January, 1990 Vol. Twenty-three, No. One

FLUORIDE

OFFICIAL QUARTERLY JOURNAL

OF

INTERNATIONAL

SOCIETY for

FLUORIDE

RESEARCH

Vol. Twenty-three, No. One January, 1990 Pages 1-50

FLUORIDE

QUARTERLY REPORTS

Issued by

The International Society for Fluoride Research

TABLE OF CONTENTS

ALL ARE INVITED

The XVIII Conference of the International Society for Fluoride Research will convene August 1-4, 1990 in magnificent Arcata, California. Arcata is situated among the mighty redwoods on California's Pacific Coast, 450 km north of San Francisco.

For participation in the program and for further information write to: **ISFR Conference, Office of Extended Education, Humboldt State University, Arcata, CA 95521.** Abstracts of original research are now due.

FLUORIDE is published quarterly by the INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH, INC.

SUBSCRIPTION RATES — Price per annum in advance, including postage: $30.00. Single copies, $8.50.

MANUSCRIPTS for publication should be submitted in English, doublespaced with generous margins. References should be arranged according to the order in which they are cited in the text, and written as follows: Author, title, journal, volume, pages and year. Each paper must contain a summary ordinarily not exceeding 15 lines. Papers are accepted for publication after favorable evaluation and recommendation by qualified reviewers.

FLUORIDE is listed in:
Current Contents/Agriculture, Biology & Enviromental Sciences

COPIES of articles from this publication are now available from the UMI Article Clearinghouse. Mail request to University Microfilms International, 300 North Zeeb Road, Box 91, Ann Arbor, Michigan 48106

THE FAILURE OF FLUORIDATION

The ultimate failure of fluoridation was inevitable because it was founded on two fallacies, namely that fluorine is an essential element for man and that water containing less than approximately 1 ppm fluoride is "fluoride deficient."

The major factor which has sustained the push for fluoridation is the widely held belief that, where it operates, it has attained its objective — that it has been shown to reduce substantially the prevalence of dental caries. This belief is based on faith in the honesty and accuracy of statements made by so-called "authorities," not because the published evidence from fluoridation trials had been investigated with the assistance of an academic statistician.

In 1978 Justice John P. Flaherty, of the Supreme Court of Pennsylvania, presided over a long (2,800 pages of testimony) court case involving fluoridation. He stated:

> I seriously believe that few responsible people have objectively reviewed the evidence (1).

An early review which pointed out many of the errors in the four main trials in Grand Rapids, Newburgh and Evanston, U.S.A. and Brantford, Canada, was published by the present author as a monograph (**Fluoridation: Errors and Omissions in Experimental Trials**, Melbourne U.P., 1959) (2).

So great was the intolerance of some officials to any criticism of fluoridation, that Cambridge University Press (the distributor of the book in America) was approached by the Nutrition Foundation Inc., and others, in an attempt to suppress the monograph in the U.S.A. Also, the printer's type at Melbourne University Press was destroyed without authority, thus almost succeeding in preventing publication of the second edition the following year.

Having failed to suppress the monograph, it was omitted from the list of recent books and pamphlets in the **Index to Dental Literature** (published by the American Dental Association) and the errors it pointed out were simply ignored. Not until fairly recently have errors in those trials been tacitly acknowledged by some fluoridation promoters. In 1987 Jackson (3) stated:

> On the question of efficacy, we do not have to rely on the inadequate studies of the past.

Emphasis was then placed on what were claimed to be approximately 100 more recent fluoridation trials which were stated to have "proved" the efficacy of fluoridation. For instance, in 1984 the most recent WHO book on this subject (4), **Environmental Health Criteria for Fluorine and Fluorides**, was written by a ten-member task group. These scientists gave as their reference, and apparently accepted without investigation, the data displayed in a poster by Murray and Rugg-Gunn in 1979 (5). They stated that ". . . 120 fluoridation studies from all continents showed a reduction in caries in the range of 50 to 75% for permanent teeth." These data obviously came from the same source as those in a table in a well-known book by the authors of that poster which listed 128 studies (6).

Mention of such a large number of studies impresses the scientifically naive, who do not realize that it is not the volume but the quality of research which counts. As the statistician Sir Ronald Fisher stated many years ago (7):

> If the design of an experiment is faulty, any method of interpretation which makes it out to be decisive must be faulty too.

In 1988, the scientific status of these studies was investigated. The preliminary results were published in January, 1989, in a letter to **Chemical and Engineering News** (8) (the official organ of the American Chemical Society) which was publishing a series of letters on fluoridation, including one from the U.S. Surgeon General. My letter stated that in 23 of those 128 fluoridation studies named by Murray and Rugg-Gunn (6) the data from the deciduous and the permanent teeth were listed separately — as 46 studies.

Two studies which included data from more than one town were listed as six studies, and in seven cases reports in different years from the same study were listed as 14 studies. Therefore, more than a quarter of the studies were recorded more than once by Murray and Rugg-Gunn (6) to give the fictitious total of 128 studies.

The most important claim made for fluoridation is that it decreases dental caries in the permanent teeth. Contrary to the statement in that WHO book, 20 studies listed did not present any data for those teeth.

This leaves 74 studies for permanent teeth, but most of these were of very poor scientific quality. One did not refer to fluoridated water, two were anonymous, three were personal communications, and eight were essentially progress reports. Fourteen were not published in a journal but were short communications in newsletters and bulletins issued by state health departments. These obvious deficiencies, not mentioned by this WHO task group (4), were revealed by merely reading the references and a table in the book by Murray and Rugg-Gunn (6).

Four of the remaining 46 studies were the original trials, all of which were mentioned prominently in this WHO book (4), although for 25 years they had been known to be faulty (2). Sixteen of the remaining studies were short reports in state dental newsletters and journals.

A further disturbing fact in the table of Murray and Rugg-Gunn (6) which lists the studies, is that one column, with 128 entries, is headed: "Nonfluoridated Community Caries Experience," implying that each of the 128 studies listed had a control. This was not the case. Even in the remaining 26 studies — now less than a quarter of the 120 mentioned by this WHO task group (4) — almost every study failed to attempt to use a control or used one which was obviously unsatisfactory. These studies were not designed to estimate examiner error or to eliminate examiner bias.

An attempt was then made to examine each of the remaining 26 studies to see whether they established the claim that fluoridation decreases the prevalence of dental caries substantially. Unfortunately four of the papers listed by Murray and Rugg-Gunn (6) could not be obtained (two could not

be found in the **Index to Dental Literature** or in the **Index Medicus.**) None of these studies was mentioned by this WHO task group.

Further examination revealed that three more of those 26 studies were obviously incapable of demonstrating that fluoridation is efficacious. A detailed examination was then made of the remaining 19 studies which could possibly have demonstrated the efficacy of fluoridation. Five were held in the U.S.A., five in Australia and New Zealand, three in the United Kingdom and six in other countries. However, on examination of these reports none of them showed in a scientifically-acceptable manner that fluoridation is efficacious.

Therefore, Murray and Rugg-Gunn (6), in what appears to have been a comprehensive world-wide search, were unable to locate even one study which demonstrated that fluoridation reduced dental caries.

In contrast, the evidence that it has failed to reduce the number of decayed teeth is mounting. In their 1972 paper (9) entitled "The Failure of Fluoridation in the United Kingdom," Schatz and Martin "graphed" the official results from the U.K. Department of Health's eleven-year study and showed that the slopes indicating increase in caries with age in the treated and control areas were almost identical. Their conclusion:

> The official report is valuable because it so clearly reveals the failure of fluoridation in Great Britain.

He added:

> The alleged benefits are thus nothing more than a statistical illusion.

More recently, Colquhoun and Mann in New Zealand (10) and Diesendorf in Australia (11) have demonstrated that fluoridation has failed in their countries. Data from a recent survey by the National Institute of Dental Research of 39,207 children aged five to seventeen years from 84 areas in the U.S.A. has shown that fluoridation has failed in America also. The number of decayed, missing and filled teeth in children who had been fluoridated all their lives was no fewer than those who had been brought up in non-fluoridated areas (12).

The original claim, made in innumerable promotional statements, was that fluoridation would reduce the prevalence of decayed teeth by about sixty percent. One of these was a WHO Press Release (WHO/45, September 4, 1957). In 1956, the authors of the Grand Rapids study (13) stated:

> In children born since fluoridation was put into effect, the caries rate for the permanent teeth was reduced on the average by about 60 per cent.

It has taken forty-five years to overcome the propaganda claim that fluoridation is very efficacious. However, in 1990 it is now clear that fluoridation has failed.

References

1. Flaherty, J.P.: Letter to the Mayor of Auckland, New Zealand, July 31, 1979.

2. Sutton, P.R.N.: **Fluoridation: Errors and Omissions in Experimental Trials.** Melbourne University Press, Melbourne, Australia, 1959.
3. Jackson, D.: Fluoridation: The Official Case. **The Ecologist,** no. 17, p. 126, 1987.
4. W.H.O.: **Environmental Health Criteria 36: Fluorine and Fluorides.** W.H.O., Geneva, 1984.
5. Murray, J.J. and Rugg-Gunn, A.J.: Additional Data on Water Fluoridation, Poster at XXVI ORCA Congress, 1979.
(Cited from W.H.O., reference 4 above.)
6. Murray, J.J. and Rugg-Gunn, A.J.: **Fluorides in Caries Prevention.** 2nd ed. Wright, Bristol, 1982.
7. Fisher, R.A.: **The Design of Experiments.** 6th ed. Oliver and Boyd, Edinburgh, 1951.
8. Sutton, P.R.N.: Fluoridation of Water. **Chemical & Engineering News,** 67(4):3, January 23, 1989.
9. Schatz, A. and Martin, J.J.: The Failure of Fluoridation in the United Kingdom. **Pakistan Dental Review,** 22:3-7, 1972.
10. Colquhoun, J. and Mann, R.: The Hastings Fluoridation Experiment: Science or Swindle? **The Ecologist,** 16:243-248, 1986.
11. Diesendorf, M.: Have the Benefits of Water Fluoridation Been Exaggerated? (in press), 1990.
12. Yiamouyiannis, J.: NIDR Study Shows No Relationship Between Fluoridation and Tooth Decay Rate. **American Laboratory,** May, 1989, p. 9-10.
13. Arnold, F.A., Jr., Dean, H.T., Jay, P. and Knutson, J.W.: Effect of Fluoridated Public Water Supplies on Dental Caries Prevalence. **Publ. Hlth. Rep.,** 71:652-658, 1956.

****

Philip R.N. Sutton
D.D.Sc., F.R.A.C.D.S.
Melbourne, Australia

THE INFLUENCES OF ELEVATED ENVIRONMENTAL FLUORIDE ON THE PHYSIOLOGY AND METABOLISM OF HIGHER PLANTS

by

James C. Pushnik* and Gene W. Miller
Logan, Utah, USA

SUMMARY: Fluoride pollution has long been documented to have adverse effects on the normal growth and development of higher plants. Although fluoride comprises at least 0.05% of the earth's crust, only minimal accumulation occurs through roots with the exception of a few accumulator species. This review focuses, therefore, on the physiologic and biochemical processes which are altered primarily due to exposure to elevated atmospheric levels of fluoride. Fluoride gases and dusts gain entry to leaf physiology via the processes of leaf gas exchange and cuticular diffusion. In the subepidermal leaf spaces, water-soluble fluorides travel with the transpirational stream toward the leaf margins where accumulation occurs. When fluoride concentrations exceed a cellular threshold level visual damage is manifested.

Disruption of fluoride-sensitive biochemical processes integrated through metabolic feedback mechanisms precede the visible foliar damage associated with prolonged exposure. The subcellular distribution of accumulated fluoride and the direct influences of fluoride on enzymatic activities prior to the display of any visual symptoms are discussed with particular emphasis on the energy metabolizing systems of the cell, the ATPases. ATPase activities indirectly regulate numerous metabolic systems and have been demonstrated to be differentially inhibited by fluoride. The varying sensitivities of these enzymes in different subcellular compartments form the basis of a proposed model which attempts to correlate in vivo and in vitro experimental observation.

KEY WORDS: Fluoride; F^- accumulation, physiolgical and biochemical processes.

Environmental Occurrence of Fluoride

Widely distributed in the earth's crust, fluorine, chemically bound as fluoride, is estimated to constitute between 0.05% and 0.09% by weight of the upper layers of the lithosphere (1). It is a component of over 80 minerals among which three are widely recognized: fluorapatite ($Ca_{10}F_2(PO_4)_6$), cryolite (Na_3AlF_6) and fluorspar (CaF_2). Fluorapatite and fluorspar are mined throughout the world. There are extensive surface deposits in Florida and Idaho which are mined for manufacturing phosphate fertilizers. In the absence of such deposits in the soil the concentrations of fluorides range from 20 to 1000 ppm (2). Micas, the source of these fluorides in the soil are relatively im-

* Department of Biological Sciences; California State University, Chico; Chico, California 95929-0515, USA.

mobile, fixed on clay-sized minerals. All soils have the capacity to fix fluoride, thereby slowing depletion by leaching or removal by crops; in addition the soil content of fluoride tends to increase with fertilizer addition (2). The water-soluble fluoride fraction of soils is generally low but may be considerably higher in saline soils, which have sodium as the dominant cation instead of calcium (2). There is little evidence of a quantitative relationship between soil fluoride and the fluoride content of plants growing on that soil, except for saline soils. In the absence of air pollution the "normal" fluoride content of plants on non-saline soils is 2 to 20 ppm. This background content is primarily attributed to uptake from the soil.

Fluorides are found in the air of all rural and urban areas. Natural sources contributing to the atmospheric content are volcanism, dusts derived from the soil, and ocean spray. During the past 100 years man has been greatly responsible for increasing fluoride concentrations of the atmosphere through industrialization. Processes which subject earthen materials to high temperatures liberate fluorides as gaseous HF and particulates which contain fluoride. Examples of such processes are burning of low-grade coals, metallurgical uses of fluorspar and cryolite as fluxes, and the use of fluorapatite in production of phosphate fertilizers. The manufacturing of pottery, glass, and bricks, as well as many other industries contribute to elevation of atmospheric fluoride.

"Natural" levels of fluoride in the atmosphere are less than 0.05 $\mu gF/m^3$, whereas industrial polluted areas may contain several $\mu g/m^3$. Concentrations exceeding 12 $\mu g/m^3$ have been measured in areas surrounding phosphate mining operations (3).

Gaseous fluoride may accumulate in the leaves of plants and produce damage, especially to sensitive species and varieties. Susceptibility to atmospheric fluoride varies greatly among plant species and varieties. Susceptibility differences are governed by genetic factors which regulate pollutant uptake and its subsequent effects (4). Weinstein (5) summarized the differential responses of species as shown in Table 1.

The age and stage of development of the plant is important in determining the relative sensitivity to fluoride injury (4). Symptoms are most likely to occur on young expanding tissue in broadleaf plants and elongating needles of conifers. It is suggested that the immature cells near the apical, marginal and sub-marginal meristems are hypersensitive. Environmental and edaphic factors such as temperature, humidity, light, soil moisture and mineral nutrition influence the sensitivity of plants to fluoride injury. Accumulation rates of corn leaves, exposed to 40 ppb HF under controlled environmental conditions, reveal a linear response to the duration of exposure (Figure 1).

Resistant plant foliage may accumulate high concentrations of fluoride (50-1000 ppm) in polluted areas and fail to display visible damage symptoms. These plants, while showing no damage, may present serious problems to herbivores.

Growth and Development Effects of Fluoride

Leaves and portions of green stems can accumulate higher concentrations of fluoride from the atmosphere than other exposed parts due to greater

Table 1

Differential Response of Plant Species

Species	Susceptible	Intermediate or Tolerant
1. Lily Family (tulips, freesia)	x	
2. Gladiolus	x*	
3. Chenepodium	x*	
4. Herbaceous annual and perennial flowering plants (petunia, zinnia, marigold)		x
5. Conifers (douglas fir, ponderosa, white pine, western larch)	x**	x***
6. Broadleaved deciduous trees		x
7. Ornamental deciduous trees and shrubs		x
8. Sweet corn, sorghum, young barley	x	
9. Other vegetable and field crops		x
10. Many fruits and berries (apricot, prune, blueberry, peach fruit, etc.)	x	
11. Other fruit (e.g. blackberry, raspberry, apple, pear)		x
12. Grasses, alfalfa, clover	****	x
13. Tobacco, soybean	****	x

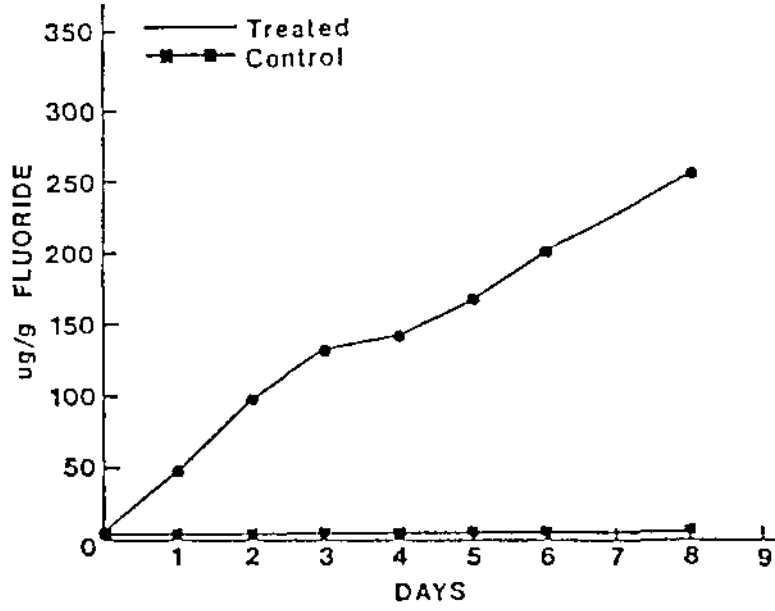
* May be injured at less than 1 $\mu gF/m^3$
** During needle elongation
*** After needle elongation
**** Personal observation

surface area, presence of stomata, and minimal retranslocation from the exposed tissue (6). Fluoride becomes concentrated in the tips of monocotyledonous and in the margins of dicotyledonous leaves which may result in extensive chlorosis or necrosis. Under such conditions the yield, growth rates and general plant vigor are adversely affected.

Physiologic and metabolic effects of fluoride have been observed to precede any visual injury symptoms. Histochemical studies of fluoride-exposed plants indicate that the initial injury occurs in the spongy mesophyll and epidermal cells of the leaf, followed by distortion of the chloroplasts in the palisade cells (7,8). Wei (9) described electron microscope studies of fluoride-fumigated soybean which showed that the spongy mesophyll cells were more susceptible to fluoride injury than adjacent palisade cells. The first noticeable cellular injury consisted of increased and aggregated endoplasmic reticulum, followed by formation of lipid droplets in the cytoplasm, mitochondrial swelling and break-up of the tonoplast membrane. Later, mitochondrial membranes exhibited signs of degeneration, while the chloroplasts remained unaffected.

Figure 1

Accumulation of fluoride in leaves of corn plants fumigated with 40 ppb HF over a 8-day period.



Conversely, electron micrographs of fluoride-fumigated corn leaves demonstrated that damage to chloroplasts in this plant occurred early in the exposure (10). The difference in response of these two plants might be explained either by their relative sensitivities or differences in chloroplast types (C_3 or C_4 photosynthesis).

Initial Whole Plant Response

Accumulation of fluorides has clearly been demonstrated to reduce plant growth and general vigor (11). Reported reductions in shoot and root growth are well correlated with exposure to airborne fluorides; these effects on growth were shown to precede visible damage expression in the foliage (12). Observations such as these have prompted the examination of whole plant physiologic responses in fluoride-impacted plants. The apparent photosynthetic rate (net CO_2 uptake) was shown to respond in proportion to the ambient fluoride concentration. Bennett and Hill (13) reported that CO_2 uptake was reduced 5% and 40% when subjected to 40 and 200 ppb HF, respectively. Photosynthetic inhibition is a rapid phenomenon occurring within 2 hours of the initial pollutant challenge.

Fluoride exposure has also been shown to alter tissue respiration patterns (14) (Figure 2). Soon after exposure, plants initially exhibit accelerated O_2 uptake, while continued exposure to even low concentrations have been shown to result in pronounced decrease in O_2 uptake (15). Respiratory inhibition

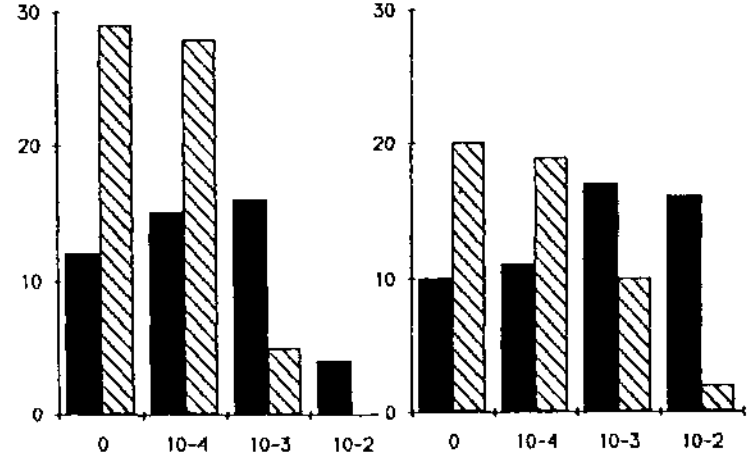
Table 2

Sugars, Organic Acids and Amino Acids in Fluoride Fumigated Soybean Leaf Tissue

Compound	Concentration Control	Fumigated
	mg/100 g fresh leaf	
Sucrose	208	55
Glucose	18	27
Fructose	22	33
	μ equiv./g fresh leaf	
Malonate	22.1	22.5
Malate	9.5	20.0
Citrate	7.9	72.5
Total	**47.5**	**76.3**
Asparagine	2.9	7.2
Aspartic	1.0	2.7
Glutamine	3.5	5.3
Total	**13.5**	**26.6**

Figure 2

Effects of NaF exposure on the rates of CO_2 fixed (■) or evolved (▨). Expressed as μg CO_2/g dry weight/min. in new and young needle tissue of white pine (adapted from McLaughlin and Barnes, 1975).



Fluoride

is generally attributed to inhibition of numerous enzyme activities, but the initial stimulation of respiration is somewhat more obscure. Respiratory responses are also known to be related to pH. Younis (16) demonstrated that radish tissue slices incubated in the presence of fluoride at pH 6.5 exhibited increased O_2 uptake, while similar slices exposed at pH 4.5 showed inhibition. Soybean leaf tissue exhibited similar responses (15).

The observed respiratory stimulation has been the subject of numerous investigations. McNulty and Lords (17) reported that higher concentrations of phosphorylated nucleotides were detectible in plants that were HF fumigated. It was speculated that the basic cellular energetics had been perturbed by the fluoride exposure. The disturbance in the energy metabolism was postulated to increase the amounts of phosphate acceptors (ADP) or phosphate donors (ATP), thereby regulating glycolytic or mitochondrial activities. Yang and Miller (18) proposed that elevated concentrations of ADP were responsible for deregulating carbohydrate metabolism, thus stimulating tissue O_2 uptake. Miller and Miller (19) studied soybean response fumigated with HF and found good correlation between tissue respiration, mitochondrial activity, and mitochondrial ATPase activity. The interpretation was that respiration was controlled by the relative ratio of ADP/ATP. In addition it was reported that leaves exposed to fluorides contained higher levels of extractible ATP (20).

Fluoride exposure has also been demonstrated to have an early influence on plant water balance. Elevated concentrations of fluoride result in wilting of numerous species (21,22). Exposure to low levels of HF decreased transpiration rates of soybeans within 2 hours (23). The reduction in transpiration, a result of stomatal closure, was correlated with increased leaf temperatures.

Accumulation and Influencing Environmental Factors

Fluoride damage becomes visual after cellular threshold levels are exceeded in leaves. The build-up of fluoride in the leaves can be obtained from two sources: through soil uptake and translocation from the roots or by direct exposure thought to be a passive process. Most of the adsorbed fluorides are readily extracted from the roots, indicating that the ion remained in the apoplastic space. Consequently, no physiologic challenge was presented to the plant (24). Plants grown in non-saline soils supplemented with high fluoride levels accumulate high levels in the roots and in subsequent shoot growth (2). However, this accumulation was not linearly correlated with soil concentration of fluorides and was attributed to passive movement in water flow via a non-selective by-pass route which circumvented the root endodermis. Fluorides traveling by this route move passively through the vascular system with the transpirational flux. Most plant species, however, do not accumulate significant levels of fluoride by root uptake, with the exception of some accumulator species. There appears to be a strong correlation between fluoride root uptake and aluminum accumulation in four horticultural varieties of Chamilla (25).

Atmospheric fluorides, gases and dusts, impact the plant foliage. Particulate fluoride deposit mainly on the leaf surface, while gases can enter directly into the substomatal chamber. Some surface-deposited fluorides can eventually diffuse through the epicuticular and cuticular layers of the leaf to gain entry into the leaf interior (26). This process is considered to be slow and consequently not a significant contributing factor to fluoride build-up, at least

not on short time scales. The primary mode of fluoride entry into the leaf is via open stomates during normal gas exchange. Environmental influences such as light, water balance, temperature, nutritional status, age and plant species could be expected to modulate entry by this route.

The influences of light and water status are closely related to the processes of photosynthesis and transpiration. Jerusalem cherry (Solanium pseudocapsian) leaves exposed to HF during a dark period resulted in lower accumulation, than with a light exposure (27). Plants growing under a water deficit, likewise, exhibited a higher resistance to fluoride exposure (28). Sunflowers, when exposed to HF fumigation during periods of elevated temperatures, showed increased rates of fluoride accumulation. Gladiolus exhibited the opposite response but displayed greater visible damage (29). Intermittent HF fumigation of soybeans at elevated temperatures resulted in enhanced damage expression even after termination of exposure (30). These observations suggest that the primary route of fluoride entry into the leaf is through the open stomates.

The nutritional status of the plant can influence accumulation and relative sensitivities to low level fluoride exposure. Bean (Phaseolus vulgaris) plants growing with potassium or iron deficiency showed increased accumulation rates of atmospheric fluorides when compared with nutrient-sufficient plants (28). Tomato had reduced fluoride injury when grown with increased supplies of magnesium; conversely, magnesium deficiency led to increased expression of damage (27). Similar results were obtained with tissue cultures (Rubus hispidus) when increased calcium, as well as magnesium partially prevented fluoride-induced depression of O_2 uptake (31). Suketa and Yamamoto (32) correlated increased foliar calcium content with decreased fluoride sensitivities in various plant species. Exposure of wheat roots to NaF induced calcium deficiency in a manner similar to known calcium chelators (33). These varied experiments demonstrated that the fluoride response is subject to the nutritional status of the plant.

Examination of fir needles which were impacted by fluoride pollution in the field confirmed that exposed leaves undergo chemical composition changes. Fluoride exposure failed to influence total nitrogen, potassium or phosphorus levels in the leaves, but reduced magnesium and manganese increased calcium content (34). The variation in mineral composition has significant impact on metabolism since numerous enzymes require these elements as enzymatic cofactors.

Leaf Movement of Substomatal Fluorides

HF and some fluoride dusts readily dissolve into the high water vapor of the substomatal cavity. Aqueous fluoride travels with the substomatal liquid phase toward the leaf margins. The driving force for this movement is the evapotranspirational pull through which water and dissolved solutes move laterally across the leaf to the margin where transpirational water evaporates. Traveling with the transpirational stream, fluorides transverse the leaf apoplast which spans the cell wall fraction to the leaf's edge. The leaf margins have the lowest evaporational boundary and therefore the highest rates of evaporation. At the leaf margin, dissolved solutes, including fluorides are deposited and accumulate. Foliar accumulated fluorides do not exhibit any mobilization to other tissues within the plant.

Fluoride is not distributed uniformly across the leaf but rather tends to accumulate in the cells which surround the point of entry into leaf and the leaf region with the highest evaporation rate. This is confirmed by electron microprobe analysis of fluoride-impacted fir needles which exhibited a steep bimodal fluoride gradient from the tip diminishing toward the leaf base. The two peaks, are at the leaf tip and the boundary between the healthy and necrotic tissue (35).

Subcellular Accumulation Sites of Fluoride

The subcellular distribution of accumulated fluoride is still largely a matter of experimental techniques. Attempts have been made to identify these sites using techniques of leaf and cellular fractionation; such procedures assume a relative immobility of the ion in question. In the case of fluoride under aqueous conditions this is a large assumption. Such investigations, while not quantitative, certainly provide qualitative information concerning the subcellular sites of fluoride buildup. Tomato plants which had been fumigated were found to have fluoride concentrations of decreasing order in the following cell fractions: cell walls, chloroplasts, soluble proteins, mitochondria and microsomes (6), results which are consistent with physiologic manifestations of fluoride exposure.

The high fluoride levels found to be associated with the cell wall fraction could be correlated with high levels of calcium found in that fraction. Accumulation in the chloroplasts was substantiated by the work of Chang and Thompson (37) and is consistent with physiologic responses (38). Electron microscopic data also indicated that the chloroplast was a subcellular site disrupted early in the expression of fluoride toxicity (9,10). The soluble-protein fraction is largely representative of the cytoplasmic enzymes, such as enolase which are known to form associations with fluorides, discussed in detail in another section of this paper. The observed respiratory responses of fluoride-fumigated plants suggested that fluoride might be influencing mitochondrial activity either directly or indirectly. The microsomal fraction encompasses membrane fragments, as well as peroxysomes and other membrane bound components.

Fluoride Influences on Enzymatic Activity

Enolase: Bonner and Wildman (39) found that the respiration of spinach brei was inhibited by the addition of 1 mg/mL fluoride. This inhibition was reversed by the addition of pyruvate, which suggested a fluoride effect on the enzyme enolase. Ross et al. (40) reported a lower C_6/C_1 ratio in fluoride-treated leaves, indicating increased use of pentose phosphate shunt in relation to the glycolytic pathway, as would be predicted if fluoride inhibits enolase activity. Warburg and Christian (41) crystallized enolase from yeast and found it to be strongly inhibited by fluoride in the presence of phosphate. Magnesium is the normal activator of enolase, and in the presence of fluoride a magnesium-fluoro-phosphate complex is formed. Miller (42) demonstrated in enolase isolated from pea seed that Mg^{+2} was required as an activator and that the observed fluoride inhibition was due to an interaction between magnesium, fluoride, and phosphate concentrations. The constant

$$k = C_{Mg} \cdot C_{PO_4} \cdot C^2_F \cdot \text{Residual Activity/Inhibited Activity}$$

was found to be 7×10^{-12}. Increasing any one of the variables and holding the others constant increased the inhibition. Over 30% inhibition was obtained when the phosphate and magnesium concentrations were 1 mM and fluoride was present at 0.5 mM in the reaction mixture.

Phosphoglucomutase: Soybean plants which were fumigated with HF showed altered metabolic processes, variations in free sugars, organic acids, and amino acids content (43) (Table 2). The carbohydrate data suggest an effect on sucrose synthesis, particularly phosphoglucomutase. The observed variations in the organic acid content point to inhibitory effects on the tricarboxylic acid cycle dehydrogenases and oxidase enzymes. The increased amino acid content was attributted to dark CO_2 fixation (43).

Detailed studies of phosphoglucomutase reveal requirements of Mg^{+2} or Mn^{+2} as a metal cofactor for enzymatic activation. The Mg^{+2} enzyme was more sensitive to fluoride inhibition than the Mn^{+2} enzyme complex. Enzymatic inhibition at exposure to 1 mM fluoride was enhanced by increasing substrate concentrations, independent of the metal cofactor concentrations. These data collectively suggest a complex interaction between fluoride and this enzyme.

Respiratory Enzymes: Fluoride effects on respiration were discussed in the section detailing the initial physiologic responses to fluoride exposure. Briefly, plants, depending on their relative sensitivity, the duration and magnitude of the fluoride exposure, exhibit an initial stimulation followed by a decrease and eventual cessation of respiratory activity in extreme exposures. The physiologic responses which result in respiratory stimulation are discussed in a subsequent section.

In cases where respiratory inhibition is observed, the obvious explanation is fluoride inhibition of various enzyme systems necessary to the respiratory process. Succinic, malic, and NADH dehydrogenases are all sensitive to fluoride; succinic dehydrogenase in the presence of phosphate is the most sensitive (44). Several oxidative enzymes such as ascorbic acid oxidase, peroxidase, and polyphenol oxidase have also been shown to be relatively sensitive to fluoride exposure (45).

Many of these fluoride-sensitive enzymes are membrane-bound. Separation of malic dehydrogenase into 3 isoenzymes from glycosomes, mitochondria, and the soluble fractions, showed that solubilized fractions were insensitive to fluoride exposure (46) suggesting that the enzyme membrane association is required for fluoride sensitivity. Further elaboration on this concept is found in the section on ATPases. The reader is referred to a recent review by Miller et al. (7) for a more complete coverage of fluoride enzyme interactions.

Fluoride Impact on ATPase Activity

Physiologic alterations in response to exposure of plants to elevated environmental fluorides must be imposed through chemical interactions explicable at the molecular level. To reach an understanding of events and processes which are fluoride-sensitive at the molecular scale, these must be studied in isolation from all other effected pathways. In vitro examination of organelles and enzymatic responses to imposed stress, by exposure to soluble

fluoride, have greatly broadened understanding of the metabolic consequences of fluoride accumulation.

Since photosynthesis is initially inhibited by a fluoride challenge, it is a good starting point to survey biochemical events that could be related to whole plant responses. A detailed systematic examination of chloroplast activity should provide an indication of biochemical causes of the observed reduction in CO_2 fixation. Gianinni et al., (47) using highly purified intact chloroplasts, ascertained that fluoride directly inhibited chloroplast function. These studies demonstrated, by using PGA dependent O_2 evolution, a marker for normal chloroplast physiology, that 10 mM NaF could inhibit this process by 30% at a physiologic pH. This observation showed that fluoride could penetrate the limiting membrane of this organelle and cause inhibition under conditions similar to those found in vivo. Ballantyne (48) demonstrated that photosynthetic electron transport was sensitive to KF exposure at pH 5.7. Re-examining this observation at physiologic pH, it was demonstrated that electron transport was not the site of a photosynthetic inhibition when fluoride was imposed under approximating cellular conditions (47). In attempt to further resolve photosynthetic inhibition, the Calvin cycle enzymes and photophosphorylation were examined. Calvin cycle enzymes were unaffected until fluoride reached 50 mM concentrations. Photophosphorylation, on the other hand, was inhibited by 30% at 10 mM fluoride (47). Detailed examination of this inhibition revealed that the site of fluoride interaction was with the ATPase. Characterisation of the inhibition indicated that fluoride affected the CF_1 part of the enzyme (49).

Fluoride effects on respiration have been studied with isolated mitochondria. Miller and Miller (19) found that mitochondria isolated from soybean plant fumigated with 9-12 ppb HF displayed an increase in respiration for the first two days of exposure but subsequent fumigation decreased respiratory rates. These authors observed an increase in ATPase activity. Pushnik and Miller (50) examined the possibility that fluoride exposure could be disrupting membrane integrity. Employing passive swelling experiments and impermeable ferricyanide probe analysis, they concluded that fluoride exposure did not disrupt membranes. Variations in O_2 uptake showed that fluoride could enter the mitochondria and alter normal respiratory patterns. Using mitochondria isolated from 5 day old etiolated corn shoots 30 mM NaF treatment resulted in increased rates of O_2 consumption but decreased the amount of energy conserved in the forms of ATP formation by 35% (50). To eliminate possible interferences in the phosphorylation reaction rate by reduced membrane transporter activity, submitochondrial particles and trypsin treated ATPases were examined. Both the submitochondrial preparation and the trypsin-solubilized ATPase showed a 30% reduction in activity when incubated with 30 mM NaF. From these observations it was concluded that fluoride did not inhibit the electron transfer process but impacted directly on the mitochondrial F_1 ATPase. These 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 (15). 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% (49). 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, similiar to DNP, and entering the mitochondria by a nonionic diffusion gradient.

Wei (9) reported that during fluoride fumigation of soybeans the initial membrane disrupted was the tonoplast. Clowes and Juniper (50) identified the tonoplast as the most fluoride-sensitive membrane. The tonoplast ATPase was also shown to be inhibited by fluoride (51). 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.

The plasmalemmal ATPase in sugarbeet (Beta vulgaris) plasma membrane vesicles were inhibited by fluoride in concentrations as low as 5 mM by 40% (52). This inhibition exhibited a relationship to the available magnesium to ATP ratio. Increasing the ratio to 3:1 (Mg/ATP) increased the inhibition of ATPase at 5 mM fluoride treatment to 60%. These authors 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.

Model of Subcellular Distribution of Fluoride and Influence on Physical Factors

The preceding discussion of the effects of fluoride on the whole plant physiologic response and the biochemical alterations induced by accumulation correlated cause and effect. However, there appear to be major differences in the concentration required to elicit the various responses between in vivo and in vitro experiments. In an attempt to equate these concentration differences, a steady state model for subcellular fluoride partitioning was postulated (54). The basis of this model resides in the weak acid characteristics of fluoride in solution. Hydrogen fluoride is a weak acid (pK_a 3.45) and would therefore be expected to present in solution two forms, F^- and HF, which are directly dependent on the pH of the immediate microenvironment for their relative concentrations. The relative distribution of the two ionic species is predicted mathematically by the Henderson-Hasselbalch equation:

$$pH = pK_a + \log[F^-]/[HF].$$

The two ionic species of fluoride differ greatly in their abilities to pass through lipid membranes (55). The charged ion has a permeability coefficient of 4.9×10^{-11} cm/sec, while the protonated ion crosses a membrane barrier at a rate of 1.4×10^{-4} cm/sec; this is approximately 6 orders of magnitude more slowly. A predictable result of these variations in membrane permeability is the differential partitioning into subcellular compartments dependent on the local pH conditions. This partitioning would be expected to occur during the movement of dissolved fluoride with the transpirational stream through the apoplast en route to the leaf margin. The result of such partitioning

would be accumulation within the plant cells and subcellular compartments. The direction of movement would be controlled by the magnitude of the pH drop across the membrane.

A number of resistances to subcellular accumulation are expected to be encountered during the passive transport of the leaf margin via transpiration. The cell wall presents the first resistance to accumulation. Cells are composed of two distinguishable spaces: the water free space (WFS) and the Donnan free space (DFS). The WFS is uncharged and presents no barrier to movement of dissolved solutes. The DFS, on the other hand, is highly charged due to the abundance of ionizable uronic acid and pectinic compounds. The charge components of the DFS preferentially sequester divalent cations electrostatically. The principal cation found associated with this fraction is calcium, bound as calcium-pectate. As fluoride moves through the DFS, calcium might be displaced due to the large binding differential between CaF_2 and Ca-pectate. Fluoride would accumulate in the cell wall fraction until all the calcium was displaced, analogous to exhaustive displacement on a cation exchange column. This calcium displacement would be expected to occur all along the DFS route but primarily in regions of high localized concentrations (e.g. leaf margins or leaf cells adjacent to the necrotic zone).

The remaining resistances to subcellular accumulation and compartmentation are transmembrane movements. As discussed earlier in this section, the two ionic species of fluoride exhibit distinct membrane permeability differences. The direction of partitioning into the various subcellular compartments is controlled by the localized concentrations of each ionic fluoride species. The ratio of F^- to HF will be dependent on the pH of the immediate environment. The diffusing ionic species is the HF which will traverse the intervening membrane barriers driven by a chemical potential gradient toward regions of lower concentrations; this process will continue until the chemical potential gradient is negated. In the case of subcellular movement, membrane compartments are known to possess differences in pH, creating a large sink for the diffusing HF molecules. To illustrate this idea, consider the movement of fluoride across any membrane in which the pH is lower on the outside relative to the inside. On the outside the fluoride concentrations will be determined by the Henderson-Hasselbach equation with the HF ions partitioning into the membrane down the concentration gradient toward the interior. On the inside of the membrane, new pH conditions will dictate a new F^-/HF ratio in favor of the F^- ion thus removing any feedback of concentration on the diffusing ion (HF). This situation results in the formation of a continuous sink for fluoride in subcellular compartments which are able to physiologically maintain transmembrane pH differences.

Movement of HF would be expected to occur from the apoplastic transpirational stream to the cytoplasm, particularily at the site of solute deposition (i.e. the effective leaf margin) where concentrations would accumulate. In the cytoplasm, accumulating fluorides would subsequently partition into the chloroplasts, mitochondria, and the vacuole driven by transmembrane pH gradients and concentration differences for the diffusing species (e.g. HF). These transmembrane movements of the HF molecule would be continuous until organelle function was disrupted due to metabolic blocks induced in compartmental enzymes. The collapse of integrated organelle and cytoplasmic functions would result in the deterioration of cellular activities and eventually be expressed as localized visible damage on the leaf surface. This proposed

model of subcellular distribution of accumulated fluoride is consistent with the physiologic expressions of fluoride exposure prior to the onset of visible damage.

References

1. Committee on Biologic Effects of Atmospheric Pollutants. National Academy of Science, Washington D.C., 1971.
2. Davison, A.W.: Uptake Transport and Accumulation of Soil and Airborne Fluorides by Vegetation. In Shupe, J.L., Peterson, H.B. and Leone, N.C., Eds., **Fluorides**, Paragon Press, Inc., Salt Lake City, Utah, 1983 pp. 61-82.
3. Smith, F.A.: Overview of Fluorides in Everday Life. In Shupe, J.L., Peterson, H.B. and Leone, N.C. Eds.: **Fluorides**, Paragon Press, Inc., Salt Lake City, Utah, 1983.
4. MacLean, D.C.: Factors which Modify the Response of Plants to Fluoride. In Shupe, J.L., Peterson, H.B. and Leone, N.C., Eds.: **Fluorides**, Paragon Press, Inc., Salt Lake City, Utah, 1983.
5. Weinstein, L.H.: Fluoride and Plant Life. **J. Occup. Med.** 19:49-78, 1977.
6. Ledbetter, M.C., Mavrodineanu, R. and Weiss, A.J.: Distribution Studies of Radioactive Fluoride^{18} and stable Fluoride^{19} in Tomato Plants. **Contrib. Boyce Thompson Inst.**, 20:331-348, 1960.
7. Miller, G.W., Yu, M.H. and Pushnik, J.C.: Basic Metabolic and Physiologic Effects of Fluoride on Vegetation. In Shupe, J.L., Peterson, H.B. and Leone, N.C., Eds.: **Fluorides**, Paragon Press Inc., Salt Lake City, Utah, 1983.
8. Solberg, R.A., Adams, D.F. and Fercham, H.A.: Some Effects of Hydrogen Fluoride on Internal Structure of *Pinus ponderosa* Needles. In **Proc. 3rd Nat. Air Pollution Symp.**, Stanford Res. Inst., Los Angles, CA, 1955.
9. Wei, L.L.: Effects of Hydrogen Fluoride on Ultrastructure of Soybean Leaf Cells. Ph.D. Thesis, Utah State University, 166 p., 1972.
10. Lhoste, A.M. and Garrec, J.P.: Fluoride Effects on Chloroplast Ultrastructure of *Zea mays*: Biometrical Study. J. **Microsc. Bio. Cell**, 24:351-364, 1975.
11. Brewer, R.F., Guillemet, F.B. and Sutherland, F.B.: The Effects of Atmospheric Fluoride on Gladiolus Growth, Flowering and Corn Production. **Proc. Amer. Soc. Hort. Sci.**, 88:208-214, 1966.
12. Stocks, D.L.: The Effects of Fluoride on the Growth and Reproduction of Bush Bean Plants. M.S. Thesis, Univ. of Utah., 1960.
13. Bennett, J.H. and Hill, A.C.: Inhibition of Apparent Photosynthesis by Air Pollution. **J. Environ. Qual.**, 2:526-530, 1973.
14. McLaughlin, S.B., Jr., and Barnes, R.L.: Effects of Fluoride on Photosynthesis and Respiration in some South-East American Forest Trees. **Envirn. Pollut.**, 8:91-96, 1975.
15. Yu, M.H. and Miller, G.W.: Effects of Fluoride on Respiration of Leaves from Higher Plants. **Plant and Cell Physiol.**, 8:483-493, 1967.
16. Younis, A.E.: Fluoride Inhibition of Respiration of Radish Root Slices. **Proc. Iraqi Sci. Soc.**, 2:19-24, 1958.
17. McNulty, I.B. and Lords, J.L.: Possible Explanation of Fluoride Induced Respiration in Chlorrella pyrenoidosa. **Sci.** 132:1553-1554, 1960.
18. Yang, S.F. and Miller, G.W.: Biochemical Studies of the Effects of Fluoride on Higher Metabolism of Carbohydrates, Organic Acids and Amino Acids. **Biochem J.**, 88:505-509, 1963.
19. Miller, J.E. and Miller, G.W.: Effects of Fluoride on Mitochondrial Activity in Higher Plants. **Physiol. Pl.**, 32:115-121, 1974.

20. Ballentyne, D.J.: Phytotoxic Air Pollutants and Oxidative Phosphorylation. In Kozoil, M.J. and Whatley, F.R., Eds.: **Gaseous Air Pollutants and Plant Metabolism.** Butterworths, pp. 528-532, 1983.
21. Brewer, R.L., Chapman, H.D., Sutherland, F.H. and McCulloch, R.B.: Effect of Fluorine Additions to Substrate on Navel Orange Trees Grown in Solution Culture. **Soil Sci.**, 87:183-187. 1959.
22. MacLean, D.C., McCune, D.L., Weinstein, L.H., Mandl, R.H. and Woodruff, G.N.: Effects of Acute Hydrogen Fluoride and Nitrogen Dioxide Exposure on Citrus and Ornamental Plants in Central Florida. **Environ. Sci. Technol.**, 2:444-449, 1968.
23. Poovaiah, B.W. and Wiebe, H.H.: Effects of Gaseous Hydrogen Fluoride on Oxidative Enzymes of Pelargonium Zonal Leaves. **Phytopathol.**, 61:1277-1279, 1971.
24. Garrec, J.P. and Letourner, L.: Fluoride Absorption by the Root and Foliar Tissues of Horse Bean (Vicia faba minor, calciole) and Lupine (Lupinus luteus; calcifuge). **Fluoride**, 14:30-38, 1981.
25. Davison, J., Takmaz-Nisancioglu, S. and Bailey, I.F.: The Dynamics of Fluoride Accumulation by Vegetation. In Susheela, A.K., Ed.: **Fluoride Toxicity**, Kalpana Printing House, New Dehli, 1985.
26. McCune, D.C., Silberman, D.H. and Weinstein, L.H.: Effect of Relative Humidity and Free Water on the Phytotoxicity of Hydrogen Fluoride and Cryolite. **Proc. Intern. Clean Air Cong.** 4:116-119, 1977.
27. MacLean, D.C., Schneider, R.L. and McCune, D.C.: Effects of Chronic Exposure to Gaseous Fluoride on the Yield of Field-Grown Bean and Tomato Plants. **J. Am. Soc. Hort. Sci.**, 102:297-299, 1977.
28. Applegate, G.H. and Adams, D.F.: Effects of Atmospheric Fluoride on the Respiration of Bush Beans. **Bot. Gaz.**, 121:223-227, 1960.
29. MacLean, D.L. and Schneider, R.E.: Effects of Gaseous Hydrogen Fluoride on Yield of Field Grown Wheat. **Environ. Pollut.** (Series A) 24:39-44, 1981.
30. Pilet, P.E. and Bajaoui, M.: Interaction between Fluoride, Calcium and Magnesium on the O_2 Uptake by Tissues Cultivated in vitro. **Biochem Physiol. Pflanz.** (BPP) 168:483-491, 1975.
31. Poovaiah, B.W. and Wiebe, H.H.: Influence of Hydrogen Fluoride on the Water Economy of Soybeans. **Pl. Physiol.**, 51:396-399, 1973.
32. Suketa, Y. and Yamamoto, T.: Effects of Atmospheric Fluorides on Plants. **Nippon Nogei Kagaku Kaishi**, 49:341-346, 1975.
33. Ramagolpal, S., Welkie, G.W. and Miller, G.W.: Fluoride Injury to Wheat Roots and Calcium Nutrition. **Plant and Cell Physiol.**, 10:675-685, 1969.
34. Garrec, J.P.: Effects of Fluoride Pollution on the Mineral Composition of Fir Needles (Abies alba Mills). **Environ. Polut.**, 13:159-168, 1977.
35. Garrec, J.P., Blanchard, B., Bruin, J.C., Bisch, A., Bliny, R. and Fourcy, A.: Etude de la repartition du fluor dans les differents tissus d'aiguilles polluses d'Abies alba. **C.R. Acad. Sci./Paris**, 277:391-394, 1973.
36. Ledbetter, M.C., Marvrodeanu, R. and Weiss, A.J.: Distribution Studies of Radioactive Fluorine[18] and Stable Fluorine[19] in Tomato Plants. **Contrib. Boyce Thompson Inst.**, 20:331-341, 1960.
37. Chang, C.W. and Thompson, C.R.: Subcellular Distribution of Fluorine in Navel Orange Leaves. **J. Air Water Pollut.**, 9:911-918, 1965.
38. Weinstein, L.H. and Alscher-Herman, R.: Effects of Gaseous Pollutants in Agriculture and Horticulture. In: Unsworth and Ormrod, Eds.: **Gaseous Pollutants Impacts on Agriculture.** Butterworths, 1982, pp. 1341-1367.
39. Bonner, J. and Wildman, S.G.: Enzymatic Mechanism in the Respiration of Spinach Leaves. **Arch. Biochem.**, 10:497-518, 1946.

40. Ross, C.W., Weibe, H.H. and Miller, G.W.: Effects of Fluoride on Glucose Catabolism in Plant Leaves. **Pl. Physiol.**, 37:305-309, 1962.
41. Warburg, O. and Christian, W.: Chemischer Mechanismus der Fluoridhemming der Garung. **Naturwissenschaften**, 29:590, 1942.
42. Miller, G.W.: Properties of Enolase in Extracts from Pea Seeds. **Pl. Physiol.**, 33:199-206, 1958.
43. Yang, S.F. and Miller, G.W.: Biochemical Studies on the Effects of Fluoride on Higher Plants. 2. The Effect of Fluoride on Sucrose-Synthesizing Enzymes From Higher Plants. **Biochem J.**, 88:509-516, 1963.
44. Lovelace, C.J. and Miller, G.W.: In vitro Effects of Fluoride on the Tricarboxylic Acid Cycle Dehyrogenases and Oxidative Phosphorylation. Part 1. **J. Histochem. Cytochem.**, 15:202-206, 1967.
45. Lee, C.J., Miller, G.W. and Welkie, G.W.: The Effect of Hydrogen Fluoride on the Wounding and Respiratory Enzymes in Soybean Leaves. **Air and Water Pollut., Intern.** J., 10:169-181, 1965.
46. Psenak, M., Miller, G.W., Yu, M.-H., and Lovelace, S.C.: Separation of Malic Dehydrogenase Isoenzymes from Soybean Tissue in Relation to Fluoride Treatment. **Fluoride**, 10:63-72, 1977.
47. Gianinni, J.L., Miller, G.W. and Pushnik, J.C.: Effects of NaF on the Biochemical Processes of Isolated Soybean Chloroplasts. **Fluoride**, 18:72-79, 1985.
48. Ballentyne, D.J.: Fluoride Inhibition of the Hill Reaction in Bean Chloroplasts. **Atmos. Environ.**, 6:267-273, 1972.
49. Gianinni, J.L., Pushnik, J.C. and Miller, G.W.: Characterization of the Effects of Fluoride Exposure on the Chloroplast Coupling Factor; CF1 particle. **J. Fluorine Chemistry**, 41:79-93, 1988.
50. Pushnik, J.C. and Miller, G.W.: Effects of Fluoride on Isolated Plant Mitochondria; Membrane Properties and Oxidative Phosphorylation. In Susheela, A.K., Ed.: **Fluoride Toxicity**, pp. 47-59.
51. Clowes, F.A.L. and Juniper, B.E.: **Plant Cells**. Blackwell Scientific Publisher, Edinborough, 1968, pp. 111-118.
52. Gianinni, J.L., Briskin, D.P. and Miller, G.W.: Effect of Fluoride on the ATPase Activity and Membrane Transport in Isolated Tonoplast Vesicles from Sugarbeet. **Pl. Physiol.**, 83:709-712, 1987.
53. Gianinni, J.L., Pushnik, J.C., Miller, G.W. and Briskin, D.P.: Effects of Fluoride on the Plasmalemmal ATPase Activity of Sugarbeet Protoplasts. **Pl. Science**, 53:39-44, 1987.
54. Miller, G.W., Pushnik, J.C. and Gianinni, J.L.: A Model for the Subcellular Distribution of Fluoride in Higher Plants: Relation to in vitro Concentrations Required for Enzyme Inhibition. In: **Fluoride Research 1985, Studies in Environmental Science**, Vol. 27, Elsevier Science Publishers, B.V., Amsterdam, 1986, pp. 241-251.
55. Gutnecht, J. and Walters, A.: Hyrofluoric and Nitric Acid Transport through Lipid Bilayer Membranes. **Biochem. Biophys. Acta**, 644:153-156, 1980.

****

THE INFLUENCE OF THE COMPOSITION OF THE TISAB SOLUTION ON THE DETERMINATION OF FLUORIDE IN TEA INFUSIONS

by

J.M. Colina*, C.F. Arias, and A. Rodríguez
Santander, Spain

SUMMARY: The fluoride ion content of eleven different kinds of tea infusions consumed in Cantabria (northern Spain) has been measured. Because most of the infusions analyzed contained interfering levels of Al^{+3}, the influence of the concentration of the complexing agent in the TISAB solution was studied. If the Al^{+3} content is no greater than 2.5 ppm, a 10^{-3} M citrate concentration in TISAB solution can be used (the TISAB solution most generally found in the literature). If, however, the Al^{+3} content ranges between 2.5 and 5 ppm, low results for F^- are obtained, and a more concentrated TISAB solution containing 0.1 M citrate is recommended.

KEY WORDS: Interfering cations; Tea infusions; TISAB composition.

Introduction

Dietary ingestion of fluoride has become a matter of great importance in recent years due to its possible adverse effects on health (1-4). Several studies on the determination of the content of this ion in various kinds of food are available (5-11), one of which is tea infusions (12-20). Currently, the determination of fluoride ion in a large variety of samples is carried out with a selective membrane electrode because of the difficulty and time consumed by classical methods, compared to the speed, simplicity, and accuracy of direct potentiometric methods.

Determination of fluoride ion by direct potentiometry with selective membrane electrode requires that the fluoride ion in the solution be free and not combined. The possible presence of interfering cations has led some authors to study the influence of the concentration of the complexing agent in the TISAB solution and its effect on the accuracy of the analysis, citrate being one of the most adequate complexing agents (21-26).

Here the fluoride ion content of eleven different kinds of tea infusion consumed in Cantabria (northern Spain) has been measured. Most of the infusions contained Al^{+3} at interfering concentrations. For this reason a study of the influence of the concentration of the complexing agent in the TISAB solution was undertaken.

Reagents and Materials

Fluoride standard solution: 0.01 M (190 ppm) prepared from NaF Merck analytical grade, oven dried.

* Department of Chemistry and Department of Applied Physics, Polytechnic School, University of Cantabria, Santander, Spain.

Fluoride dilute standards: 5 x 10^{-4} M (9.5 ppm), 10^{-4} M (1.9 ppm) and 5 x 10^{-5} M (0.95 ppm) were prepared by several dilutions of the previous standard.

TISAB II (A-D) Solutions: Sodium chloride, 58.5 g/L. Glacial acetic acid, 15 mL/L. Sodium acetate trihydrate, 102 g/L. Sodium citrate dihydrate: A — 0.3 g/L. B — 3 g/L. C — 30 g/L. D — 75 g/L.

TISAB III Solution: Sodium chloride, 58.5 g/L. Glacial acetic acid, 57 mL/L. CDTA, 4 g/L. Sodium hydroxide, 5 M to obtain pH 5-5.5.

Plastic volumetric material. Digital active ion meter CRISON MICROPH 2002. Fluoride ion selective electrode INGOLD: electrode with INGOLD reference Ag/ClK (3M)/AgCl of simple junction. Electromagnetic stirrer SELECTA. AA Spectrophotometer: PERKIN ELMER Model 306.

Procedure

Calibration is effected by adding 10 mL of TISAB solution to 10 mL of the standard 0.95 ppm F standard. The clean dry electrodes are introducted into the magnetically stirred mixture; the active ion meter is connected and a continuous measure taken, reading the E_1 (mV) measure once the reading is stable.

This operation is repeated with the standard 9.5 ppm and obtaining the E_2 value. The slope of the indicator electrode is $S = E_1 - E_2$, its value for this study being taken as in the range of 55 ±1 mV/decade.

To determine the fluoride ion in a sample, E is established using the same process. The conversion of E in the concentration of the fluoride ion is obtained by this equation:

$$pF = \frac{E - E_2}{S} + pF_2$$

where $pF = -\log (F^-)$; $pF_2 = -\log 5 \times 10^{-4} = 3.3$; $ppmF = 10^{-pF} \times 19{,}000$

Tea infusions are prepared by putting a small bag (2 g) into 200 mL (about one cup) of boiling drinking water and leaving it in the water for 5 to 10 minutes (the usual time in Spain).

All measurements in this work are average values from five determinations. The concentrations of cations in the infusions was determined by atomic absorption spectrophotometry.

Results and Conclusions

When the commonly employed TISAB II A solution was used, the fluoride content found in tea infusions was about 1-2 ppm (Table 1). The analysis of interfering cations in these infusions showed a maximum concentration of Al^{+3} of 5 ppm and slightly smaller concentrations of Fe^{+3}, Ca^{+2} and Mg^{+2}. The analysis of fluoride in drinking water gave a reading of less than 0.2 ppm, and the Al^{+3} value found was 0.28 ppm. With the 1.9 ppm standard

Table 1

F^- Concentrations Obtained in Tea Infusions When Using TISAB II A Solution

Tea	t (minutes)	F^- (ppm)
1	5	1.55
	10	1.93
2	5	1.00
	10	1.01
3	5	1.11
	10	1.16
4	5	0.92
	10	0.93
5	5	0.97
	10	1.24
6	5	1.22
	10	1.27
7	5	2.01
	10	2.36
8	5	1.75
	10	2.09
9	5	1.13
	10	1.13
10	5	1.70
	10	1.94
11	5	0.93
	10	0.92

Table 2

F^- Concentrations Obtained in the Standard 1.9 ppm With Several Contents of Al^{+3} When Using Various TISAB Solutions

Al^{+3} (ppm)	TISAB solution	F^- (ppm)
0	II A	1.87
2.5	II A	1.12
5	II A	0.77
5	II B	1.34
5	III	1.53
5	II C	1.81

of fluoride to which Al^{+3} was added along with various TISAB solutions, the F values shown in Table 2 were obtained.

The results indicate 1. The greater the concentration of Al^{+3} present in the sample, the greater error in determination of fluoride using TISAB II A solution. 2. The greater the concentration of citrate in TISAB II solutions, the better the results. 3. Results with TISAB III solution, also frequently used, are better than those obtained with TISAB II A and II B solutions, but those obtained by TISAB II C solution are better. The above conclusions led us to test the determination of fluoride in infusions using TISAB II C solution with the results shown in Table 3.

Since it was possible that a greater concentration of citrate might reveal higher readings of fluoride concentration, tests were carried out with TISAB II D solution on infusions Tea 1 and Tea 7, since these readings and TISAB II C solution did not differ significantly. Comparison of results of Tables 1 and 2 follows. If the content of Al^{+3} is not greater than 2.5 ppm, no significant differences are found when using TISAB solutions with a greater concen-

Table 3

F^- Concentrations Obtained in Tea Infusions With Several Contents in Al^{+3} When Using TISAB II C Solution.

Tea	t (minutes)	Al^{+3} (ppm)	F^- (ppm)
1	5	5.0	2.54
	10	5.0	2.61
2	5	2.8	1.17
	10	2.8	1.28
3	5	4.4	1.67
	10	4.4	2.07
4	5	3.2	1.09
	10	3.2	1.20
5	5	3.8	1.39
	10	3.8	1.63
6	5	2.2	1.06
	10	2.2	1.31
7	5	4.5	2.63
	10	4.5	2.70
8	5	2.4	1.59
	10	2.4	2.04
9	5	1.5	1.11
	10	1.5	1.17
10	5	3.4	2.11
	10	3.4	2.30
11	5	1.4	0.98
	10	1.4	0.99

tration of citrate than that used in TISAB II A solution, but in tea infusions containing 2.5-5 ppm of Al^{+3} the fluoride content measured rises with the concentration of citrate in TISAB solutions.

Taking into account the fluoride readings found in infusions shown in Table 3 and the maximum daily permitted dosage of this ion (about 1.82 mg) (5), the contribution of tea infusions to total ingestion of fluoride in the alimentary diet can be calculated (Table 4).

Conclusion

1. In determining fluoride in tea infusions, one must know the concentration of interfering cations in the sample, since their high content will cause the fluoride readings to be lower than actual unless the fluoride is chelated appropriately.

2. It is customary in Spain to drink one or two cups of tea daily. Hence, according to readings reported in column V of Table 4, the average

Table 4

The Contribution of Ingesting One Cup of Tea to Maximum Daily Permitted Dosage of Fluoride

Tea	t (minutes)	F^- (ppm)	F^- (mg/cup)	% Maximum Daily Permitted Dosage
1	5	2.54	0.508	27.9
	10	2.61	0.522	28.7
2	5	1.17	0.234	12.8
	10	1.28	0.256	14.1
3	5	1.67	0.334	18.4
	10	2.07	0.414	22.8
4	5	1.09	0.218	12.0
	10	1.20	0.240	13.2
5	5	1.39	0.278	15.3
	10	1.63	0.326	17.9
6	5	1.06	0.212	11.7
	10	1.31	0.262	14.4
7	5	2.63	0.526	28.9
	10	2.70	0.540	29.7
8	5	1.59	0.318	17.5
	10	2.04	0.408	22.4
9	5	1.11	0.222	12.2
	10	1.17	0.234	12.9
10	5	2.11	0.422	23.2
	10	2.30	0.460	25.3
11	5	0.98	0.196	10.8
	10	0.99	0.198	10.9

quantity of fluoride ingested from teas is not in itself likely to be dangerous, since it is less than the maximum daily permitted dosage. This amount of fluoride from tea can, however, contribute to a disturbingly high total ingestion when added to other sources of fluoride ion (toothpaste, fluoride water, wine, etc.).

References

1. Cook, H.A.: Crippling Arthritis Related to Fluoride Intake. **Fluoride**, 5:209-213, 1972.
2. Yanyu-Fu, Wanfeng-gen and Zhaurong-Shun: Analysis of Fluoride Content in 1376 Water Samples and Endemic Fluorosis in Jian Xi Province. **Fluoride**, 20:75-78, 1987.
3. Del Olmo, J.A., Sanmartí, R., Alba, R. et al: Fluorosis por Agua Mineral. **Medicina Clínica**, 85:560-561, 1985.
4. Wang, F.: The Effects of Different Fluoride Concentrations in Drinking Water on Dental Fluorosis and Dental Caries Incidence Among 12-14 Year Old Adolescents in Wuhan. **Fluoride**, 20:144, 1987. (Abstract)
5. Walters, C.B., Sherlock, J.C., Evans, W.H. et al: Dietary Intake of Fluoride in the United Kingdom and Fluoride Content of Some Foodstuffs. **J. Sci. Food Agric.**, 34:523-528, 1983.
6. Taves, D.R.: Dietary Intake of Fluoride Ashed (Total Fluoride) v. Unashed (Inorganic Fluoride) Analysis of Individual Foods. **Br. J. Nutr.**, 49:295-301, 1983.
7. Skorkowska-Zieleniewska, J.: In Heavy Tea and Coffee Drinkers "Nutritional Status" of Fluoride in Relation to That of Other Mineral Elements. **Fluoride**, 16:20-23, 1983.
8. Farkas, C.S.: Potential Fluoride Intake of Northern Canadian Indians. **Fluoride**, 10:137-141, 1977.
9. Mazarrasa, O. and Lazuen, J.A.: Fluoride in Spanish Bottled Waters. **Fluoride**, 9:201-203, 1976.
10. Sadowski, S. and Skorkowska-Zieleniewska, J.: Fluorine in Selected Beverages in the Domestic Market. **Rocz. Panstw. Zakl. Hig.**, 31:413-417, 1980.
11. Schmidt-Hebbel, H., Pennacchiotti, I., Truhaut, R. et al: Content of Fluoride in Some Foods and Beverages Consumed in Chile. **Fluoride**, 5:82-84, 1972.
12. Effendi, I. and Wibowo, D.: Fluoride in Tea. **Odonto-Stomatologie Tropicale**, 7:163-167, 1984.
13. King, N.M. and Tsang, M.C.K.: Fluoride Content of Chinese and Black Teas Available in Hong Kong. **Fluoride**, 20:18-23, 1987.
14. Srebrnik-Friszman, S. and Van der Mijnsbrugge, F.: The Fluoride Content of Some Varieties of Tea Obtained at the Belgian Markets and in Their Infusions. **Arch. Belges de Med. Soc. Hyg., Med. du Travail et Med. Leg.**, 33:551-556, 1975.
15. Duckworth, S.C. and Duckworth, R.: The Ingestion of Fluoride in Tea. **Br. Dent. J.**, 145:368-370, 1978.
16. Moody, G.J. Ong, B., Quinlan, K. et al: The Determination of Fluorine in Coffee and Tea Using a Microprocessor Coupled with a Fluoride Ion-Selective Electrode. **J. Food Technol.**, 15:335-343, 1980.
17. Farsam, H. and Ahmadi, N.: Fluorine Content of Teas Consumed in Iran. **J. of Food Science**, 43:274-275, 1978.

18. Pérez-Olmos, R.: Determinación del Contenido de Fluoruros en Tés. **Alimentaria**, 22:57-61, 1985.
19. Struebig W. and Guelzow, H.J.: Fluoride Content of Various Varieties of Tea. **Dtsch. Zahnarztl. Z.** 36:379-381, 1981.
20. Smid, J.R. and Kruger, B.J.: The Fluoride Content of Some Teas Available in Australia. **Aust. Dent. J.**, 30:25-28, 1985.
21. Harwood, J.E.: The Use of an Ion-Selective Electrode for Routine Fluoride Analyses on Water Samples. **Water Research**, 3:273-280, 1969.
22. Frant, M.S. and Ross, J.W.: Use of a Total Ionic Strength Adjustment Buffer for Electrode Determination of Fluoride in Water Supplies. **Anal. Chem.**, 40:1169-1171, 1968.
23. **Handbook of Electrode Technology.** Orion Research, 1982.
24. Yuchi, A. Ueda, K., Wada, H. et al: Equilibrium Study on the Masking of Aluminum Ion in the Determination of Fluoride with Ion-Selective Electrodes. **Anal. Chim. Acta**, 186:313-318, 1986.
25. Kauranen, P.: The Use of Buffers in the Determination of Fluoride by an Ion-Selective Electrode at Low Concentrations and in the Presence of Aluminum. **Anal. Lett.**, 10:451-465, 1977.
26. Vickery, B. and Vickery, M.L.: Suppression of Interfering Ions in the Analysis of Plants to Determine Fluoride Using the Fluoride Ion-Selective Electrode. **Analyst**, 101:445-454, 1976.

****

THE ORIENTATION OF GLYCOSAMINOGLYCANS IN OSTEOCYTE CAPSULES OF FLUORIDE TREATED RATS

by

J. Frege, C. Gräf, and G.W. Dominok*
Cottbus, GDR

SUMMARY: The oriented microstructure of the extracellular matrix in osteocyte capsules shows an enhanced birefringence after anisotopic staining procedures. The anisotopic staining of the functional groups of glycosaminoglycans has been performed on rat bone tissue sections with and without fluoride treatment of rats. The number of birefringent osteocyte capsules is reduced in fluoride-exposed rat bone tissue in relation to the bone fluoride content, indicating a fluoride-induced decrease of the spatial orientation of the glycosaminoglycan macromolecules in osteocyte capsules.

KEY WORDS: Birefringence; Fluoride content; Glycosaminoglycans; Osteocyte capsules; Rat bone.

Introduction

Fluoride-induced alterations of the collagen metabolism have been reported (1,2) in the literature. Bély (1986) described a reduced spacial orientation of collagen in the bone matrix of rats after fluoride treatment, by use of topo-optical reactions and polarization microscopical analysis. Oriented microstructures of the extracellular matrix show an enhanced birefringence after aniosotopic stain procedures (oriented binding of reagents to oriented microstructures), called topo-optical reactions (3). Osteocyte capsules (OC) consist of uncalcified bone matrix (4). We found a decreased spatial ordering of collagen of OC in rats in relation to the fluoride content of bone. The question arises: are there also changes in the steric orientation of glycosaminoglycan macromolecules (GAG) in the OC of fluoride-treated rats, detectable by polarization microscopical investigation.

Materials and Methods

Forty-five rats (female Wistar rats, body weight: 200 g) were divided into 3 groups and treated with NaF in 2 different concentrations by i.p. application: 0.5 mg NaF per day (low exposure group); 5.0 mg NaF per day (high exposure group); 2 mL physiological sodium chloride solution per day (control group). After 12 weeks of treatment all animals were sacrificed. The fluoride content of bone ash (femur, tibia, humerus of the left side of the body were measured by fluoride sensitive electrode.

Following histotechnical preparations of bone tissue samples of the right femur, tibia and humerus were performed: decalcification (formic acid + 70% ethanol 1:1), paraffin sections (thickness: 10 µm), deparaffination (xylene 56 °C, 16 h) stainings: 0.1% toluidine blue pH 5.0 10 min for staining carboxyl and

* Prof. Dr. G.W. Dominok, Institute of Pathology, County Hospital of Cottbus, German Democratic Republic, 7500 Cottbus, Thiemstr. 111.

sulfate groups; 0.1% toluidine blue pH 6.0 10 min after blocking the carboxyl groups by CH_3J for staining the sulfate groups (4,5). The stained OC were counted in the cortical bone and the number of birefringent OC among 500 stained OC was determined in each histological section; expressed as percentage by use of a polarization microscope "Amplival pol d Carl Zeiss Jena."

The significance of differences in the number of birefringent OC and fluoride level among the bones and groups was calculated by Student's t-test.

Results

In the control group different bones show different fluoride content: the highest fluoride level was found in the femur, the lowest in the tibia. The fluoride content is increased in bone tissue samples of both fluoride-treated animal groups compared to the control group; it was different between the exposed groups, depending upon the fluoride level of treatment. The GAG orientation in OC is depressed by fluoride treatment: the number of birefringent OC is reduced significantly in both fluoride-exposed animals groups, in relation to controls.

The decrease of birefringent OC is higher in the group exposed to higher levels of fluoride than in those exposed to the lower level (Table 1; Figures 1-6).

Table 1

Fluoride Content of Bone and Number of Birefringent OC of Fluoride-treated Rats

Bone	Fluoride Content (ppm)		Birefringent OC (%) Anionic Groups		Sulfate Group	
	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.
High Exposure Group						
Femur	11,854	798	20.89	8.89	26.20	7.29
Tibia	11,047	654	20.39	7.39	26.76	6.92
Humerus	12,224	539	19.87	7.19	24.57	7.90
Low Exposure Group						
Femur	2,827	214	86.63	3.92	53.88	10.16
Tibia	2,791	217	86.51	2.72	54.78	12.14
Humerus	2,861	157	81.53	2.86	53.77	12.94
Control Group						
Femur	1,415	113	94.67	1.91	90.48	3.21
Tibia	1,288	105	94.45	1.55	87.47	2.66
Humerus	1,370	132	94.78	1.37	88.66	2.86

Figure 1

Cortical bone (humerus of rat No. 112 – control group). Intensive metachromatic (red) staining of OC. Topo-optical reaction: Blocking of the carboxyl groups by CH_3J + staining of the sulfate groups by Toluidine blue pH 6.0. Bright field. x 520

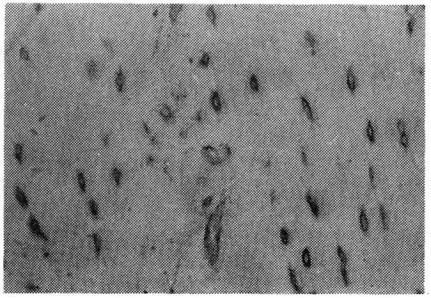


Figure 2

The same area and magnification as in Figure 1 between crossed polars. Numerous birefringent OC.

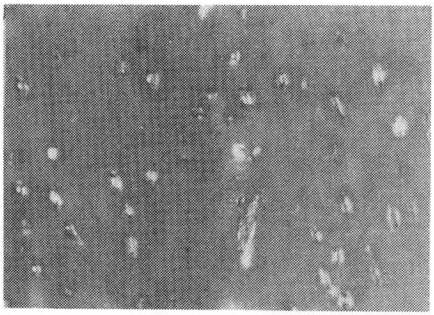


Figure 3

Cortical bone (femur of rat No. 210 – lower exposure group). Intensive staining of OC. Same topo-optical reaction as in Figure 1. Bright field. x 520

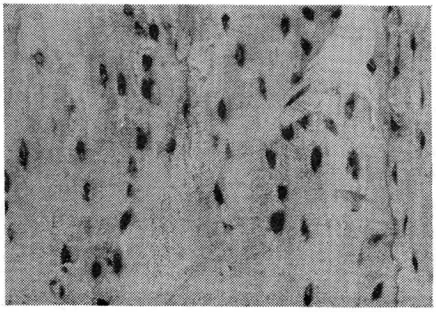
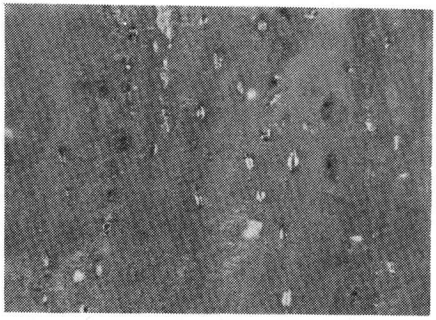


Figure 4

Same area and magnification as in Figure 3 between crossed polars. Low decreased number of birefringent OC.



Conclusions

The GAG orientation in the bone matrix is altered on the basis of our findings on the birefringence of OC, caused by fluoride. The decrease of birefringent OC seems to be a sensitive indicator of the fluoride-induced biological condition of bone cells and bone tissue, because there is a strong

Figure 5

Cortical bone (femur of rat No. 115 – control group) between crossed polars. Numerous birefringent OC. Toluidine blue pH 5.0. x 260

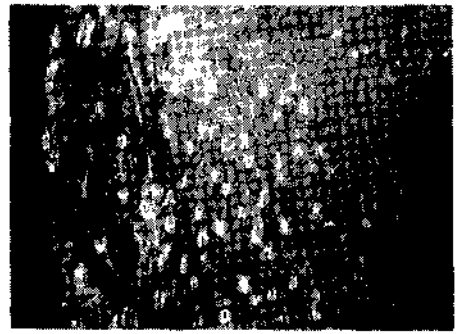


Figure 6

Cortical bone (femur of rat No. 313 – high exposure group) between crossed polars. Decreased number of birefringent OC. Toluidine blue pH 5.0 x 260

correlation between the decreased number of birefringent OC and the level of bone fluoride content. It is not a specific one, because other experiments have also reduced the number of birefringent OC (4). The impairment of the submicroscopic structure of OC alters the transport activity (4). Probably fluoride depresses the bone cell metabolism in 2 different ways, namely 1] it influences various enzyme activities directly and 2] it indirectly alters cell metabolism by changes in the extracellular transport activity, indicated by a reduced steric orientation of collagen and GAG.

Acknowledgement

We are grateful to DC. K. Seifert from the County Hygiene Institute of Cottbus for the chemical investigations.

References

1. Sharma, Y.D.: Variations in the Metabolism and Maturation of Collagen after Fluoride Ingestion. **Biochim. et Biophys. Acta**, 715:137-141, 1982.
2. Uslu, B.: Effect of Fluoride on Collagen Synthesis in the Rat. **Res. Exp. Med.**, 182:7-12, 1983.
3. Romhanyi, G.: Über die submikroskopische strukturelle Grundlage der metachromatischen Reaction. 1966, [cited by Modis, L., 1974.]
4. Modis, L.: Topo-optical Investigations of Mucopolysaccharides (Acid Glycosaminoglycans), In: Graumann, W. and Neumann, K., Eds.: **Handbuch der Histochemie**, Vol. II, Part 4, Gustav Fischer Verlag, Stuttgart, 1974.
5. Adany, R.: A Selective Topo-optical Reaction of Sulfated Glycosaminoglycans. VII European Symposium on Connective Tissue Research, Prague, 1980.

****

FLUORIDE CONTENT IN RIVER WATER
A LONGITUDINAL PROFILE FROM SOURCE TO MOUTH

by

C.W. Schmidt* and W. Leuschke
Heidenau, GDR

SUMMARY: A longitudinal profile of rivers can provide information not only about the natural-geologic fluoride load, but it can also detect artificial fluoride sources. In this way it can fulfill a contribution to ecological research and to communal hygienic sanitation.

KEY WORDS: Fluoride, artificial, natural; Ecological research.

Introduction

Fluoride in natural waters is of concern because of its impact on the environment. Fluoride in river waters can affect drinking water (1), agriculture, animal nutrition and the entire surrounding ecosystem (2). A toxic substance for the skeletal system (3) and the teeth (4) fluoride has been utilized to alleviate osteoporosis (5).

A fluoride investigation of river waters from source to mouth including subsidiary rivers provides information regarding deposits of fluoride as well as the discovery of artificial fluoride sources such as industrial emitters (6).

Materials and Methods

Twenty-three river water samples were collected in summer and thirteen in winter, respectively, from a river and several of its subsidiary rivulets from source to mouth, a distance of about 40 kilometers. The fluoride content in all samples was measured by means of a fluoride-sensitive electrode (Krytur ISE/CSSR) after addition of TISAB (7).

Since at the lower course of the river at a little town extremely high fluoride levels were found, additional samples of water were collected upstream, downstream, and also in the middle of the town daily during the months from March to August. Downstream from the town during one week, daily samples were taken at the same time each day.

Results

Results (Table 1) show that fluoride values varied widely from 0.16 to 69 ppm along the river.

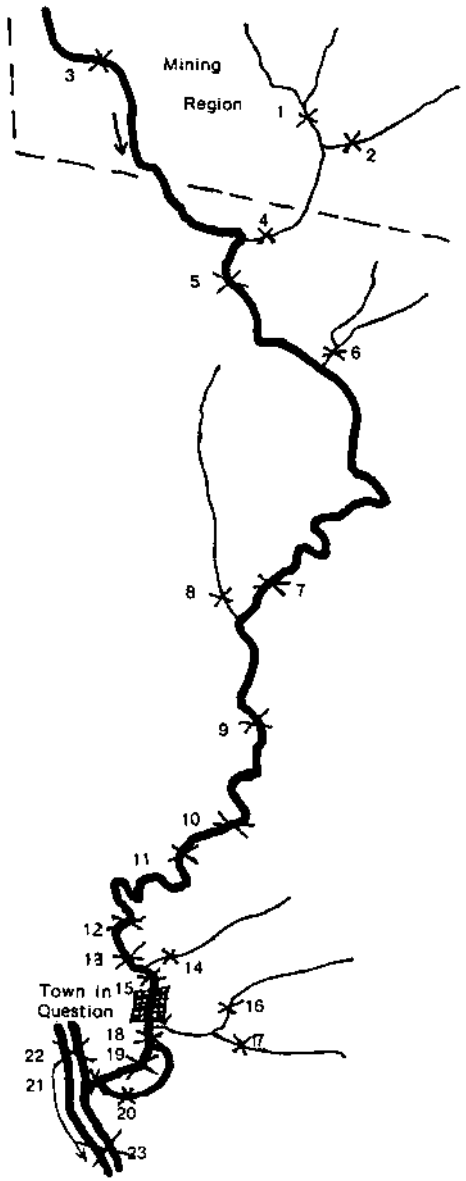
They fall into the following groups:

1. Main river from source upstream to the town in question near the

* Department of Internal Medicine, General District Hospital, Heidenau, DDR-8312, GDR

Figure 1

River from source to mouth with subsidiary rivulets and points of water sample collecting.



mouth (19 samples): fluoride values averaged 2.38 ppm, ranging from 1.0 to 11.0 ppm.

2. Tributary rivulets (8 samples): fluoride values ranged from 0.16 to 0.83 ppm (average 0.53 ppm).

3. Main river downstream from the town in question to the mouth (7 samples): fluoride content averaged 44.7 ppm, ranging from 17 to 69 ppm.

The river into which the river sampled by us empied showed fluoride values upstream at the mouth of only 0.8 ppm; 100 meters downstream from the river's mouth values were only 4.8 ppm.

Fluoride values were measured monthly in river water above and below the town in question (Table 2): Above the town the average was 2.5 ppm; within the town the fluoride content was 49.6 ppm and directly below the town 50.8 ppm.

Fluoride values in river water below the town in question varied widely during one week of sampling (the average was 43.0 ppm). On a daily basis, fluoride values (ppm) were as follows: Monday, 28.0; Tuesday, 17.0; Wednesday, 42.0; Thursday, 125.0 and Friday, 39.0; Saturday, 35.0; and Sunday, 15.0.

Discussion

The groups of fluoride values with similar levels in the river can be explained as follows:

Group 1: The upper source of the river flows through mountains, rich in ore and minerals where until now mining activities have been in operation. Rock containing tin ore mined from the depths of the earth is pulverized and later washed with

Table 1

Fluoride Content in River Water Samples (ppm)

Sample Collecting Point	Summer	Winter
1	1.15	2.3
2	0.83	0.56
3	1.7	
4	0.5	
5	1.0	1.0
6	1.45	1.15
7	1.6	11.0
8	0.38	0.16
9	1.5	3.35
10	1.7	2.45
11	1.7	2.4
12	3.1	2.07
13	2.3	2.2
14	0.75	
15	62.0	26.0
16	0.52	
17	0.5	
18	51.0	
19	69.0	17.0
20	46.0	
21	42.0	
22	0.8	
23	4.8	

great amounts of river water. This procedure caused the river water to become red and to contain, from rock minerals, considerable amounts of fluoride (8).

Group 2: All small tributary rivers, also coming from the ore-mountains, have a fluoride content below 1 ppm since they do not pass through the mining region.

Group 3: Above the town, at the lower course of the river, the fluoride content is as high as in the upper course. Therefore it is also influenced by the mining activities. Within and below the town the fluoride values increase at such an extraordinarily high rate that unanticipated fluoride sources had to be identified. Three industrial fluoride emitters within the town have been operating; they send their fluorine-containing waste waters into the river. A hydrofluoric acid factory (3) the main emitter, an aluminum smelter (9) and a graphite mill which uses concentrated hydrofluoric acid for purification processes, drains the acid into the river after use once weekly.

That the extremely high fluoride levels in the river water below the town originate from the above-mentioned three factories is substantiated by the fact that the fluoride values in the river are lowest on Sunday. The highest value of 125 ppm fluoride seen on Thursday is caused by the graphite mill dumping into the river. On this day only the factory uses hydrofluoric acid for "purification," really an anachronism. In previous years, drinking water for the town was obtained from local wells situated near the river banks downstream from the town. Therefore, drinking water was also highly contaminated with fluoride; severe cases of dental fluorosis were observed in children (4). A few cases of fluorosis in inhabitants who had been living for decades in the town without occupational fluoride contact (3) are rare cases of so-called neighborhood skeletal fluorosis.

The town's drinking water wells have been closed. Now the inhabitants

Table 2

Fluoride Content (ppm) in River Water Samples from Upstream, Downstream and Middle of the Town in Question.

Month of Sampling	Upstream from Town	Center of Town	Downstream from Town
March	1.12	56.0	44.0
April	1.25	11.2	10.9
May	1.64	28.8	34.6
June (begin.)	2.30	62.0	69.0
June (end)	2.40	45.0	71.0
July	5.80	94.0	76.0
August	2.65	50.0	50.0

obtain their water from a distant drinking water storage reservoir in which the fluoride content is low (0.25 ppm).

References

1. Schmidt, C.W. and Funke, U.: Trinkwasserfluorkataster des Kreises Pirna/ Bezirk Dresden. **Stomatol. DDR**, 32:550-553, 1982.
2. Yu, M.-H. and Tsunoda, H.: Environmental Fluoride Problems in China. **Fluoride**, 21:163-166, 1988.
3. Schmidt, C.W.: **Untersuchungen über die Entwicklung einer nicht berufsbedingten Skelettfluorose – Nachbarschaftsfluorose – mit Berücksichtigung spezieller Morbiditätsfragen im Territorium.** Bromotion B, Halle/Saale, 1983.
4. Binder, G., Jackisch, H. and Thun, I.: **Die Dentalfluorose in Dohna/Bezirk Dresden.** Promotion A, Dresden, 1979.
5. Franke, J.: **Our Experience in the Treatment of Osteoporosis with Relatively Low Doses of Sodium Fluoride.** Editions Médicine et Hygiène, Geneva, 1978, p. 256-262.
6. Schmidt, C.W.: Auftreten von Nachbarschaftsfluorose unter der Bevölkerung einer sächsischen Kleinstadt. **Dt. Gesundh.-Wesen**, 31:1271-1274, 1976.
7. Leuschke, W. and Schmidt, C.W.: Fluoridbestimmung in der Knochenasche nach Beckenkammstanzbiopsie. **Z. klin. Med.**, 41:231-232, 1986.
8. Auermann, E. and Borris, W.: Fluorgehalt in einigen erzgebirgischen Trinkwässern. **Z. gesamte Hyg.**, 7:484-492, 1961.
9. Schmidt, C.W.: Nachbarschaftsfluorose – Untersuchungen in der Umgebung einger Aluminiumdruckgiesserei. **Dt. Gesundh.-Wesen**, 31:1700-1703, 1976.

****

ALUM PLANT WASTE: APPLICATION IN TREATMENT OF FLUORIDE POLLUTED WATER

by

Shyama Nair, Geeta Jallan and G.S. Pandey*
Raipur, India

SUMMARY: In a study of the removal of fluoride by alum waste, discharged by an alum manufacturing plant, it was observed that the presence of Ca hardness in the treatment medium is advantageous to its total removal. The optimum pH of the treatment medium was 7.64. One gram quantity of alum waste removed 10 mg of soluble fluoride in three days. Alum waste can thus be economically used for removal of fluoride from potable and industrial waste waters.

KEY WORDS: Alum waste; Ca hardness; Fluoride; Waste waters.

Introduction

The effects of ingestion of fluorides are widely reported. Indian ground waters are often characterized by high basicity and fluorides. For removal fluoride requires an alum treatment. However, the allowable concentration of sulphate without affecting the potability of water is 400 mg/L. Bulusu (1) described the use of $AlCl_3$ in place of $Al_2(SO_4)_3$ for the removal of fluoride, keeping in view the sulphate limit in potable water. Waters heavily polluted with fluoride are also discharged by several manufacturing industries such as phosphate fertilizers (2), semiconductors, and alumina (3). In this connection, we report a study of the application of alum-plant waste for the removal of soluble fluorides in fluoride contaminated water. About 71 kg of alum waste is discharged by an alum manufacturing plant per ton of alum (hydrated aluminum sulfate) manufactured. The manufacturing process involves treatment of crushed bauxite ore with diluted sulphuric acid (80%).

Materials and Methods

Sample Collection: Three samples (each 1 kg) of alum waste were collected from three different points of the stock pile of waste matter of an alum manufacturing plant, and then composited.

Sample Preparation: The air dried sample was powdered to 100 mesh size.

Sample Analysis: The alum plant waste was chemically analyzed by recommended procedure (4,5) and the percentages of major components (%) were as follows: SiO_2, 12.4%; Al_2O_3, 53.4%; Fe_2O_3, 3.2%; CaO, 0.1%; Na_2O, 0.7%; K_2O, 0.2%; TiO_2, 24.0%; $SO_4^=$, 2.2%; Cl^-, 1.2%; S, 1.0%; solubility (g/100 mL at 32 °C), 1.42.

Treatment Procedure: The following series of reaction mixtures were prepared: **1.]** 100 mL NaF solution (100 mg F^-/L) + 100 mL hard water (hardness 965 mg $CaCO_3$/L); **2.]** alum waste (1 g) + 100 mL NaF solution (100 mg F^-/L);

* Department of Chemistry, Ravishankar University, Raipur, India.

3.] alum waste (1 g) + 100 mL NaF solution (100 mg F^-/L) + 100 mL hard water (hardness 965 mg $CaCO_3$/L).

Three sets of pH values 3.10, 5.89, and 7.64 were used for each series. Chemically pure sulphuric acid and sodium hydroxide solutions were used for the pH adjustment. The fluoride levels in nine reaction mixtures of the three series were determined spectrophotometrically (6) using zirconyl-alizarin red S reagent, measuring absorbance values at 495 nm. Standard solutions of fluoride were prepared using anhydrous sodium fluoride (analytical grade 99.99% pure) for obtaining the calibration graph. The fluoride levels were measured at intervals of one day until constant values were obtained.

Results and Discussion

The results obtained led to the following conclusions: 1.] The Ca hardness, pH value of the medium and the presence of alum waste, separately and in combination showed positive effects on the extent of removal of fluoride. 2.] In all cases, increasing acidity of the medium resulted in a decreasing effect on removal of fluoride. 3.] In the presence of alum waste alone, fluoride was partially removed (64%). 4.] Ca hardness alone produced a partial but greater removal of fluoride (82%). 5.] The combination of alum waste and Ca hardness induced slow fluoride removal during the first day. It became faster thereafter, resulting in total removal in the three-day period.

Ca hardness is almost always present in potable and industrial waste waters. It presence is, therefore, a built-in advantage for decreasing the fluoride level. It has been observed that if fluoride polluted potable or industrial waste water is subjected to a treatment of alum plant waste, the fluoride can be completely removed by devising a suitable dose rate of alum waste. These findings are of additional value to manufacturers who produce both alum and phosphate fertilizers at the same location; their alum waste discharge can be diverted for removal of fluorides from heavily polluted effluents.

References

1. Bulusu, K.R.: Defluoridation of Waters Using Combination of $AlCl_3$ and $Al_2(SO_4)_3$. **J. of Inst. of Engineers** (India), Env. Eng. Div., 65:22-26, 1984.
2. Agerwal, K.M., Sharma, H.C. and Agarwal, A.L.: Fluoride Accumulation Characteristics of Soil and Vegetation in the Vicinity of a Phosphate Fertilizer Plant. **Asian Environ.**, 10:53-62, 1988.
3. Hitachi Plant Engineering and Construction Co., Ltd.: **Fluoride Removal from Waste Water.** Japan Kokai, Tokyo, n.d., [1984] p. 373.
4. Bassett, J., Denny, R.C. Jeffery, G.M. and Mendham, J.: **Vogel's Text Book of Quantitative Inorganic Analysis**, 4th ed. ELBS and Longmann, London, 1975.
5. Furman, N.H.: **Standard Methods of Chemical Analysis.** 6th Ed. D. Van Nostrand Co., Inc., London, 1962, Vol. 1, p. 1100-1101.
6. Patel, C.B. and Pandey, G.S.: Fluoride Content of Effluent and By-Products of an Aluminum Industry in India. **Fluoride**, 19:10-13, 1986.

****

THE ANTI-FLUORIDE EFFECTS OF ZHONGHUA MAIFANSHI
An Experimental Study

by

Guang-Sheng Li, Jian-Xue Wang, and Ling Jing
Changchun, Jilin, China

SUMMARY: The anti-fluoride effect of Zhonghua Maifanshi (China medical stone, CMS) was studied using osteomalacic skeletal fluorosis of rats as an animal model. When CMS powder was added either to the diet or to drinking water, at the ratio of 1000 or 2000 ppm respectively, the toxic action of excess fluoride on rats was alleviated. Compared to the control, the degree of osteomalacia induced by fluoride was reduced; the activity of serum ALP decreased; the serum corticosterone level increased; the serum content of T_4 declined; the lowered ratio of Ca/P in serum was restored to normal. The foregoing results indicate that CMS serves as an antidote to fluoride. The mechanism however remains to be clarified.

KEY WORDS: Anti-fluoride effect; Maifanshi; Osteomalacia; Skeletal fluorosis.

Introduction

Fluorosis is a serious problem in some areas of the world. Scientists have been searching for a suitable antidote to fluoride for a long time. Since 1975, Rao and his co-workers (1) have found serpentine is useful in the treatment of skeletal fluorosis. Because some kinds of serpentine minerals contain asbestos, known as a carcinogen, the potential risk of extensive use of serpentine may not be safe (2).

Zhonghua maifanshi (China medical stone, CMS) which can be found in Inner Mongolia is a kind of intrusion of grandiorite porphyry composed of aluminosilicates. Maifanshi was documented as a medical stone by a famous medical scientist Li Shi-Zhen in his **Compendium of Materia Medica** about 400 years ago. In recent years it has been used in producing artificial mineral water and refigerator odor killer in Japan. In the present paper, the anti-fluoride effects of CMS is reported.

Materials and Methods

Forty-eight adult rats, the mean weight of which was about 100 g with 1:1 male-female sex ratio, were equally divided into four groups. All groups were kept on a diet using maize as a staple (maize, 89%; soybean, 10%; table salt, 1%). The drinking water was supplemented with 200 ppm sodium fluoride in all groups except Group I. In Group II, CMS powder was added to the diet at a ratio of 1000 ppm, in Group IV the drinking water was pretreated with 2000 ppm CMS powder prior to supplementation by fluoride.

* From the Institute of Endemic Diseases, Norman Bethune University of Medical Sciences, Changchun, Jillin, People's Republic of China.

After 50 days the rats were sacrificed for blood, bone and kidney assays. The morphological changes of the skeletal system were observed with the naked eye and under the microscope; the content of copper, calcium, zinc in bone (femur) and kidney were analyzed with atomic absorption spectrophotometry; the activities of alkaline phosphatase (ALP), lactate dehydrogenase (LDH), glutamic-oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) in serum and cartilage and the serum contents of calcium and phosphorus were determined by Abbot Biochromatic Analyzer 100; the levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), corticosterone, thyroid-stimulating hormone (TSH), and thyroxine (T_4) were analyzed by radioimmunoassay.

Results

Pathologic Changes: The rats treated with fluoride (Group II) showed typical changes of osteomalacic skeletal fluorosis, such as enlargement of the epiphyseal ends of the long bones and fusiform or moniliform swelling at the middle of the ribs (3). In Group III and IV with additioon of CMS, the degree of osteomalacia was reduced (Tables 1 and 2).

Table 1

Pathologic Changes of Ribs in Different Groups

Groups	Degrees of Pathologic Changes				
	-	+	++	+++	++++
II	0	3	5	2	2
III	1	3	5	0	0
IV	1	2	7	0	0

Table 2

Areas* of Hypertrophic Chondrocyte Layer of Growth Plate of Tibiae (Upper End)

Groups	N	Areas of Hypertrophic Chondrocyte Layer
II	7	176.14 ±40.00
IV	6	94.50 ±45.96

Group II : Group IV, $p < 0.05$

* Mean values measured under microscope with an eyepiece check micrometer at 10 x 10 magnification.

Endocrinologic Examination: Table 3 shows that the content of T_4 was markedly increased in Group II treated with fluoride. With the administration of CMS in Group IV, the increased content was decreased. In Table 4, following the addition of CMS, the declined level of corticosterone (as shown in Group II) was raised (Group III and IV).

Table 3

The Contents* of T_4 and TSH in Group I, II, IV

Groups	T_4(ng/mL)	TSH(ng/mL)
I	88.70 ±56.08	0.18 ±0.08
II	162.27 ±51.85**	0.51 ±0.55
IV	105.88 ±21.08***	0.38 ±0.25

* Mean values ±S.D.
** Group I : Group II, $p < 0.01$
*** Group II : Group IV, $p < 0.05$

Table 4

The Level* of Corticosterone, LH and FSH in Serum in Different Groups

Groups	Corticosterone (ng/mL)	LH (ng/mL)	FSH (ng/mL)
I	254.6 ±107.2	0.1169 ±0.0460	2.6562 ±0.4285
II	101.1 ±52.8**	0.1405 ±0.0660	2.6708 ±0.5894
III	288.5 ±127.4***	0.0700 ±0.0094	2.9842 ±0.9182
IV	157.2 ±53.4****	0.1157 ±0.0864	3.3167 ±1.0382

* Mean Values ±S.D.
** Group I : Group II, $p < 0.05$
*** Group II : Group III, $p < 0.01$
**** Group II : Group IV, $p < 0.05$

Element Content: The content of copper, calcium and zinc is summarized in Table 5. No significant differences were observed between different groups, although the copper content in bone was slightly higher in Groups III and IV than that in Group II.

Table 5

Element Content (Mean ±S.D.) in Kidney and Bone

Organs	Groups	Cu (ppm)	Ca	Zn (ppm)
Femur	I	5.15 ±0.85	9.61 ±0.45 (mg/g)	3.26 ±0.94
	II	4.67 ±0.56	8.44 ±1.11 (mg/g)	2.52 ±0.59
	III	6.16 ±2.67	8.28 ±0.92 (mg/g)	2.98 ±1.20
	IV	9.26 ±7.91	9.18 ±1.42 (mg/g)	2.60 ±1.93
Kidney	I	11.51 ±4.65	71.92 ±25.95 (ppm)	8.63 ±2.25
	II	9.17 ±1.55	89.05 ±44.11 (ppm)	9.48 ±4.42
	III	9.57 ±1.66	64.59 ±17.59 (ppm)	10.06 ±4.33
	IV	9.42 ±1.05	87.75 ±40.04 (ppm)	7.54 ±2.03

Biochemical Examination: As shown in Tables 6 and 7, the ratio of Ca/P in serum was lowered in Group II and raised to the Control level in both Groups III and IV. The activity of LDH in cartilage was increased in Group II and decreased to the Control level in Group IV.

Table 6

The Activities (Mean ±S.D.) of ALP, GPT, GOT and the ratio of Ca and P in Serum

Groups	Ca/P	ALP (IU/L)	GPT (IU/L)	GOT (IU/L)
I	0.62 ±0.10	615.25 ±137.93	87.50 ±22.63	228.25 ±18.08
II	0.44 ±0.05	701.80 ±92.92	73.30 ±15.80	226.30 ±27.71
III	0.63 ±0.18	620.70 ±90.57	98.30 ±12.51	203.75 ±30.65
IV	0.65 ±0.28	546.50 ±118.27	90.56 ±50.55	199.44 ±30.47

Ca/P Group I : Group II, $p < 0.05$; Group III : Group II, $p < 0.05$; Group IV: Group II, $p < 0.05$
ALP Group IV : Group II, $p < 0.05$

Table 7

The Activities of LDH, ALP, GOT in Rib Cartilage

Groups	LDH (IU/mg pr)	ALP (IU/mg pr)	GOT (IU/mg pr)
I	0.82 ±0.20	0.46 ±0.24	0.47 ±0.04
II	1.11 ±0.12*	0.63 ±0.14	0.43 ±0.08
IV	0.89 ±0.20**	0.53 ±0.12	0.52 ±0.04

* Group I : Group II, $p < 0.05$
** Group IV : Group II, $p < 0.05$

Discussion

The above-mentioned results indicate that CMS definitely reduces fluoride toxicity as reflected by the morphologic changes, the serum ALP activity, the serum leves of T_4 and corticosterone, the ratio of Ca/P in serum, etc.

It is of interest that the serum T_4 rose in Group II treated with fluoride and dropped markedly after administration of CMS. Scientists disagree concerning the effect of excess fluoride upon the thyroid function. The disagreement may be related to the doses of fluoride. When experimental animals are treated with relatively small doses of fluoride, the secretion of thyroxine may be suppressed (4). When high doses of fluoride (more than 100 ppm of F^- in drinking water) were given, the serum thyroxine increased (5). The present experimental result is the same as the latter situation. Therefore, the decrease of T_4 in the CMS treated group is a manifestation of alleviation of fluoride toxicity although the mechanism is unclear.

In view of the facts that tissue copper decreased under exposure to excess fluoride (6) and copper deficiency may lead to a decreased formation of adrenal steroid hormone (7), the serum corticosterone was tested in the present study. Suppresion of corticosterone was revealed in this study and was repeated many times in our laboratory. Whether this suppression is due to secondary copper deficiency or to the direct action of fluoride on the endocrine system remains to be clarified. It should be mentioned that, in a previous experiment, the corticosterone level also rose in rats given CMS without adding fluoride to the drinking water (8). So CMS itself can increase adrenal steroid synthesis rather than through its anti-fluoride effect only. Further study is needed for elucidation of the real mechanism.

The chemical composition (weight %) of CMS was as follows (9): SiO_2, 62.17; Al_2O_3, 17.75; Na_2O, 5.00; CaO, 3.87; K_2O, 3.24; FeO, 2.27; Fe_2O_3, 1.89; MgO, 1.39; BO_3, 1.13; TiO_2, 0.76; P_2O_5, 0.36. Essential trace elements such as Zn, Cu, Mn, Co, Cr, Mo, Se, V, Ni, Sn and rare-earth elements, were also found in it. Additional observations in our laboratory showed that the solubilities of Ca, Al, Mg, Fe and Cu of CMS were markedly increased in acid solution (ph = 2) under 37 °C, a mimic of conditions in the human stomach (10). It is well-known that calcium can inhibit fluoride toxicity. As the calcium content of CMS is very limited and 2000 ppm of $CaCO_3$ is necessary for elimination of the osteomalacic effect of fluoride under experimental conditions (3), it is unlikely that calcium alone can play an important role in the anti-fluoride effect of CMS. More attention should be paid to the action of some trace elements in CMS.

As a natural substance, CMS has its proper ratio of various elements and compounds. It might be more helpful and safe for clinical usage than a single element. According to the experience of Chinese traditional medicine and the current research work, CMS has no harmful effect on human health in a therapeutic dosage. Therefore CMS may be valuable in the prevention and treatment of fluorosis, although its effective components and the exact mechanism of fluorosis, although its effective components and the exact mechanism of its anti-fluoride role require further investigation.

References

1. Rao, S.R., Murty, K.J.R., Murty, T.V.S.G. et al.: Treatment of Fluorosis in Humans. **Fluoride**, 8:12-24, 1975.
2. Liu, H.B.: The Effect and Security of Serpentine in Treating Fluorosis. [in Chinese], **Endemic Diseases Bulletin**, 3:75-77, 1988.
3. Jing, L. et al.: An Experimental Model of Osteomalacic Skeletal Fluorosis. [in Chinese], **J. Norman Bethune Univ. Med. Sci.**, 13:79-82, 1987.
4. Yu, Y.N. et al.: The Effects of Chronic Fluorosis on the Thyroid Gland. **National Medical Journal of China**, 65:747-749, 1985.
5. Tsuchida, D.: Effects of Fluoride Toxicity on Serum and Urine Components – An Experimental Study on Rats Kept on Drinking Water Containing 100 ppm F^- for One Year. [in Japanese], **Fluoride Research**, 7:20-25, 1986.
6. Singh, M. and Kanwar, K.C.: Copper and Iron in Tissue Following Experimental Fluorosis. **Fluoride**, 14:107-111, 1981.
7. Kenkin, R.I.: Trace Metals in Endocrinology. **Med. Clin. North Am.**, 60:779-797, 1976.

8. Jing, L. et al.: Zhonghua Maifanshi as an Antidote in Fluoride Intoxication in Rats. [in Chinese], **Chin. J. Control Endemic Dis.**, 4(Fluorosis Suppl.):19-20, 1989.
9. (Anonymous): **Zhonghua Maifanshi — A Health Gem.** China Foodstuff Publishing House, Beijing, 1987, pp. 35-42.
10. Wang, J.X. et al.: The Mechanism of Anti-fluoride Role of Zhonghua Maifanshi — A Preliminary Experiment. [in Chinese], **Chin. J. Control Endemic Dis.**, 4(Fluorosis Suppl.):17-18, 1989.

****

FLUORIDATION: A FIFTY-YEAR-OLD ACCEPTED BUT UNCONFIRMED HYPOTHESIS

by

Philip R.N. Sutton

(Abstracted from **Medical Hypotheses**, 27:153-156, 1988)

The fluoridation hypothesis, now fifty-years-old, has never been confirmed. Nevertheless, millions of people are still being medicated with fluoride by government decree, on the assumption that this process has been proven entirely safe, and very efficacious in reducing the incidence of dental caries whereas no scientifically-acceptable evidence has ever been presented. Promotion is mainly based on emotion-based endorsement.

Relying on endorsements of USPHS, American Medical Association, and American Dental Association, without investigating, numerous bodies, including service organizations, mothers' clubs and even the Boy Scouts of America, hastened to endorse this process.

After more than thirty years, claims made by authors of five faulty studies still remain the main basis for the contention that fluoridation greatly reduces dental caries. Projects termed "demonstrations" were commenced in the U.K. in 1955/56 and in New Zealand in 1954. The term "demonstration" was used to conceal the true situation — since practically nothing was known of the health effects of prolonged medication with fluoride. These, like the American trials, were experiments on large numbers of human subjects without their consent. For many years it was simply assumed that it was safe, a myth finally shattered in 1972-1975, when Hans Moolenburgh, M.D. of Haarlem, Holland, and his research team of physicians, demonstrated by double-blind tests that fluoridation can produce many harmful side-effects. Moolenburgh explained "Instead of simply prohibiting the use of fluorides, as they [the authorities] had done with cyclamates," they used every statistical and judicial gimmick to keep their defeat and loss of face at bay.

When, in 1977, a U.S. Congressional inquiry into the fluoride/cancer link revealed the National Cancer Institute had done no original studies to support its twenty-five year endorsement of fluoridation, the N.C.I. was forced to undertake an animal study — which according to the N.C.I. would require three years. After ten years the results of the "three year" study have yet to be reported. Politicians and bureaucrats are reluctant to acknowledge their mistakes — if indeed they realize that fluoridation has been widely rejected because it has been shown to be both ineffective and unsafe.

"Fluoridation must not continue to be accepted on the basis of 'endorsements,'" the authors states "merely because it is convenient for politicians and emotionally attractive to the dental profession."

KEY WORDS: Efficacy; Endorsements; Fluoridation; Harmful side effects; Safety.

REPRINTS: 163A New Street, Brighton, Victoria 3186, Australia.

****

COMPARISON OF DIFFERENT METHODS FOR ESTIMATING HUMAN TOOTH-ERUPTION TIME ON ONE SET OF DANISH NATIONAL DATA

by

J. Heidmann
Aarhus, Denmark

(Abstract from Archs. Oral Biol., 31:815-817, 1986)

The prevalence of congenitally missing teeth and the eruption times for the teeth in the human permanent dentition defined as the mean age at which the teeth emerge in the mouth, were calculated from cross-sectional records of 760,108 children. A computation method based on probit analysis and Kärber's method based on dosage/response curves were compared and their estimates related to figures obtained by direct interpolation in the data. Kärber's method was less precise than probit analysis especially when data were not corrected for frequencey of congenitally missing teeth or the number of observation points decreased. As the advantage of Kärber's method of easy computation is now reduced by the use of computers, probit analysis is recommended. —Author's Abstract

KEY WORDS: Denmark; Kärber's method; Probit analysis; Teeth, computational method, congenitally missing.

REPRINTS: Department of Child Dental Health and Community Dentistry, Royal Dental College, Aarhus, Denmark.

****

BLOOD PLASMA FLUORIDE IN HAEMODIALYSED PATIENTS

by

D. Chaleil, P. Simon, B. Tessier, F. Cartier and P. Allain
Angers-Cedex, France

(Abstracted from Clinica Chimica Acta, 156:105-108, 1986)

Blood plasma fluoride was determined in 15 chronic hemodialysed patients (60.2 ±7.2 yrs. old) before and after a 4 h dialysis using dialysates with very low fluoride level, and in two control groups; the first, 20 healthy younger subjects (45.9 ±3.4 yrs. old); the second, 8 healthy older subjects (69.1 ±6.8 yrs. old).

During dialysis, the mean fluoride concentration fell to 0.94 ±0.26 μmol/L, remaining, however, significantly higher than the effect of renal impairment since plasma fluoride is only moderately increased in these patients. Plasma fluoride concentration in the younger group of healthy subjects (0.35 ±0.16

µmol/L) was lower than in the older group (0.44 ±0.16 µmol/L) but the differences were not significant.

In patients, before dialysis, blood plasma fluoride concentrations were at least three times higher than in control subjects (Mann-Whitney U test significant at $p = 0.01$). At the end of dialysis, the fluoride concentration decreased (Wilcoxon test significant at $p = 0.01$) to 0.94 ±0.48 µmol/L, still remaining higher (Mann-Whitney U test significant at $p = 0.01$) than in control subjects.

The increase in blood plasma fluoride due to renal impairment is reduced by dialysis when very low fluoride level dialysates are used.

KEY WORDS: Fluoride; Free fluoride dialysates; Hemodialysis.

REPRINTS: Laboratoire de Pharmacologie, Centre Hospitalier Universitaire F, 49040 Angers-Cedex, France.

****

MANAGEMENT OF OSTEOPOROSIS

by

R. Lindsay
West Haverstraw, New York, USA

(Abstracted from **Baillieres Clin. Endocrinol. Metab.**, 2:103-124, 1988)

Estrogens, the most effective therapeutic agent for prevention of postmenopausal bone loss, must be used judiciously with attention to reduction in other nutritional and lifestyle risk factors. When given to women with an intact uterus, estrogens should be accompanied by progestogens, several of which may also reduce bone loss. All individuals should obtain an adequate calcium intake (100-500 mg/day) and continue to be active. Regarding the clinically overt disorder, therapy should follow similar guidelines, with therapeutic options including calcitonin androgens and diphosphonates as well as estrogens as antiresorptive agents.

Methods of stimulating new bone formation are limited. One of the best documented is that of fluoride. However, care must be taken in use of fluoride, the efficacy of which has not been fully established. Other modalities are as yet experimental. Reduction in risk of falls, and thus fracture, is as important as attempts to modify the skeleton.

KEY WORDS: Estrogen therapy; Fluoride therapy; Osteoporosis, female.

REPRINTS: Helen Hays Hospital Research; Route 9W, West Haverstraw, NY 10993, USA.

****

FLUORIDE DISTRIBUTION AND HISTOLOGICAL STRUCTURE OF HUMAN CEMENTUM

by

H. Nakagaki*, K. Kawai, T. Murakami, Y. Sakakibara,
N. Ohno, J.A. Weatherel and C. Robinson
Nagoya, Japan

(Abstracted from **Archs. Oral Biol.**, 33:257-264, 1988)

Thirty-one teeth taken post-mortem from 10 subjects 40 to 66 years old showed a close relationship between fluoride (F) distribution and histological structure. As in all mineralized tissues, F concentration tended to be highest towards the external surface. Nevertheless, individual patterns of F distribution likewise seemed to reflect the histological pattern, especially the distribution of cellular or acellular cementum. In general, F concentrations were high in acellular and low in cellular cementum.

KEY WORDS: Cementum histology; Fluoride distribution; Human cementum

REPRINTS: H. Nakagaki, Department of Preventive Dentistry and Dental Public Health, School of Dentistry, Aichi-Gakuin University, 1-100 Kusumoto-cho, Chikusa-ku, Nagoya 464, Japan.

****

DISTRIBUTION OF FLUORIDE ACROSS HUMAN DENTAL ENAMEL, DENTINE AND CEMENTUM

by

H. Nakagaki, Y. Koyama, Y. Sakakibara, J.A. Weatherell and C. Robinson
Nagoya, Japan

(Abstracted from **Archs. Oral Biol.**, 32:651-654, 1987)

Samples of coronal dentine, root dentine and cementum were obtained from 20 mandibular premolars in a single experiment. Fluoride concentration was precisely related to the position of the tissue sample. Increase in the fluoride content of coronal and root dentine was marked at least until about 50 years of age. Uptake of fluoride by root dentine and cementum had taken place throughout the life of the tooth. No evidence of change in fluoride content of enamel with age was observed.

KEY WORDS: F^- in cementum; F^- in dental enamel; F^- in dentine.

REPRINTS: H. Nakagaki, Department of Preventive Dentistry and Dental Public Health, School of Dentistry, Aichi-Gakuin University, 1-100 Kusumoto-cho, Chikusa-ku, Nagoya 464, Japan.

****

BONE AND JOINT PATHOLOGY IN FLUORIDE-EXPOSED WORKERS

by

Edward Czerwinski, Jozef Nowak, Danuta Dabrowska,
Artur Skolarczyk, Bartlomiej Kita, Marek Ksiezyk
Cracow, Poland

(Abstracted from **Archieves of Environmental Health**, 43:340-343, 1988)

Clinical radiological investigation performed on 2258 aluminum workers exposed to fluoride for an average of 17.6 years showed that the occurrence of fluorosis and the time and degree of fluoride exposure were closely related. The difficulties in diagnosing skeletal fluorosis result from the questionable sensitivity of x-ray techniques and from the non-specificity of the associated symptoms. For this reason a quantitative method to assess osteosclerosis and bone structure alteration is needed.

Worker's main complaints were limitation of movement and tenderness of cervical, thorasic and lumbar spine, shoulder, elbow, hip and knee. The most frequent symptoms in those exposed i 6 yr were low back pain (92.6%); painful knee (73.8%), elbow (64.8%), and hip (49.4%). Osteosclerosis was found in 5.1% of radiographs of the forearm, 3.9% of the leg, 4.2% of the pelvis, and 3.2% of the lumbar spine in workers exposed for a longer time. Analysis showed an increase in the incidence of fluorosis in the ratio of 1:2:3 in the groups of workers with the index of exposure-years of 1-10, 11-20, and 21-30 yr. Potmen and anodemen were adversely affected three times more frequently than were clerical workers.

Radiological changes were crucial findings in the diagnosis of osteofluorosis. Unfortunately, there is general confusion about osteosclerosis and the alterations in bone structure that have been accepted as essential criterion of fluorosis. Although fluorosis was rare in our cases, only 15.7% of those examined could be assessed as free from any changes in bones and joints. Based on radiologic findings, the incidence of pelvic ossification was vastly higher than in forearem, lower leg or spine, in all age groups.

KEY WORDS: Aluminum workers; Fluorosis, radiological and clinical.

REPRINTS: Edward Czerwinski, M.D., Cracow Academy of Medicine, Department of Orthopedics, ul. Kpernika 19a, 31-501 Cracow, Poland.

****

GASTROINTESTINAL ABSORPTION OF FLUORIDE IN HUMANS — A COMPARATIVE STUDY OF NaF AND CaF_2

by

Noriko Tsunoda, Shiro Sakurai and Humio Tsunoda
Morioka, Japan

(Abstracted from **Fluoride Research 1985; Studies in Environmental Science 27**, pp. 389-394)

Differences in gastrointestinal absorption of fluoride based on changes in serum and urinary fluoride concentrations were examined in 23 men subsequent to ingestion of NaF or CaF_2 two fluoride compounds with contrasting solubilities.

With NaF, fluoride absorption was rapid; serum fluoride concentration showed a rapid rise immediately following NaF administration; it reached a peak 0.341 ±0.076 ppm 30 minutes after administration. Although fluoride levels diminished subsequently, normal values were not restored until 24 hours later. On the other hand, the rise in serum fluoride level of the group given CaF_2 (10 mg F) was not pronounced. However it varied widely among different individuals. Its peak, after 2 hours, was only 10.7% of that exhibited by the NaF group. In 2 subjects, peak concentrations averaged 0.045 ±0.021 ppm.

Regarding 24 hour fluoride excretion in urine, in the CaF_2 group fluoride absorption rate was estimated to be only 10%, although in some instances the rate was estimated to be as high as 20-30% of the corresponding value of NaF. The reason for these differences is not known. Possibly the pH of the gastric juice and disturbance of the gastric mucosa — namely inflammation or ulcers — may be involved. The influence of host factors requires further study.

Recently a critical problem associated with fluoride air contamination has arisen in Japan regarding fluoride accumulation in agricultural products. To evaluate the influence of polluted farm products on humans, it is important to establish both their fluoride content and their chemical properties including solubility.

KEY WORDS: Calcium fluoride; Fluoride absorption in men; Gastrointestinal F^- absorption; Sodium fluoride.

REPRINTS: Department of Hygene and Public Health, School of Medicine, Iwate Medical University, Morioka 020, Japan.

****

RISK-BENEFIT RATIO OF SODIUM FLUORIDE TREATMENT IN PRIMARY VERTEBRAL OSTEOPOROSIS

by

N. Mamelle, P.J. Meunier, R. Dusan, M. Guillaume, J.L. Martin, A. Gaucher, A. Prost, G. Zeigler, P. Netter
Lyons, France

(Abstracted from Lancet, 2:361-365, 1988)

The risk-benefit ratio of combined fluoride-calcium therapy in primary vertebral osteoporosis was examined prospectively in patients with at least one vertebral fracture. 257 patients received at random 25 mg sodium fluoride twice daily plus 1 g elemental calcium daily and a vitamin D2 supplement; 209, on the other hand, received one of the alternative therapies usually prescribed in France.

After 24 months in the fluoride-calcium group new vertebral fractures were significantly lower; a higher incidence of osteoarticular pains in ankle and foot was the main adverse effect. The risk of non-vertebral fractures did not increase; digestive disorders were equally frequent in both groups.

KEY WORDS: Fluoride-calcium therapy; Osteoporosis; Vertebrae.

REPRINTS: Inserm, U 265, Etude Traitments osteoporose Primit. Groupe, 151 Cours à Thomas, F-69424 Lyons 03, France.

****

FLUORINE THERAPY IN OSTEOPOROSIS: ACUTE EFFECTS ON PARATHYROID AND MINERAL HOMEOSTASIS

by

T.C. Stamp, M.V. Jenkins, N. Lovereridge, P.W. Saphier
M. Katakity, S.E. MacArthur
Stanmore, England, UK

(Abstracted from Clin. Sci., 143-146, 1988)

Analysis of acute metabolic effects of sodium fluoride therapy on 41 osteoporotic patients receiving large calcium supplements, 33 of whom underwent simultaneous metabolic balance studies showed mean serum calcium fell transiently within 24-48 h by 0.03 +/- 0.07 (SD) mmol/L ($p < 0.001$). In a subgroup, ionized calcium fell and biologically active parathyroid hormone (bio-PTH) rose more than fivefold ($p < 0.01$). Urine calcium rose after an insignificant fall. Pretreatment calcium and phosphorus balances were significantly positive; they failed to change overall during the first 8 days of treatment. However, when balances in two groups analyzed relative to serum

changes in patients whose serum levels changed least – sodium fluoride increased fecal calcium ($p < 0.025$) and phosphorus ($p < 0.01$) and reduced calcium balance ($p < 0.01$): a mean balance difference between the two groups of 2.1 mmol daily ($p < 0.001$) occurred. Very small changes in serum levels, therefore, indicate well-marked metabolic responses: sodium fluoride which stimulates bio-PTH activity must also enhance mineral uptake from circulation into tissue(s). By separate and opposing action(s) it inhibits intestinal calcium and phosphorus absorption, predominantly in those whose serum levels remain stable. These effects may be relevant to long-term therapeutic results.

KEY WORDS: Calcium balances; Fluoride therapy; Mineral homeostasis; Osteoporosis; Parathyroid hormone.

REPRINTS: Royal National Orthopaedic Hospital, Metabolic Unit, Stanmore HA7 4LP, Middlesex, England.

****

TRENDS IN DENTAL FLUOROSIS AND DENTAL CARIES PREVALENCES IN NEWBURGH AND KINGSTON, N.Y.

by

J.V. Kumar, E.L. Green, W. Wallace, T. Carnahan
Albany, NY, USA

(Abstracted from **Am. J. Public Health**, 79:565-569, 1989)

In a New York State study to determine changes in dental fluorosis prevalence from 1955 to 1986 in fluoridated Newburgh and non-fluoridated Kingston children, the frequency and severity of dental fluorosis among 884 7-14-year-old children were measured by two dentists utilizing Dean's Index. Among Newburgh residents, the prevalence of dental fluorosis (very mild to moderate) varied from a low of 5 percent for the 9-10-year-old group to a high of 9.4 percent for 11-12-year-olds. Except for the 13-14-year-old group, the children in non-fluoridated Kingston had the lowest dental fluorosis prevalence rates. The changes are apparent for Kingston residents, indicating the availability of fluorides in non-fluoridated areas. The increased risk for dental fluorosis for Kingston residents appears to be from the use of fluoride tablets. Analysis of dental caries data revealed that caries prevalence declined substantially in both fluoridated and non-fluoridated areas.

KEY WORDS: Dental caries; Dental fluorosis; Fluoridation; Fluoride tablets; Kingston, NY; Newburgh, NY.

REPRINTS: Bureau of Dental Health, NY State Dept. of Health, Albany, NY 12237, USA.

****

INSTRUCTIONS TO AUTHORS

Fluoride, the official journal of the International Society for Fluoride Research (ISFR) is published quarterly (January, April, July, October). Its scope is the publication of papers and reports on the biological, chemical, ecological, industrial, toxicological and clinical aspects of inorganic and organic fluoride compounds. Papers presented at the annual ISFR conference are published in *Fluoride*. Submission of a paper implies that it presents original investigations and relevant bio-medical observations. Review papers are also accepted.

PREPARATION OF PAPERS

1. **General** – No precise limit is given on the length of the paper; it should be written concisely in English, submitted in two copies, doublespaced with generous margins. Measures are given in metric system (SI).

2. **Title** – A concise but informative title should be followed by the name of author(s), the location and state (country) where the research was carried out. The name and address of the institution where the work was done should appear at the bottom of the first page.

3. **Summary** – The paper should begin with a brief, factual summary.

4. **Introduction** – Following the summary, a short introduction should state the reason for the work with a brief review of previous works on the subject. References are given by numbers in parentheses.

5. **Materials and Methods** – should be condensed; however if the methodology is new or developed by the author(s) it can be more detailed.

6. **Results** – should contain the *direct conclusions* of the experimental work.

7. **Discussion** – should deal with the *general conclusions*. Reference should be made to other work on the subject with an indication whether the experimental results agree or disagree with previous work. In short papers, results and discussion can be combined.

8. **Abbreviations or Acronyms** – must be defined either parenthetically or in a footnote when they first appear.

9. **Bibliography** – should be arranged according to the order in which the articles are cited in the text (not alphabetically). An example follows:

> Fiske, C.H. and Subba Row, Y.: The Colorimetric Determination of Phosphorus. J. *Biol. Chem.*, 66:375-400, 1925.

For books, the title, editor, publisher, location and year of publication, and pages should be given.

THE INTERNATIONAL SOCIETY for FLUORIDE RESEARCH

P.O. BOX 692 WARREN, MICHIGAN 48090