phosphorylation, increased the respiration of control tissue but had little or no effect on fluoride-treated tissue (47). This observation prompted the examination of the membrane potential, a coordinating factor between oxidative phosphorylation and mitochondrial electron transport. Using a fluorescent probe of membrane potential, (1-anilinonaphthalene-8-sulfonic acid), it was demonstrated that fluoride exposure reduced the membrane potential by 10% (22). This reduction would reduce the electrochemical back-pressure on the respiratory chain, allowing it to function more rapidly than under control conditions. It was proposed that fluoride was acting as weak classic uncoupler, similar to DNP, and entering the mitochondria by a nonionic diffusion gradient (22).

Wei (10) reported that during fluoride fumigation of soybeans the initial membrane disrupted was the tonoplast. Clowes and Juniper (54) identified the tonoplast as the most fluoride-sensitive membrane. The tonoplast ATPase was also shown to be inhibited by fluoride (31). It appears that fluoride at 10 mM inhibits the maintenance of the

transmembrane pH gradient. The cause of this disruption is blockage of chloride antiport movement inwardly during proton extrusion. At higher concentrations the ATPase is inhibited directly. It was postulated that the nature of the blockage of the chloride movement was a competitive exclusion in the transport channel by bound fluoride (Figure 11).

FIGURE 11. Effects of fluoride on pH gradient formation and ATPase activity in membrane vesicles isolated from sugarbeet taproot. Proton gradient formation was measured in sealed vesicles using quinacrine fluorescence quenching (———) while ATPase activity was measured in vesicles made leaky by treatment with 5 µM gramicidin D (- - - - - -). The control rate of ATPase activity was 13.1 µmol/h mg^{-1} protein in the presence of µM vanadate.



The plasmalemma ATPase in sugarbeet (*Beta vulgaris*) plasma membrane vesicles was inhibited by fluoride in concentrations as low as 5 mM by 40% (30). This inhibition exhibited a relationship to the available magnesium to ATPase ratio. Increasing the ration to 3:1 (Mg/ATP) increased the inhibition of ATPase at 5 mM fluoride treatment to 60%. We suggest that increased sensitivity may be due to the formation of a MgF_2 complex at the active site of the enzyme, preventing binding of the normal substrate, Mg-ATP (Figures 12, 13).

At early stages of fluoride injury the nuclear structure showed two types of intranuclear inclusions. One of them possessed an electron transparent nuclear core; the other had an outer circular rim composed of very closely packed electron dense particles. Severely injured leaf cells had nuclei that showed marked changes. In addition to increased nuclear volume chromatin material lost their granularity, shrank into compact electron dense clumps and gradually the masses collected mainly on the inner surface of the nuclear envelope leaving a relatively less electron dense central area (Figure 14). The central role of the nucleus in controlling cellular inheritance and metabolism is well documented.

FIGURE 12. Plasmalemma ATPase activity as a function of fluoride concentration at various Mg^{2+} and ATP concentrations; ATP to Mg^{2+} ratios were maintained at 1. Plots are as follows: A (9 mM ATP, 9 mM Mg^{2+}), B (6 mM ATP, 6 mM Mg^{2+}) and C (3 mM ATP, 3 mM Mg^{2+}). Data points are means ± S.D. of 4 replicates. Control activity (no fluoride, 3 mM ATP, 3 mM Mg^{2+}) was 21.2 µmol/h (mg protein).

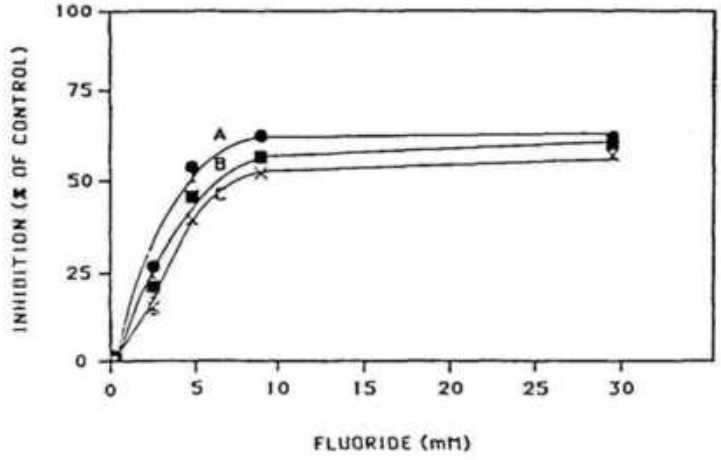


FIGURE 13. Plasmalemma ATPase activity as a function of fluoride concentration at various Mg^{2+} and ATP concentrations: A (9 mM Mg^{2+}, 3 mM ATP), B (6 mM Mg^{2+}, 6 mM ATP) and C (3 mM Mg^{2+}, 3 mM ATP). Data points are means ± S.D. of 4 replicates. Control activity (no fluoride, 3 mM ATP, 3 mM Mg^{2+}) was 18 µmol/h (mg protein)

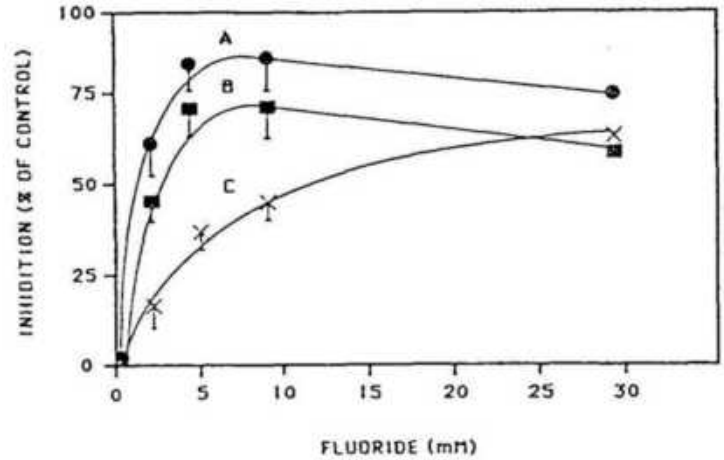


FIGURE 14 (opposite page) Nuclei from injured leaves after exposure to 40-50 ppb of HF for 5 days

31A. Chromatin materials clumped into dark masses and loss of their granularity (double arrows). Single arrow indicates the inter-chromatin granules. A type II circular nuclear inclusion is also found in these nuclei (x13,800).

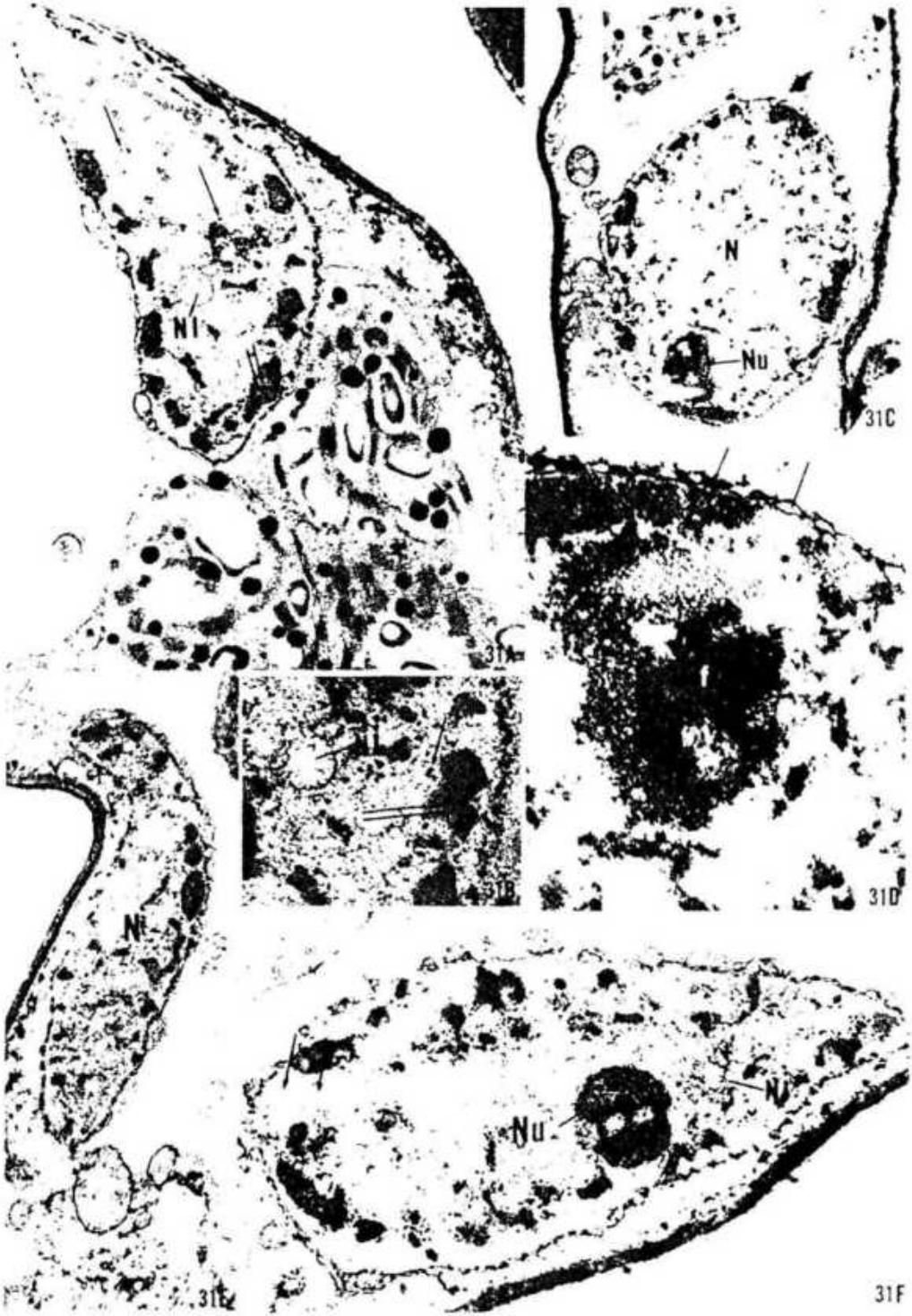
31B. An enlarged circular nuclear inclusion (x23,000).

31C. Clumped chromatins at the periphery of the nucleus, resulting in a relatively electron-transparent central region (x9,200).

31D. A nucleus with dilated nuclear envelope and accumulation of electron-dense particles are shown by arrows (x40,000).

31E. Another altered nucleus with clumped chromatins (x13,800).

31F. A nucleus containing nucleolus and a type II nuclear inclusion. Also shown by arrows are tonofibril-like fibers (x16,000).


N
Nu
31C
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31E
31F

Little information had been available on the cytogenetic effects of air pollutants on the genetic systems of either plants or animals until Mohamed *et al* (55) reported the cytological effect of sodium fluoride on onion (*Allium cepa*) root tip chromosomes. These investigators demonstrated that vigorously growing young onion roots treated with 1×10^{-2} M NaF showed chromosomal aberrations including anaphase bridges and fragments. Frequency of chromosome aberrations and treatment duration were positively correlated. In addition, tetraploid nuclei and multipolar anaphases were observed. These results indicated that sodium fluoride solutions had a specific action on onion root tip chromosomes as well as on the spindle.

Similar results were obtained from experiments with tomato plants (55,56) and with maize (57). Phenotypic abnormalities in tomato plants consisted of 1, 3 or 4 cotyledons, deformed cotyledons, fasciated petioles, wiry seedlings, double-stalked plants and dwarf seedlings (56). Chromosomal aberrations resulting from hydrogen fluoride treatment included bridges, fragments and bridges plus fragments. All of these aberrations were observed during mitosis and meiosis of the experimental plants. It was believed that hydrogen fluoride had caused the chromosomes to become sticky and/or the formation of decentric chromosomes. These effects of hydrogen fluoride did not persist. Some degree of recovery could be attained following removal of the plants from the source of fluoride (55). The presence of bridges plus fragments in meiosis during the recovery was thought to be due to crossing over in heterozygous paracentric inversions. These findings suggest that hydrogen fluoride may be a mutagenic agent (56). Hydrogen fluoride was thought to block the replication of the DNA directly or indirectly. Using maize seedlings of the genotype C'ShWx, fumigated with hydrogen fluoride, Mohamed (58) further demonstrated that the chemical not only was a mutagenic agent, but was able to reduce crossing over in certain chromosome segments. These findings were confirmed in part by Bale and Hart (59), who treated barley (*Hordium vulgare*) seedling root tips with 1×10^{-2} M of either sodium fluoride or hydrogen fluoride and noted a markedly slowed rate of seedling growth. In addition, they observed induction of chromosomal aberration and mitosis inhibition. Bridges, fragments, chromosomal gaps, binucleate cells and micronuclei characterized the aberrations. Temple and Weinstein (60), on the other hand, observed neither visible mutations nor obvious aberrations in any of the seedlings from tomato plants exposed to hydrogen fluoride.

Gritsan (45) studied the cytogenetic effects of gaseous fluoride on winter wheat and spring barley growing in a highly polluted area in the South-East Ukraine. Ambient air fluoride levels were up to $0.2 mg/m^3$. The grain had no visible injury but had leaf fluoride levels of over 500 mg/kg dry weight within 0.2 km of the industrial source. The frequency of chromosomal aberrations in root tips was 6.7 and 4.9 times as high as the control level in wheat and barley, respectively.

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CYTOGENETIC EFFECTS OF GASEOUS FLUORIDES ON GRAIN CROPS

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SUMMARY: Atmospheric pollution of fluoride from a non-ferrous industrial plant has been studied for its effect on the frequency of chromosome aberrations in root tips and shoot tips of wheat and barley. The anaphase-test was used. The percentage of mutations in the meristematic cells of plants growing in polluted areas was 2-6 times higher than in the control and the spectrum of chromosome abberations showed changes. The testing of hydrogen fluoride (HF) for its mutagenic activity by fumigation of barley seedlings showed that the mutation rate was linear with dose. It was found that the cytogenic effects of gaseous fluoride on grain crops was correlated with the fluoride content in plant tissue.

Key words: Environmental pollution; Gaseous fluorides; Grain crops; Mutagenic effects; Ukraine.

Introduction

The interest in environmental mutagenesis has strengthened considerably following understanding of the broad overlap between mutagens and carcinogens. Also alterations in environmental mutagenicity lead to increases in the mutability of living organisms. Little however is known concerning mutagenic effects of gaseous fluoride, in particular fluorine containing emissions from industrial plants.

Previous studies have shown that grain crops in areas surrounding fluoride-emitting industries and in fumigation experiments have been adversely affected in growth rate, apparent photosynthesis, respiration rate, and total yield of plants. There is evidence that if enough metabolic sites are affected or the inhibition of a major pathway becomes sufficiently great, alterations in the genetic material can occur. That is why it is suggested that fluoride in its gaseous form may be a mutagen. Moreover, Mohamed observed chromosomal aberrations in tomato and corn, and in onion roots after fumigation with HF or treatment with sodium fluoride solutions (1).

Objectives of this study were to determine whether gaseous fluorides can induce chromosome aberrations in meristematic cells of plants. Thus, we have considered:

1. The mutation rates in grain crops in zones of chronic pollution from fluorine-containing industrial emissions and the spontaneous background level of the mutations.
2. Testing gaseous fluorides for their mutagenic activity by fumigation of barley seedlings in growth chambers.
3. The relationship between the chromosome aberrations and fluoride content in plant tissue.
4. The spectrum of chromosome aberrations in root tips and shoot tips of wheat and barley.
5. A comparison of the mutability in wheat and barley.

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The data in this paper were presented to the XIXth ISFR Conference by Professor Gene W Miller.

Materials and Methods

The species of grain crops chosen for this study were winter wheat (Odesskaja semi-dwarf) and spring barley (Zernogradskii 73). The crops were sampled in the vicinity of the biggest non-ferrous metallurgy plant in Europe (Nikopo, Dniepropetrovsk region in the South-East of the Ukraine). The area used for control was situated 60 km from this plant and was free of industrial pollution of any type. The control area was agriculturally similar to the study area. It was possible at both sides to collect wheat and barley species, for comparison.

Determination of the frequency of mutations and the spectrum of chromosome aberrations was carried out using meristematic cells of the vegetative cones. They were collected together with root tips from seeds of wheat and barley and fixed using the techniques of Pausheva (2). Seeds were collected at the end of July at different distances from the plant and were grown in the laboratory. Their root tips were cut off and fixed.

The testing of gaseous fluorides for their mutagenic activity was made by fumigation of barley seedlings with 0.02 to 0.2 mg HF/m^3 for 1 hour daily for 10 days in polyethylene chambers (0.15 m^3). After harvesting, the shoot tips (apical cones) of seedlings were cut off and fixed. The seedlings had no visible injuries. This method of seedling fumigation may be used for the testing of cytogenetic effects of various atmospheric pollutants and barley seedlings may be used as sensitive and effective cytogenic monitors.

The anaphase-test was used. More than 1000 anaphases were studied for each variant. The genetic materials were fixed in acetic acid-alcohol 1:3, and then were colored by Fëlgen (2). The samples were analyzed for fluoride using a fluoride selective ion electrode (3).

Results and Discussion

The fluoride levels found in the plants at each sample site in the study and control areas and the percentage of chromosome aberrations in root tips and shoot tips of grain crops are given in Table 1. It was established that the background mutation rate of plants growing in non-polluted areas was relatively low, but some mutations occurred mainly due to the use of fertilizers and pesticides.

A significant increase in the rate of chromosome aberrations in root tips of wheat and barley were found in plants near the fluoride source. The frequency of mutations in root tips from plants in this area was 2-6 times higher than in the control. The spectrum of chromosome aberrations was also changed to a great extent. Thus, in zones of chronic pollution with fluorine containing emissions from industry the percentage of the complex types of aberrations (chromosomal bridge, chromatic bridge, bridge with fragments, etc.) was increased and the amount of the simple ones (single fragments, twin fragments, etc.) was decreased (Figure 1). The relationship between the percentage of fragments and the percentage bridges in root tips of plants was 10:5 in control areas and it was 10:11 in polluted areas. The microphotographs of the main types of mutation are illustrated in Figure 2 and Figure 3.

It was established that the frequency of chromosome aberrations in shoot tips (apical cones) of wheat and barley of the polluted populations was 6.7 and 4.9 times as high as the control level, respectively (Table 1). The rate of mutation in wheat and barley was correlated with the distance the crops were located from the plant. The types of mutations in apical cones of the crops are illustrated in Figure 4.

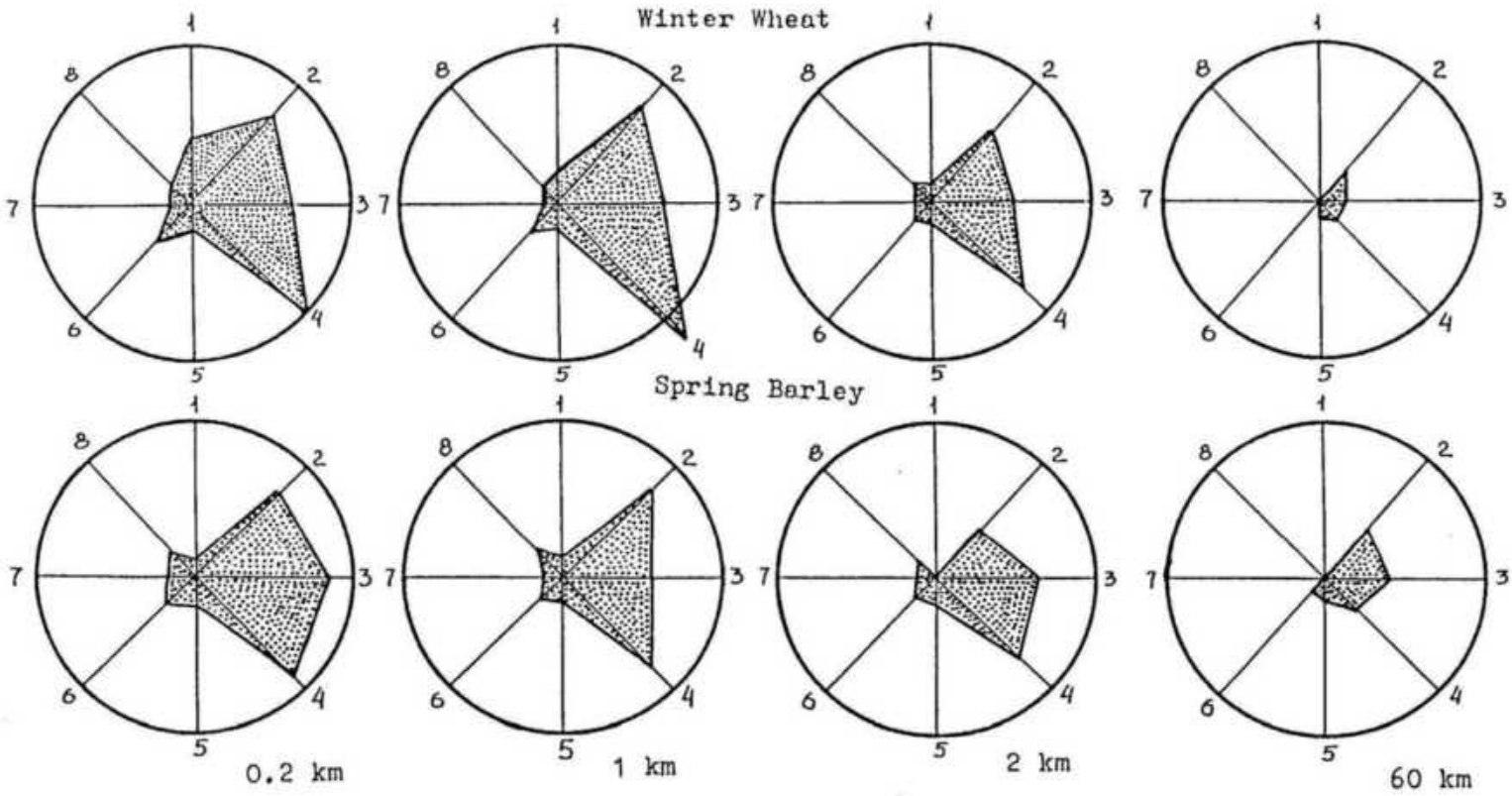
Although the highest fluoride content in the grain of wheat and barley was markedly less than that obtained from the green tissue of plants, the mutation rates in shoot tips and root tips of grain crops at similar locations were comparable. It may not be out of place to touch upon the problem of potential alterations (4,5). Results showed that grain crops, growing near the industrial plant, accumulated fluoride in high concentrations that were 5-120 times higher than in control areas (Table 1), which could lead to the beginning of potential alterations. Some of these alterations are present as chromosome aberrations in the vegetative cones, which are evident (Figures 1-4). The others remain until harvesting. These are the long-living potential alterations (4). It is probable that the potential alterations are induced by biochemical changes. The relationship between the chromosome aberrations and fluoride content in wheat and barley is shown in Figure 5.

TABLE 1

FLUORIDE ACCUMULATION IN PLANTS AND INDUCTION OF CHROMOSOME ABERRATIONS IN MERISTEMATIC CELLS FROM THE ROOT TIPS AND SHOOT TIPS OF GRAIN CROPS

Distance from plant	F^- accumulation (mg/kg dry weight)		No. of studied anaphases	Chromosomal aberrations	
(km)	Grain	Straw		Number	%
WINTER WHEAT	Root tips:-				
0.2	2.8 ±0.02	514.0 ±0.27	1003	134	13.4 ±0.93
1	1.9 ±0.02	79.0 ±0.76	1213	156	12.9 ±0.87
2	0.8 ±0.04	58.7 ±0.94	1144	86	7.5 ±0.95
60(control)	0.0	3.8 ±0.02	1097	24	2.2 ±0.13
	Shoot tips:-				
0.2		104.0 ±0.45	1001	127	12.7 ±0.81
1		14.1 ±0.28	1045	127	12.2 ±0.53
2		10.2 ±0.07	1204	100	8.3 ±0.12
60(control)		1.8 ±0.01	1050	20	1.9 ±0.10
SPRING BARLEY	Root tips:-				
0.2	6.2 ±0.03	276.0 ±0.14	1234	157	12.7 ±0.83
1	2.4 ±0.02	105.0 ±0.09	1117	105	9.4 ±0.91
2	1.3 ±0.02	87.2 ±0.13	962	76	7.9 ±0.54
60(control)	0.0	4.1 ±0.01	1084	40	3.7 ±0.25
	Shoot tips:-				
0.2		45.0 ±0.28	956	124	13.0 ±0.84
1		36.8 ±0.09	1078	97	9.0 ±0.92
2		19.9 ±0.14	1015	69	6.8 ±0.23
60(control)		0.8 ±0.01	1008	27	2.7 ±0.08

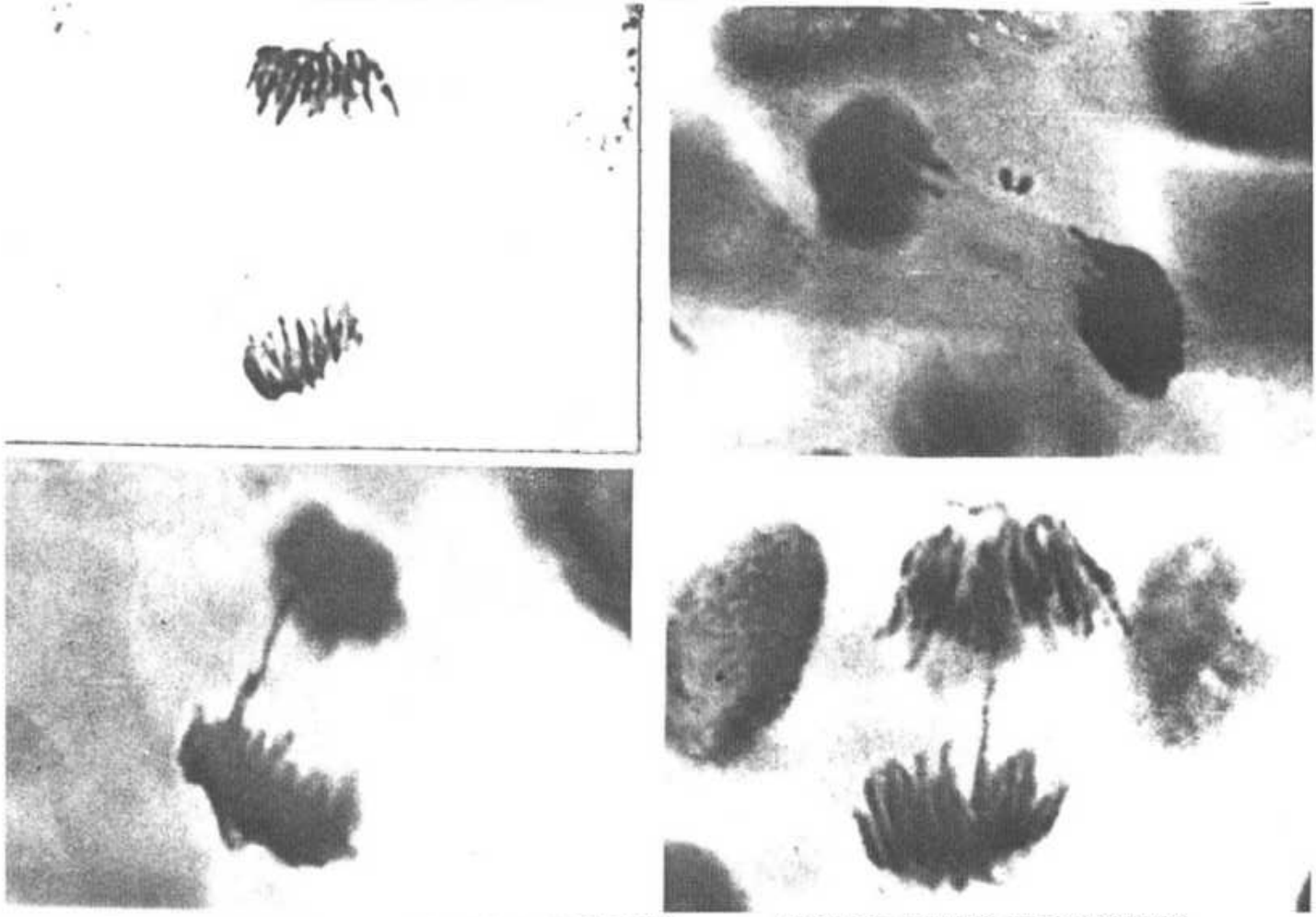
FIGURE 1. SPECTRUM OF CHROMOSOME ABERRATIONS IN ROOT TIPS OF GRAIN CROPS



RADIUS IS 4%. ZERO IS THE CENTRE OF THE CIRCLE.

1 - TWIN FRAGMENTS 2 - SINGLE FRAGMENT 3 - PLURAL FRAGMENTS 4 - CHROMATID BRIDGE 5 - CHROMOSOMAL BRIDGE
6 - SEVERAL BRIDGES IN THE CELL 7 - BRIDGE(S) WITH FRAGMENT(S) 8 - OTHER ABERRATIONS

FIGURE 2. SOME TYPES OF CHROMOSOME ABERRATIONS

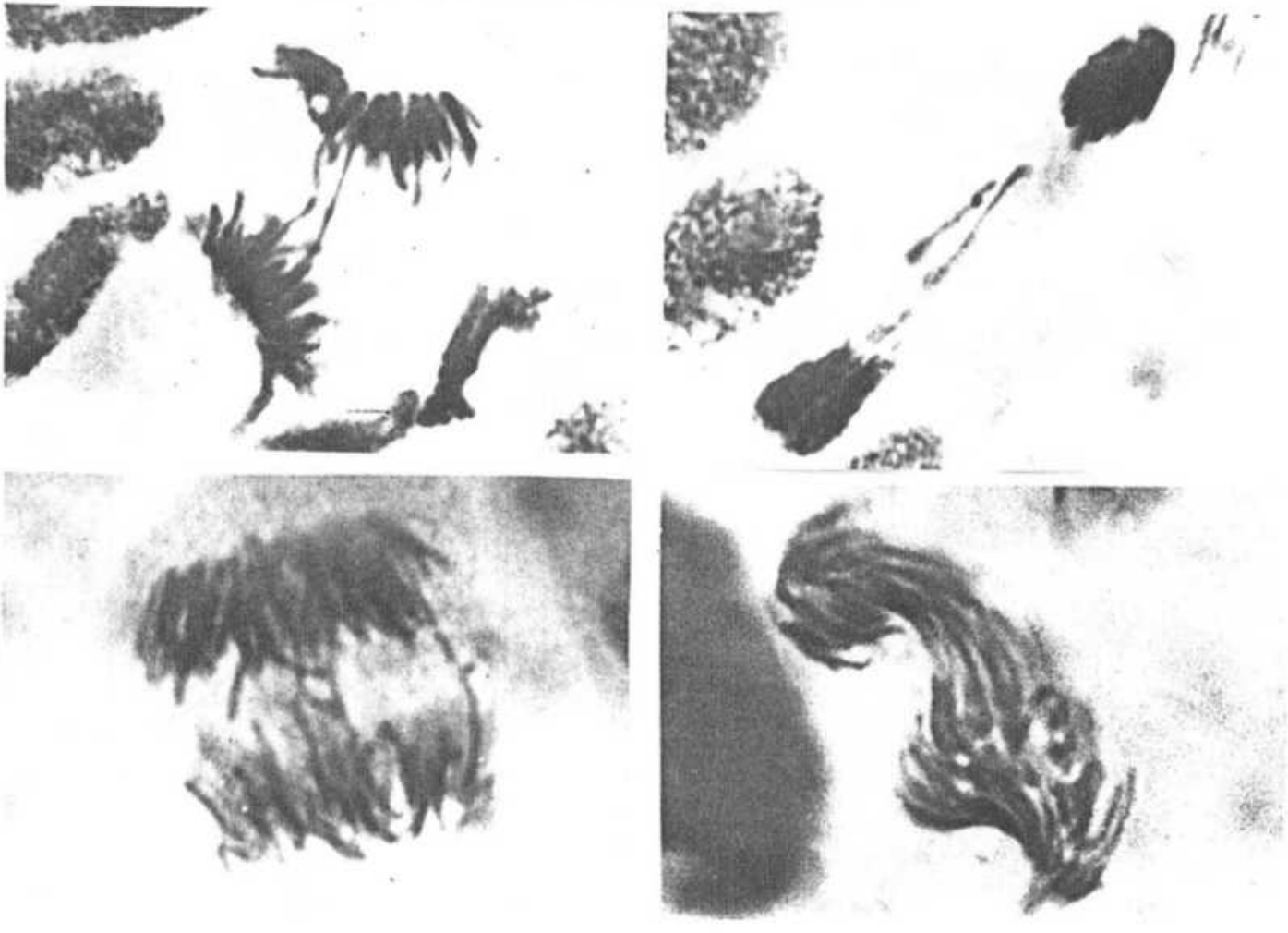


TOP LEFT: NORMAL ANAPHASE

TOP RIGHT: TWIN FRAGMENTS

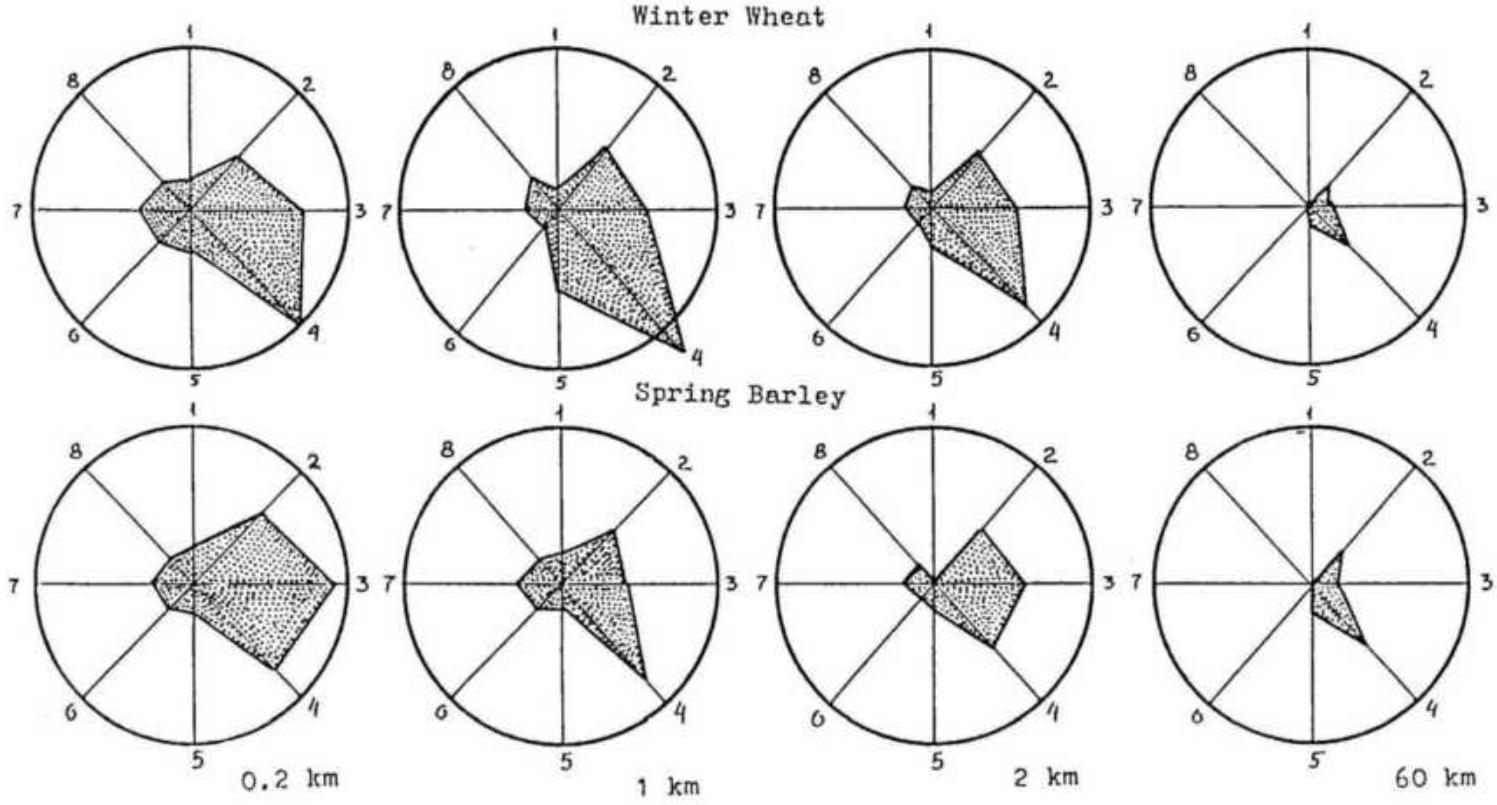
LOWER LEFT AND RIGHT: CHROMATID BRIDGES

FIGURE 3. SOME TYPES OF CHROMOSOME ABERRATIONS



TOP LEFT: CHROMOSOMAL BRIDGE TOP RIGHT: BACKWARD CHROMOSOME LOWER LEFT: SEVERAL ABERRATIONS LOWER RIGHT: OTHER ABERRATIONS

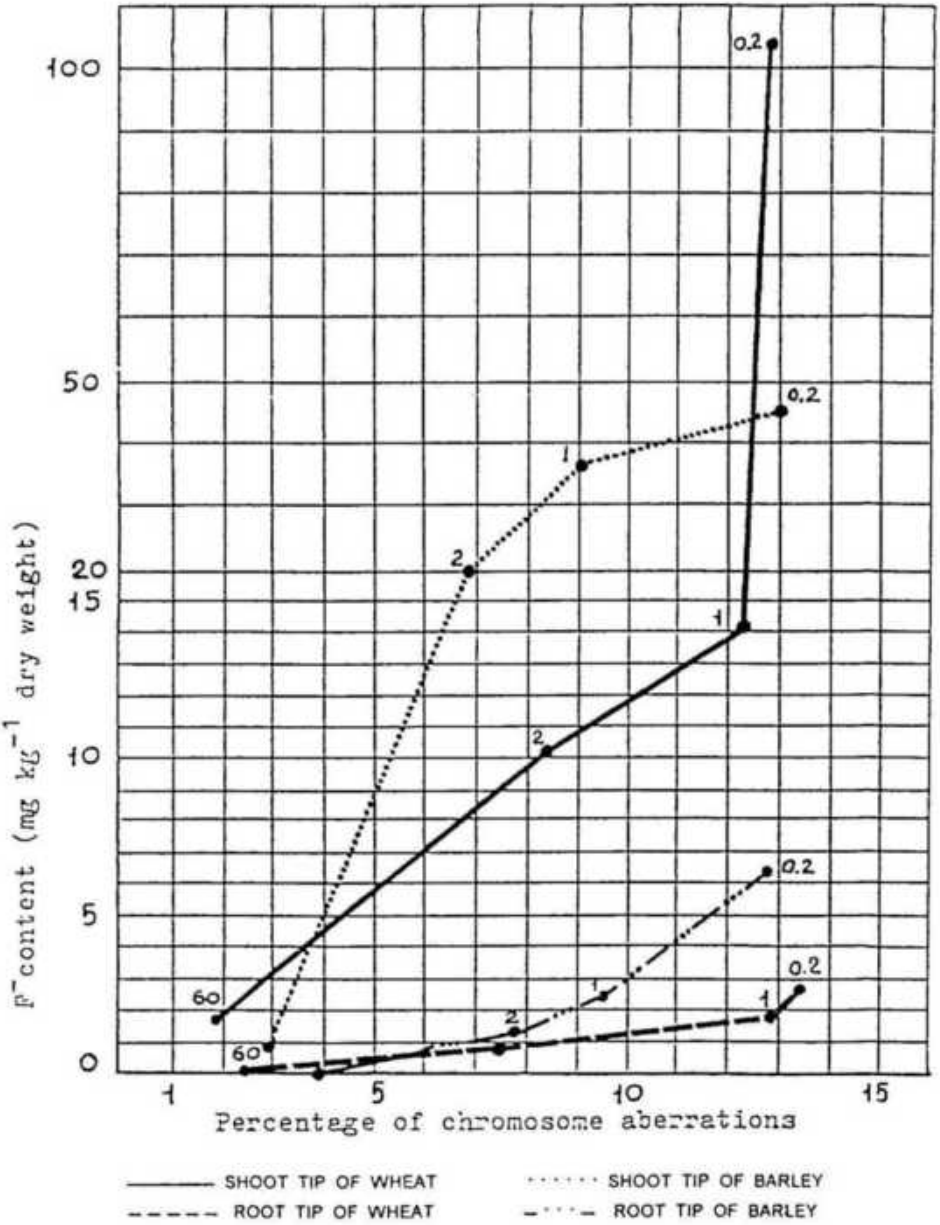
FIGURE 4. SPECTRUM OF CHROMOSOME ABERRATIONS IN SHOOT TIPS OF GRAIN CROPS



RADIUS IS 4%. ZERO IS THE CENTRE OF THE CIRCLE.

1 - TWIN FRAGMENTS 2 - SINGLE FRAGMENT 3 - PLURAL FRAGMENTS 4 - CHROMATID BRIDGE 5 - CHROMOSOMAL BRIDGE
6 - SEVERAL BRIDGES IN THE CELL 7 - BRIDGE(S) WITH FRAGMENT(S) 8 - OTHER ABERRATIONS

FIGURE 5.
RELATIONSHIP BETWEEN THE CHROMOSOME ABERRATIONS AND FLUORIDE CONTENT IN PLANTS

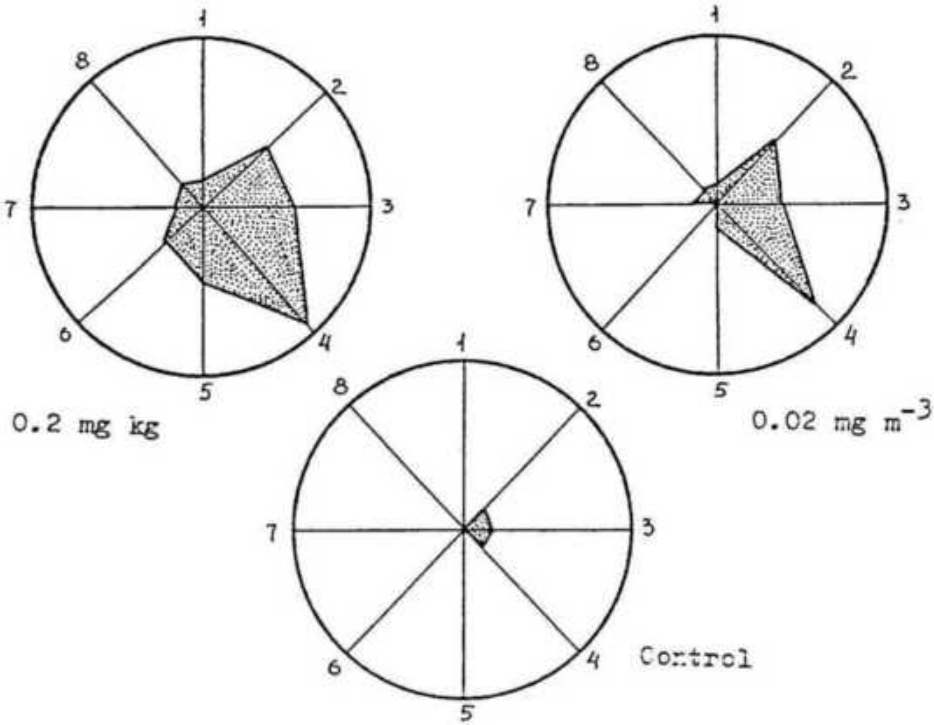


THE FIGURES 0.2, 1, 2, AND 60 ARE THE DISTANCE FROM THE PLANT IN KILOMOTERS

TABLE 2
FLUORIDE ACCUMULATION AND CYTOGENETIC EFFECT OF HF FUMIGATION ON BARLEY SEEDLINGS

HF concentration ($mg\ m^{-3}$)	F^- accumulation (mg/kg^{-1} dry weight)	No. of studied anaphases	Chromosomal aberrations	
			Number	%
0.2	25.3 ±1.11	1086	122	11.2 ±0.76
0.02	2.7 ±0.08	1115	58	5.2 ±0.08
Control	0.0	1112	13	1.2 ±0.03

FIGURE 6. SPECTRUM OF CHROMOSOME ABERRATIONS IN VEGETATIVE CONES OF BARLEY SEEDLINGS



RADIUS IS 4%. ZERO IS THE CENTRE OF THE CIRCLE.
1 - TWIN FRAGMENTS 2 - SINGLE FRAGMENT 3 - PLURAL FRAGMENTS
4 - CHROMATID BRIDGE 5 - CHROMOSOMAL BRIDGE 6 - SEVERAL BRIDGES IN THE CELL
7 - BRIDGE(S) WITH FRAGMENT(S) 8 - OTHER ABERRATIONS

Our calculations have indicated that the speed of the mutations in the meristems of winter wheat was on average 2.3 times lower than in spring barley. It should be emphasized that winter wheat has a 7-month growing season (without 2 winter months) whereas spring barley has a 3-month season. The percentage of chromosome aberrations in the wheat, however, is similar to that found in barley. Winter wheat is much more resistant than spring barley due to the hexaploidy of its genome (wheat has 42 chromosomes and barley has 14 chromosomes). It is evident that fluoride mutagenicity depends largely upon the plant species.

Results of laboratory experiments testing HF for its mutagenic activity by fumigation of barley seedlings in chambers are given in Table 2. The percentage of chromosome aberrations in apical cones of barley seedlings for the 0.2 mg/m^3 HF-treated groups was 9.3 times higher than in the control. HF fumigation induced not only a high mutation rate, but also alterations in the spectrum of chromosome aberrations (Figure 6). The HF-induced mutation rate was correlated linearly with dose of pollutant.

Barley seedlings are highly sensitive to mutagens in gaseous forms such as HF and may be used for the screening of mutagens and as cytogenetic monitors for chemical agents. Thus, gaseous fluorides (HF) resulting from industrial emissions are highly mutagenic for grain crops.

Plant responses to the widespread atmospheric pollutant, fluoride, have been documented in detail (6-9), but the mechanism of the mutations induced by HF is unknown. Several possibilities based on previous studies of researchers suggested the mechanism of HF mutagenicity was at the biochemical level, but further experimental studies are needed to elucidate the mechanisms involved.

Acknowledgement

The author is sincerely grateful to Dr G W Miller of Utah State University for his assistance in preparation of this manuscript.

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EFFECT OF CARBON TREATMENT ON AQUEOUS FLUORIDE DETERMINATIONS

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SUMMARY: Various aqueous samples (*e.g.*, tap water, tea infusions, coffee extracts, fruit and vegetable juices) were stirred with four kinds of laboratory grades of decolorizing carbon and analyzed for fluoride by the ion-selective electrode method. Depending on the nature of the sample and the type and activity of the carbon, small decreases in the fluoride content (generally on the order of 5 to 15 percent, but sometimes considerably more, especially with Norit-A (Alkaline) and Nuchar C-190N), were observed compared to the results without carbon treatment.

Keywords: Aqueous samples; Carbon treatment; Decolorizing carbon; Fluoride analysis.

Introduction

During the last several decades, concern over potential health hazards from increased sources and uses of fluoride has stimulated closer monitoring of fluoride levels in food and beverages (1). Unfortunately, the analysis of water-soluble ionic fluoride in such items is frequently complicated by the highly colored and oftentimes cloudy nature of the samples and extracts. Under these circumstances, the determination of fluoride by direct colorimetric methods is not feasible, but the fluoride content can usually be measured satisfactorily by the LaF_3 ion-selective electrode.

In this connection, we undertook a brief investigation of the effect of prior carbon treatment of water and beverage samples on the ion-selective fluoride analysis. It is, of course, well known that carbon mixed with alum (2), alumina (3), or phosphate bone minerals (3,4) has a strong tendency to remove fluoride from water. But, as far as we are aware, the extent to which laboratory grades of decolorizing carbon might alter the fluoride content of aqueous samples has not been studied in any comparative way.

Experimental Procedure

Fluoride determinations were performed in triplicate with an Orion Model 94-09 fluoride ion electrode and a Model 90-01 reference electrode connected to an Ion Analyzer, Model 407A, calibrated against standard 10.0, 1.00, and 0.100 ppm aqueous F^- solutions of sodium fluoride. All samples were first analyzed without carbon treatment.

Tea infusions and coffee extracts were prepared according to directions on the product labels. For example, in the case of tea, approximately 2.5 g of the leaves were steeped for 3 to 5 min in 250 mL of hot (80-90°C) tap water.

For each determination, a 5.0-mL portion of the aqueous sample was mixed with 5.0 mL of TISAB (Total Ionic Strength Adjustment Buffer containing 1,2-cyclohexylenedinitrilotetraacetic acid to prevent interference by aluminum ions). Readings were taken with the electrodes immersed into the buffered sample in a small plastic beaker and became constant after about 7 min. This amount of time was therefore used for each determination.

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Presented at the XIXth Conference of the International Society for Fluoride Research, Kyoto, Japan, September 8-11 1992, and to the Analytical Division at the 27th Midwest Regional Meeting of the American Chemical Society, the University of Kansas, November 4-6 1992.

After measurement of the fluoride concentration, 50-mL portions of the sample were stirred magnetically for 2 min at room temperature (20-25°C) with the following brands and amounts of decolorizing carbon that had been heated previously under reduced pressure (20 mm/Hg) for 24 hr at 110°C:

Norit RO 0.8 Pellets (1.0 g)
Norit-A (Neutral) (0.5 g)
Norit-A (Alkaline) (0.5 g)
Nuchar C-190N (0.5 g)

When mixed with distilled water having a pH of 5.2, these mildly activated carbons, in the order listed, changed the pH to 7.7, 4.9, 7.9, and 6.7, respectively. When heated to 400°C for 15 min in an open dish, the carbons produced comparable pH readings in distilled water, except Norit-A (Neutral), which then raised the pH to 7.5.

Before the actual fluoride determination, the carbon was separated from the slurry by aspirator suction through two pieces of 4.25-cm diameter Whatman No. 1 filter paper that had been pre-rinsed with distilled water. A 5.0-mL portion of the filtrate was then analyzed for fluoride as already described.

The paper used in the above filtration was found to introduce at most only about 0.01 ppm fluoride into the filtrate. However, filtration of the samples or the carbon slurries through filter aid (Hyflo Supercel or Celite), even when pre-washed, did introduce appreciable amounts of exogenous fluoride and was therefore avoided.

Results and Discussion

Changes in the fluoride concentration of typical aqueous samples after treatment with the carbon activated at 110°C are summarized in the Table and in the Figures. Interestingly, the initial fluoride readings after carbon treatment were sometimes considerably lower than those reached after several minutes, suggesting that fluoride ions were being released from metal complexes (most probably from aluminum) by the TISAB reagent.

Except for the very low fluoride bottled water samples treated with Nuchar C-190N, all carbon treatments resulted in reduced fluoride levels. The largest decreases were produced by Norit-A (Alkaline), and, when this occurred, to a lesser extent by Nuchar C-190N. One notable exception was the local (Lawrence, Kansas) fluoridated tap water, on which Norit-A (Neutral) had the greatest effect. As seen in the Table, this same pattern was also observed with the carbons activated at 400°C (tested only on the tap water).

Besides the items in the Table, a number of other beverages such as carbonated drinks, beers, wines, and various brand-name bottled waters were examined but less systematically. The results were similar to those recorded in the Table and illustrated in the Figures.

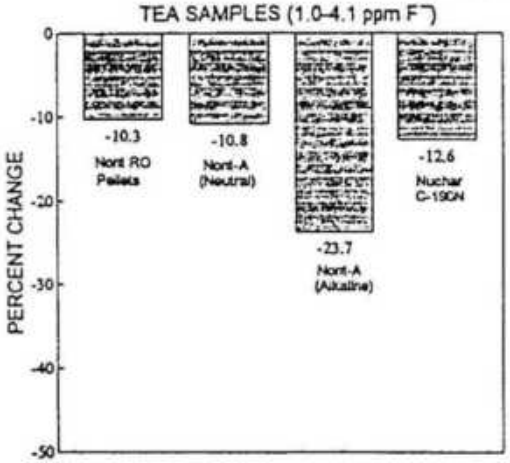
Conclusion

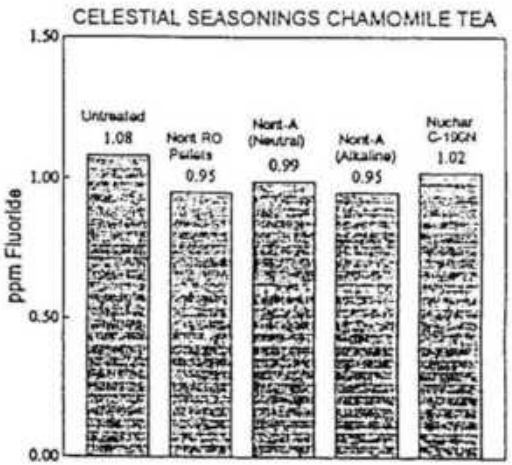
This work has shown that four kinds of decolorizing carbon commonly used in the laboratory can appreciably reduce the fluoride concentration in a wide variety of aqueous samples, including tap water, tea infusions, coffee extracts, fruit and vegetable juices, beers, wines, and carbonated beverages.

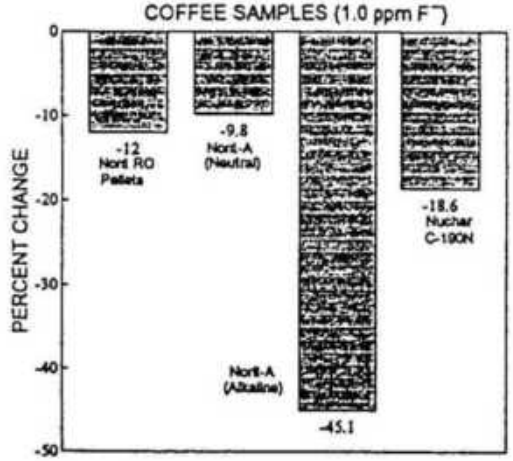
Acknowledgements

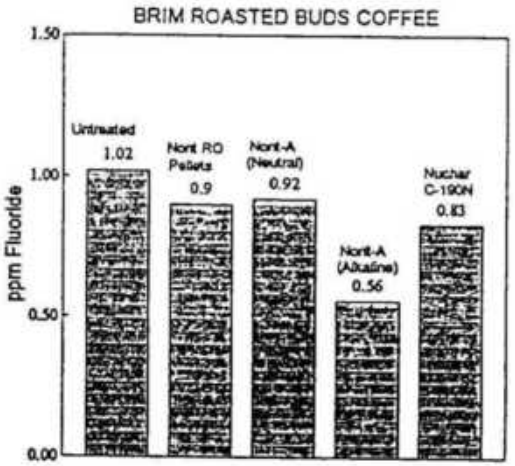
We thank the University of Kansas for support (to J.S.L.) from the General Research Fund and Kok Thai Yoong for assistance in revising the Figures.

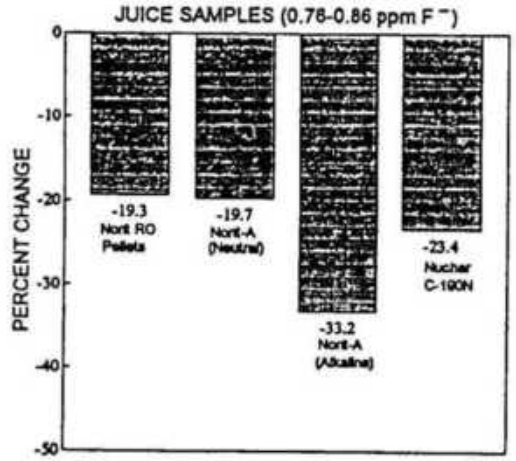
FIGURES. RESULTS OF 110°C-ACTIVATED CARBON TREATMENT ON AQUEOUS FLUORIDE DETERMINATIONS
(Tea and coffee samples prepared with 1.0-ppm fluoridated water)











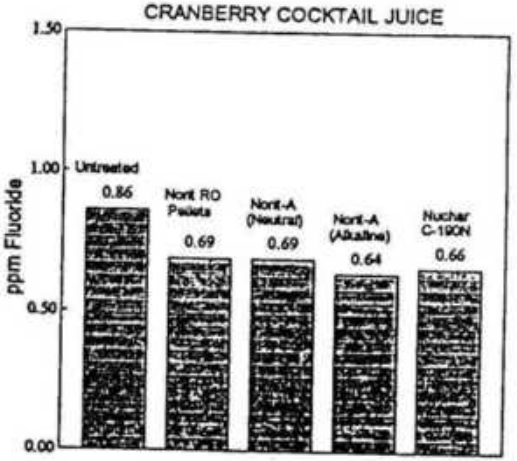


TABLE. Fluoride concentration (ppm)[a] of aqueous samples

Sample	Untreated	110°C-Activated Carbon Treated			
		Norit RO Pellets	Norit-A Neutral	Norit-A Alkaline	Nuchar C-190N
Tea infusions (tap water)					
Jasmine Spice	1.65	1.52	1.51	1.42	1.57
Sassafras (Red Mountain)	1.11	1.01	0.98	0.96	1.03
Chamomile (Celestial Seasonings)	1.08	0.95	0.99	0.95	1.02
Blackberry (Celestial Seasonings)	1.10	0.98	0.94	0.56	0.81
Lipton Temple of Spice	1.06	0.94	0.92	0.58	0.82
Lipton Iced Tea Mix	2.49	2.01	2.01	1.49	1.89
Royal House	4.11	3.96	4.00	3.90	4.04
Royal House (dist. water)	3.38	3.10	3.10	3.02	3.10
Coffee brew (tap water)					
Brim Roasted Buds	1.02	0.90	0.92	0.56	0.83
Juice Beverages					
Cranberry Cocktail	0.86	0.69	0.69	0.64	0.66
Gatorade	0.76	0.62	0.61	0.45	0.58
Water					
Hinckley and Schmidt (bottled water)	0.10	0.094	0.092	0.082	0.11
Lawrence municipal (tap water)	0.95	0.87 0.84[b]	0.72 0.72[b]	0.82 0.80[b]	0.86 0.86[b]

[a] Means of triplicate determinations.
[b] After treatment with 400°C-activated carbon.

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BONE STRUCTURE ASSESSMENT ON RADIOGRAPHS OF DISTAL RADIAL METAPHYSIS IN CHILDREN WITH DENTAL FLUOROSIS

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Lodz and Cracow, Poland

SUMMARY: The effect of fluoride on bone structure was assessed in children with endemic dental fluorosis by use of radiographs of the distal radial metaphysis of test children and those of unfluoridated age-matched controls. Bone structure assessment was based on the computerized image analysis of the radiographs. In addition, the quantitative description of bone structure was analyzed in terms of correlation with age, sex, and serum calcium, magnesium, and alkaline phosphatase levels. Our findings indicate greater trabecular height and area in children with dental fluorosis than in controls. Detailed analysis of the results, in relation to age and gender, showed that the significant differences were between younger age groups and between boys. This finding indicates a stronger influence of fluoride on children of younger developmental age.

Key words: Bone; Dental fluorosis; Fluoride; Image analysis; Mottled enamel.

Introduction

Assessment of fluoride influence on bone in children is both interesting and difficult. Fluoride exerts an effect on bones during the period of fast growth and the continuous remodelling of bone structure (1-3). In this study we analyzed bone structure of children with endemic fluorosis and of non-fluoridated age-matched children, using computerized image analysis which allowed quantitative evaluation of bone structure differences.

Materials and Methods

The investigation involved 43 children aged 11 to 15 years who had lived since birth in the village of Blaszki near Sieradz, an area in which drinking water contained naturally occurring fluoride at 2.7 mg/liter. Mottled enamel was evident in all children of this group. An age-matched control group of children without evident dental fluorosis was chosen from an area with water fluoride < 0.1 mg/liter. Clinical evaluation of the children of both groups was otherwise unremarkable. The children were divided into two sub-groups: AG-I, aged 11-12 years (average 11.8) and AG-II, aged 13.5-15.0 years (average 14.6). In both sub-groups, children were also identified by gender.

A radiograph of the left distal radial metaphysis of each child was used for bone structure assessment. High-quality film (Roentgen XS) with a TUR 1000 apparatus was used. Processing was standardized in terms of constant composition of reagents and development time (5 minutes at 20°C). All radiographs were taken and processed by the same technician.

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[2] Department of Orthopaedics, Cracow Academy of Medicine, Poland.

Bone structure evaluation was based on computerized radiograph image analysis with a VFG-512-8BC (Visionetics) analyzer with resolution power of 512 x 512 pixals and 256 grey levels. Optical density was measured at 128 points in the middle of the metaphysis. The distance between these points was 0.096 mm. Microdensitometric curves obtained this way were the subject of computer analyses carried out according to the program worked out by Czerwinski (4). This program defined "radiologic trabeculae" and provided the following quantitative description of bone structure: the number of trabeculae, their width, height, area, and trabeculation density. Trabecular width is given in millimetres and density in the percentage of the cross-section enclosed by the trabeculae. Trabecular height is expressed as a percentage of the minimum and maximum of the optical density on the given radiograph. Trabecular area is expressed as a percentage of the area enclosed by the whole microdensitometric curve. The compatibility of the results of the analysis was checked together with the diagram of the curve and the diagram, in turn, with the radiologic image of the evaluated area.

In all children with dental fluorosis (DF) and in 34 children of the control group (CG), the following blood tests were performed:
- serum calcium concentration (Ca) by the method of Kovacs and Tarnoky (5);
- serum magnesium concentration (Mg) by the Lachen firm's photocolorimetric method (6);
- serum alkaline phosphatase activity (AP) by the method of King and Armstrong.

Statistical differences between the groups were assessed by variance analysis and test F according to Fisher-Snedecor. Differences of $p < 0.05$ were regarded as significant. In addition, linear correlation coefficients (r) by Pearson (significant at $p < 0.05$) were determined among some variables (7,8). Statistical calculations were performed by the Institute of Sociology of the University of Lodz.

Results

The results of analyzed bone structure parameters are included in Table 1. Children with DF were found to demonstrate a statistically significant greater trabecular height and area (averaged and complete) than the control group (CG) children. No significant differences were found for trabecular number, width, or density.

When the age and gender sub-groups were compared, it was found that the significantly greater trabecular height and area occurred only in DF boys in AG-I. No significant trabecular differences were found in subgroup AG-II or among girls (Tables 2 and 3). Figures 1 and 2 show microdensitometric curves of digitalized radiographs (including the relative diagram of radiologic trabeculae) in a child with DF and from the control group. As Table 4 shows, the average serum calcium was higher and the alkaline phosphatase activity and serum magnesium were lower among DF children than CG children, though both sets of Ca and AP values were in the normal range and only the Mg value of DF children was slightly less than the lower limit of normal range. Table 5 contains linear correlation coefficients and their levels of significance calculated between bone structure parameters and concentration of Ca, Mg and AP blood levels. Statistically significant correlation coefficients were noted only in the DF group where the increases of trabecular height and area correlated with lower serum calcium and higher alkaline phosphatase activity levels. The detailed analysis of differentiation between groups which could suggest a direct influence of fluoride on specific ions and enzymes is not, however, the subject of this report.

TABLE 1. Results of bone structure analysis in children with dental fluorosis (DF) and in the control group (CG).

		No. of trabeculae	Width	Height	Area av.	Area complete	Density
			mm	%	%	%	%
DF	x	17.7	0.42	26.9	0.59	10.3	59.6
n=43	(SD)	(1.6)	(0.03)	(4.9)	(0.12)	(1.9)	(2.6)
CG	x	17.2	0.42	24.2	0.54	9.3	58.9
n=75	(SD)	(1.9)	(0.04)	(5.1)	(0.1)	(2.2)	(3.1)
	F	1.96	0.78	8.03	4.28	5.98	1.58
	p	0.16	0.38	0.005	0.04	0.02	0.21

TABLE 2. Statistical analysis of bone structure assessment in age groups with dental fluorosis (DF) and control group (CG). (AG-I average age 11.8 yrs. AG-II average age 14.6 yrs)

		No. of trabeculae	Width	Height	Area av.	Area complete	Density
			mm	%	%	%	%
AG-I							
DF	x	17.4	0.42	27.4	0.61	10.5	59.7
n=22	(SD)	(1.4)	(0.03)	(5.4)	(0.12)	(2.1)	(2.9)
CG	x	16.9	0.43	23.2	0.53	8.8	58.7
n=34	(SD)	(2.2)	(0.05)	(3.9)	(0.1)	(8.8)	(3.3)
	F	0.79	0.21	11.38	6.78	9.25	1.61
	p	0.38	0.65	0.001	0.01	0.004	0.23
AG-II							
DF	x	17.9	0.41	26.4	0.57	10.05	59.6
n=21	(SD)	(1.8)	(0.04)	(4.2)	(0.13)	(1.72)	(2.9)
CG	x	17.3	0.42	25.03	0.55	9.6	59.6
n=41	(SD)	(1.7)	(0.03)	(5.8)	(0.13)	(2.51)	(2.9)
	F	1.44	0.86	0.90	0.34	0.57	0.40
	p	0.23	0.36	0.35	0.56	0.45	0.53

TABLE 3. Results of statistical analysis of bone structure assessment in boys and girls with dental fluorosis (DF) and in control group (CG).

		No. of trabeculae	Width	Height	Area av.	Area complete	Density
			mm	%	%	%	%
BOYS							
DF	x	17.4	0.43	28.9	0.64	11.0	59.6
n=22	(SD)	(1.5)	(0.03)	(4.4)	(0.12)	(2.2)	(2.9)
CG	x	17.2	0.43	24.8	0.55	9.5	59.0
n=38	(SD)	(2.1)	(0.05)	(5.2)	(0.1)	(2.2)	(2.9)
	F	0.24	0.05	9.82	8.56	7.67	0.55
	p	0.63	0.82	0.003	0.005	0.007	0.46
GIRLS							
DF	x	17.9	0.41	24.8	0.54	9.5	59.7
n=21	(SD)	(1.7)	(0.04)	(4.5)	(0.11)	(1.7)	(2.6)
CG	x	17.2	0.42	23.6	0.53	9.1	59.8
n=37	(SD)	(1.8)	(0.03)	(5.0)	(0.1)	(2.2)	(3.3)
	F	2.39	1.33	0.87	0.02	0.47	1.02
	p	0.13	0.25	0.36	0.90	0.90	0.31

All Tables: F = value of the Fischer-Snedecor Test. p = level of statistical significance

FIGURE 1A.
RADIOGRAPH OF DISTAL RADIAL METAPHYSIS OF 11.5-YR-OLD BOY WITH DENTAL FLUOROSIS

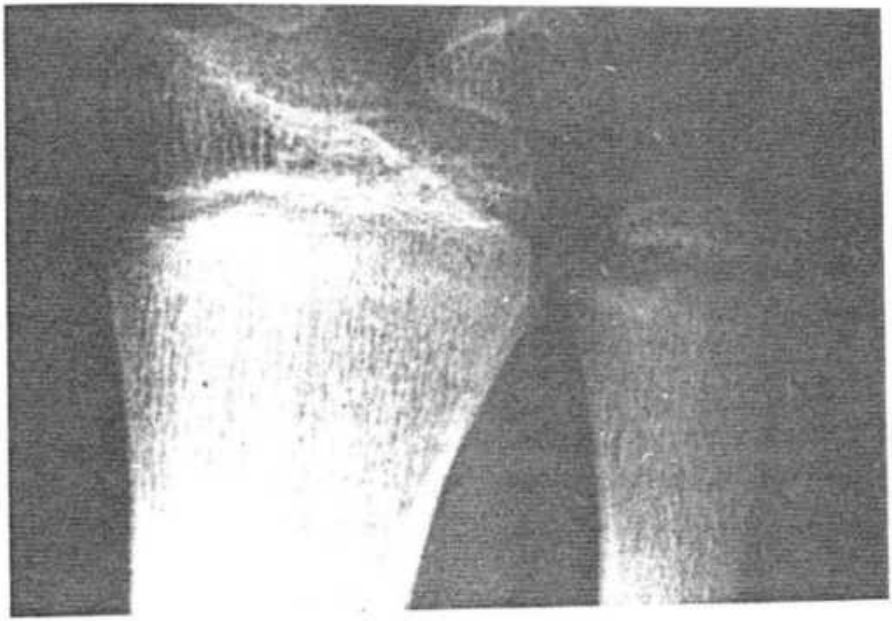

FIGURE 1B.
MICRODENSITOMETRIC CURVE (ABOVE) AND DEFINED TRABECULAE IN RECTANGLES (BELOW)
BONE STRUCTURE ANALYSIS: NO. OF TRABECULAE 19; AV. WIDTH 0.42 MM; AV. HEIGHT 37.5%
AV. AREA 0.80%; COMPLETE AREA 15.1%; DENSITY 65.6%.

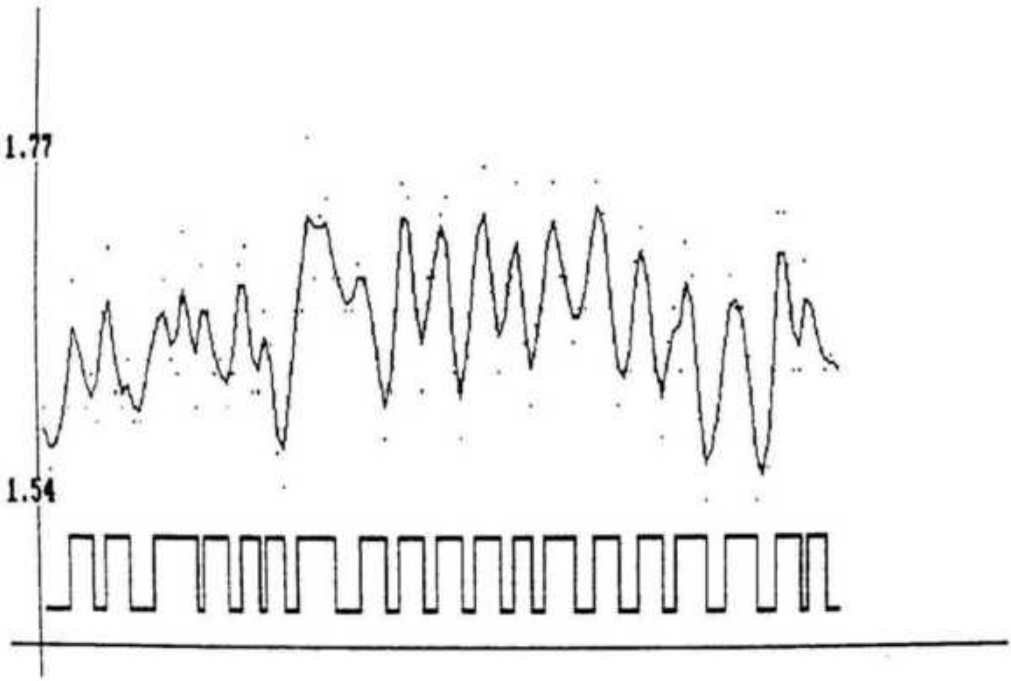

FIGURE 2A.
RADIOGRAPH OF DISTAL RADIAL METAPHYSIS OF 14-YR-OLD BOY FROM THE CONTROL GROUP

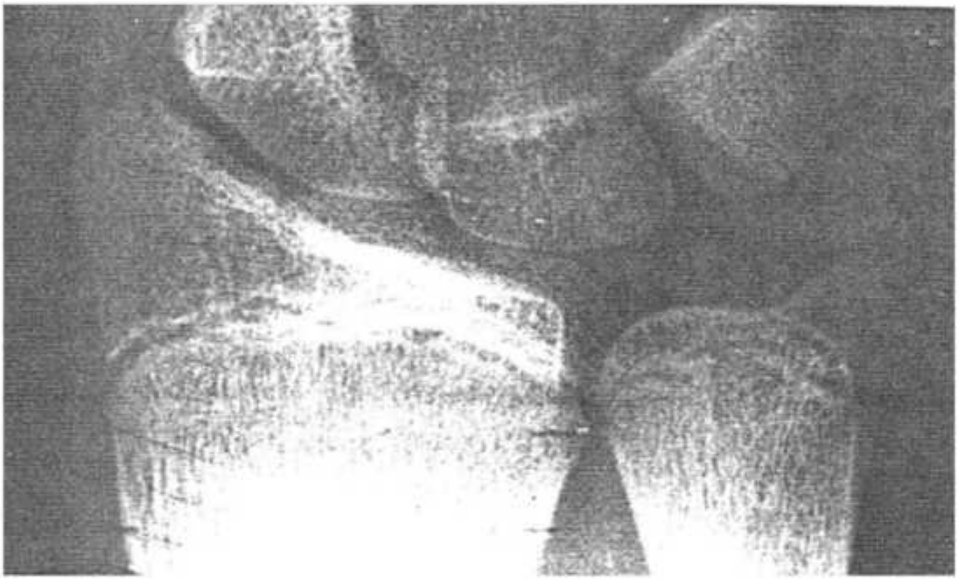

FIGURE 2B.
MICRODENSITOMETRIC CURVE (ABOVE) AND DEFINED TRABECULAE IN RECTANGLES (BELOW).
BONE STRUCTURE ANALYSIS: NO. OF TRABECULAE 17; AV. WIDTH 0.41 MM; AV. HEIGHT 17.24%;
AV. AREA 0.34%; COMPLETE AREA 5.7%; DENSITY 57.03%.

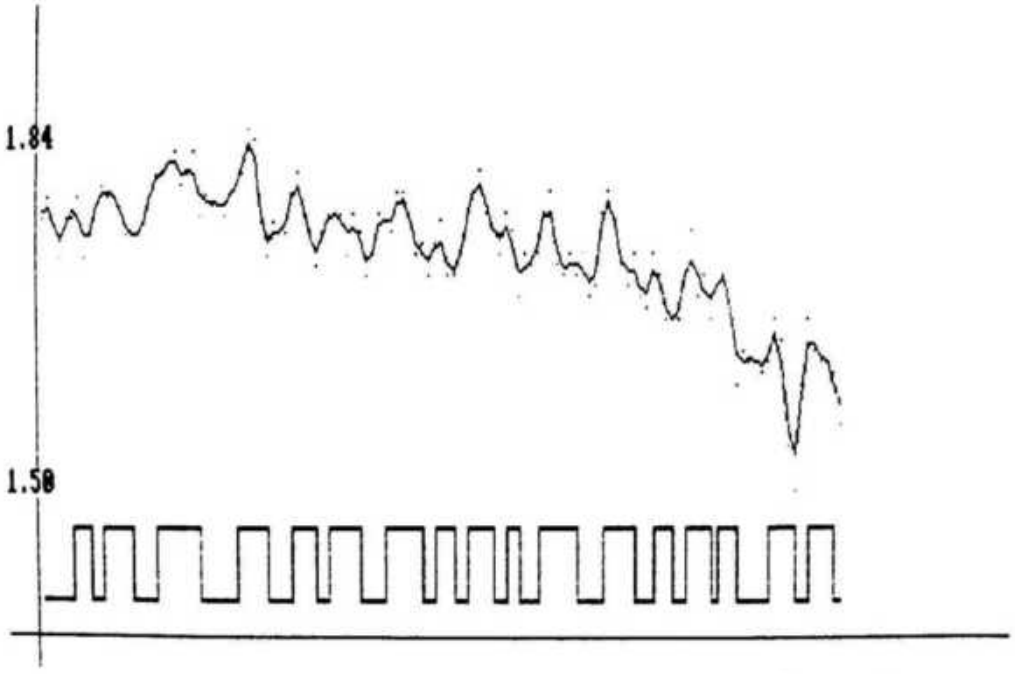

TABLE 4. Statistical analysis of serum calcium (Ca), magnesium (Mg) and alkaline phosphatase (AP) in children with dental fluorosis (DF) and in the control group (CG)

		Ca	Mg	AP
	normal =	2.24-2.61 (mmol/l)	0.78-1.19 (mmol/l)	59.5-193.5 (UI)
Group	No.	x (SD)	x (SD)	x (SD)
DF	43	2.48 (0.12)	0.73 (0.06)	107.9 (41.8)
CG	34	2.39 (0.11)	0.81 (0.05)	154.2 (77.9)
	F	13.0	16.8	12.4
	p	0.001	0.001	0.001

(F = value of the Fischer-Snedecor Test. p = level of statistical significance)

TABLE 5. Linear correlation coefficients (r) and their level of statistical significance (p) of bone structure parameters with some biochemical tests in children with dental fluorosis (DF) and in the control group (CG)

Analyzed parameters		Ca		Mg		AP	
	Group	r	(p)	r	(p)	r	(p)
No. of trabeculae	DF	0.366	(0.009)	0.335	(0.016)	-0.156	(0.159)
	CG	0.171	(0.197)	0.280	(0.057)	0.188	(0.143)
Width	DF	-0.348	(0.012)	-0.298	(0.029)	-0.213	(0.085)
	CG	-0.092	(0.302)	-0.254	(0.077)	-0.154	(0.193)
Height	DF	-0.285	(0.034)	-0.286	(0.051)	0.354	(0.010)
	CG	0.008	(0.483)	0.065	(0.360)	0.109	(0.270)
Average area	DF	-0.405	(0.004)	-0.315	(0.022)	0.343	(0.012)
	CG	-0.155	(0.191)	-0.018	(0.461)	-0.077	(0.333)
Complete area	DF	-0.314	(0.021)	-0.242	(0.063)	0.356	(0.010)
	CG	-0.008	(0.482)	0.146	(0.208)	0.051	(0.388)

Discussion

Previous histomorphometric examinations have shown that fluoride causes an increase of mineralized bone mass by the increase of trabecular volume and width. This is shown by the increased absorption of radiologic radiation which increases the density shadow on radiographs (9). Because of the relatively small fluoride dosage of the children in this study, we expected to find only the initial changes of bone structure. For this evaluation, we used a computer image analysis of distal radial metaphysis to quantify bone structure parameters.

Comparing children with dental fluorosis to an age-matched control group, we found statistically significant increases in trabecular height and area in children with dental fluorosis. In addition, mean serum calcium was increased but alkaline phosphatase and serum magnesium were decreased in the DF group compared to controls. However, within the DF group, individual case analysis found a negative correlation between serum calcium and trabecular height, area and width. The decrease in alkaline phosphatase activity correlated with a decline in trabecular height and area.

Czerwinski has shown that an increase in trabecular height and area is proof of an increase in bone mineral content (10). The same process is likely to occur in children with dental fluorosis. An increase of bone mineralization could be the consequence of retarded bone resorption due to formation of less soluble fluorapatite and osteoclastic activity inhibition by fluoride ions (11-13). The decreased AP activity in children with DF indicates lower metabolic turn-over in the bone and its growth retardation (14). The statistically significant positive correlation between calcium concentration and the number of trabeculae, and the negative correlations in reference to the remaining bone structure parameters, found in children with dental fluorosis, show the slight bone structure disturbances found in this group - probably the result of the relatively small fluoride doses. It has previously been estimated that initial skeletal changes begin when the concentration of fluoride in drinking water is 8 mg/liter (15,16).

The finding of greater trabecular height and area of trabeculation occurred only in younger boys with DF (average age 11.8 years) and not among the older children (average age 14.6 years) nor among girls of both groups. This would suggest the greater influence of fluoride on the mineralization process in the earlier period of development (AG-I and boys). Boys at this time of their life are always in a younger development stage than girls (17). Similar results, from experimental examinations on rats by Bialas (18), show that the earlier the development period, the stronger is the influence of fluoride. The above suggestions agree with the results of earlier examinations which showed that an excess of fluoride in a child may inhibit growth, especially in younger boys (19).

Conclusions

With this technique of evaluating the bone structure of the distal radial metaphysis, significantly greater height and area of trabeculae were found in children with dental fluorosis than in the control group. These differences were not observed for number and width of trabeculae. The results indicate the effects of chronic supraoptimal doses of fluoride in increasing bone tissue mineralization. This fluoride effect is stronger in younger children.

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BENEFICIAL EFFECTS OF ASCORBIC ACID AND CALCIUM ON REVERSAL OF FLUORIDE TOXICITY IN MALE RATS

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Ahmedabad, India.

SUMMARY: The present study was undertaken to investigate the therapeutic effects of simultaneous ascorbic acid and calcium ingestion along with sodium fluoride (NaF) treatment on the structure and metabolism of vital organs like liver, adrenal, gastrocnemius muscle and serum parameters of male rats. The decrease in muscle and serum proteins suggested inhibition of protein synthesis by fluoride and a possible change in osmotic balance. Alterations in the activities of succinate dehydrogenase (SDH), adenosine triphosphatase (ATPase) and cholinesterase (ChE) in gastrocnemius muscle elucidate disturbances in its oxidative metabolism, neuromuscular transmission and contractility. The accumulation of glycogen in liver and muscle indicate reduction in the activities of enzymes of glycolytic pathway. The total ascorbic acid in liver and adrenal were enhanced to overcome the stress imposed by the treatment. However, the fluorotic rats did not suffer from hypercholesterolemia. The levels of Na^+ and K^+ in serum showed a significant increase, thus suggesting alteration in the electrolyte balance of the body due to fluoride intake. The liver of fluoride treated rats revealed zonal necrosis and pycnosis of nucleus of the hepatocytes. The enhanced levels of serum transaminases, which are considered as markers for liver structure and function, also support this observation. The altered histology of the adrenal gland is related to adrenal hypofunction in NaF treated rats. However, simultaneous administration of ascorbic acid (AA) and calcium (Ca^{+2}) to the NaF treated rats revealed marked recovery from fluoride toxicity in all the parameters. The recovery was more pronounced in the NaF + AA treated rats than in NaF + Ca^{+2} treated ones. We consider the data of the present study are significant since the beneficial therapeutic effects of AA and Ca^{+2} in overcoming and reversing fluoride toxicity have far reaching implications the world over.

Key Words: Ascorbic acid; Calcium; Rat; Sodium fluoride; Soft tissues.

Introduction

Fluoride is a cumulative poison under conditions of continuous exposure to sub-acute doses. Thus the absorbtion of relatively small quantities of fluoride causes chronic intoxication. Earlier work fom this laboratory has revealed that administration of low doses of sodium fluoride (NaF) to mice and rats altered the structure and function of some of their soft tissues and reproductive organs (1-5). Fluoride treated rabbits manifested the same changes (6). A block in the conversion of glycogen to glucose occurred in liver, muscles and vas deferens of NaF treated rodents which might be related to the decreased activity of phosphorylase but enhanced catecholamines (1).

The fluoride-induced changes were transient and reversible by withdrawal of treatment (7) and by feeding ascorbic acid (AA) and/or calcium (Ca^{+2}) during the withdrawal period or simultaneously with NaF (6,8,9). Hence fluoride-induced toxicity was overcome by these therapeutic treatments, wherein combined

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AA + Ca^{+2} ingestion manifested a synergistic effect (6,8). Therefore, the present study was designed to investigate further the therapeutic effects of AA + Ca^{+2}, administered simultaneously to fluoride ingesting rats, on some soft tissues and their metabolism in the light of earlier data.

Materials and Methods

Healthy prepubertal male rats (23-25 days old) of Charles Foster Strain (*Rattus norvegicus*) weighing about 45-55 gm were used for the study. The animals were maintained on a standard chow and water was given *ad libitum*. The animals were categorized in four groups and treatments were given as shown in Table 1.

TABLE 1. SUMMARY OF TREATMENT

Group	Treatment	No. of animals	Duration of treatment	Autopsy day
I	Control	20	–	Sacrificed along with treated groups
II	NaF (10 mg/kg body wt./rat/day)	20	30 days	31st day
III	NaF + AA (10 mg NaF/kg body wt./rat/day + 50 mg AA/animal/day)	20	30 days	31st day
IV	NaF + Ca^{+2} (10 mg NaF/kg body wt./rat/day + 62.5 mg Ca^{+2}/day/animal)	20	30 days	31st day

After the respective treatments, the animals were weighed and sacrified by cervical dislocation. The liver, adrenals and gastrocnemius muscle were excised, blotted free of blood and utilized for various parameters. Fresh blood was collected from the heart and serum was separated and used for biochemical estimations. The parameters studied were:

1. *Whole body weight*: Normal and treated animals were weighed before sacrificing and their body weights were recorded to the nearest gram.
2. *Protein levels in muscle and serum*: Estimated by the method of Gornall *et al* (10) and expressed as mg/100 mg fresh tissue weight and mg/100 ml serum respectively.
3. *Glycogen*: Levels in muscle and liver were determined by the method of Seifter *et al* (11). The concentration of glycogen was expressed as μg/100 mg fresh tissue weight.
4. *Succinate dehydrogenase* (SDH) *in muscle*: Activity was assayed by the modified terazolium reduction method of Beatty *et al* (12) and expressed as μg formazan/30 minutes/100 mg tissue weight.
5. *Adenosine triphosphatase* (ATPase): Activity in gastrocnemius muscle was determined by the method of Quinn and White (13) and expressed as μ moles of ip released/mg/30 minutes.

6. *Cholinesterase* (ChE): The activity in muscle was estimated by the method of Huerga *et al* (14) and expressed as activity of ChE/100 mg fresh tissue weight.
7. *Ascorbic acid*: Levels in liver and adrenal gland were determined by the method of Roe and Küther (15). The concentrations were expressed as mg ascorbic acid/gm fresh tissue weight.
8. *Cholesterol*: Estimation in serum was carried out by the method of Pearson *et al* (16) and expressed as mg/ml serum.
9. *Serum glutamate pyruvate transaminase* (SGPT) *and serum glutamate oxaloacetate transaminase* (SGOT): Activities were assayed by the method of Reitman and Frankel (17) and expressed as mU/ml serum.
10. Na^+ *and* K^+ *levels in serum*: were estimated by using the Systronics Flame Photometer, Digital Unit Type 125, according to the method of Dean (18) and espressed as ppm.
11. *Histology of the liver and adrenal gland*: was carried out by the standard haematoxylene-eosin staining.
12. *Histochemistry*: The histochemical localization of muscle SDH and sudanophilic lipids were carried out by the methods of George and Talesara (19) and Baker (20) respectively.

Results

Body weight

The total body weight of rats treated with NaF for 30 days showed a decrease compared to the control. The body weights of NaF + AA treated rats were significantly recovered compared to the NaF alone treated ones ($P < 0.001$). The recovery in body weights of NaF + Ca^{+2} treated rats was less ($P < 0.02$) compared to that obtained by ascorbic acid (Table 2).

TABLE 2. BODY WEIGHTS OF CONTROL AND EXPERIMENTAL RATS

PARAMETER	CONTROL GROUP	SODIUM FLUORIDE ALONE (30 days)	SODIUM FLUORIDE + ASCORBIC ACID (30 days)	SODIUM FLUORIDE + CALCIUM (30 days)
Body weight (Grams)	152.14 ±4.49	139.28 ±8.51	149.23 ±4.84	141.28 ±3.99

Values are mean ± S.E. * $P < 0.001$

Succinate dehydrogenase in muscle

The SDH activity in muscle showed significant decrease with NaF treatment ($P < 0.001$). However, a significant recovery occurred with AA and Ca^{+2} treatments ($P < 0.001$). The recovery was more by AA feeding than by Ca^{+2} (Table 3).

Adenosine Triphosphatase

ATPase activity in gastrocnemius muscle of NaF fed rats was decreased significantly ($P < 0.001$) in comparison with control. On the other hand, significant recovery ($P < 0.001$) was observed in AA and Ca^{+2} treated groups (Table 3).

Cholinesterase

The ChE activity in muscle was significantly enhanced with NaF treatment. While the enzyme activity showed significant recovery with NaF + AA and NaF + Ca^{+2} treatments ($P < 0.001$) (Table 3).

Protein

The protein levels of muscle were decreased by NaF treatment ($P < 0.01$). But a significant recovery was observed by ascorbic acid ($P < 0.001$) as well as NaF + Ca^{+2} combined treatments (Table 3).

Glycogen

The glycogen concentration in liver and muscle were enhanced significantly in NaF fed rats, whereas, a significant recovery occurred by both NaF + AA ($P < 0.001$) and NaF + Ca^{+2} treatments (Table 4).

TABLE 3.

THE ACTIVITIES OF SUCCINATE DEHYDROGENASE (SDH), ADENOSINE TRIPHOSPHATASE (ATPase) CHOLINESTERASE (ChE) AND PROTEIN IN GASTROCNEMIUS MUSCLE OF CONTROL AND EXPERIMENTAL RATS

PARAMETER	CONTROL GROUP	SODIUM FLUORIDE ALONE (30 days)	SODIUM FLUORIDE + ASCORBIC ACID (30 days)	SODIUM FLUORIDE + CALCIUM (30 days)
SDH (µg formazan /100 mg fresh tissue wt./30 min)	383 ± 8.07	215.6 ± 7.24*	348 ± 13.01*	302 ± 9.16
ATPase (µ moles of ip released/100 mg fresh tissue wt./30 min)	19.20 ± 0.13	12.22 ± 0.42*	18.72 ± 0.58*	18.28 ± 0.15*
Cholinesterase (activity/100 mg fresh tissue wt./hr)	1.37 ± 0.07	3.08 ± 0.11*	2.16 ± 0.07*	2.70 ± 0.50
Protein	16.35 ± 2.05	11.15 ± 0.89**	15.63 ± 0.28*	15.48 ± 0.89

Values are mean ± S.E. * $P < 0.001$ ** $P < 0.01$

TABLE 4

LEVELS OF GLYCOGEN IN GASTROCNEMIUS MUSCLE AND LIVER AS WELL AS ASCORBIC ACID IN LIVER AND ADRENAL GLAND OF CONTROL AND EXPERIMENTAL RATS

PARAMETER	ORGAN	CONTROL GROUP	SODIUM FLUORIDE ALONE (30 days)	SODIUM FLUORIDE + ASCORBIC ACID (30 days)	SODIUM FLUORIDE + CALCIUM (30 days)
GLYCOGEN (µg/100 mg tissue wt.)	Muscle	327 ± 14.52	735 ± 6.21*	536 ± 26.79*	659 ± 10.56*
	Liver	1282 ± 14.50	1501 ± 19.92*	1289 ± 41.41*	1386.9 ± 38.87
ASCORBIC ACID (mg/gm fresh tissue wt.)	Liver	5.42 ± 0.24	7.66 ± 0.09*	6.30 ± 0.18	7.14 ± 0.05
	Adrenal	2.97 ± 0.16	5.54 ± 0.13*	3.58 ± 0.70*	3.84 ± 0.54*

Values are mean ± S.E. * $P < 0.001$

Total ascorbic acid

The total ascorbic acid in liver was significantly increased in NaF treated rats ($P < 0.001$). An insignificant recovery was observed in NaF + AA treated animals. In adrenal gland also, the total AA levels were increased significantly while in NaF + AA and NaF + Ca^{+2} treated rats a significant recovery was observed ($P < 0.001$) (Table 4).

Serum parameters

Protein: serum levels showed a significant decline with NaF treatment ($P < 0.001$), while a more significant recovery was observed with AA ($P < 0.001$) than with Ca^{+2} treatment (Table 5).

Cholesterol: levels in serum remained insignificantly affected after NaF treatment. The combined NaF + AA and NaF + Ca^{+2} treatments resulted in a decline in comparison to both control as well as NaF alone treated rats (Table 5).

Serum glutamate pyruvate transaminase

The SGPT levels were significantly increased by NaF treatment ($P < 0.001$) compared to control. The recovery was more significant with AA than with Ca^{+2} (Table 5).

Serum glutamate oxaloacetate transaminase

The SGOT levels showed a significant increase in NaF treated rats ($P < 0.001$), but significant recovery occurred with ascorbic acid ($P < 0.001$) and calcium treatments (Table 5).

Na^+ *and* K^+ *levels*

The Na^+ and K^+ levels in serum significantly increased ($P < 0.001$) with fluoride treatment, while a significant recovery was observed in ascorbic acid and calcium fed rats ($P < 0.001$). On the whole, the recovery in K^+ levels was more marked than Na^+ and was more significant by NaF + AA than by NaF + Ca^{+2} treatment (Table 5).

TABLE 5
PROTEIN, CHOLESTEROL, SGOT, SGPT, AND Na^+ AND K^+ LEVELS OF CONTROL AND EXPERIMENTAL RATS

PARAMETER	CONTROL GROUP	SODIUM FLUORIDE ALONE (30 DAYS)	SODIUM FLUORIDE + ASCORBIC ACID (30 DAYS)	SODIUM FLUORIDE + CALCIUM (30 DAYS)
PROTEIN (mg/ml serum)	64.35 ± 0.74	49.81 ± 4.17*	57.62 ± 1.53*	51.02 ± 1.16
Cholesterol (mg/ml serum)	0.71 ± 0.02	0.64 ± 0.02	0.61 ± 0.01	0.57 ± 0.01
SGPT (mU/ml serum)	11.08 ± 0.40	21.88 ± 0.16*	13.3 ± 1.16*	15.5 ± 3.15
SGOT (mU/ml serum)	86.54 ± 5.87	107.66 ± 8.6*	88.18 ± 2.94*	98.61 ± 8.4
Na^+ level (ppm)	103.48 ± 9.05	173.58 ± 5.25*	144.00 ± 3.45*	153.31 ± 3.60
K^+ level (ppm)	6.45 ± 0.36	24.43 ± 0.53*	13.23 ± 0.24*	16.49 ± 0.33

Values are mean ± S.E. * $P < 0.001$

Histology

The liver histology was affected by NaF treatment, zonal necrosis being the most common feature. The shape of the hepatocyte nuclei was irregular and they were pycnotic. The arrangement of hepatic cords was also disturbed. The zonal necrosis persisted although to a lesser extent in NaF + Ca^{+2} treated rats. However, significant recovery was observed in the liver histology of rats treated with NaF + AA which was almost similar to that of the control rats (Figures 1-3).

The adrenals

The adrenal gland consists of outer cortex and inner medulla. The cortex is made up of three distinct layers of cells, while adrenal medulla is made up of granular chromaffin cells. The histology of the adrenal gland manifested some changes with administration of fluoride. The cells were less compactly arranged and the structure of the adrenal medulla was altered showing vacuolization and hypertrophy. In the case of the NaF + calcium treated group, the histology of the adrenal gland did not show much change compared with control. However, the adrenal of NaF + AA treated rats revealed the structure similar to that of control animals.

Histochemistry

Histochemical localization of SDH: The normal rat gastrocnemius muscle stained for the activity of succinate dehydrogenase showed its localization most intensely in the red narrow fibres. However, there was a marked decrease in the staining intensity of the enzyme in case of NaF treated rat muscle compared to the control (Figures 4-7). In NaF + AA and NaF + Ca^{+2} treated rats, the staining pattern of the enzyme showed marked recovery in comparison to NaF alone treated ones (Figures 8-11).

Sudan Black B staining for lipids: The transverse sections of NaF treated rat gastrocnemius muscle showed a decrease in the staining intensity in comparison to control, while the staining pattern showed recovery in case of NaF + AA as well as NaF + Ca^{+2} treated rats.

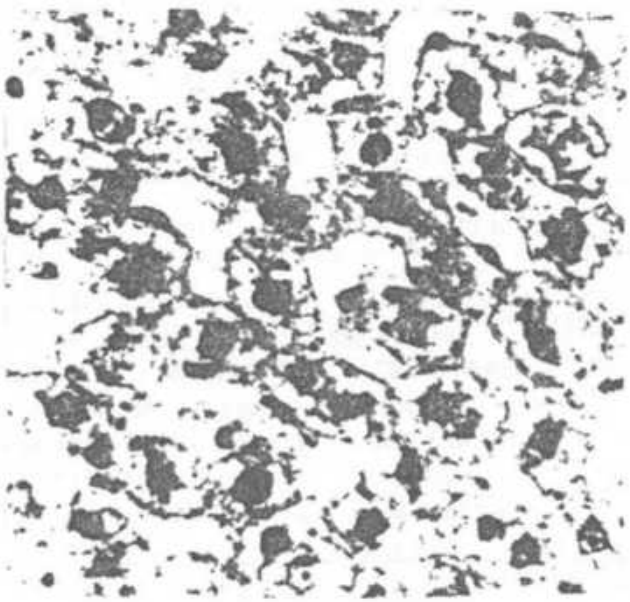
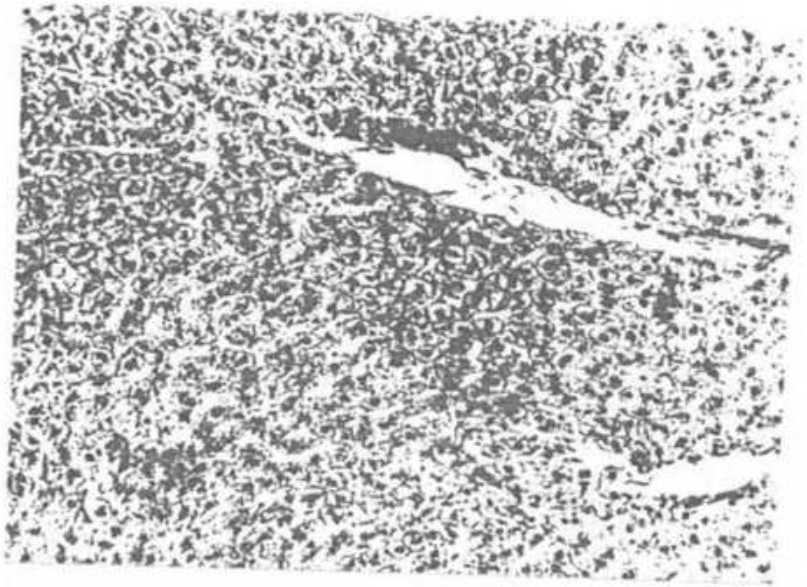


FIGURE 1
T.S. OF LIVER OF CONTROL RAT.
H & E x 600



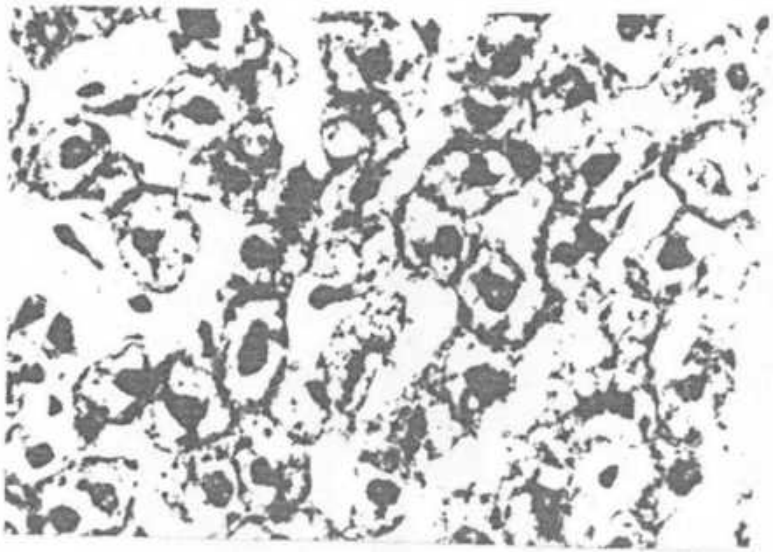


FIGURE 2 (ABOVE) T.S. OF LIVER OF 10 mg/kg BODY WEIGHT NaF TREATED RAT SHOWING ZONAL NECROSIS. H & E X 150.

FIGURE 3 (BELOW) MAGNIFIED VIEW OF FIGURE 2. H & E x 600.

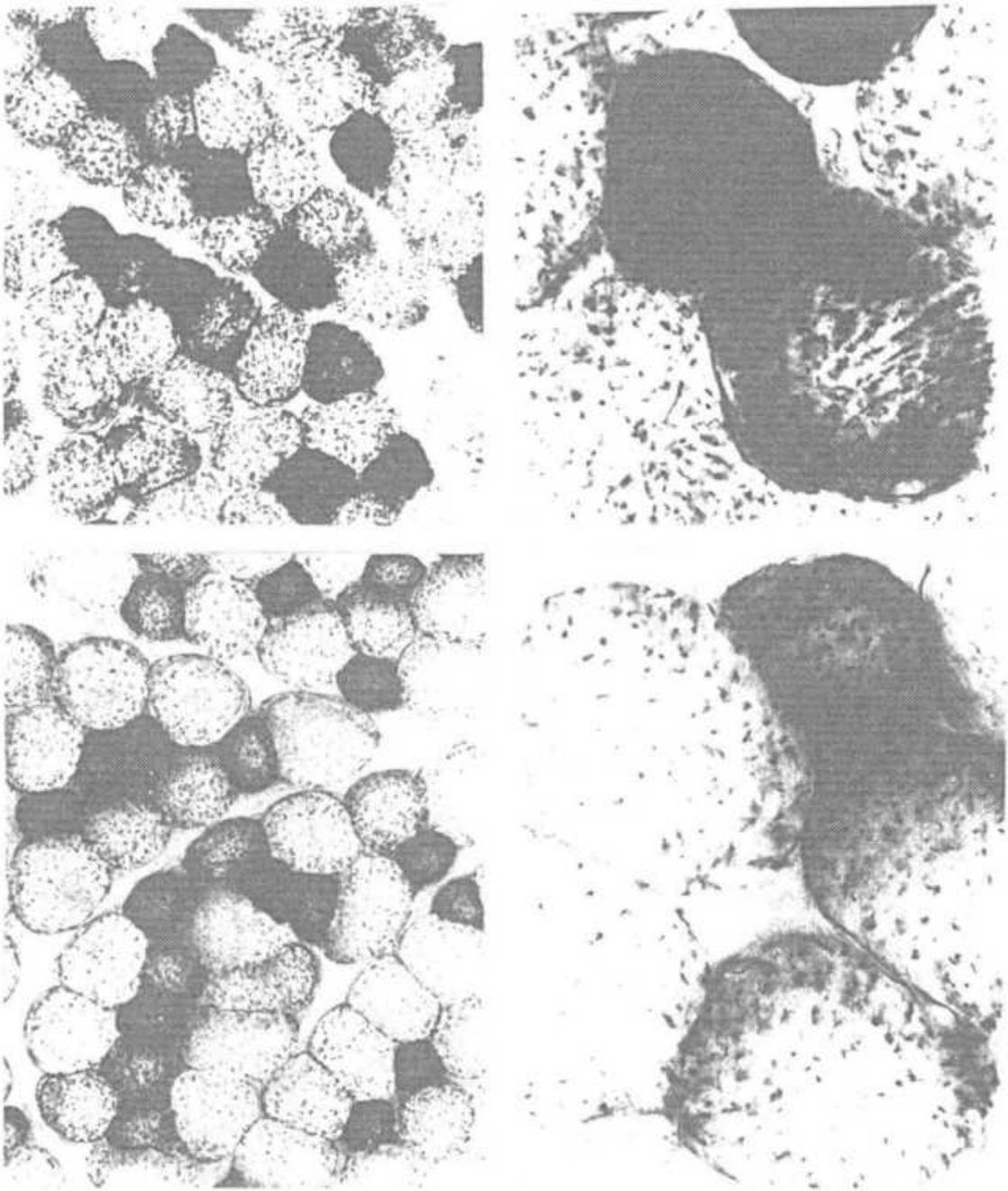


FIGURE 4 (TOP LEFT) T.S. OF CONTROL RAT GASTROCNEMIUS MUSCLE. NOTE THE CHARACTERISTIC FIBRE PATTERN. SDH IS LOCALIZED MOST INTENSELY IN THE RED NARROW FIBRES. x 150.

FIGURE 5 (TOP RIGHT) MAGNIFIED VIEW OF FIGURE 4. x 600.

FIGURE 6 (BOTTOM LEFT) T.S. OF NaF TREATED RAT GASTROCNEMIUS MUSCLE. NOTE THE DECREASE IN STAINING INTENSITY. x 150.

FIGURE 7 (BOTTOM RIGHT) MAGNIFIED VIEW OF FIGURE 6. x 700.

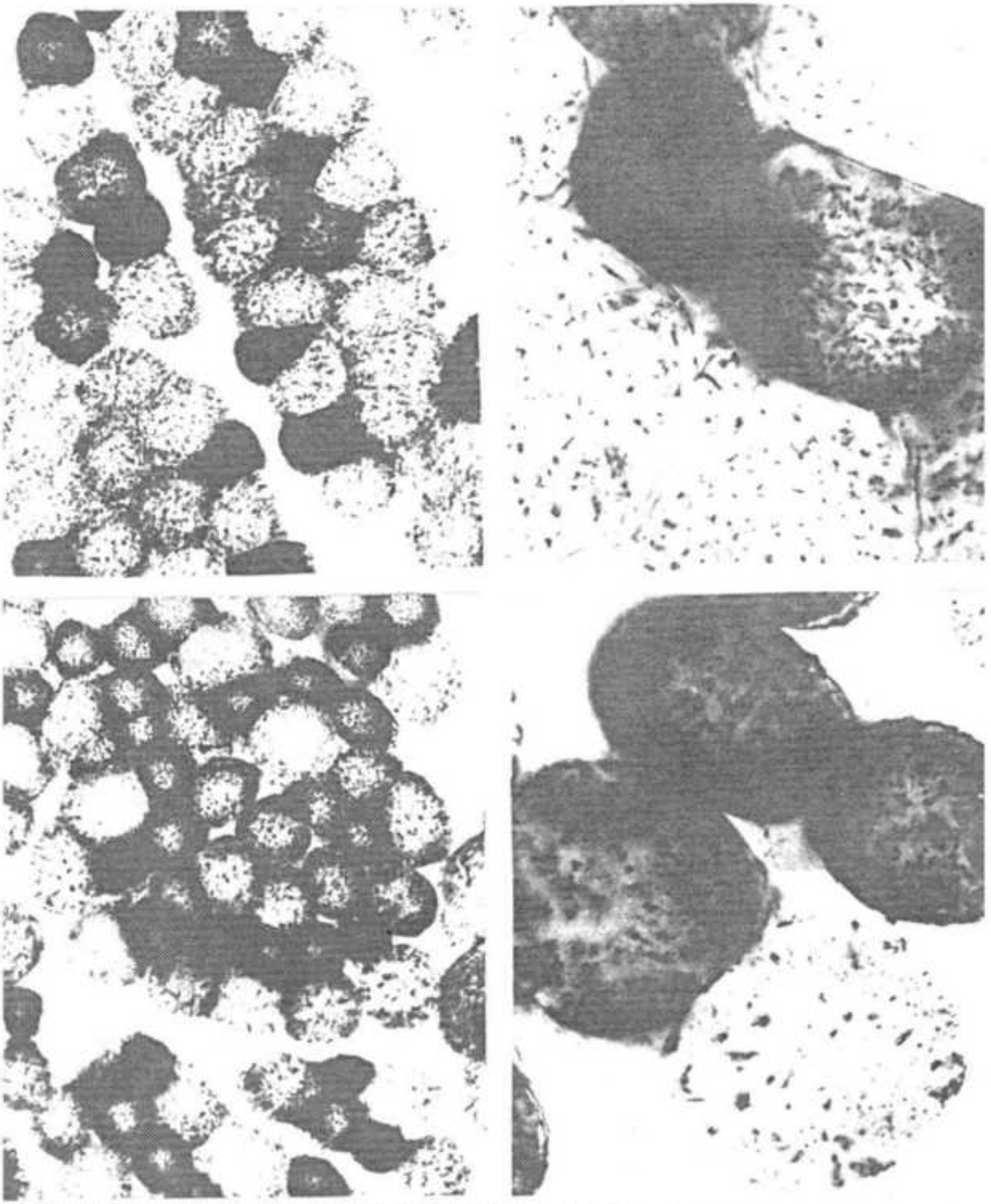


FIGURE 8 (TOP LEFT) T.S. OF GASTROCNEMIUS MUSCLE OF NaF AND ASCORBIC ACID TREATED RAT. NOTE SIGNIFICANT RECOVERY IN THE STAINING PATTERN COMPARED WITH NaF TREATMENT. X 150.

FIGURE 9 (TOP RIGHT) MAGNIFIED VIEW OF FIGURE 8. X 600.

FIGURE 10 (BOTTOM LEFT) T.S. OF GASTROCNEMIUS MUSCLE OF NaF AND CALCIUM TREATED RAT SHOWING RECOVERY IN STAINING PATTERN. X 150.

FIGURE 11 (BOTTOM RIGHT) MAGNIFIED VIEW OF FIGURE 10. X 640.

Discussion

The present study was undertaken to investigate the possible therapeutic effects of ascorbic acid and calcium ingestion simultaneously with NaF treatment on the structure and metabolism of some vital organs like liver, adrenal, gastrocnemius muscle and serum of male rats in the light of our earlier data in rabbits (6).

The results revealed that NaF treatment for 30 days resulted in a decrease in total body weight. This might be due to the reduced food intake and protein metabolism as a result of fluoride ingestion (1). The significantly enhanced levels of serum Na^+ and K^+, which are strong electrolytes, may lead to a disturbance in electrolyte balance, causing water loss from cells and tissues, resulting in decreased body and organ weights. Similarly, several authors have observed that excess of fluoride either in the diet or in drinking water retards growth and causes decrease in body weight (1,3).

The chronic fluoride intoxication seems to alter muscle function and to damage muscle cells (21,22). In the present study a significant accumulation of glycogen occurred in muscle and liver as a result of fluoride treatment. Since glycogen is considered to be one of the main fuels for muscle contraction (23), its accumulation would affect muscle function. The enhancement of glycogen might be due to the reduction of phosphorylase activity (1), an enzyme which catalyses the conversion of glycogen into glucose-1-phosphate. These changes might also be influenced by the increase in catecholamine levels, *i.e.* hormones which regulate carbohydrate metabolism. The increased catecholamines and glycogen could have a negative feedback for glycolysis, since we have previously observed that fluorotic mice suffered from hypoglycemia. Earlier studies from our laboratory (1) corroborate the present data.

The muscle protein was decreased significantly in the present study as a result of fluoride ingestion. This might be related to inhibition of protein synthesis by fluoride and would affect contractile proteins of muscle and hence its function.

The muscle succinate dehydrogenase (SDH) is an oxidative enzyme involved in the contractile mechanism of muscle fibres (23). The data of the present study revealed a significant decline in this enzymatic activity. Thus the conversion of succinate to fumarate would be slow and cause a block in the Krebs Cycle. Moreover, SDH is a mitochondrial enzyme and its decreased activity indicates a possible alteration in mitochondrial structure and function as a result of fluoride ingestion. The reduced staining intensity of SDH and sudanophilic lipids in mitochondria of gastrocnemius muscle of NaF treated rats also corroborates the above observation. Machoy (24) has also reported inhibitory action of fluoride on SDH activity in animals. Hence detailed studies on ultrastructure of muscle, especially its mitochondria, are called for.

It is known that acetylcholine, a neuromuscular transmitter, is released from the vesicles present at the neuromuscular junctions upon stimulation of muscle for contraction. The released acetylcholine is acted upon by the enzyme, acetylcholin-esterase, and converted into acetyl and choline moities. Chitra *et al* (25) found an increase in AChE levels in blood of NaF treated *Channa punctatus*. In the present study too, the muscle AChE showed a significant increase compared to the control. This increase might be due to the alteration in Ca^{+2} ions concentration in muscle, which are essential for the release of acetylcholine from the vesicles.

Fluoride, even at low concentration, inhibits a number of important enzymes and biochemical processes including the activity of adenosine triphosphatase (ATPase). In the present study, the ATPase activity of gastrocnemius muscle was significantly decreased in the rats treated with NaF, which might be one of the causative factors leading to change in contractile mechanism of muscle as a result of reduced energy

liberation for contraction. The decrease in ATPase could probably also be related to the changes in Ca^{+2} levels, since they are necessary for activation of ATPase. All these results clearly indicate that fluoride affects muscle structure, metabolism, neuromuscular transmission and finally contractility. These data make clear that prolonged fluoride ingestion leads to severe muscular atrophy/dystrophy.

The liver of fluoride treated rats revealed zonal necrosis and pycnosis of hepatocyte nuclei. The significantly enhanced levels of serum transaminases (SGOT and SGPT) indicate altered liver function since these enzymes are considered to be specific markers.

It has been reported that ingestion of inorganic fluoride in rats causes promotion of ascorbic acid (AA) synthesis (26). In the present study also, levels of AA in liver and adrenal gland were enhanced after treatment. The adrenal gland is known to be involved in the stress mechanism and helps in overcoming stress by an increased utilization and mobilization of ascorbic acid from the bound form (27). The increase in AA level might be related to augmented synthesis of the vitamin in the liver, in order to overcome the imposed stress by fluoride (27).

The histology of the adrenal gland revealed pycnosis of cortical cell nuclei in some regions. The chromaffin cells of the medulla also showed extensive cytoplasmic vacuolization and hypertrophy in comparison to control. Das and Susheela (28) have reported adrenal hypofunction in fluorotic individuals.

Sodium fluoride treatment did not cause any alteration in the levels of serum cholesterol. Saralakumari *et al* (29) observed similar results in rats supplemented with 100 ppm fluoride in drinking water for two months. Unaltered cholesterol levels in testis and serum testosterone in NaF treated rodents (rats, mice) as well as in serum of fluorotic cases from fluoride endemic regions of Mehsana District, North Gujarat, have also been reported earlier (1,30). These data make clear that NaF causes neither hypercholesterolemia nor androgen deficiency.

In the present study administration of NaF + AA and NaF + Ca^{+2} resulted in significant recovery of fluoride induced effects. The role of ascorbic acid in overcoming the induced physiological stress conditions are well known and it is recognized as an anti-stress factor (27). The supplementation of ascorbic acid along with NaF showed significant recovery in the various enzymatic and metabolic parameters of muscle, liver, adrenal and serum. The administration of Ca^{+2} along with NaF also showed significant recovery. However, the extent of recovery was on the whole more pronounced by ascorbic acid treatment than by calcium. This finding might be related to the prophylactic action of ascorbic acid following administration of NaF, similar to such action of several drugs as reported earlier (27). Further, as suggested by Chinoy *et al* (6,8), the effects of administration of a combined dose of ascorbic acid and calcium might be highly beneficial in the recovery of fluoride induced alterations as they manifest a synergistic effect. The possible mechanism of action might be via inhibition of phosphodiesterase by both Ca^{+2} and AA and thereby augmentation of cyclic AMP levels (8) - which is involved in activation of several phosphokinases in muscle, liver *etc* during hormonal regulation of their metabolism, especially by catecholamines. Ascorbic acid may also bring about recovery from fluoride toxicity through its involvement in the detoxification process in the liver (27).

The present study elucidates the role of ascorbic acid and calcium as therapeutic agents against fluoride induced toxic effects. Therefore they could be used for preventing and/or combating fluorosis in endemic areas the world over. Further studies in this direction are under way.

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CHANGES OF SEROTONIN CONTENT AND TURNOVER RATE IN HYPOTHALAMUS OF FEMALE RAT DURING FLUOROSIS

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SUMMARY: Animal models of subacute and chronic fluorosis in female rats were developed with injection of large doses of NaF(IP) and with drinking water containing 100 ppm F^-, respectively. The serotonin or 5-hydroxytryptamine (5-HT) content and turnover rate in the hypothalamus were determined with spectrofluorometry combined with degradation blockade. The 5-HT turnover rate decreased during both subacute and chronic fluorosis. The 5-HT content increased during subacute fluorosis, but decreased during chronic fluorosis. These results suggest that the influences on 5-HT metabolism of the two kinds of fluorosis are not completely identical.

The decrease of 5-HT turnover rate in hypothalamus may be one of possible mechanisms of deficiency of pituitary prolactin (PRL) and milk secretion during fluorosis.

Key words: Female rat; 5-hydroxytryptamine; Fluorosis; Hypothalamus; Seratonin.

Introduction

We have reported that both subacute and chronic fluorosis inhibited milk secretion and that chronic fluorosis blocked pituitary prolactin (PRL) release in non-lactating female rats (1-3). However, the mechanisms were not understood. It was well known that central neuro-transmitters are involved in modulation of PRL secretion, that dopamine (DA) inhibits PRL secretion and that seratonin (5-hydroxytryptamine or 5-HT) stimulates it. The 5-HT content and turnover rate in the hypothalamus of non-lactating rats, during both subacute and chronic fluorosis, were determined with spectrofluorometry combined with degradation blockade, in order to study the mechanisms by which fluorosis influences PRL and milk secretion.

Materials and Methods

1. Animal models of subacute and chronic fluorosis.

Experiment 1. Subacute fluorosis:

26 mature Wistar female rats were divided into two groups: experimental (n=13) and control (n=13). The experimental group was given NaF 10mg/ml/kg (IP), QD over 5 successive days. The control was given normal saline (NS) in equal volume and in the same way. Animals were sacrificed on the 6th day.

Experiment 2. Chronic fluorosis:

29 young Wistar female rats were divided into two groups,: experimental (n=14) and control (n=15). The experimental group drank water containing 100 ppm F^- freely. The control drank tap water (containing 0.26 ppm F^-).

After sacrifice, trunk blood was collected. Blood F^- concentration was determined with a F^- selective electrode (Corning) and pH meter (Metrohm-654 type). Incisors of rats with chronic fluorosis were observed.

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2. Determination of 5-HT content and turnover rate in hypothalamus.

Rats of the experimental and control groups were further divided into two subgroups on the day of sacrifice. One group was injected with MAOI pargyline (100 mg/ml/kg, IP) 4 hrs before sacrifice. The other group was injected with NS in the same volume and way. The hypothalamus was cut and frozen rapidly, and 5-HT was determined by Curzon's method (4). The calculation of 5-HT turnover rate was as follows: The original concentration of 5-HT was designated as Co; the concentration 4 hrs after pargyline injection was designated as Ct, then

$$b = \frac{\log Co - \log Ct}{t} \qquad k = \frac{b}{0.434} \ (\mu g/g/hr)$$

Results

1. The changes of blood level F^- and incisors after fluoride administration.

Blood F^- levels of experimental and control groups were 0.46 ± 0.15 (n=6) and 0.04 ± 0.01 (n=12) μg/ml respectively ($p<0.01$), during the subacute experiment and 0.11 ± 0.04 (n=8) and 0.04 ± 0.01 (n=12)mg/ml, respectively ($p<0.05$), during the chronic experiment. Incisors of normal rats were of orange colour and semitransparent, but those of chronic fluorosis were mottled. These results indicated that rats in the two experimental groups suffered from subacute and chronic fluorosis, respectively.

2. The influences of fluorosis on hypothalamic 5-HT content and turnover rate (Table 1).

The Table shows that the hypothalamic 5-HT turnover rate decreased during both subacute and chronic fluorosis. Hypothalamic 5-HT content also decreased during chronic fluorosis, but increased during subacute fluorosis.

TABLE 1. Changes of rat hypothalamus 5-HT content and turnover rate in fluorosis

		Group of NS injection (mg/g)	Group of pargyline injection (m/g)	k (mg/g/hr)
Subacute fluorosis	Experimental Group	2.02 ± 0.41(7)	2.96 ± 0.40(6)	0.193
	Control Group	1.11 ± 0.11(6)	3.12 ± 0.24(7)	0.287
Chronic fluorosis	Experimental Group	1.40 ± 0.07*(7)	4.54 ± 0.26(7)	0.410
	Control Group	2.06 ± 0.18(8)	6.76 ± 0.24(7)	0.612

The data in brackets represent the number of rats in each group
* $p < 0.01$ vs Control

Discussion

Until now there has been only a modicum of reports on the influence of fluorosis on milk secretion. Maylin *et al* reported that commercial feed of high fluoride content decreased milk production of cows and silver fox and that the pollution of herbage by industrial fluoride also decreased milk production of cows (5-7). We reported that both subacute and chronic experimental fluorosis decreased milk secretion in lactating rats (1-3). Because the rat is a PRL-dependent animal, PRL is involved in both initiation and maintenance of lactation. Accordingly, we propose that deficiency of pituitary PRL secretion is possibly the mechanism for inhibition of milk secretion during fluorosis. We have demonstrated that both subacute and chronic fluorosis decreased the serum PRL level in lactating rats and that chronic fluorosis also inhibited pituitary PRL secretion in non-lactating female rats (1-3). However, the mechanism of fluoride-inhibited PRL secretion remains to be elucidated.

It is well known that pituitary PRL secretion is controlled by hypothalamic hormones and central neurotransmitters. Therefore the site of fluorosis-inhibited PRL secretion may possibly be the pituitary and/or the hypothalamus. In an *in vitro* semipituitary culture study we did not find a direct inhibitory effect of fluoride on pituitary PRL secretion (to be published). Therefore it is highly possible that the site of fluoride-inhibited PRL secretion is the hypothalamus. Much research has demonstrated that 5-HT is a factor stimulating pituitary PRL secretion. However, very little has been reported about the relationship between 5-HT and milk secretion. Because 5-HT is involved in the regulation of PRL secretion, it can theoretically be inferred that it has a regulatory effect on milk secretion. Wei Wang and QiWen Xie in our laboratory reported that the chemical lesion of central serotoninergic neurons caused decrease of milk secretion in lactating rats (8). So it is quite possible that hypothalamic 5-HT is involved in the inhibitory effect of fluorosis on PRL and milk secretion.

The results of this investigation indicated that the turnover rate of hypothalamic 5-HT decreased during both subacute and chronic fluorosis, which suggests that the inhibitory effect of fluorosis on central 5-HT metabolism may be one of the mechanisms in fluorosis-decreased pituitary PRL secretion.

Turnover rate of hypothalamic 5-HT decreased during both subacute and chronic fluorosis, but the change of hypothalamic 5-HT content was different under the two situations. The content of 5-HT in the hypothalamus increased during subacute fluorosis, but decreased during chronic fluorosis. These results suggest that release of 5-HT may have been blocked in fluorosis of short duration, but synthesis of 5-HT was apparently affected after prolonged fluorosis. Geeraerts reported that single administration of a large dose of NaF decreased excretion of metabolic products like tryptophan, 5-HT, *etc.* in the urine of rabbits (9). This also suggests that fluoride may influence 5-HT metabolism by means of an effect on some step of tryptophan metabolism.

It was well known that DA was a central factor controlling PRL secretion. So it is essential to study the change of DA content and turnover rate during fluorosis, in order to eventually understand how fluorosis affects pituitary PRL and milk secretion.

Besides involvement in endocrine function of the pituitary, the central serotoninergic system also possesses a variety of physiologic functions, one of which is pain perception. Much research indicates that the decrease of central serotoninergic activity lowers pain

threshold, whereas increase of 5-HT activity elevates pain threshold (10). A vast amount of clinical observations has indicated that one of the most common symptoms of fluorosis is general or local pain and hyperalgesia caused by decrease of pain threshold. Up to now this phenomena has not been interpreted rationally, so effective treatment has been absent.

In our study, decrease of seratoninergic activity in the hypothalamus was observed during chronic fluorosis. It is desirable to explore the possible change of serotoninergic activity in other parts of the central nervous system such as the descending or ascending serotoninergic system and its significance in the pathogenisis of hyperalgesia.

Conclusions

Hypothalamic 5-HT turnover rate decreased during both subacute and chronic fluorosis. Hypothalamic 5-HT content increased during subacute fluorosis, but decreased during chronic fluorosis. The decrease of hypothalamic 5-HT activity may be one of the mechanisms inhibiting pituitary PRL and milk secretion during fluorosis.

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AN EXPERIMENTAL STUDY FOR EARLY DIAGNOSTIC FEATURES IN FLUOROSIS

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SUMMARY: Creatine phosphokinase (CPK) activity and fluoride contents in serum, hair and various bones were determined at different periods in 28 experimental rabbits with subacute fluorosis induced by different orally-fed dosages of sodium fluoride. CPK activity, and fluoride content in serum, hair and bone increased in relation to exposure time or the dose of fluoride. These changes in fluoride content may be valuable for early diagnosis of fluorosis.

Keywords: Creatine phosphokinase; Early diagnosis; Fluoride content; Fluorosis; Rabbits.

Introduction

The diagnosis of fluorosis relies mainly upon X-ray changes in bone. But minor X-ray changes of bone are often nonspecific. The diagnosis of fluorosis therefore is still difficult. When the X-ray changes of bone become significant, the disease has advanced too far for recovery.

In 1974 Susheela reported that CPK activity in serum was affected by fluorosis resulting from ingestion of excessive fluorides (1). It was believed that the fluoride ion could act directly on muscle fibres, damage the chondrosomes, and release CPK, and also increase the permeability of cell membranes. Increase of CPK activity in serum indicates muscle damage. Opinions vary regarding use of fluoride content in serum, hair and bone as evidence for the early diagnosis of fluorosis. This experiment is an attempt to determine whether his evidence is trustworthy for the early diagnosis of fluorosis.

Materials and Methods

1. Experimental animals: 28 healthy adult rabbits, with equal numbers of males and females, and weight range between 1.75 and 3.2 kg. They were randomly divided into 3 groups of 6 and a high dosage group of 10.

2. Dosage and administration: One group of 6 was the control, the others were experimental. The experimental groups were fed sodium fluoride (NaF) solution, with dosages of 10, 25 and 50 mg/kg/BW/day respectively, for 40 days. The drinking water contained 0.4 ppm fluoride and the diet 16.4 mg/kg fluoride.

3. Observation items:

1) CPK activity in serum was assayed by the method of Hughes (2). It was measured 3 times before the experiment to obtain an average, and then once every 10 days until the end of the experiment.

2) Fluoride content in serum was determined with the fluoride ion electrode, once before the experiment and twice during the experiment. In tissues, fluoride contents in ribs 1-5, in lumbar vertebra 3-5, and in bones of the legs were also determined with the fluoride ion electrode.

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3) Histopathological exaninations: At the end of the experiment all the animals were killed by injecting air into the ear veins. We then took out the heart, liver, kidney, stomach and muscles of the legs for gross examination. Later, all the samples were fixed in 10% formalin for histopathololgical study. Animals which died during the period of experiment were also treated the same way.

4) Changes of body weight: All the animals were weighed before the experiment, and then every 10 days during the experiment.

Results

1. Table 1 shows the results of CPK activity in serum of all the experimental animals. The increase of CPK activity varies with the increase of dosage and exposure time. In the low-dosage group the increase after exposure for 40 days is highly significant compared with that before the experiment ($p < 0.01$). In the middle dosage group the increase occurred from the 20th to the 40th day after exposure and was significantly higher than that determined before exposure, and 10 days after exposure ($p < 0.05$). In the high dosage group the increase began on the 10th day after exposure, continuing to the 20th day. It was significantly higher than that before the experiment ($p < 0.05$) and up to the 30th day. It was very significantly higher than that before exposure and 10 days after exposure ($p < 0.01$), while up to the 40th day of exposure it was also very significantly higher than after different exposure times ($p < 0.01$).

CPK of the high dosage group on the 30th day of exposure is increased highly above all the other groups. When compared with the low dosage group the difference is significant ($p < 0.05$). On the 40th day of exposure CPK of the high dosage group is significantly higher than all the other groups ($p < 0.05$).

CPK activity is positively correlated with exposure time. The ratios are 0.5202, 0.5953 and 0.7562 respectively. The difference between them is highly significant. CPK activity is also positively correlated with dosage. On the 20th, 30th and 40th day after exposure the ratios are 0.5293, 0.6307 and 0.6272 respectively. The differences again are highly significant.

TABLE 1

THE RESULTS OF CPK ACTIVITY IN EACH GROUP FOR DIFFERENT PERIODS AFTER EXPOSURE

Group	Untreated (X)	Days after NaF treated				F	P
		10	20	30	40		
10mg/kg	13.32(6)	10.50(6)	16.76(6)	14.27(6)	31.61(6)**	33.27	<0.01
25mg/kg	13.47(6)	15.62(6)	20.14(6)	28.92(6)	38.74(6)*	3.80	<0.05
50mg/kg	13.53(10)	20.40(10)	32.31(9)*	42.50(9)**	65.10(5)**	14.27	<0.01

Number in parenthesis shows number of animals.
* Statistically significant compared with untreated.
** Statistically very significant compared with untreated.

2. Table 2 shows the content of fluoride in serum determined for all groups at different exposure times. For each experimental group and exposure time the serum fluoride content is very significantly different ($p < 0.01$) from that before the experiment, except for the middle dosage group on the 20th day of exposure, which was still significant ($p < 0.05$)

When the serum fluoride content of all the experimental groups are compared with that of the control group at different exposure times, the differences are significant. The serum fluoride content for the high dosage group is very significantly higher ($p<0.01$) than that of all the other groups. The correlation coefficients for blood fluoride and exposure time are 0.7360, 0.8140 and 0.8250 respectively - all significantly different. On the 20th and 40th day the serum fluoride content is related to the dosage, the correlation coefficients being 0.8477 and 0.7966, and are significantly different.

In the animals of the experimental groups, CPK activity compares with the fluoride content in serum. The correlation coefficients on the 20th and the 40th day are 0.8477 and 0.7966, respectively. The difference is highly significant. The increase of fluoride content in serum is related to the increase of CPK activity in serum.

TABLE 2

THE SERUM FLUORIDE CONTENT IN EACH GROUP FOR DIFFERENT PERIODS AFTER EXPOSURE

Group	Untreated	Day after NaF treated		F	P
		20	40		
Control	12.58(6)	13.70(6)	12.64(5)	0.136	>0.05
10mg/kg	14.98(6)	40.92(6)**	45.96(5)**	11.68	<0.01
25mg/kg	16.08(6)	77.97(6)*	133.45(6)**	14.78	<0.01
50mg/kg	14.95(10)	283.04(9)**	421.88(5)**	24.90	<0.01

Number in parenthesis shows number of animals.
* Statistically significant compared with untreated.
** Statistically very significant compared with untreated.

3. Fluoride content in tissues:

a) Table 3 shows mean values of differences between fluoride contents determined in hair before and after exposure to fluoride. In the low dosage group the difference is small ($p>0.05$), but in the middle dosage group is significant ($p<0.05$), and in the high dosage group is highly significant ($p<0.005$). The fluoride content in hair is closely correlated with dosages ($r = 0.6455$, $p<0.002$).

TABLE 3

MEAN VALUES OF DIFFERENCES (X) BETWEEN FLUORIDE CONTENT OF HAIR DETERMINED BEFORE AND AFTER EXPOSURE TO FLUORIDE

	10mg/kg	25mg/kg	50mg/kg
X	1.800	3.742	15.08
t	1.174	3.070	4.281
p	>0.05	<0.05	<0.005

b) Table 4 shows fluoride content measured in different bones. In all the experi-mental groups the fluoride content, in different parts of the skeleton, is higher after exposure than in the control group. In the high dosage group the increase is significantly higher ($p<0.05$). The correlation coefficients for fluoride content in different bones and dosages are 0.6282, 0.5618 and 0.5843 respectively - all differences very significant.

TABLE 4
FLUORIDE CONTENT (MG/KG) MEASURED IN DIFFERENT BONES FOR ALL DOSAGE GROUPS

Bone	Control (5)	10mg/kg (6)	25mg/kg (6)	50mg/kg (6)	F	p
Ribs	637.44	2533.81	3477.55	6111.26*	3.25	<0.05
Front legs	486.62	1677.15	3254.16	5433.48*	3.4	<0.05
Hind legs	447.15	1844.75	3954.59	6260.75*	3.18	<0.05

* Statistically significant compared with control

4. Histopathological observations:

a) Gross Observations. In the middle and high dosage groups the periosteum became rough, there was osseous hyperplasia, and the bones became more easily broken. The mucous membrane of the stomach presented hyperaemia and erosions. The liver of some animals had extravasated blood. The body weight of the high dosage group decreased with the increase of exposure time.

b) Microscopic examination. In the middle and high dosage groups, some necrotic areas were seen in the liver cells, associated with vacuolar changes and proliferation of connective tissue fibres. Some of the superficial cells of the renal tubes showed swollen, aqueous changes and proliferation of connective tissue fibres. But in the heart and skeletal muscles nothing abnormal was found under the common microscope.

Discussion

CPK in the body exists mainly (90%) in skeletal muscles, heart muscle and brain tissue. It is a kind of specific enzyme (3) which can promote the change-over of adenosine diphosphate (ADP) and the phosphoric acid radicals. Therefore it plays an important role in the phosphorylating procedure of high energy substances in tissue. In recent years muscles have been recognized as the target tissue of fluoride poisoning (4). When muscle fibres are damaged by fluoride CPK is released into the blood stream. Therefore, by determining CPK activity in serum we can understand the condition of the damaged muscle fibres (1). The results of our experiment show that CPK in serum of experiental animals was successively increased during the 40 days of exposure. This finding is consistent with results obtained by Susheela. Our results also show that the increase of CPK activity in serum is positively related to the dosage of fluoride, exposure time, and fluoride content in serum. These results identify one important point: the amount of CPK activity in serum is correlated with the degree of fluoride poisoning. They also accord with what we found in poisoned workers who were constantly in contact with fluoride.

However, the increase in CPK activity is affected by many other factors, such as progressive muscular disease, necrosis of heart muscle due to infarction, acute infections in brain and shock due to trauma (5). But those diseases often have an acute onset and a clear pathogenesis. Therefore, if we can rule out the above factors, using CPK activity in serum as evidence for early diagnosis of chronic fluorosis would be valuable. Whether it can be so used is still in dispute among different authors. According to our previous animal experiments and observations of workers in contact with fluorides (6), we consider that fluoride in serum is a reliable index. The results of this present study further confirm that fluoride in serum is positively correlated with fluoride dosage, exposure time and CPK

activity, and the proposal of Dinman *et al* that serum fluoride could be an index for diagnosing fluorosis (7). We consider that serum fluoride may indeed be such a helpful index for diagnosing chronic fluorosis, but that it needs further study to ascertain its normal value, or normal value in different areas, because it may be affected by different conditions of fluoride action.

Fluoride content of bone as a diagnostic index has often been reported, always with a tendency to affirm the conclusions (8). Our experimental results showed that fluoride content of bone is related to the clinical features (9). In the present study the fluoride content of bone in all experimental animals exceeded that in control animals and was positively correlated with the disease. So fluoride in bone is undoubtedly useful for diagnosing chronic fluorosis. However, the materials for measuring it are not easily obtained so it is not readily accepted by clinical physicians. Some authors have suggested using horny tissues instead of bone (9). But the results observed in our study are not in accord with that suggestion, because fluoride contents of blood, bone and hair are not correlated, probably correlating only with exposure time. Further study is needed.

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TIME TRENDS FOR BONE AND JOINT CANCERS AND OSTEOSARCOMAS

IN THE SURVEILLANCE, EPIDEMIOLOGY AND END RESULTS (SEER) PROGRAM NATIONAL CANCER INSTITUTE

Robert N Hoover, Susan Devesa, Kenneth Cantor and Joseph F Fraumeni Jr

Abstracted and reviewed by John Colquhoun from
Review of Fluoride Benefits and Risks
Public Health Service, USA 1991 pp F1-F7

This study presents incidence rates for bone and joint cancers and osteosarcomas for two time periods (1973-1980 and 1981-1987) collected by the National Cancer Institute from its "SEER" program, a network of cancer registries covering 10% of the USA population. The incidence of all bone and joint cancers over all ages increased only slightly between the two periods. But when examined by age, the rates for under 20-year-olds increased 18% for the sexes combined, reflecting a 23% rise in males and a 13% rise in females. The rise in osteosarcomas among males under 20 was 53%. This rise in bone and joint cancers among males under 20 occurred in fluoridated counties (rise of 39%) but not in non-fluoridated counties (decline of 5%).

Osteosarcoma is the rare bone cancer (around one case per 100,000 of population) which originates most often at the growing ends of bones and is most prevalent among males aged 10 to 19 years. In an earlier study confined to two cancer registries these authors reported "When restricted to persons under age 20, the rates for bone and joint cancers in both sexes rose 47% from 1973-80 to 1981-87 in the fluoridated areas of Seattle and Iowa and declined 34% in the non-fluoridated areas. For osteosarcomas in males under 20, the rates increased 79% in the fluoridated areas and decreased 4% in the non-fluoridated areas."

However, they claimed that "there was no evidence of an increase in the incidence ratios with increasing duration of fluoridation." That is, the increase in fluoridated areas had occurred whether the young males had lived all or only a part of their lives in a fluoridated area. Applying the same approach to their larger study of all cancer registries, they arrived at the same conclusion that "these increases are unrelated to the timing of fluoridation, and thus are not linked to the fluoridation of water supplies."

They admit, however, that in the earlier study "the observed numbers on which some of these incidence ratios are based are relatively small." To compensate for this problem in their expanded analysis of all registries, they added data to the fluoridated areas (from Detroit, Atlanta, San Francisco and Connecticut) and to the non-fluoridated areas (mainly from Utah and New Mexico), commenting: "The resulting comparison of trends between these different areas could be suspect." Nonetheless they proceeded with their analysis and concluded: "For none of the categories revealing differences in time trends between fluoridated and non-fluoridated areas is there any evidence of an increase in incidence ratios by duration of fluoridation. For osteosarcomas, there is even some evidence of a decline in the ratio with duration of fluoridation."

It is possible to interpret these data quite differently. Many will question the assumption, in the first place, that "duration of fluoridation" is an appropriate measure of dose relationship, given the small numbers involved in this extended part of the analysis. Nowhere in their report do the authors attempt to explain *why* there is a consistent large increase in bone and joint cancers, especially osteosarcomas among young men, in fluoridated areas but not in non-fluoridated areas.

A BRIEF REPORT ON THE ASSOCIATION OF DRINKING WATER FLUORIDATION AND THE INCIDENCE OF OSTEOSARCOMA AMONG YOUNG MALES

Perry D Cohn

New Jersey Department of Health, November 8,1992.
(The following is the Executive Summary)

It is well known that fluoride provides important public health benefits by effectively preventing dental caries in children. The Public Health Service (1991) endorses artificial fluoridation of drinking water at a concentration of 0.7-1.2 milligrams of fluoride per liter of water (or parts per million) as the optimally beneficial level for preventing dental caries. The U.S. Environmental Protection Agency (USEPA) allows up to 2 parts per million for artificial fluoridation and up to 4 parts per million for naturally occurring fluoride (National Primary Drinking Water Regulations, 40 CFR 141.11 and 143.3). Other potential sources of fluoride ingestion include food, vitamins, and swallowed toothpaste.

Recently, a national study of drinking water fluoridation at the county level found a significant association with osteosarcoma incidence among males under 20 years of age (Hoover *et al* 1991). However, the meaning of the association was questioned by the authors because of the absence of a linear trend of association with the duration of time for which the water supplies were fluoridated. Furthermore, the simple study design used did not have individual information on the average amount of water ingested daily, use of dental fluoride supplements, long term residence, other potentially confounding (or causal) exposures, or genetic involvement.

As a follow-up to the study by Hoover *et al*, a small study of similar design was initiated by the New Jersey Department of Health to compare drinking water fluoridation at the municipal level with the municipal residence of osteosarcoma cases at the time of diagnosis. No interviews were conducted and data on individual residential history, average amount of water ingested, use of dental fluoride supplements, exposure to other carcinogens and familial cancer history were not available. In addition, the total number of cases was small. Therfore, observations should be interpreted causiously because: 1) exposure misclassification could lead to under- or overestimation of effects, 2) unmeasured confounding by other potential causes of osteosarcomas could introduce bias leading to under- or overestimation of effects of exposure, and 3) an observed association could be due to chance.

Osteosarcoma incidence between 1979 and 1987 was compared by ecologic epidemiology methods to water supply fluoridation in seven counties in central New Jersey. Twelve cases were diagnosed among males under age 20 in fluoridated municipalities *vs* eight cases in non-fluoridated municipalities. The rate ratio of incidence in fluoridated *vs* non-fluoridated municipalities was 3.4 with a 95% statistical confidence interval (95%CI) between 1.8 and 6.0. All twelve cases in fluoridated municipalities resided in a three county area with the greatest prevalence of fluoridation. The rate ratio of incidence in fluoridated *vs* non-fluoridated municipalities in the three county area was 5.1 (95%CI 2.7-9.0). Among 10-19 year old males in those three counties, the rate ratio was 6.9 (95%CI 3.3-13). No other age/sex groups exhibited significant association with fluoridation.

Because of the limitations of the study design and the small numbers of cases that occurred, this analysis does not imply a causal connection between fluoridation and osteosarcoma. From the public health perspective, the findings are not sufficient to recommend that fluoridation of water supplies be halted, but do support the importance of investigating the possible link between osteosarcoma and overall ingestion of fluoride. In addition, it is recommended that dentists identify whether children reside in fluoridated communities and appropriately advise on fluoride supplementation.

Reprints: State of New Jersey Department of Health, Trenton NJ 08625 0360, USA.

(Editorial comment: The "limitations" of the above report were fewer, and the "small numbers" greater, than those of earlier small-sample studies abstracted on the following page. A review of all these studies will be published in our next issue.)

IS THERE A LINK BETWEEN FLUORIDATED WATER AND OSTEOSARCOMA?

S M McGuire, E D Vanable, M H McGuire, J A Buckwalter and C W Douglass
Boston MA, USA

Abstract from *Journal of the American Dental Association 122* 39-45, 1991

To test the hypothesis that fluoride is a risk factor for osteosarcoma, a case control study compared the complete residential fluoride histories of osteosarcoma patients with matched hospital-based controls. Fluoridation was not found to be a risk factor for osteosarcoma in the study population. The trend in the data from this small sample study suggests the hypothesis that a protective effect may exist against the formation of osteosarcoma for individuals consuming fluoridated water.

Key words: Fluoridated water; Osteosarcoma.

Reprints: Dr Sheila M McGuire, Oral Epidemiology Fellow, Harvard School of Dental Medicine, 188 Longwood Ave., Boston MA 02115, USA.

BONE CANCER INCIDENCE RATES IN NEW YORK STATE: TIME TRENDS AND FLUORIDATED DRINKING WATER

Martin C Mahoney, Philip C Nasca, William S Burnett and James M Melius
Albany NY, USA

Abstract from: *American Journal of Public Health 81*, 475-479 1991

Background: Recent animal studies of the potential carcinogenicity of fluoride prompted an examination of bone cancer incidence rates.

Methods: Trends in the incidence of primary bone cancers, including the incidence of osteosarcomas were examined among residents of New York State, exclusive of New York City. Average annual osteosarcoma incidence rates in fluoridated and non-fluoridated areas were also compared.

Results: Among persons less than 30 years of age at diagnosis, bone cancer incidence among males demonstrated a significant increase since 1955, while incidence among females has remained unchanged. A significant decrease in bone cancer incidence rates since 1955 was observed among both males and females age 30 years and over at time of diagnosis. Osteosarcoma incidence rates have remained essentially unchanged since 1970, among both younger and older males and females. The average annual age adjusted incidence of osteosarcomas (1976-1987) in areas served by fluoridated water supplies was not found to differ from osteosarcoma incidence rates in non-fluoridated areas.

Conclusions: These data do not support an association between fluoride in drinking water and the occurrence of cancer of the bone.

Key words: Fluoridation; Osteosarcoma.

Reprints: Dr Martin C Mahoney, Bureau of Cancer Epidemiology, NY State Department of Health, Empire State Plaza, Tower 565, Albany NY 12237-0683, USA.

INTERNATIONAL TRENDS IN THE INCIDENCE OF BONE CANCER ARE NOT RELATED TO DRINKING WATER FLUORIDATION

S C Freni and D W Gaylor
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Abstract from *Cancer 70* 611-618 1992

Background. Because osteosarcomas may develop in rats exposed to fluoridated water, water fluoridation might pose a cancer risk to humans.

Methods. A time trend analysis of the cumulative risk (CR) of bone cancer for the period 1958-1987 for 40 cancer registry areas showed an increased risk for young males in Canada, Europe, and the United States, and a decreased lifetime risk for either sex in Europe.

Results. This was unrelated to water fluoridation and may have resulted from changes in coding practices. Bone cancer risk was inversely related to the incidence of cancers of unknown origin, suggesting that bone metastases were erroneously coded as primary bone cancer. In 1968-1972, most areas recorded more bone cancer deaths than new cases of the disease.

Conclusions. The mortality/incidence ratio, but not the incidence rate (IR), has dropped sharply since then, which erodes the basis of past inferences relating cancer mortality to fluoridation.

Key words: Bone cancer risk; Cancer incidence; Cancer mortality; Fluoride; Trend analysis; Water fluoridation.

Reprints: S C Freni, US FDA, National Center for Toxicological Research, Division of Biometry and Risk Assessment, Jefferson AR 72079, USA.

EVALUATION OF THE NATIONAL TOXICOLOGY PROGRAM (NTP) CANCER BIOASSAY ON SODIUM FLUORIDE

Edward J Calabrese*
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Abstracted and reviewed by John R Lee M.D.

Professor Calabrese was requested by the East Bay Municipal Utility District (water provider for Oakland and environs) to conduct an independant appraisal of the 1990 NTP report (NTP Technical Report Series 393). Of the NTP findings, Professor Calabrese stated "The principal finding of the NTP study was the occurance of a *significant* dose response trend in male rats of osteosarcoma (malignant bone cancer)." (emphasis added) Concerning this, he found the NTP's choice of the word "equivocal" in classifying the evidence for fluoride's carcinogenicity to be "confusing", "inappropriate", and "not consistent with what most people would call equivocal." The reasons he gave are the following:

1) Its own definition of equivocal is in disagreement with the generally accepted definition of equivocal.

2) The findings with the male rat clearly exceeded marginal increases and are *biologically plausible* given the capacity for fluoride to both concentrate and be biologically active in bone. (emphasis added)

3) The statistical analysis for trend effects is stronger than pair-wise comparisons since it uses all available data not just data from two comparison groups; yet this point is never acknowledged.

4) The basic reality is that *humans can be exposed in critical target tissue to as much fluoride as the high dose rats while consuming water at the EPA maximum contaminant level of 4 mg liter.* (emphasis added)

In his "Overview of NTP Bioassay Procedure", Dr Calabrese points out that, in the NTP test, the fluoride exposure covered only the time after weaning and not during the embryonic, fetal and neonatal stages, contrary to the experience of humans reared in a fluoridated community. This lack of exposure during the critical development stages is *an important limitation of the NTP bioassay* which must be taken into consideration when interpreting the findings of such studies. (emphasis added)

Dr. Calabrese also points our that the NTP decision to use only 50 animals per sex per dosage level represents a "practical compromise" between scientific requirements and practical (*e.g.* cost) constraints. He states: "If a tumor incidence were to occur in one out of 250 animals, it would not be detectable in this study. Yet such an incidence in the U.S. population would be of considerable public health concern."

Thirdly, Dr Calabrese points out that the common practice of using historical controls to determine "natural" tumor incidence is not applicable in this NTP test since such data is derived over different time intervals, often at different locations, using different diets and possibly different histological criteria for diagnosing tumors. He adds that "past historical control diets often had appreciable amounts of fluoride thereby providing fluoride ingesting levels between the low (25 ppm) and mid (100 ppm) group range (of the NTP study)". Furthermore, "the most appropriate historical control is the experience of the Battelle Columbus Laboratory where the actual NTP NaF study took place." Of these, only 0.6% displayed osteosarcoma. (compared to 5% in the high dose

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NTP male rats). Dr Calabrese also notes that the issue of "sex specific response" (osteosarcome in male rats, osteofluorosis in females) can not be used to "discount the significant trend effect in the males". He points out that the only previous chemical (*i.e.* acromycin) determined by NTP to be a bone carcinogen (osteosarcoma) likewise displayed its response in male but not in female rats (NCI, 1978)".

Lastly, Dr Calabrese reminds us "of particular concern and *in great need of emphasis* is that the bone fluoride levels observed in the NTP bioassay at the high dose group were similar to human bone samples taken from people who lived for at least 10 years in an area with average fluoride level of 4 ppm in drinking water, the USEPA national primary drinking water standard for fluoride. (emphasis added) This information is striking since most NTP bioassays would be expected to use dosage levels that grossly exceed expected human exposures. For example, exposure to TCE in the NTP bioassay exceeded typical human exposures by about 100,000-fold!"

Dr. Calabrese concludes that the word "equivocal" should not be applied to findings (such as the NTP study) where statistically significant trend relationships exist and where the response has substantial biological plausibility. He states that "the fact that fluoride is a potential mutagen/clastogen, concentrates in the bone, and is known to stimulate bone development, suggests that bone alterations need to be carefully considered. The linkage of the site of deposition and biological activity with bone cancer outcome *all speak to the plausibility issue.*" (emphasis added) Several times he states that "these collective findings indicate that the decision by the NTP to classify the male F344 rat osteosarcoms findings as equivocal is inappropriate." He notes that all members of the task force convened to review the findings (and who concurred with the interpretation of the NTP) were from PHS agencies.

In discussing the human epidemiologic bone cancer data from NCI, Dr Calabrese cautions that the negative appraisal, admittedly highly debated and contentious, "is not particularly re-assuring that a problem is not present." He points out that "the entire cadre of available studies are ecological in nature", *i.e.* that "the unit of analysis was the community and not the individual." In no study is it known which people drank the community water or how much, whether they smoked cigarettes or how often, had X-rays and how many, how long specific residents lived in the community, or had other risk factors. Such studies may be useful for hypothesis generation but "not for determining cause-effect relationship." The assertion by PHS that "optimal fluoridation of drinking water does not pose a detectable cancer risk to humans as evidenced by extensive human epidemiological data ... does not reflect the limitations of ecological epidemiological methodology used to address the hypothesis."

Despite the impossibility of determining to what extend the NTP rat model can be extrapolated to humans, it is possible that "the animal bioassay in male rats would predict that exposure to NaF at normally fluoridated levels in drinking water would pose *a highly significant risk in males.*" Since other agents, (*e.g.* bone seeking radioisotopes, radiotherapy, alkylating agents, familial factors, and loss or inactivation of a tumor suppressor gene) can induce or cause osteosarcoma, the NTP male rat cannot be literally used to estimate human cancer risk. That is, "while the information available indicates that NaF is a *bone carcinogen for the male rats*, it is not possible to confidently use this qualitative judgement to define what fraction of the annual cases of osteosarcomas, if any, can be attributed to the consumption of NaF." (emphasis added) What is needed now, Dr Calabrese states, is "more powerful epidemiologic protocols."

FLUORIDES AND OSTEOPOROSIS

Michael Kleerekoper and Raphaella Balena
Detroit MI, USA

Abstract from *Annual Review of Nutrition 11* 309-324 1991

Sodium fluoride has clearly been shown to have pronounced affects on the skeleton, probably more than any other currently available therapeutic agent. Unfortunately, these effects appear to be both beneficial and potentially toxic at the same time. A more clear understanding is needed of the basic mechanisms whereby these effects (both beneficial and detrimental) are exerted. When such data are forthcoming, it may be possible to modify the therapeutic use of fluoride in osteoporosis and other brittle bone diseases such that the beneficial effects outweigh the toxic effects much more completely than is currently the case. Until such time, and despite thirty years of meaningful clinical investigation, we must conclude that sodium fluoride has no role in clinical medicine outside the confines of properly conducted clinical research studies.

Key words: Bone histology; Bone mass; Fluoride; Fluorosis; Fractures; Osteoporosis.

Reprints: Dr M Kleerekoper, Bone and Mineral Division, Henry Ford Hospital, Detroit MI 48202, USA.

THE EFFECT OF FLUORIDE ON OSTEOGENESIS *IN VITRO*

H C Tenebaum, J Richards, D Holmyard, H Mamujee and M D Grynpas
Toronto, Ontario, Canada

Abstract from *Cells and Materials 1* 317-327 1991

In order to study the effects of fluoride on bone formation, a model system in which *de novo* osteodifferentiation and bone formation occurs was utilized; the chick periosteal osteogenesis (CPO) model. Various biochemical methods including measurement of alkaline phosphatase activity (bone cell phenotype marker), ^{3}H-thymidine incorporation (cellular proliferation), protein content (culture size), calcium and phosphorus uptake (mineralization) were used to analyze the effects of fluoride on osteogenesis. In addition, the use of electron microcospy, electron diffraction and x-ray analysis facilitated localization and measurement of early mineral deposits.

The data indicated that fluoride treatment induced an increase in alkaline phosphatase activity of bone cells as well as increased cellular proliferation. Mineral accumulation as assessed biochemically was apparently decreased or slowed. Ultrastructural studies using both conventional fixation and cryo-fixation methods suggested that the bone-mineral produced in the presence of fluoride was less soluble whereas crystal size remained constant.

These studies indicate that fluoride may stimulate osteogenic cell activity directly but could retard mineralization. However, the mineral produced is less susceptible to dissolution and possibly resorption.

Key words: Alkaline phosphatase; Apatite; Aqueous fixation; Cell-proliferation; Cryo-fixation; Mineralization; Osteogenesis *in vitro*.

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SIDE EFFECTS OF FLUORIDE THERAPY

Michel Laroche and Bernard Mazières
Toulouse, France

Abstract from *Balliere's Clinical Rheumatology 5* 61-76 1991

Fluoride stimulates bone formation and is used in the treatment of vertebral osteoporosis. Epidemiological and experimental studies have shown that fluoride bone is denser but not always stronger than normal bone, and the main side-effects attributed to this drug involve bone tissue. True fractures, with cortical rupture, are few (near 5%); their rate seems to correspond to that generally found in osteoporotic patients without treatment. On the other hand, stress fractures and arthralgia of the lower limbs are much more frequent (near 30%), but they often follow a benign course. Digestive tolerance of fluoride is now good, improved by the introduction of gastro-resistant industrial galenicals. However, the question of whether the risk/benefit ratio is positive is always under discussion.

Key words: Bone; Fluoride therapy; Fractures; Side effects.

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THE EFFECT OF PROLONGED INGESTION OF HIGH LEVELS (100 PPM) OF FLUORINE ON OSSEUS TISSUES OF RATS

Mitsuru Tsuchida, Isao Okayasu, Kayo Teraoka, Yasuyo Hiruta and Edward K Fujimoto
Tokyo, Japan

Abstract from: *Environmental Sciences* 1,2 63-72, 1991

In order to study the behavior of fluorine and calcium *in vivo*, highly concentrated (100 ppm) fluorine was given to rats orally *ad libitum* for a period of one year, and transversely cut surfaces of their thigh bones were examined for histological and biochemical changes. Serum concentrations of fluorine increased following administration of concentrated fluorine drink, but to a much lower level than expected. No difference in growth was seen, over the one-year period of observation, between the fluorine-treated group and control rats not receiving fluorine. While the serum total calcium concentration did not change, increases in the urinary excretion of both fluorine and calcium were seen in observations made at 6 months and 12 months, and their density in the bone shank also increased. Thus, the fluorine administration appears to have caused changes in the calcium metabolism, centering around the bone. After a year of fluorine ingestion, rat thigh bone was cut tranversely, and the cut surfaces were examined to determine the cross-sectional area. For the fluorine-administered group, the total cross-sectional area of the cut surface was found to have decreased and the bone cortex to have shrunk. The histological findings obtained indicated inhibition of osteogenesis and acceleration of bone resorption. In contrast, urinary and serum biochemical values at the end of the one-year period of fluorine administration indicate that bone resorption was in an inhibitory trend. From the foregoing, it appears that at the initial stages of fluorine ingestion, bone resorption is accelerated, but the subsequent continual ingestion of fluorine inhibits the bone resorption.

Key words: 100 ppm fluorine; Bone; Rats.

Reprints: M Tsuchida, Division of Social Medicine, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan.

THE EFFECT OF FLUORIDE TREATMENT ON BONE MINERAL CRYSTALS IN THE RAT

M D Grynpas and C Rey
Toronto. Canada

Abstract from *Bone 13* 423-429 1992)

In order to investigate the effect of fluoride on bone mineral crystals, we gave groups of female rats 8 mM NaF/L water and distilled water to control groups. The rats were sacrificed at six weeks, three months, and six months. The fluor content of the bone was determined by neutron in bone crystal size/strain with fluoride treatment. Fourier transform Infrared Spectroscopy (FTIR) showed an increased crystallinity in with a decrease of labile phosphate environment. Three carbonate bands have been found in fluoridated and normal bone samples. The distribution of carbonate ions on type A and B sites is strongly affected by fluoride. Type A carbonate is always present in bone, but decreases with increasing bone fluoride content. A carbonate band found at 866 cm-1 may correspond to a fluoride interaction with type B carbonate ions. Lastly, phosphate bands have been found to be shifted towards high wave number, which is probably related to the change in unit cell size induced by the fluoride ion. All these changes induced by fluoride reduce the solubility of bone crystals by direct incorporation of fluoride ions in the apatite lattice and by decreasing the labile phosphate environments.

Key words: Bone crystals; Bone minerals; Carbonate; Carbonate Crystals; Fluoride; Infrared; Phosphate.

Reprints: M D Grynpas, Mt Sinai Hospital, Samuel Lunenfeld Research Institute, 600 University Ave, Room 984, Toronto M5G 1X5, Ontario, Canada.

SODIUM FLUORIDE PREVENTS BONE LOSS IN PRIMARY BILIARY CIRRHOSIS

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Abstract from *Journal of Hepatology 15* 345-349 1992

Low-bone-turnover osteoporosis is a common complication of primary biliary cirrhosis (PBC). Since sodium fluoride stimulates bone formation we assessed the effect of this drug on bone mass in a 2-year, prospective, double-blind trial including 22 women with PBC who were randomly assigned to receive sodium fluoride (50 mg/day) or placebo. All received calcium supplements and low doses of vitamin D. Bone mineral density of the lumbar spine was measured by dual-photon absorptiometry initially and every 6 months. Vertebral fractures were evaluated in thoracic and lumbar spine initially, and after 1 and 2 years. Seven patients in the fluoride group and eight in the placebo group completed the trial. In the fluoride group, bone mineral density did not change after 2 years (initial 1.05 ± 0.07, final 1.07 ± 0.06 g/cm^2; p = n.s.). In the placebo group, however, bone mineral density decreased significantly (initial 1.00 ± 0.07, final 0.93 ± 0.06 g/cm^2; p = 0.03). Moreover, in the fluoride group bone mineral density increased by 2.9 ± 3.6%, and in the placebo group decreased by 6.6 ± 2.6% (p = 0.04). None of the patients developed new vertebral or non-vertebral fractures. Treatment with sodium fluoride did not impair liver function or cholestasis in PBC. These results indicate that sodium fluoride prevents bone loss in PBC and therefore might be considered as a possible therapeutic agent for osteoporosis associated with this liver disease. Since a small number of patients completed the trial, further studies are required.

Key words: Biliary cirrhosis; Osteopenia; Osteoporosis.

Reprints: N Guanabens, University of Barcelona Hospital, Villarroel 170, E-08036 Barcelona, Spain

DO ESTROGENS IMPROVE BONE MINERAL DENSITY IN OSTEOPOROTIC WOMEN OVER AGE 65

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Abstract from *Journal of Bone and Mineral Research* 7 1275-1279 1992

A retrospective analysis of our experience with estrogen and fluoride treatment in 91 patients with postmenopausal osteopenia followed for 6-47 months has been performed. Treatment included calcium (1000 mg/day) and either conjugated estrogens (0.625 mg/day) or sodium fluoride (50 mg/day), or both. All patients had at least two serial dual-photon spinal bone mineral density measurements performed 6 months or more apart. Estrogen treatment was associated with increased bone mineral density (5.3%/year), as was fluoride alone (7.5%/year). Estrogen and fluoride together were additive (9.6%/year). In women over age 65 the estrogen effect was just as great (6.9%/year) as in younger women. Estrogen benefit occurred predominantly in the first 18 months of treatment (7.0%/year), after which time changes in bone mineral density were similar to those in untreated controls, who showed stable bone mineral density. We conclude that aggressive estrogen and fluoride treatment tailored to the severity of the individual's postmenopausal osteopenia results in short-term improvement in spinal bone mineral density. These data further support that elderly women respond to estrogen replacement therapy with absolute and relative increments in bone density similar to those in younger women.

Key words: Calcium; Double-blind; Fluoride treatment; Fractures; Gestagen; Hip; Mass;Postmenopausal osteoporosis; Prevention; Replacement therapy.

Reprints: G E Dailey, Scripps Clinical and Research Foundation,10666 N Torrey Pines Road, La Jolla, CA 92037, USA.

EFFECTS OF EXPERIMENTAL FLUORIDE POISONING ON AL-P ACTIVITY AND cAMP LEVEL IN PLASMA AND BONE IN RATS: STUDIES WITH PAIR FEEDING CONTROLS

Takayuki Iwasaki, Tsutomu Sato and Moto Niwa
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Abstract from *Journal of Dental Health 42* 333-347 1992

Alkaline phosphatase (Al-P) activity and 3', 5' cyclic AMP (cAMP) levels, in plasma and bone in rats experimentally poisoned with fluoride (F), were determined and compared with the results of pair feeding.

1) As F doses increased, the uneaten food in F intake groups increased. This was about 30% in 4 mg F groups and about 50% in 6 mg F groups. Body weight was lower in F intake groups than in control groups. The change in body weight in pair feeding control groups showed the same patterns as in F intake groups
2) Al-P activity in plasma was significantly greater in 4 mg F groups and 6 mg F groups than in pair feeding control groups ($P < 0.05$ and $P < 0.01$, respectively).
3) Al-P activity in bone appeared to increase in F intake groups compared to control groups. Al-P activity in bone was lower in pair feeding groups used as controls to 4 mg F groups or 6 mg F groups than in the respective F intake groups.
4) Cyclic AMP level in plasma was higher in F intake groups than in control groups. There was significant difference between control groups and both 4 mg F and 6 mg F groups ($P < 0.01$). Plasma cAMP level in pair feeding control groups was about the same level as control groups.
5) Cyclic AMP level in bone tended to be higher in F intake groups than in control groups. There was a significant difference between control groups and 4 mg F groups ($P < 0.01$). Bone cAMP level in pair feeding groups used as controls to 4 mg F or mg F groups was similar to the level in control groups.
6) F concentration in both plasma and bone increased in proportion to the dose of F. There was a significant positive correlation between Al-P activity and F concentration.

Key words: Alkaline phosphatase; Cyclic AMP; Fluoride; Pair feeding.

Reprints: T Iwasaki, Nippon Dental University, School of Dentistry, Tokyo, Japan.

SURVEY OF BOTTLED DRINKING WATER SOLD IN CANADA .1. LEAD, CADMIUM, ARSENIC, ALUMINUM AND FLUORIDE

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Ottawa, Canada

Abstract from *Journal of Aoac International 75* 949-953 1992

Samples of bottled water (n = 172) offered for sale in Canada were analyzed for lead, cadmium, arsenic, aluminum, and fluoride: means and ranges (mug/g) found were, respectively, 0.0026 (<0.0010-0.074), 0.00018 (<0.0001-0.0004), 0.0030 (<0.001-0.048), 0.027 (<0.010-0.568), and 0.543 (<0.050-5.85). Comparison of levels among mineral waters (n = 64), spring waters (n = 77), and miscellaneous waters (n = 31) indicated appreciable differences only in the case of fluoride. For fluoride, the means and medians (mug/g) for mineral, spring, and miscellaneous waters were 1.179 and 0.455, 0.152 and 0.090, and 0.201 and <0.050, respectively. No samples were found in violation of the tolerances in the Canadian Food and Drug Regulations; however, 1 sample (in a lead-soldered can) contained lead and 15 samples contained fluoride at levels above the limits recommended by the Guidelines for Canadian Drinking Water (tap-water) Quality.

Key words: Aluminum; Arsenic; Bottled drinking water; Cadmium; Canada; Fluoride; Survey.

Reprints: R W Dabeka, Department of Health and Welfare Canada, Division of Food Research, Ottawa K1A 0L2, Ontario, Canada.

EFFECT OF HARD CHEESE EXPOSURE, WITH AND WITHOUT FLUORIDE PRERINSE, ON THE REHARDENING OF SOFTENED HUMAN ENAMEL

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Jerusalem, Israel

Abstract from *Caries Research 26* 290-292 1992

The in situ rehardening effect was compared between Cheddar cheese consumption and saliva secretion with and without a fluoride pretreatment on softened human enamel. Ten volunteers wearing prostheses which held slices of human enamel participated in this study. Average microhardness of enamel was determined on the surface at baseline, after exposing to an acidic beverage, after exposing to saliva and mastication of cheese, with and without a mouth F prerinse (10 ml Meridol containing 0.025% F). The rehardening was increased in the groups consuming cheese compared to the saliva controls. The effect was increased by an F prerinse; the initial hardness of the intact enamel surface, however, was not reached.

Key words Fluoride; Hard Cheese; Saliva; Tooth remineralization..

Reprints: I Gedalia, Hebrew University of Jerusalem, Hadassah Faculty of Dentistry, Dental Research Unit, IL-91120 Jerusalem, Israel

DOES THE NAKED FLUORIDE ION EXIST?

Konrad Seppelt
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Abstracted from *Angewandte Chemie 31* 292-293 1992

Of couse the naked fluoride ion does not exist in a chemical environment, just as no free proton is stable in a chemical environment. The potential usefulness of a naked fluoride ion is apparent: whereas H^+ or the closest to it, the magic acids, are extremely acidic, F^- would be extremely basic with immense catalytic properties. Methods of approximating a naked fluoride ion are described and discussed. Recently two fluorides that can easily be prepared in every laboratory became accessible. Even more important, the chemical properties of the cations of these fluorides are complimentary. The field is

condensation reactions, silylation and desilylation, and cyclization, each with now wide open - studies on C-C bond formation, formation of carbanions, elimination and stoichiometric or catalytic amounts of F^-, is possible. Finally it is noted that many physicochemical properties of F^- need to be redetermined, since without doubt the almost naked F^- ion differs substantially from the hydrated F^- ion.

Key words: Cations; Cesium effect; Fluoride ion

Reprints: Prof Dr K Seppelt, Institut für Anorganische und Analytische Chemie der Freien Universitat Berlin, Fabeckstrasse 34-36, D-W-1000 Berlin 33, Germany.

CHRONIC FLUORIDE TOXICITY AND PITUITARY-ADRENAL FUNCTION

Taposh K Das and A K Susheela
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Abstract from *Environmental Sciences 1* 56-62 1991

Some of the least-studied calcium-regulating hormones in chronic fluoride toxicity and fluorosis are the glucocorticoids. They are produced by the adrenal cortex and are known to play an important role in bone metabolism by regulating the calcium homeostasis. Hypocortisolemia in fluorosis patients and in experimental animals subjected to long-term fluoride administration has been reported. The present study was undertaken to determine the cause of adrenal insufficiency in chronic fluoride toxicity. Single-dose metyrapone and ACTH stimulation tests were applied. Both of the tests clearly demonstrated that in chronic fluoride toxicity, there is secondary adrenal insufficiency due to suppressed ACTH release system.

Key words: ACTH; Adrenal; Fluoride toxicity; Metyrapone tests; Pituitary.

Reprints: T K Das, All India Institute of Medical Sciences, New Delhi 110 029, India.

EFFECTS OF FLUORIDE, LITHIUM, AND STRONTIUM ON GROWTH AND ACID PRODUCTION OF MUTANS STREPTOCOCCI AND *ACTINOMYCES VISCOSUS*

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Abstract from *Caries Research 25* 179-184 1991

Eight strains of mutans streptococci and two strains of *Actinomices viscosus* were studied to determine 1) their relative sensitivities to fluoride, lithium, and strontium and 2) whether lithium or strontium interact to enhance the known antimicrobial effects of fluoride. Analyses of variance of the data revealed a major inhibition of growth and acid production by fluoride, but not by lithium or strontium. Fluoride (5 mg/l) inhibited cell growth of all strains tested by a mean of 17% and total titratable acid production by a mean of 31%. However, there were marked differences between the strains. Mean total titratable acid was inhibited by fluoride least with the A. *viscosus* strains (15%) and most with the mutans streptococci (34%). Although interactions among the elements were statistically significant, they were generally slight in magnitude.

Key words: *Actinomices viscosus*; Fluoride; Lithium; Mutans streptococci; Strontium.

Reprints: A D Eisenberg, Eastman Dental Center, Rochester NY, USA.

THE PATHOLOGY OF CHRONIC BOVINE FLUOROSIS - A REVIEW

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Abstract from *Toxicologic Pathology 20* 274-285 1992

Clinical, pathologic, and analytical records from 200 cattle were reviewed to etermine if long-term exposures to elevated fluorides resulted in previously unrecognized or unreported pathologic changes, especially skeletal neoplasia. Animals were part of comprehensive field and laboratory investigations of bovine fluorosis conducted by the Utah State University Agricultural Experiment Station over a 25-year period. Records indicated that over 170 cattle included in this review were exposed to dietary fluorides levels in excess of 25 ppm (dry wt), for most of their life span, and these animals exhibited bone fluoride concentrations ranging between 2,000 and 12,500 ppm (dry wt). Although dental and/or skeletal changes were present in most animals, significant soft tissue damage or neoplasia was not observed in any organ system. Renal degeneration and mineralization were slightly more prevalent in range cattle ingesting high fluoride levels, but these changes were not recognized in animals that received high experimental fluoride doses. The absence of significant soft tissue damage or neoplasia in these cattle combined with results of an extensive literature review suggests that environmental fluorides are not significant factors in mammalian carcinogenesis.

Key words: Cattle;Chronic; Fluoride; Non-Neoplastic; Toxicity.

Reprints: R H Bruner, Pathology Associates Inc, 6217 Central Park Drive, W Chester OH 45069, USA.

INVITRO IMMUNE MODULATION OF POLYMORPHONUCLEAR LEUKOCYTE ADHESIVENESS BY SODIUM FLUORIDE

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Abstract from *European Journal of Clinical Investigation 22* (10) 659-661 1992

We investigated the influence of sodium fluoride on polymorphonuclear leukocyte (PMN) adhesiveness in a healthy subject with low serum levels of fluoride. The PMN were separated from venous blood, and the percentages of adhered and unadhered cells were determined *in vitro* in plastic culture plates. The cells were cultured with five different fluoride concentrations ranging from 6.25 10(-2) muM to 4.0 muM in the presence and absence of autologous serum.

PMN adhesiveness in both the presence and in the absence of autologous serum was 98.5%; the addition of fluoride had no effect on the results in the absence of serum. However, in the presence of autologous serum, PMN adhesiveness decreased significantly with the addition of fluoride ($P < 0.05$) from 0.5 muM. The decrease was smaller (1.1%) at a concentration of 0.125 muM, and larger at 12 times this concentration of fluoride (52.7%). We conclude that sodium fluoride reduces PMN adhesiveness in a dose-dependent manner. The effect is not direct, but should be modulated by a seric factor.

Key words: Adhesiveness; Fluoride; Polymorphonuclear leukocyte.

Reprints: J L Gomezubric, Granada Faculty of Medicine, Department of Microbiology, Avda Madrid 11, E-18071 Granada, Spain.

SERUM FLUORIDE AS AN INDICATOR OF OCCUPATIONAL HYDROFLUORIC ACID EXPOSURE

K Kono, Y Yoshida, M Watanabe, Y Tanioka, Y Orita, T Dote, Y Bessho, Y Takahashi, J Yoshida and Y Sumi
Osaka, Japan

Abstract from *International Archives of Occupational and Environmental Health 64* 343-346 1992

To define the relationship between ionic fluoride concentration in the serum of workers and the amount of hydrofluoric acid (HF) in the work environment, pre- and postshift serum and urine samples of 142 HF workers and 270 unexposed workers were examined. The maximum and minimum concentrations of HF in the air in each workshop varied from the mean by less than 30%. The preexposure levels of serum and urinary fluoride in HF workers were higher ($P < 0.001$) than the control values. This suggests that fluoride excretion from the body continues for at least 12 h. The postshift serum and urinary fluoride concentrations of these workers were significantly higher ($P < 0.001$) than the preshift concentrations. A good correlation ($r = 0.64$) was obtained between postshift serum fluoride and postshift urine fluoride. There was a linear relationship between mean serum fluoride concentration and HF concentration in the workshop. A mean fluoride concentration of 82.3 mug/l with a lower fiducial limit (95 %, $P = 0.05$) of 57.9 mug/l was estimated to correspond to an atmospheric HF concentration of 3 ppm. This is the maximum allowable environmental concentration recommended by the Japanese Association of Industrial Health, and it is also the threshold limit value suggested by the American Conference of Governmental Industrial Hygienists. The results demonstrate that exposure to HF can be monitored by determining the serum fluoride concentration.

Key words: Atmospheric hydrofluoric acid concentration; Biological monitoring; Hydrofluoric acid worker; Serum fluoride.

Reprints: K Kono, Osaka Medical College, Department of Hygiene and Public Health, 2-7 Daigakumachi, Takatsuki, Osaka 569, Japan.

EFFECT OF DIETARY FLUORIDE ON SELENITE TOXICITY IN THE RAT

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Corvallis, Oregon, USA

Abstact from *Biological Trace Element Research 34* (3) 265-278 1992

Three factorial experiments were conducted to determine if high dietary fluoride (F) would inhibit selenite toxicity in rats. Initially, three levels of selenite (0.05, 3, and 5 mg/kg diet) were matched against three levels of F (2, 75, and 150 mg/kg diet). Fluoride failed to prevent the depressive effect of selenite on 8-wk food intake and body wt gain. Selenium (Se) concentration of plasma and kidney and enzymatic activity of whole blood glutathione peroxidase (GSH-Px) were also unaffected by F. Liver Se concentration, however, was slightly (12%) but significantly ($P < 0.025$) reduced when the highest F and Se levels were combined. Fluoride (150 mg/kg) appeared to reduce liver selenite toxicity (5 mg/kg). Therefore, further study focused on liver histology with treatments that eliminated the middle levels of selenite and F. Fluoride prevented the hepatic necrosis seen in selenite-toxic rats. Similar histological lesions were not observed for kidney or heart. Fluoride partially (26%) but significantly ($P < 0.025$) reduced thiobarbituric-reactive substances in selenite-toxic rats, but there was no F effect on intracellular distribution of liver Se, glutathione levels in liver and kidney, or on liver xanthine oxidase activity. Overall, the protective effect of F on selenite toxicity appears to be confined to liver pathology. The exact mechanism for this effect, however, remains unclear.

Key words: Fluoride; Glutathione peroxidase; .Liver necrosis; Reduced glutathione; Selenite toxicity; Xanthine oxidase.

Reprints: F L Cerklewski, Oregon State University, Department of Nutrition and Food Management, Corvallis, OR 97331, USA.

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