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CONTENTS

The program for the 12th I.S.F.R. Conference on May 16-18, 1982 at the Colonial Gateway Inn, St. Petersburg Beach (not the Hilton Hotel as previously announced) promises to be one of the most instructive ones of our organization.

In addition to several clinical papers on human fluorosis, one session will deal with the cyto-chemical effects of fluoride in relation to bones, soft tissues and blood cells. New data on fluoride metabolism will highlight another session. Several papers will be concerned with the ecological aspect of fluoride research.

The final program will be available by mid-April.

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MANUSCRIPTS for publication should be submitted in English, double-spaced 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 of not more than 12 lines.

FLUORIDE is listed in
Current Contents Agricultural
Food and Veterinary Sciences

EDITORIAL

CLASSIFICATION OF DENTAL FLUOROSIS

In 1934 H.T. Dean (1) proposed a classification of mottled teeth which he related to the fluoride content of drinking water, for which he received wide recognition. His five categories, which range from "Questionable" to "Severe", are based on the macroscopic appearance of the denture. In one of his articles (2), he expressed the need for a better understanding of the histopathology of dental fluorosis when he wrote: "It would be highly desirable if future reports relating to the histopathology of human teeth would describe in detail for each tooth included in the report, the macroscopic appearance including diagnosis of the clinical degree of severity."

By placing the entire denture into a certain category, the Dean index fails to distinguish between children, only two of whose teeth are affected and those whose entire denture is pitted.

This deficiency was remedied by Takamori and his collaborator Kawahara who devised a classification of dental fluorosis which describes in detail the defect of every single tooth (3). Their classification includes four "systems", each of which was subdivided into 5 degrees according to the intensity of the changes. System #1 records the presence of streaks "S-System" on the enamel surface. The "P-System" (porcelain) refers to the lustrous marble appearance of the enamel. The "B-System" (brown) designates degree of staining of the enamel defect. For instance, a tooth characterized as $P_4B_3D_1$ falls into degree four respecting enamel luster, into degree three for brown stain and its enamel defect is minimal. By this method, Takamori delineates more than 300 different combinations which immediately convey a clear picture of each tooth of a fluorotic denture.

More recently in 1978, Thylstrup and Fejeskov (4) devised a classification which has made a greater impact upon investigators. They describe the degree of dental fluorosis on the buccal, occlusal and lingual surface of each tooth. Scores 1-5 pertain to the degree of opacity whereas scores 6-9 indicate the extent of enamel defect (pits). Teeth in group 9 have lost the main part of enamel with change in the anatomic appearance of the surface.

By means of this classification, these authors make certain important observations as, for instance, the fact that the enamel thickness rather than the length of exposure to fluoride-containing body fluids determines the degree of dental fluorosis (4).

Each of these three classifications has its own merit depending on the nature of the study which an investigator wishes to undertake: The epidemiologist, interested in the overall picture of mottling, might adhere to the Dean classification, the biochemist might prefer the one by Takamori, for the clinician the classification by Thylstrup et al. would be more valuable.

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Ream, L.J., and Principato, R.: Glycogen Accumulation in the Parathyroid Gland of the Rat After Fluoride Ingestion. Cell Tissue Res., 220:125-130, 1981.

> Rats given 150 ppm fluoride in their drinking water for 10 weeks accumulated glycogen in the parathyroid chief cells. Glycogen was also seen within intercellular spaces. This condition was accompanied by a rise in the number and development of the organelles associated with protein synthesis and secretion, similar to what is observed in hyperparathyroidism.

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Mayhew, R.R., and Brown, L.R.: Comparative Effect of SnF_2, NaF, and $SnCl_2$ on the Growth of Streptococcus Mutans. J. Dent. Res., 60:1809-1814, 1981.

> Sodium fluoride showed some bactericidal activity in vitro at 150 and 300 ppm F^-, and was totally bactericidal at 600 ppm F^-, while stannous fluoride (SnF_2) suppressed the rate of growth at 75 ppm F^-.

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DRUG-INDUCED SKELETAL FLUOROSIS

by

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Geneva, Switzerland

SUMMARY: Fluoride quantitative analysis and histological observations are reported in two new cases of niflumic acid induced bone fluorosis. The bone fluoride content is higher than in normal compact bone. Microradiographs and stained sections of compact and cancellous bone tissue show the presence of mottled lacunae, linear formation defects, extended and thick osteoid seams. The existence of a skeletal fluorosis induced by a prolonged and continuous treatment with niflumic acid, is confirmed.

Introduction

Recently four cases of skeletal fluorosis have been described due to side effect of fluoride treatment. The patients who were suffering from rheumatoid arthritis received uninterrupted and prolonged treatment with niflumic acid (1-3). Fluoride assay and histological studies of bone tissue in two new cases of niflumic acid induced bone fluorosis are herewith presented.

Material and Methods

Material was obtained from two women suffering from rheumatoid arthritis who had been treated orally for ten years with a daily dose of three capsules of 250 mg of niflumic acid (Nifluril, UPSA Laboratories, France). The first patient was 28-years old and had also received a low-dose corticotherapy. The second patient, 62-years old, who had not received corticotherapy showed moderate renal failure. In the first case, the bone samples were obtained from an iliac crest biopsy and in the second case, a fragment of the tibial distal metaphysis was taken close to a fracture callus.

The bone fluoride content was measured in calcinated compact bone with a specific electrode according to the method of McCann (4). The values obtained appear very different in the two cases of drug-induced bone fluorosis (0.263% and 0.856% respectively), but they were higher than in compact bone tissue of control subjects of the same age and sex and residing in a low fluoride geographic area (0.028±0.006% in women of 30 years old, and 0.079±0.013% in women of 60 years old). The bone fluoride content obtained in case #1 corresponds to that reported in the literature in cases of niflumic acid induced fluorosis (1, 3), that in case #2 to that observed in cases of industrial fluorosis (5) with about 10 years' fluoride exposure, wherein the period between the end of exposure and the biopsy (average fluoride content 0.751±0.109%) was of no consequence.

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Presented at the 11th annual conference of the I.S.F.R. April 8-10, 1981, Dresden, G.D.R.

The samples of undecalcified compact and cancellous bone were fixed in alcohol, embedded in methyl-methacrylate, cut into sections, microradiographed, and stained with basic fuchsin. Qualitative and, when possible, quantitative histological studies were performed using both microradiographs as well as stained sections.

In the two cases treated for a prolonged period with niflumic acid, the histological study revealed pronounced signs of skeletal fluorosis, namely the presence of mottled bone with mottled periosteocytic lacunae, numerous linear formation defects, extended and thick osteoid seams. These lesions are observed both in compact and cancellous bone tissue, and appear more frequent when the bone fluoride content is high, as in the second case. Hypervascularization was only observed in the second case.

Certain histological aspects of bone fluorosis, such as cortical porosity, were not observed in the two cases of niflumic acid induced bone fluorosis recorded here. A quantitative histological study was only possible in the first case. In contrast to results in other bone fluorosis, the values obtained show that the trabecular bone volume is low, whereas the cortical porosity and the periosteocytic lacunar surface are normal. The osteoid parameters confirm the observed aspect of hyperosteoidosis.

Results

The results obtained in this study confirm that prolonged and continuous treatment with niflumic acid induces skeletal fluorosis, but individual variations can be observed. The induced fluorosis is due to an excess of fluoride ions in the blood; each patient had in fact received, during 10 years, a daily dose of 151.5 mg of fluoride. The individual variations can be partially explained by: a) the individual variations in the enzymatic cleavage of the carbon-fluoride bonds; b) in the first case, the association of two treatments, niflumic acid and corticotherapy; c) in the second case, the moderate renal failure as well as the increased remodeling in a zone close to a fracture callus.

In order to better understand the effects of niflumic acid on bone tissue, an experimental study in rats was undertaken in collaboration with UPSA laboratories in France. In young and adult rats, the effects of niflumic acid and sodium fluoride on bone tissue were compared. The preliminary results confirm the stability of the C-F bond, but emphasize the fact that a certain percentage of these bonds is broken, provoking an increase in the blood ionized fluoride level as well as in the bone fluoride content.

Acknowledgement

The authors wish to thank Dr. F. Texier-Bardon (UPSA Laboratories, France) and Dr. G. Cournot (Hôspital des Enfants Malades, Paris, France) who provided the bone material used in the present study.

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FLUORIDE IN SMALL MAMMALS AND THEIR POTENTIAL FOOD SOURCES IN CONTAMINATED GRASSLANDS

by

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SUMMARY: Systematic sampling of vegetation, macroinvertebrates and small mammals was carried out from a grassland established on a reclaimed fluorspar tailings dam. This facilitated the study of the transfer of fluoride through the food chain. In particular the fluoride concentrations in two species of small mammals, Microtis agrestis L. (Short-tailed field vole) and Sorex araneus L. (Common shrew) and their diets were investigated.

In Microtis agrestis, a herbivorous species, feeding upon green vegetation, concentrations were significantly elevated when compared with controls. On the dam surface, a comparison of the total body fluoride concentration of the two species of small mammal showed that it was significantly higher in Sorex araneus, a carnivore, compared to M. agrestis which reflected the higher F^- concentration in its diet of macroinvertebrate species. This increase in total body concentration is probably a consequence of the higher skeletal values, with significantly higher femur and pelvic girdle concentrations having been measured. The fluoride levels of the soft tissues in the two species did

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not differ. This may suggest some mechanism regulating soft tissue concentrations in S. araneus with excess fluoride being incorporated into the skeleton.

Introduction

International concern for the prevention of environmental pollution due to dispersal of toxic residues produced by metalliferous mining operations has led to paying increased attention to the aftercare and management of reclaimed, contaminated land. Historically, land reclamation schemes have concentrated on landscape reconstruction for amenity improvement and abatement of pollution. More recently, emphasis has been placed on recreating the wilderness character of semi-natural habitats on derelict mineral workings. This change in policy is connected with maximizing the wildlife potential of abandoned sites and is an attempt to compensate for losses of natural and semi-natural ecosystems. Complex land restoration techniques have been devised to achieve this objective but only superficial attention has been given to the significance of residual contamination to the structure and functioning of ecosystems on reclaimed sites.

It is well established that mercury, cadmium, lead and other nonessential trace metals are subject to food chain accumulation in terrestrial ecosystems (1,2). Toxicological effects in plants and animals have been documented (3,4) but the long term ecological consequences for wildlife are largely unknown. The behavior of other trace elements known to adversely affect livestock and man has not been investigated in any detail in the semi-natural environment.

Fluoride, for example, a pollutant associated with aluminum smelting, glass and fertilizer manufacturing has been studied as a factor in the health of farm animals grazing contaminated pastures (5) but little is known about its accumulation, trophic level transfer and magnification within food webs of semi-natural ecosystems. This paper describes the preliminary results of a study of food-consumer relationships for fluoride in grassland communities established on derelict tailings dams enclosing the mineral waste from mining and processing of fluorspar ores in Derbyshire, England.

Study Area Characteristics

Fluorspar (CaF_2), an important commercial mineral used in the production of aerosol propellants, steel and a range of fluoride compounds used in industry, occurs together with calcite, barytes, lead and zinc ores in fissures within the Carboniferous Limestones of the Peak District in Central England. During the separation of fluorspar and other minerals of commercial value from the associated, valueless minerals, large quantities of waste are produced. These are discarded as a slurry into tailings dams which are eventually subject to land reclamation works in order to minimize dispersal of the contaminated residues by wind and water erosion (6). Recent projects have concentrated on maximizing the wildlife potential of these derelict sites by a combination of grassland establishment and woodland planting schemes. Recolonization of these areas by wildlife is sur-

prisingly rapid and within a few years of revegetation the density and diversity of avifauna, small mammals and invertebrates is comparable to that of adjacent undisturbed grassland and moorland communities.

Materials and Methods

A grassland ecosystem comprises a number of interconnected components. The living component is a complex of producers, consumers and decomposers interrelated and organized into a food web. There are two basic interlocking parts of the food web: a grazing subdivision of herbivorous species that form the food supply for carnivores; and a detritus subdivision of decomposer organisms and their predators. Any investigation of food chain relationships and transference of fluoride must, therefore, involve a systematic sampling of the major components of the ecosystem. However, each component of the ecosystem can be subdivided into distinct units which represent alternative food sources. Thus, for example, in terms of producer-consumer relationships, herbaceous vegetation provides a range of potential food sources, namely green shoots and leaves, stems, seeds, flowers, roots, senescent tissue, each of which must be sampled and analyzed separately.

In the present study some of the major components and subdivisions of the grassland ecosystem were sampled for analysis on (a) a reclaimed fluorspar tailings dam; (b) a semipolluted site 1-2 km from the main study area; and (c) a control site. Particular attention was given to the food-consumer relationship for two small mammals; the herbivore, Microtis agrestis L. (Field vole); and the carnivore, Sorex araneus L. (Common shrew). The sampling program, in which soil, vegetation, macroinvertebrates and small mammals were collected from the study areas was carried out from July 1980, 7th - 28th.

Composite samples of ground cover vegetation were collected and soil blocks taken for separation of roots. The above-ground vegetation was sorted into subsamples consisting of grass, clover, flowering stalks and seed heads, dead and living tissue being separated within each subsample. All vegetation was oven dried at 60°C for 48 hours and ground to pass a 0.5 mm mesh in a Cyclotec mill.

Small mammals were caught using Longworth traps and were killed with ether. Each animal was identified, sexed and weighed in the field and then stored at -10°C until analysis. After thawing, muscle, liver and kidneys, were dissected and freeze-dried before wet oxidation analysis. The femurs were also excised and the muscle attached to them removed by soaking overnight at 60°C in a 10% w/v solution of papain. The clean bone and carcasses were also freeze-dried. The latter were then ground to <1mm.

Invertebrates were sampled using a sweepnet and pitfall traps containing 0.1% v/v detergent in 2% formalin which were inserted into the substrate in the vicinity of the Longworth traps. Samples were sorted into taxonomic groups and stored in 5% formalin. Prior to analysis they were oven-dried at 60°C for 48 hours and carefully ground using an agate pestle and mortar.

Table 1

Fluoride ($\mu g\ g^{-1}$ ± standard error)+ in Soil, Vegetation and Grasses in the Three Study Sites

	Dam Surface	Semi-polluted Site	Control Site
Surface Soil (0-5 cm)	8905±1101 (6)	359±67 (6)	100±11 (6)
Composite ground-cover vegetation	332±46 (6)	34±4.6 (7)	8.1±1.3 (9)
Fine-leaved grasses	293±28 (6)	35.3±5.8 (3)	4.8±1.1 (6)

+ Values expressed on a dry weight basis; Number of replicates in parenthesis.

Table 2

Fluoride ($\mu g\ g^{-1}$ ± standard error)+ in the Major Components of the Grass Sward on the Dam Surface

Components of Grass Sward		Fluoride Concentration
Leaf blades	Live	335±50 (6)
	Dead	838±45 (6)
Stems	Live	108±10 (6)
	Dead	576±227 (6)
Panicles	Immature	246±23 (6)
	Mature	311±57 (6)
Roots		5422±589 (6)

+ Values expressed on a dry weight basis; Number of replicates in parenthesis.

The fluoride concentration of all soils, vegetation and animal tissues was determined after wet oxidation with concentrated nitric acid (Analar) for 1 hour at 100°C. Digests were analyzed using a fluoride ion-specific electrode with trisodium citrate (final concentration 1.2M) as a total ionic strength adjustment buffer.

Results

Fluoride concentrations in surface soil and unwashed vegetation are shown in Table 1 from which it is apparent that concentrations in vegeta-

tion reflect substrate fluoride values thus confirming previous observations for these sites (7). There were no significant differences($p>0.05$) between composite samples of ground-cover vegetation and fine-leaved grasses (predominantly Festuca rubra). Fluoride concentrations in the major components of the standing ground-cover vegetation are given in Table 2. There were marked differences between the various subcomponents of the grass sward, with root concentrations being higher than in green or dead foliage. This follows the usual pattern of distribution in plants where accumulation is from the soil (8). Fluoride levels in senescent and dead stems and leaf blades were consistently higher than in green tissue suggesting retranslocation of fluoride into senescing tissue (9). These differences between subcomponents of the grass sward are particularly important when considering the dietary intake of fluoride by grazing herbivores (e.g. M. agrestis) which have selective feeding strategies.

Table 3 gives the concentrations of fluoride in the dominant invertebrate groups at the three study sites. These can be used as a broad index of the fluoride in the diet of the carnivore, S. araneus. Between site differences in fluoride concentrations for each of the major groups of invertebrates reflect the comparative fluoride status of soil and vegetation in each of the three locations. The magnitude of the differences in fluoride concentration between taxonomic groups again emphasizes the importance of detailed sampling and analysis when estimating dietary intake by S. araneus which has known preferences in invertebrate prey selection (10).

Table 3

Fluoride ($\mu g\ g^{-1}$ ± standard error)+ in Four Major Groups of Invertebrates which form the Diet of Sorex araneus

Invertebrate Group	Dam Surface	Semi-Polluted Site	Control Site
Large ground beetles(Carabidae)	321±37 (30)	40±11.6 (14)	12±3 (5)
Spiders (Araneida)	1527±297(6)	77±24 (6)	15±3 (6)
Flies(Diptera)	1804±176(4)	76±15 (5)	8.8±1.6 (4)
Earthworms (Lumbricus spp)	3204±1178(3)	60 (1)	5.3±1.4 (6)

+ Values expressed on a dry weight basis; Number of replicates in parenthesis.

Fluoride concentrations in selected soft and hard tissues of M. agrestis for each of the study sites are given in Table 4. Concentrations in the femur were significantly higher ($p < 0.001$) than in the soft tissues, at all sites. Femur concentrations were at least 10-fold higher than kid-

ney, liver and muscle in each case. The concentrations followed the order: femur > muscle > kidney > liver for the two polluted sites. For all tissues, the fluoride concentrations were significantly higher at the polluted sites, compared to the control area.

Table 4

Fluoride ($\mu g\ g^{-1}$ ± standard error)$^{+}$ in Body Tissues of *Microtus agrestis*

Tissue	Dam Surface	Semi-polluted Site	Control Site
Femur	554±43 (10) ***	212±15 (11) *	117±18 (9)
Kidney	22.7±2.9 (13) **	13.8±0.7 (9) **	6.7±0.78 (9)
Liver	15.7±1.0 (13) ***	12.3±1.3 (9) **	5.4±0.48 (9)
Muscle	41.2±7.0 (12) ***	20.2±2.2 (9) ***	4.2±0.30 (9)

*, **, *** denote significant difference compared with control site at $p<0.05$, 0.01, 0.001 respectively

+ Values expressed on a dry weight basis; Numbers of replicates in parenthesis.

Table 5 compares the fluoride concentration in a range of soft tissues and components of the skeleton for *M. agrestis* and *S. araneus* caught on the dam surface. The values for the soft tissues were similar for both species ($p > 0.05$), but for the femur and pelvic girdle higher values($p < 0.05$) were obtained for *S. araneus*. Total body concentrations were also higher for *S. araneus* ($p < 0.05$), reflecting the over-riding influence of the skeleton as the major site for fluoride accumulation. The skeletal values also contrast with the soft tissues in that they reflect the differences in the fluoride concentrations of the estimated diets of the two species.

Discussion

This study has attempted to analyze the food-consumer relationships in natural populations of two small mammals, *Microtis agrestis*, a herbivorous species and *Sorex araneus*, a carnivorous species inhabiting polluted grasslands. There is little information on absorption and accumulation in natural populations with particular regard to the mobility and transfer of fluoride through food chains. However, a few studies have catalogued the fluoride concentrations in a wide range of wild species from uncontaminated ecosystems (13) and from areas near sources of atmospheric pollution (14).

In the present study, tissue distribution in *Microtis agrestis* conformed to the general pattern of high skeletal fluoride and low soft tissue

Table 5

Fluoride ($\mu g\ g^{-1}$ ± standard error)+ in Body Tissues and Estimated Diets from the Dam Surface

Tissue	Microtis agrestis	Sorex araneus	
Femur	554±43 (10)	1283±252 (7)	*
Pelvic Girdle	585±63 (9)	1298±245 (9)	*
Skull	517±37 (10)	714±120 (7)	NS
Kidney	22.7±2.9 (13)	22.5±3.6 (9)	NS
Liver	15.7±1.0 (13)	15.9±1.4 (9)	NS
Muscle	41.2±7.0 (12)	27.6±4.3 (8)	NS
Total body concentration	332	1063	

+ Values expressed on a dry weight basis.
* Significant ($p<0.05$) or NS not significant differences between species
** Estimated Diet: M. agrestis (ground-cover vegetation); S. araneus (invertebrates) based on components of the diet and ratios described by Godfrey (11), Chitty et al. (12) and Rudge (10)
Number of replicates in parenthesis

fluoride. However, soft tissue concentrations in the polluted sites responded to increased fluoride concentrations in the diets, producing significantly elevated levels in kidney, liver and muscle. This confirms previous data for these sites (7) but contrasts with experimental investigations where no significant elevation of soft tissue fluoride concentrations occurred with up to 100 mg/kg fluoride in the diet (15). However, numerous factors including the higher fluoride content of the diet and its composition, and interspecific variation in the rate of food ingestion, could contribute to the differences in the concentrations observed in this field study.

In S. araneus higher dietary concentrations were not reflected in a further increase in soft tissue levels when compared with M. agrestis on the polluted site (Table 5). However, the pelvic girdle and femur fluoride concentrations do respond to the differences in dietary fluoride levels of the two species. This may suggest homeostatic control mechanisms acting to maintain equilibrium concentrations in the soft tissues, with excess fluoride being incorporated into the skeleton. The total body concentration in S. araneus, representing a higher trophic level, was significantly higher ($p < 0.05$) than for M. agrestis. This mainly reflects the differences in skeletal fluoride concentrations. Because it is within the skeleton this fraction of the total body burden may prove less available for absorption and retention by higher predators. The toxicological significance of these increased soft and hard tissue fluoride concentrations in the animals from the contaminated sites is at present unknown.

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FLUORIDE BRIEF

SF_6 sulfahexafluoride is a stable inert gas which is very slowly absorbed by the mucous membranes of the middle ear. By injecting SF_6 through the tympanic membrane of the auditory canal in 32 patients, the authors reestablished the desired pressure and eliminated retraction of the drum head, adhesions and middle ear infections.

Koch, U., and Becker, W.: Treatment in Cases of Retractions of the Tympanic Membrane, Adhesive Processes and Middle Ear Effusions. Laryng. Rhinol. 60:198-204, 1981.

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MECHANISM OF FLUORIDE ACTION AND FLUOROSIS

by

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SUMMARY: Particles of enamel dissected from rat mandibular incisors were analyzed in order to establish the fluoride distribution in developing enamel in relation to the chemical changes occurring during the period of development. The findings indicate that fluoride concentrations decreased as the tissue mineralized. There was usually a relatively high concentration of fluoride, however, in the stage of development corresponding to the period of late formation or early maturation, when the tissue was porous. Some of the fluoride in this area appeared to be labile and might influence nearby cells, thereby affecting the tissue's development and mineralization. Such a mechanism might explain the recognized sensitivity of enamel to dental fluorosis.

Introduction

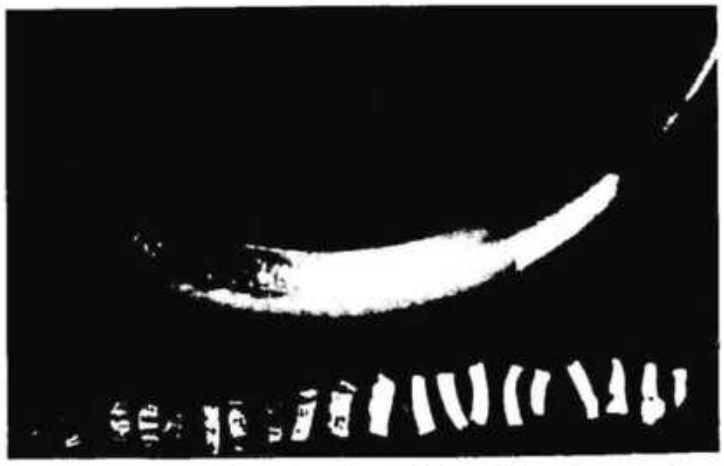
Hodge (1) made the now classical remark that fluoride locked in the mineral lattice was not harmful per se, it being generally assumed that the fluoride is incorporated in bones and teeth in the form of a more stable fluoridated apatite. However, it has long been known, that a fraction of the fluoride trapped by the skeleton is mobile (2) and that some of it is rapidly lost again in a matter of hours (1, 3-5). The suggestion was made (6) that this labile fraction might affect nearby cells. In other words, mineralizing tissues not only absorb the element but might at the same time raise their extracellular concentrations of fluoride locally. This could explain how fluoride can influence the cells of mineralizing tissues even when the fluoride concentration of the blood is relatively low and why such effects are largely limited to bones and teeth. It would also explain why fluorosis chiefly involves sites of high metabolic activity,e.g. cancellous bones,tendon and muscle attachments, surface regions of bones and developing enamel (7-9), since the recently incorporated fluoride from which the labile fraction is presumably derived is chiefly found in such metabolically active areas (3, 10, 11).

Recent attempts have been made to obtain evidence for or against this possibility by determining the distribution of fluoride in bones and teeth. This paper describes some observations on fluoride uptake and distribution in developing enamel and relates these findings to changes occurring in the enamel's chemistry while the cells associated with the tissue are still active.

From the Dept. of Oral Biology, School of Dentistry, University of Leeds, Clarendon Way, Leeds England. Presented at the 11th I.S.F.R. Conference, April 8-10, 1981, Dresden.

Figure 1

Enamel Particles Dissected from a Mandibular Rat Incisor



From the thin forming enamel at the continuously forming root tip (left) to the hard, mineralized enamel in the maturing regions (right) (x 5).

Progressive Mineralization of Enamel

The work was chiefly carried out on the continuously growing incisor of the rat (11, 12). Wistar rats reared on a stock diet (Oxoid rat cake, fluoride concentration 10-14 ppm per dry weight) were killed at about 250g body weight. The lower incisors were removed and a series of about 20 enamel particles weighing 10-100 ug were dissected from each tooth (Fig. 1). This formed a series of enamel particles representing the different stages of development from partially mineralized tissue near the root apex to the highly mineralized enamel of the maturing region. Fluoride in the particles was determined by means of the Orion electrode (14), phosphorus by the method of Chen et al. (15) and calcium by spectrophotometry (16).

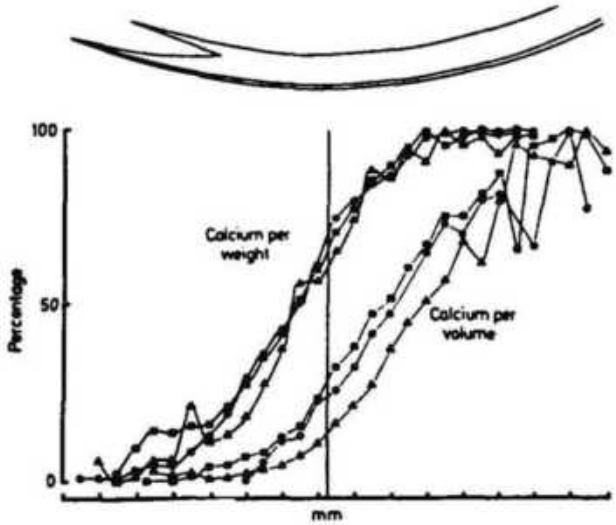
Analysis showed that by the time the enamel had achieved its full thickness, the mineral content of the tissue - when expressed on a dry weight basis - had already begun to increase (Fig. 2 [11]). This was not primarily due to increased mineral in the tissue but to the removal of organic matrix which begins at a fairly early stage of development (17). When the mineral content was measured per volume of tissue, however, it was found (Fig. 2) that the absolute increase in the mineral content occurred at a later stage of development. The displacement between the two curves (calcium per weight and calcium per volume) represented the presence of space or porosity within the tissue. Presumably, the developing enamel had lost its organic matrix and not yet acquired enough mineral to occlude the resulting porosity, this space in vivo being filled with tissue fluid. These findings supported the earlier description of enamel formation by Deakins (18) who found that, chronologically, the organic matrix was displaced first and the water later, this water being eventually displaced by mineral.

Uptake of Fluoride by the Developing Rat Enamel

When fluoride was administered in vivo to rats, either for prolonged periods in their drinking water (100 ppm ad libitum) (Fig. 3), or for shorter periods via the atmosphere (Fig. 4) a relatively high concentration of

Figure 2

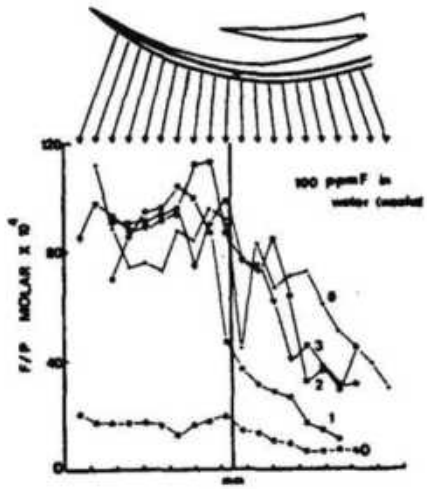
Comparison Weight-Related and Volume-Related Ca^{++} concentrations Along the Developing Enamel.



Three pairs of adjecent rat incisors analyzed. In one tooth of the pair, Ca^{++} content was related to enamel weight; in the other, to enamel volume. Graphs have been 'normalized' i.e. each result has been plotted as a % of the final Ca^{++} content per weight or volume of enamel (12).

Figure 3

F^- Content (F/P Molar) from Forming Enamel at the Root Apex to the Maturing Enamel



From lower incisors of rats given 100 ppm F^- as NaF in drinking water for 0, 1, 2, 3, and 6 weeks ad libitum.

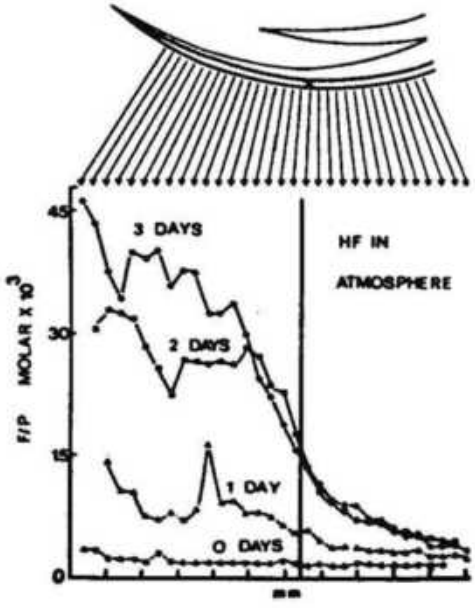
fluoride was acquired by the porous enamel in the areas after late formation or early maturation, these being described by Robinson et al. (19) (and referred to subsequently) as stages 2 and 3. The high F/P values towards the root tip largely reflect the very low concentrations of P in this region and a more defined picture is obtained when the total fluoride in the enamel is measured (Fig. 5). Even in normal animals, the concentration of fluoride was usually relatively high in stages 2 and 3 (Fig. 6), the sole source of fluoride in this case being that in the diet. Studies on the uptake of ^{32}P-labelled phosphate (19,20) in vivo and in vitro revealed the similar tendency for the phosphate ion to be taken up preferentially at this stage of development.

Fluoride Concentration per Volume of Tissue

There was almost invariably a marked fall in fluoride concentrations as the tissue mineralized. Calculated on the basis of F/P ratios, this fall could have been due to a loss of fluoride and/or to the observed increase in phosphate content of the tissue as it mineralized, fluoride acquired during the early stages of development being diluted by the incoming mineral.

Figure 4

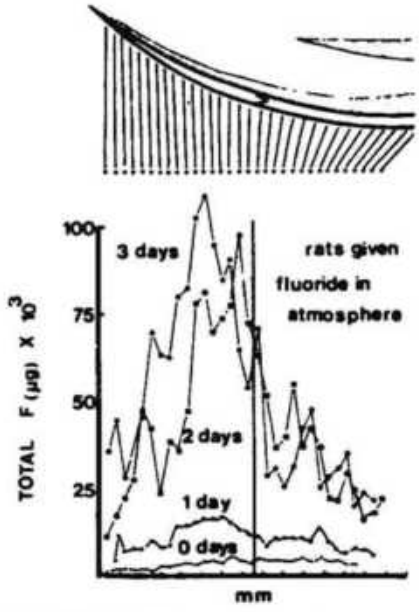
F/P Molar content from Forming Enamel at Root Apex to Maturing Enamel



From lower incisors of rats given F^- as HF in the atmosphere (0.01 µg F/ml air) for 0, 2, 3, and 6 days (13).

Figure 5

Variations in the Total Fluoride (per mm of Enamel) of Developing Rat Enamel



Rats given F^- as atmospheric HF (0.01 g F/ml air) for 0, 1, 2, and 3 days (13).

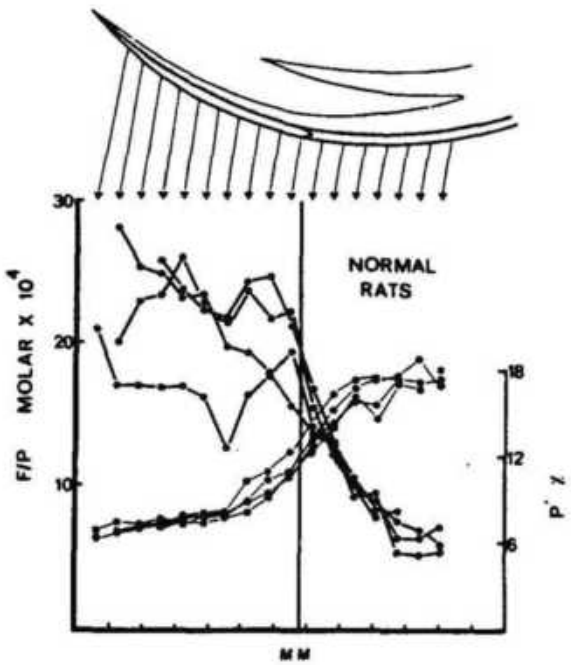
An attempt was made to determine the concentration of fluoride on the basis of tissue volume in order to establish whether there had been any loss of fluoride from the tissue. To do this accurately proved very difficult on the very small particles because some of their weights were of the order 5-10 µg and the very small amounts of fluoride were also difficult to measure, fluoride concentrations in the analytical solutions (about 0.05ppm) sometimes approaching the limits of sensitivity for the fluoride electrode. Despite this, the results (Fig. 7) usually suggested on a volume basis that fluoride concentrations tended to be relatively high in stages 2 and 3 and that some of the fluoride disappeared from the tissue as it mineralized. The work of Speirs (21, 22) on pig enamel supported this view. He found that fluoride concentrations tended to fall on a volume basis as the enamel mineralized. The overall picture of fluoride distribution in the developing enamel also seemed to be essentially similar in deciduous cow incisors (23).

Conclusions

It therefore appears that fluoride concentrations tend to be relatively high during the period of enamel development. This probably reflects the tendency for ions to be taken up preferentially by the enamel during the

Figure 6

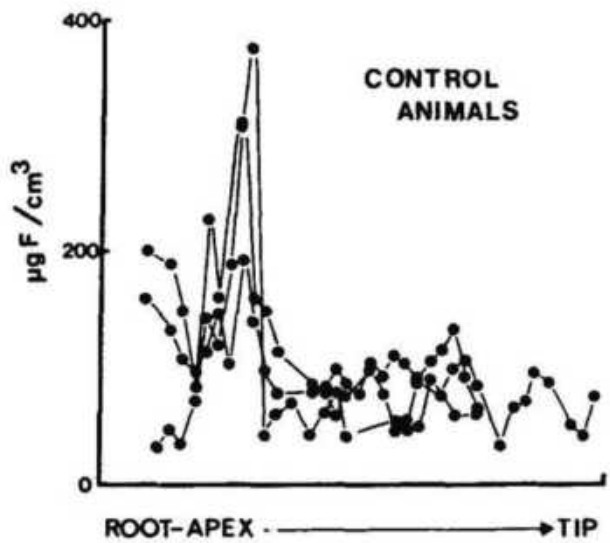
F^- and P Distribution Along Developing Enamel of Continuously Growing Incisors



From control animals incisors (F/P molar) (13).

Figure 7

Variations in F^- Concentration ($\mu gF/cm^3$) of Developing Enamel



From the root apex to the mature erupted region of incisor teeth from control rats (13).

period of late formation or early maturation (stages 2 and 3) when the tissue is porous. Some of this fluoride then seems to disappear again as the enamel mineralizes. This labile fluoride is probably part of that fraction lost from the mineralizing tissues during the hours following fluoride ingestion. The site at which this occurs in developing enamel corresponds to the porous stage of late formation or early maturation and it also seems to coincide with the site where injected fluoride can cause changes in enamel pigmentation in the rat incisor (24). To this extent, the observations made on the uptake and distribution of fluoride in developing enamel support the original hypothesis i.e. that the tissue not only absorbs fluoride but may raise the extracellular concentration of fluoride ion locally, influencing nearby cells and affecting the tissue's development and mineralization. Perhaps the other mineralizing tissues are involved in a similar way in the mechanism of dental and skeletal fluorosis.

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A STUDY OF FACTORS AGGRAVATING DENTAL FLUOROSIS

by

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SUMMARY: In school children born and reared in a central location in Poland with 2.0 to 3.6 ppm of fluoride in drinking water, fluorosis of the permanent teeth was determined according to the index proposed by Thylstrup. Data on breast feeding and on the daily water intake were collected. Of the children under study, 97.5% had dental fluorosis, only 19.5% of which were categorized mild (1st and 2nd scores according to Thylstrup's index.) A direct correlation was found between the severity of fluorosis and the degree of physical development based on weight and height measurement.

Introduction

Enamel fluorosis of the permanent teeth results from ingestion of water containing about 1 part fluoride per million parts of water (ppm) and above during the first 10 years of life (1). Mottling therefore can only occur when the fluoride is absorbed before eruption, while the tissue is still developing. The degree of mottling varies from small opaque white spots covered by an intact, hard and shiny surface to gross dark brown lesions with a pitted surface. Fluorotic enamel is characterized by increased porosity which in severe grades is present as a distinct hypomineralized subsurface lesion (2-4). The severity of dental fluorosis is directly related to the fluoride level in the drinking water (1,5,6). Other factors, however, influence its occurrence and severity (7-9).

The aim of the present study was to determine what factors, other than the concentration of fluoride in water supplies, affect the degree of dental fluorosis.

Material and Methods

A population of 121 children and youths aged 7 to 18 (49 boys and 72 girls) born and reared in the small town of Blaszki (5000 inhabitants) in central Poland was examined. The fluoride concentration in water supplies in this town ranges from 2.4 to 3.6 ppm. The individuals involved in the study were divided in 4 age groups (Table 1).

In permanent dentition, caries experience as well as enamel fluorosis were assessed. The examination was carried out in the dental chair in artificial light using a mirror and a probe. Prior to examination the teeth were dried. Caries experience was determined by means of the DMF-s index. Dental fluorosis was recorded with the help of a sensitive classification

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Table 1

Age Groups

Group	Age	No. of Children
I	7-9	30
II	10-12	29
III	13-15	31
IV	16-18	31

Table 2

Caries Experience

Group	Boys	Girls	∓
I	0.1	1.0	0.5
II	1.8	2.5	2.1
III	3.7	4.4	4.0
IV	7.2	7.3	7.2

system developed by Thylstrup and Fejerskov (6). Enamel changes on the buccal, occlusal and lingual surfaces of each tooth were estimated, the degree of dental fluorosis was expressed according to the 10 scores of the classification. Physical development of the individuals was evaluated by measuring height and weight. Consideration was given to the amount and quality of the beverages and food consumed by the children.

Results

Caries experience based on mean values of DMF-s is presented in Table 2. The indices are relatively low, but they increase with age. In the oldest age group, the DMF-s value for boys and girls are similar.

In 92% of the individuals under study, enamel changes characteristic of fluorosis were found. They varied widely, however, in intensity, from narrow white lines of opacity (1° according to Thylstrup) to pits regularly arranged in horizontal bands (<2 mm in vertical extension) with marked attrition of occlusal surfaces.

Table 3

Occurrence and Degree of Dental Fluorosis

	Normal enamel		Mild fluorosis		Severe Fluorosis	
Group	Number	%	Number	%	Number	%
I	4	13.3	16	53.4	10	33.3
II	3	10.3	11	30.1	15	58.6
III	1	3.2	10	32.3	20	64.5
IV	1	3.2	13	42.0	17	54.8

Frequency and intensity of dental fluorosis in the particular age group are presented in Table 3. Among the 121 children under study only 9 were found to have normal enamel. Cases were regarded normal when the index value for all the teeth equalled 0, or if no more than 2 teeth scored at 1. Cases classified as severe fluorosis were those whose scores were 5 or higher for 2 or more teeth. Of 62 individuals belonging to this group, only two teeth in 24 were severely affected whereas, in the remaining 38, the changes were more pronounced and more teeth were affected.

No differences were noted in fluorosis incidence with respect to the sex of the studied children. Severe fluorosis affected 33% of the youngest children and more than 50% in the other groups: the highest percentage(64.5%) was found in children aged 13-15 years.

FIG. I.

The mean Fluorosis Index Scores

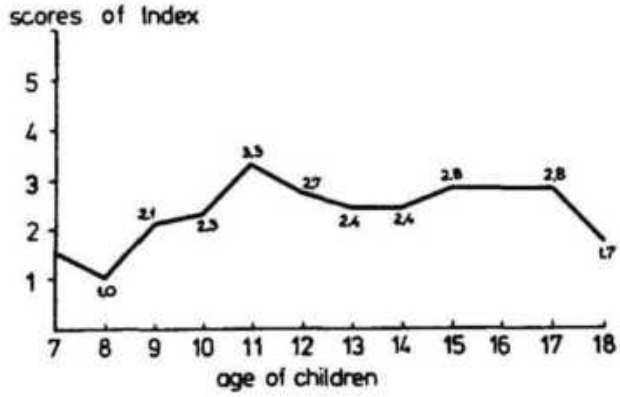

FIG. II.

The mean Fluorosis Index Scores for the particular teeth.

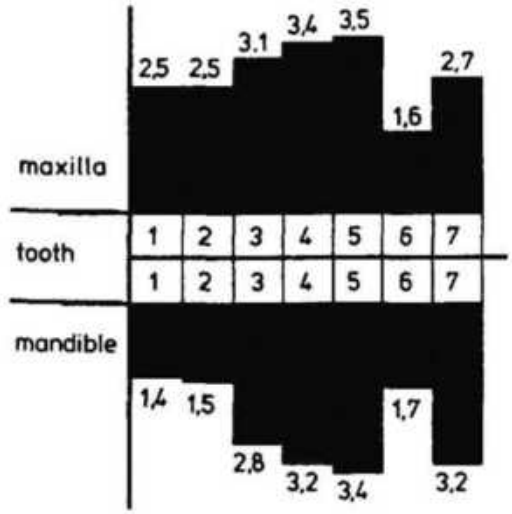

Fig. 1 represents index scores according to Thylstrup with respect to the particular years of life of the studied individuals. The scores rise until the 11th year; subsequently, whereas they decline slightly, they still remain at a high level. A comparison of homologous pairs of teeth revealed a symmetry in the distribution of fluorotic changes on the particular tooth surfaces.

With respect to intensity of mottling (Fig. 2), the most pronounced changes occurred in the second upper and lower premolars. Slightly lower scores were found in the first premolars as well as in the second lower molars and in the upper cuspids. The least changes in enamel were found in the lower incisors and the first molars.

In children with severe fluorosis, height and weight was slightly lower than in the others. According to Table 4, individuals with severe mottling were, on an average, 2.5 cm shorter and weighed, on an average,1.2 kg less (Table 5). Nevertheless the children appeared to be normally developed and well nourished. In the town of Blaszki, situated in an agricultural region, the children's diet contains all basic foodstuffs, such as milk, cheese, eggs, etc.

Table 4

Height Deficiency - Severe Fluorosis

Group	Boys	Girls	$\bar{x}$
I	4.0cm	1.6cm	2.8cm
II	4.2cm	2.8cm	3.5cm
III	3.0cm	0.8cm	1.9cm
IV	1.4cm	2.0cm	1.7cm
			$\bar{x}$ 2.5cm

Table 5

Weight Deficiency - Severe Fluorosis

Group	Boys	Girls	$\bar{x}$
I	0.5kg	0.2kg	0.3kg
II	4.2kg	0.4kg	2.3kg
III	5.2kg	0.4kg	2.8kg
IV	1.2kg	1.9kg	0.7kg
			$\bar{x}$ 1.2kg

An inquiry concerning the intake of beverages showed that their volume was within the normal range for these age groups. However, children with severe fluorosis drank about 250 ml per day more than the others.

Discussion

Children and youth born and reared in the locality where the fluoride concentration in water supplies is 2.4-3.6 ppm showed marked differences in the appearance of the enamel. The classification of dental fluorosis established by Thylstrup and Fejerskov permitted a more precise differentiation of the changes than might have been obtained by means of Dean's index. It is commonly recognized that with the latter's index it is not possible to distinguish between children with pitting of only two teeth and those all of whose teeth are pitted to a varying degree.

Among the children under study, only 7% had normal enamel, mainly those of the two youngest age groups. In the remaining 92%, the enamel was mottled, in most of them there was marked opacity with pitting (scores:5-6) and definite impairment of their general appearance.

The highest percentage of severe fluorosis was found in children 13-14 years old, the age associated with eruption of the premolars, cuspids and second molars. These are the teeth that, as a rule, are most severely affected. In the children under study a progressive increase in severity was noted from the anterior to the posterior teeth, with the exception of the first molar.

Mottling increased until age 11. Subsequently, there was a slight decrease, the reason for which is not fully clear. Probably, the scores for teeth which erupt after the 11th year of life, i.e. the second molar as well as the cuspids, are affected somewhat less than premolars. Least affected by fluorosis were the incisors, especially those in the lower jaw, as reported by other authors. Thylstrup and Fejerskov concluded that the severity of mottling is determined by enamel thickness rather than by the length of exposure to body fluids (3,4,6). Such an opinion is valid in the case of mild mottling on incisors, but does not explain why the first molars are only slightly affected. It may be assumed, therefore, that the length of time that has elapsed between the full formation of the tooth crown and eruption of the tooth must be important, a period which may last as long as 4 to 5 years in the case of teeth most severely affected with fluorosis. Weatherell and coworkers (10) have expressed the hypothesis that dental fluorosis develops because the forming enamel remains incompletely mineralized long enough to be affected by a relatively high concentration of labile fluoride. Recent research (3) suggests that dental fluorosis results from impairment of enamel maturation.

Individuals with severe dental fluorosis showed deficiencies in height and weight, as compared to others of equal age. On the whole, however, they were well-nourished and adequately developed. The prevalent habit of drinking more beverages per day (250 ml) appears to be of greater significance since above 3 ppm fluoride in the drinking water cannot be without consequences.

This investigation does not bear out the view that the weak and undernourished children are chiefly affected with dental fluorosis. Intake of fluoride with beverages, especially tea, may play an important part (11).

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ACTION OF BORON UPON FLUOROSIS: AN EXPERIMENTAL STUDY

by

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SUMMARY: The effect of boron as a possible antidote to fluoride was studied in fluorosed rabbits which received fluoride in drinking water (1). Boron was administered at a constant F/B ratio,1) at the beginning of fluoride intoxication (preventive [p]), 2) midway during intoxication (prevento-curative [pc]), and 3) after discontinuance of fluoride administration (curative [c]). In the first two experiments, the animals were divided into groups F+Bp, F+Bpc, and Bc. They were compared to the F group and to a group after fluoride administration was discontinued (spontaneous detoxification). Boron was also administered alone. All groups were compared to normal controls (N). The effect of boron upon different metabolic pathways was explored (2,3). Boron can be employed in the prevention of human fluorosis, but it must be done cautiously.

I. Acute Intoxication

In our first series of experiments fluoride, calcium and phosphorus balances were studied during massive and short term fluoride intoxication (60 mg F/kg/day for 2 months) with and without boron as a preventive, (F+Bp), prevento-curative (F+Bpc) or curative (bc) (3-5).

1. Blood and Urinary Fluoride, Calcium and Phosphorus: Fluoremia was very high in lots F and F+Bp ($p < 0.01$). It returned to normal 11 days after interruption of the intoxication without and with boron. Hypocalcemia was present in lot F ($p < 0.05$) but was corrected in lot F+Bp. Later, a hyperparathyroid reaction occurred during the first part of the spontaneous detoxification with hypercalcemia, hypophosphatemia and a decrease of the renal phosphorus reabsorption coefficient ($p < 0.05$ or $p < 0.01$). This condition improved when boron was administered curatively.

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2. Fluoride Balance: Retention of fluoride was very pronounced; it was equal in lots F and F+Bp (p 0.01) because of an increase of the fluoride digestive utilization coefficient (p 0.01) and of the fluoride content of bones (p 0.01) regardless of marked urinary fluoride excretion (p 0.01). Boron, as preventive, did not modify the turnover of fluoride. It produced the same turnover, but fluoride became less toxic. However, when boron was administered curatively, after fluoride intake was interrupted, the excretion of fluoride increased and spontaneous detoxification was enhanced. Upon interruption of fluoride on day 11, the fluoride balance was strongly negative with Bc (p 0.01) and slightly negative in lot F (p 0.10). The fluoride content returned to normal in lot Bc on day 45, but remained high (p 0.01) during spontaneous detoxification.

3. Calcium and Phosphorus Balances: Calcium and phosphorus retention decreases considerably with F (p 0.05), because of reduction of the calcium and phosphorus digestive utilization (pduc). Boron, given as preventive, normalizes the toxic effect of fluoride upon the calcium-phosphorus metabolism. It corrects the hypocalcemia, the calcium and pduc, as well as calcium and phosphorus balances. After interruption of fluoride intake, the fluoride balances are normal with or without boron. However, boron given as curative corrects secondary hyperparathyroidism and increases the sequestration of fluoride from bone.

II. Subacute Intoxication

Our second series of experiments was concerned with the toxicological effects during subacute fluoride intoxication (40 mg F/kg/day for 7 to 10 months), with and without boron (preventive F+B; and F).

1. Calcium-Phosphorus Metabolism and Skeletal Radiography: As in the above experiments, a low dose of fluoride given for a prolonged period caused fluoremia in the two lots and hypocalcemia in lot F (p 0.01). In lot F+Bp, the blood calcium remained normal (3). Posterior pad radiography showed an increase of cortical thickness in lot F which was less pronounced in lot F+Bp. These findings indicate that boron may serve as a preventive antidote.

2. Hepatic Disease: After 7 months, blood values for proteins, lipids, cytolytic enzymes and the histological findings were normal in the two lots (5). However, respiration of hepatic tissue measured at the time of fluoride administration increased in lots F and F+Bp (p 0.05) because of possible liver damage. In vitro, there was a difference with or without boron when a single large dose of fluoride or fluoride plus boron was added. Fluoride caused a biphasic curve of tissue VO_2. At first, oxygen consumption increased (p 0.05) then it declined (p 0.01). Fluoride + boron eliminated the initial increase and reduced the secondary decline (p 0.05).

3. Hemostasis: Vascular resistance, the quality and number of platelets and the fibrinogen rate did not vary from normal values in the two lots (6). Howell and coagulation times were prolonged in lot F (p 0.10), but became normal in lot F+B. The rate of the prothrombin complex decreased in lot F (p 0.10) but was normal in lot F+B.

There are two explanations for the changes in coagulation: a) Chelation of ionized calcium by fluoride as indicated by normalization of the coagulation time after a 20 day interruption of fluoride and b) slight hepatic damage as indicated by an abnormal prothrombin complex rate.

III. Low-Grade Subacute Intoxication

A third series of experiments pertained to the toxicological effects during subacute, less severe, fluoride intoxication (15 to 30 mg F/kg/day for 8 months) with or without boron supplementation: preventive, preventive-curative or curative (7). In this experiment, changes in the calcium phosphorus metabolism and hemostasis were minor during fluoride intoxication without boron (F lot) or, with boron given as preventive, and as preventive-curative antidote (F+Bp, F+Bpc). Boron administered alone did not appear to be toxic.

An antidote effect on the fluoride and calcium phosphorus metabolism was obtained with boron administered curatively (Bc) compared with fluoride by itself. Urinary fluoride excretion was still elevated four months after spontaneous detoxification but was greater in lot Bc(p 0.01) in which fluoride excretion was induced by the antidote.

The fluoride content of bones and claws was lower in lot Bc, compared with fluoride alone (p 0.10). Claws, but not hair may be utilized as a parameter for fluoride intoxication and for the effect of antidotes. The radiography of the posterior pads remained abnormal four months after fluoride was discontinued, but appeared to be normal when boron was administered for four months. The content of skeletal calcium did not vary in any of the lots with or without boron.

Discussion

If we consider, in endemic fluorosis areas, a mean amount of 30 mg F/day consumed with food and water - more in summer and less in winter - the F/B ratio of 1.73 (8) would require a daily supply of boron of 17.3mg. The minimal daily dose which causes borism after prolonged use is about 100 mg. The ratio 7.3 to 100 or about 1 to 5 would not be sufficient to prevent boron intoxication.

For prevention of hydrofluorosis it is desirable to make deep drillings of wells and for industrial fluorosis to apply protective measures rather than to consider a concommitant antidote. However, when fluoride intoxication is interrupted, a boron might be used as an antidote provided that it is given only on a short-term basis in order to avoid toxicity.

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FLUORIDE IN AIR, SALIVA, URINE, AND ENAMEL IN SCHOOL CHILDREN

by

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SUMMARY: The aim of this study was to reveal the fluoride level in saliva, urine, and enamel in 123 school children, aged 7 and 10 years, of both sexes. The children under study resided in Police where the concentration of fluoride in air was several times above the allowable limit. The level of fluoride in saliva was significantly higher in the exposed area than in the control. The fluoride content of the enamel and the urine was similar in both groups. A higher value of DMFS index was observed in children aged 7 and 10 living in the area exposed to fluoride.

Introduction

With increasing development of the chemical industry various chemical compounds are emitted into the air. In the vicinity of a chemical plant in Police near Szczecin, fluoride, sulphur, and ammonium compounds were emitted as dusts, vapors, and gases.

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The purpose of this paper is to determine the level of fluoride in saliva, urine, and tooth enamel of school children residing in various regions of Szczecin.

Material and Methods

The investigation was conducted in 123 school children, chosen at random at ages of 7 and 10 of both sexes. Group I consisted of the children from the vicinity of the chemical plant in Police, near Szczecin, where the concentration of fluoride in air was several times above the permissible limit. Group II was composed of children from Podjuchy, the region of Szczecin where only trace amounts of fluoride were found in the air. All the investigated children had been residing in those regions for at least five years.

A total of 377 samples were analyzed. Saliva, urine and enamel were included among the samples. Samples of 10 ml of saliva were taken during the morning. The urine sample was obtained in the morning upon awakening and the children brought it from home.

The microsamples of enamel were taken from the first upper incisor according to the method of Larsen et al. (1). The tooth to be biopsied was isolated with cotton rolls and soft debris was removed. The labial surface was then abraded by means of a sterile rubber cup and a slurry consisting of quartzite in glycerol with sampled enamel was collected and placed into a plastic tube. The time of biopsy was 40 seconds at 2000 cycles per one minute.

The chemical analysis was made by using the ion selective fluoride electrode produced by Orion Research, Inc. as described in another paper (2). The DMFS index was estimated in both groups. The results underwent statistical analysis on the basis of test "u".

Results

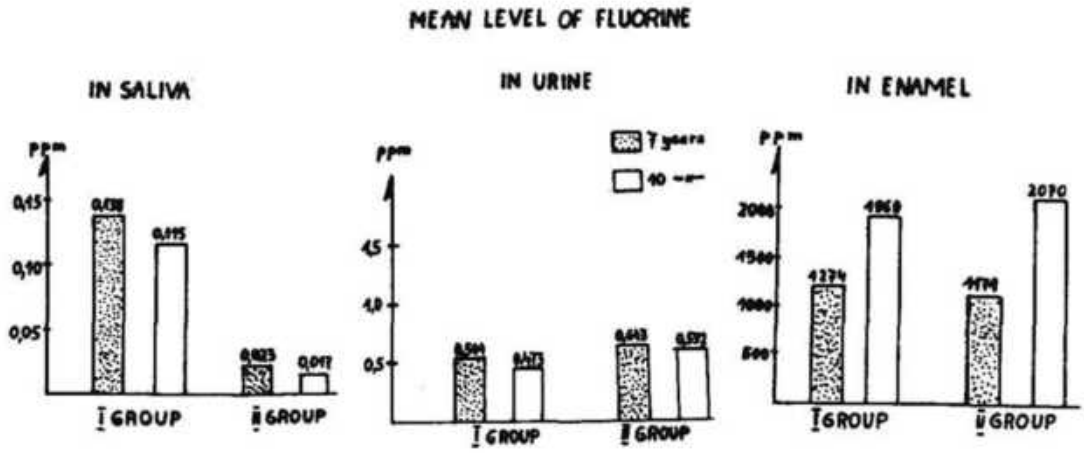
The results of the chemical analysis of saliva, urine, and enamel are summarized in Figures 1, 2, and 3.

The mean level of fluoride in the saliva in the respective groups is shown in Figure 1. The level of fluoride in saliva of children in Group I was 6 times higher than that in the saliva of Group II. The differences appeared to be statistically significant.

The mean levels of fluoride in urine (Figure 2) are similar in both groups and are not statistically significant.

In Figure 3 the mean level of fluoride in enamel was similar in both 7 and 10 year old children. Differences were not statistically significant.

The results of DMFS index are presented in Table II. A higher value of the DMFS index was observed in the children residing in the exposed



area compared with the DMFS of children in Group II (unexposed). It is difficult to compare the results of this investigation with earlier results because this time the observed groups consisted solely of children (3-5).

Table 1

Samples for Chemical Analysis

Region	7 yrs.	10 yrs.	Total
Group I-Police	99	93	192
Group II-Podjuchy	94	91	185

Table 2

DMFS-Index for Both Groups

Region	7 yrs. No.	7 yrs. DMFS	10 yrs. No.	10 yrs. DMFS
Group I	252	2.4	225	4.9
Group II	236	2.0	236	3.9

Conclusions

1. The excessive concentration of fluoride in the air was accompanied by an increased level of fluoride in saliva.

2. Neither a higher level of fluoride in urine and enamel nor a better state of dentition was observed in children who were exposed to a high level of fluoride in air.

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COPPER, ZINC AND MAGNESIUM IN BREAST MILK OF WOMEN RESIDING IN ENDEMIC FLUOROSIS AREAS OF SOUTHERN INDIA

by

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SUMMARY: Breast milk of 229 nursing mothers in villages where genu valgum was endemic was assayed for copper, zinc and manganese. The drinking water contained 6-11 ppm.Milk of 226 mothers in a village with 5-10 ppm in water where genu valgum was not prevalent was also analyzed. The mother's milk of 258 women residing in a rural, nonfluorotic area (1-1.4 ppm) served as control. In all three study groups the copper concentration was high during the first weeks of lactation but fell steeply during the fourth week. The concentration of manganese was several times higher than that of copper and zinc and remained unchanged for 18 months.

Introduction

Endemic skeletal fluorosis is a public health problem in parts of India where the fluoride content of drinking water is high. In some regions of Southern India, endemic genu valgum has been identified as a manifestation of environmental fluoride toxicity (1-2). Epidemiological, hormonal and metabolic aspects of endemic genu valgum have already been described (3-4).

Subjects affected by endemic genu valgum are relatively young with rapidly growing bones. Radiological evidence of osteoporosis of the ends of long bones in addition to osteosclerosis of other bones are characteristic features. Earlier studies indicate that altered trace element metabolism may play a role in the etiology of endemic genu valgum (5). Cortical bone

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samples, analyzed at the time of surgical correction of deformity in subjects with endemic genu valgum, showed that levels of copper, magnesium and manganese were significantly lower compared to levels in bone samples obtained from the comparable sites of normal subjects residing in nonendemic fluorosis areas (6). Epidemiological studies indicated that genu valgum does not occur in all endemic fluorosis areas. Therefore it was considered important to look for special attributes of population groups that are exposed to high intakes of fluoride in whom genu valgum is prevalent, in contrast to population groups exposed to high fluoride in whom genu valgum does not occur. Earlier, a significant inverse correlation between the percent prevalence of genu valgum in a village of endemic fluorosis of India and the concentration of copper in drinking water was observed (7). In view of known trace elements such as copper and zinc and of the macronutrient magnesium in bone metabolism, an attempt was made to study the contents of these elements in breast milk of nursing mothers residing in endemic fluorosis areas with and without genu valgum. The results of this study are herewith presented.

Materials and Methods

Cross-section studies: Two hundred and twenty-nine nursing mothers residing in villages where the fluoride content of drinking water ranged between 6.0 and 11.0 mg per liter and where genu valgum was endemic were investigated. In addition, 226 lactating mothers who resided in villages where the fluoride content of drinking water was between 5.0 and 10.0 mg per liter, but genu valgum was not endemic were likewise studied. A group of 258 nursing women residing in nonfluorotic areas of rural Andhra Pradesh (fluoride content of drinking water between 1.0 and 1.4 mg per liter) constituted the control group.

All mothers were contacted at their homes between 9:00 A.M. and 12:00 noon. Maternal age, duration of lactation (months) and parity were recorded. Breast milk samples were collected directly into polythene screw-capped vials which had been thoroughly washed in acid and deionized water, all precautions against contamination being taken in the laboratory. Milk samples were immediately frozen at the field center and transported on ice to the laboratory.

The samples were centrifuged, fat separated and the fat-free solution appropriately diluted with deionized water (dilution factor was 2, 5, and 100 for Cu, Zn, and Mg respectively) and Cu and Zn and Mg were estimated by atomic absorption spectrophotometry (model 100 Varian Techtron) in duplicate. Results were expressed as mg per liter.

Longitudinal studies: Twelve lactating mothers residing in endemic genu valgum villages were investigated longitudinally. For eleven weeks milk samples were obtained every week; subsequently, monthly for ten months. Samples were processed as indicated earlier.

Daily variation: In twelve other lactating women who were residing in genu valgum area, daily milk samples were collected for seven consecutive days and analyzed for their Cu, Zn, and Mg concentrations to deter-

mine the day-to-day variation. Analysis of variance and normal proportion test was used wherever necessary.

Results

Data on the analysis of milk samples collected on seven consecutive days indicated that daily variations in Cu, Zn, and Mg, were not significant for the same individual. Inter-individual variations, however, agreed with observations made earlier by Rajalakshmi and Srikantia in urban mothers (8).

Cross-sectional studies: In all three study groups, the concentration of copper was high during the first few weeks of lactation. It fell steeply during the 4th week, followed by a slow but steady downward trend. Even at 18 months of lactation, concentrations of copper remained at fifty per cent of that observed at the 4th week. The concentration of zinc was 6 to 8 times that of copper during the entire period of lactation. As in the case of copper, zinc concentrations also showed a steady decline as lactation continued and, at 18 months, levels were between 25 and 36 per cent of those of the 4th week. The concentration of Mg was several times higher than that of Cu and Zn and it remained essentially unchanged even at 18 months.

It was significant that the mean values for copper were significantly lower in breast milk samples of women in the endemic genu valgum group whose parity was either 3 or below. Also the mean values were lower in mothers under 30 years of age.

The frequency distribution for the values for copper in relation to the duration of lactation in the three groups is presented in Table 1. Whereas in 28.5% of the milk samples in the endemic genu valgum groups values were below 100/µg/l, only in 8 and 12% of the samples in the other two groups were values as low. In contrast, the proportion of samples of values of milk over 250/µg/l was significantly less in the endemic genu valgum group (15.4%) compared to those in the other two groups (23.6 and 30.0% respectively).

Distribution of breast milk copper concentration in relation to parity and maternal age in the three groups is presented in Tables 2 and 3 respectively. Among mothers of parity 1-3 who were less than 30 years old a significantly higher proportion of samples in the endemic genu valgum group were in the range of <100/µg/l compared with the other two groups. Also, a significantly lower percentage of values were distributed in the range of over 300 µg/l in the endemic genu valgum group.

Frequency distribution of zinc and magnesium values, in contrast, did not show any significant difference between the endemic genu valgum group and the other two groups either in relation to parity or in relation to maternal age.

Longitudinal studies: The pattern of change in breast milk concentration of Cu, Zn, and Mg with time in twelve mothers who were followed longitudinally was similar to that observed in cross sectional studies.

Table 1

Distribution of Copper (ug/1) in Breast Milk at Different Lactation Periods in Rural Mothers

Copper ug/1 / Duration of Lactation Months	Endemic Genu Valgum (221)						Endemic Fluorosis without Genu Valgum (226)						Non-Fluorosis Control (254)					
	<100	101-150	151-200	201-250	251-299	>300	<100	101-150	151-200	201-250	251->300		<100	101-150	151-200	201-250	251-299	>300
<1	-	-	-	2	-	3	-	-	-	-		11	-	-	-	-	-	9
1-3	2	8	4	3	2	8	1	1	9	4	9	16	-	1	5	2	4	13
4-6	4	2	6	4	2	2	1	6	12	4	5	4	6	10	13	9	1	10
7-9	4	4	2	-	-	2	1	13	7	2	6	1	2	5	8	2	-	2
10-12	14	15	14	7	2	7	4	15	8	2	3	2	7	28	10	7	6	5
13-18	9	12	5	-	2	1	4	11	5	4	3	1	7	17	10	2	3	2
>18	30	21	11	4	3	-	7	13	17	5	2	3	8	22	12	1	2	3
TOTAL	63[ab]	62	42	20	11[a]	23[ab]	18	59	58	21	29[c]	38	30	83	58	23	16	44
%	28.5	28.0	19.0	9.1	5.0	10.4	8.1	26.4	26.0	9.4	13.0	17.1	11.8	32.7	22.8	9.1	6.3	17.3

Table 3

Distribution of Copper (ug/l) in Breast Milk in Relation to Maternal Age in Rural Mothers

Copper (ug/l)	Endemic Genu Valgum (229)					Endemic Fluorosis without Genu Valgum (226					Non-Fluorosis Control (254)				
	<100	101-150	151-200	200-300	>301	<100	101-150	151-200	201-300	>301	<100	101-150	151-200	201-300	>301
Maternal Age Yrs.															
<20	16	14	12	6	2	8	10	11	24	15	8	12	8	2	10
21-25	26	25	18	12	9	11	15	20	9	13	18	27	22	17	21
26-30	20	16	3	8	9	11	10	18	10	6	14	17	16	11	13
31-35	9	3	3	4	2	4	8	6	4	3	8	3	9	5	-
>35	6	2	1	2	1	1	3	1	2	3	3	2	3	3	2
Total	77[ab]	60	37[a]	32[a]	23[ab]	35	46	56	49	40	51	61	58	38	46
%	33.6	26.2	16.1	13.9	10.0	15.5	20.3	24.7	21.7	17.7	20.0	24.0	22.8	14.96	18.1

Table 2

Distribution of Copper (ug/l) in Breast Milk in Relation to Parity in Rural Mothers

Copper (ug/l)	Endemic Genu Valgum (229)					Endemic Fluorosis without Genu Valgum (226)					Non-Fluorosis Control (256)				
	<100	101-150	151-200	200-300	>301	<100	101-150	151-200	201-300	>301	<100	101-150	151-200	201-300	>301
Parity															
1	16	12	11	8	3	8	11	16	21	18	10	21	7	8	14
2	17	23	8	6	6	9	10	12	14	7	12	14	20	11	7
3	25	12	11	5	8	12	7	12	8	5	5	11	13	7	14
4	9	8	6	8	1	3	9	9	7	6	8	9	13	2	8
5	6	4	-	2	3	1	3	2	2	3	8	2	3	5	4
6	1	-	1	1	2	1	2	4	-	-	4	1	1	2	1
7	3	1	-	2	-	-	2	1	-	1	2	3	1	5	-
Total	77[ab]	60	37[a]	32[a]	23[ab]	34	44	56	52	40	49	61[c]	58	40	48
%	33.6	26.2	16.1	13.9	10.2	15.2	19.4	24.7	34.0	17.7	19.1	23.8	22.6	15.7	18.8

Discussion

Most significant was the observation that in a high proportion of mothers residing in endemic genu valgum areas copper values in breast milk were low. This was particularly true among young mothers and in those who had one to three previous pregnancies. As levels of zinc and magnesium did not show such changes, the low copper levels in breast milk appear to be a special attribute of mothers residing in endemic genu valgum villages. Whether the low values of copper in breast milk among young mothers represent an environmental inadequacy of copper in endemic genu valgum areas is yet to be ascertained. Earlier studies by Deosthale, et al. (5) indicated the possibility that subjects residing in endemic genu valgum areas whose staple is sorghum, consume higher amounts of molybdenum in their diets as compared to those residing in control areas. In view of the well-known metabolic interaction between molybdenum and copper in the body, the observed low values in breast milk appear to be significant since endemic genu valgum is prevalent in the younger generation of the population.

The pattern of changes in relation to time in breast milk Cu and Zn obtained in cross sectional and longitudinal studies in this investigation are essentially similar to the observations made earlier by Rajalakshmi and Srikantia (8) in urban women in Hyderabad.

In endemic fluorosis areas, high amounts of fluoride are ingested through water by children, as soon as their diet is supplemented with food in addition to milk. It is not known whether subtle differences in the intake of micronutrients such as copper and molybdenum during the rapidly growing phase of a child's life, modify the metabolic aspects of fluoride toxicity in children. Copper is known to be essential for the maturation of collagen including that of bone, particularly in growing children. In children suffering from endemic genu valgum, osteoporosis in addition to osteomalacia and osteosclerosis is known to occur in the long bones.

In view of the specific changes seen in the copper content of breast milk in endemic genu valgum areas, systematic studies to determine the intake of micronutrients by children and adolescents residing in these areas may help to understand the role of the trace elements - copper and molybdenum, in the genesis of genu valgum deformity in endemic fluorosis areas in India.

Acknowledgement

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BIOCHEMISTRY OF FLUOROSIS X - COMPARATIVE STUDY OF THE FLUORIDE LEVELS IN BIOLOGICAL FLUIDS

by

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SUMMARY: The fluoride content of drinking water is directly correlated with the serum and saliva fluoride. Serum fluoride levels are also directly related to those of saliva, but not to urinary fluoride.

These interrelations are so close that a mathematical formula can be devised for the calculation of fluoride in body fluids. For instance, a sample of saliva may serve for the quantification of serum fluoride. Whereas these interrelations are observed in general population groups they do not always apply to individuals.

Introduction

The object of our investigation was to determine up to what point the evaluation of fluoride levels in blood can be replaced by its quantification in other organ fluids. By this method venipuncture might be eliminated, the follow-up of patients and periodic studies on populations might be simplified We therefore analyzed the fluoride levels of serum, urine,

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and saliva in individuals residing in areas of low, medium and high fluoride concentrations in their drinking water and correlated the results.

Material and Methods

a) Methodology and instruments, for the sake of clarity, will be described for serum, urine, and saliva separately.

b) The biological samples were classified in accordance with the fluoride content of the drinking water of the 3 population groups, namely 10 - 13 healthy male and female adults, aged 20 to 60 years, residing in high fluoride zone 1: 2.6-8 ppm (area of Buena Esperanza in the South of the Province of San Luis, Argentina); medium fluoride zone 2: 1 ppm in the City of San Luis, Argentina and low fluoride zone 3: 0.1 ppm in the city of Buenos Aires, Argentina.

All subjects were well nourished and adhered to a balanced diet with respect to animal proteins, fats, and vitamins A and C. They had been residing for at least 6 years in their respective areas, the majority were born in area 1. Fasting blood samples of urine specimens and saliva were obtained simultaneously in every case. The serum was separated in polyethylene containers. Urine specimens were those first voided in the morning which we found equivalent to the 24-hour samples.

Saliva was also obtained in a fasting state, without stimulation, as follows: after thoroughly rinsing the mouth with deionized water, approximately 5 ml of total saliva was collected in a polyethylene container, during a period of approximately 10-15 min. All subjects were cautioned against the use of fluoridated toothpastes.

The difficulty of obtaining the samples (blood, saliva, and urine) simultaneously in each individual in order to correlate them with the fourth element, i.e.: the drinking water, necessitated a reduction in the number of subjects.

Experimental Data and Results

I. Serum Fluoride

Blood serum or plasma are adequate for fluoride determinations; they are more accurate than those obtained with whole blood. The level of fluoride in serum remains practically constant due to the action of regulating mechanisms (extracellular fluid, bone fixation, urinary excretion, etc.) (1-4). Most authors observed similar fluoride values in individuals drinking water, the fluoride content of which is low or medium (0.1 - 1 ppm)(5). However, the determination of the fluoride values in serum has generated much controversy among investigators (6-8).

We postulated that, depending upon the treatment to which the sample is submitted for the separation of fluoride, this element bound to proteins will be liberated to a greater or lesser degree and bring about an increase in the ionizable fraction (9). This, in our opinion, is the ex-

planation for the discordant results.

Methodology: Our modus operandi, was a reproduction of that proposed by different authors (10-16), adapted to our requirements. This enabled us to carry out determinations of ionized fluoride in blood by means of a selective electrode without prior blood deproteinization or microdiffusion.

Reagents and Apparatus: Standard F^- solution of 100 ppm F^-: dissolve 2.2105 g of NaF in distilled water and dilute to 1 liter.

Working F^- solutions: prepare solutions containing 0.1, 1, and 10ppm F^-. - Buffer solution to adjust ionic strength: TISAB (Orion), μ= 0.05; pH = 4.75 (20). - Sodium hydroxide 2.5 N. - Saturated solution of silicone fluid in hydrochloric acid 6N (Dow Corning 740 Fluid, 39 centistokes, Dow Corning, Midland, Michigan (14). - Magnesium oxide p.a. - Combination ion F^- electrode, model 96-09 (Orion). - Digital pH meter model 701 (Orion).- Lucite Conway cells (40 mm diameter) with covers of the same material.

Ionic F^- Determination: 1 ml serum is mixed with a similar value of TISAB in a small plastic tray. Evaluation is made with the electrode and the aid of calibration curves.

Table 1

Bueno Esperenza		San Luis		Bueno Aires	
Fluoride Content (ppm)					
Water	Serum	Water	Serum	Water	Serum
8.0	0.14	Home Water Supply 1 ppm	0.064	Home Water Supply 0.1 ppm	0.090
5.0	0.10		0.070		0.042
2.6	0.12		0.065		0.040
3.0	0.17		0.060		0.044
4.5	0.21		0.060		0.052
6.2	0.20		0.070		0.044
4.0	0.13		0.075		0.040
5.2	0.12		0.080		0.040
6.4	0.14		0.072		0.072
4.8	0.14		0.080		0.050
-	-	-	-	-	0.170
-	-	-	-	-	0.120
-	-	-	-	-	0.220
X=4.97	X=0.147	X-1	X=0.0696	X=0.1	X=0.078

Table 2

Bueno Esperenza		San Luis		Bueno Aires	
Fluoride Content (ppm)					
Water	Serum	Water	Serum	Water	Serum
8.0	0.25	Home Water Supply 1 ppm	0.13	Home Water Supply 0.1 ppm	0.18
5.0	0.20		0.12		0.10
2.6	0.24		0.12		0.12
3.0	0.30		0.10		0.10
4.5	0.38		0.12		0.10
6.2	0.38		0.13		0.12
4.0	0.23		0.15		0.10
5.2	0.24		0.16		0.12
6.4	0.25		0.13		0.18
4.8	0.26		0.15		0.12
-	-	-	-	-	0.30
-	-	-	-	-	0.29
-	-	-	-	-	0.40
$\bar{X}$=4.97	$\bar{X}$=0.273	$\bar{X}$=1	$\bar{X}$-0.131	$\bar{X}$=0.1	$\bar{X}$=0.171

In tables 1 - 5 all subjects had over 6 years residence in San Luis before the analysis and since birth in Buena Esperanza and Buenos Aires.

Total F^- Determination: Fundamentally the method consists of a) calcination, in order to destroy organic matter; b) microdiffusion, in order to separate fluoride; c) evaluation of separated fluoride by means of the selective electrode.

a) Calcination: 5 ml of serum and 75 mg magnesium oxide are placed in a platinum capsule. They are heated gently until volume is reduced by one-half, and placed in a 100°C. oven until dry, then heated in furnace for 30 minutes at 150°C., followed by 15 minutes at 300°C. and finally 90 minutes at 500°C.

b) Microdiffusion: Work is undertaken with lucite Conway cells prepared as follows:

Central chamber: 0.5 ml NaOH 2.5N. External chamber: add to all the ashes obtained during calcination 0.5 ml silicone solution in HCl 6N and 2 ml 50% perchloric acid. Diffusion time: 3 hrs. at room temperature.

c) Selective electrode readings: After microdiffusion, the contents of the central chamber are transferred quantitatively to a plastic tray, aided by 2-3 rinses with deionized water. Adjust pH to 7-7.2 with HCl (concentrated, IN and 0.IN, successively), arriving at final volume of 5ml with deionized water. Add 5 ml of TISAB, mix and proceed as in the case of ionic fluoride.

The values obtained for ionic and total F^- are shown in Tables 3 and 4.

Table 3

Buena Esperanza		San Luis		Bueno Aires	
Fluoride Content (ppm)					
Water	Saliva	Water	Saliva	Water	Saliva
8.0	0.12		0.055		0.079
5.0	0.10		0.062		0.058
2.6	0.10		0.058		0.040
3.0	0.14		0.050		0.050
4.5	0.15		0.060		0.100
6.2	0.16		0.060		0.060
4.0	0.10		0.065		0.060
5.2	0.15		0.065		0.032
6.4	0.15		0.075		0.050
4.8	0.15				0.080
-	-				0.150
-	-				0.078
$\bar{X}$=4.97	$\bar{X}$=0.132	$\bar{X}$=1	$\bar{X}$=0.060	$\bar{X}$=0.1	$\bar{X}$=0.068

Table 4

Sample N°	First Urine	24-hr. Urine
1	2.10	1.70
2	1.50	1.40
3	1.80	1.40
4	1.40	1.00
5	0.94	1.10
6	3.30	3.00
7	1.60	1.50
8	1.50	1.20
9	1.70	1.60
10	2.00	1.80
	$\bar{X}$=1.78	$\bar{X}$=1.57

II. Fluoride in Saliva

There is a wide discrepancy between the values given in the literature for salivary fluoride which range between 0.00 and 0.75 ppm (21-25). Some authors have used total saliva, others parotid saliva after stimulation. Table 3 presents the values obtained by us. Some investigators, however, have found no appreciable difference in fluoride excretion under normal conditions or after stimulation (26).

We worked with samples of spontaneously eliminated total saliva in order to facilitate extraction and in order to abide by the aim of our investigation i.e. to simplify the determinations and to render them useful for large scale population controls. For the serum we refer to ionic and total fluoride content, for saliva we refer solely to ionic fluoride. Because of the low protein content of saliva, equivalent values were obtained in the direct determinations with the selective electrode as compared with those established after calcination.

The method used was that of Gron et al. (27), the reagents and instruments were the same as those used for the determination of F^- in blood. Five ml of saliva is mixed with an equal amount of TISAB in a plastic dish and shaken.

III. Urinary Fluoride

Urinary fluoride excretion is utilized to determine the degree of danger to which workmen are being exposed. Here again the values reported in the literature are highly variable since, in addition to the analytical methods employed, certain factors affect the results such as former and duration of exposure, age, pregnancy, kidney disease, etc. Fluoride concentrations in urine vary from hour to hour and from subject to subject. The influence of the factors responsible for the inconsistent values, has been confirmed once more in our investigation, and we were unable to find a mathematical formula to express the urine/blood/saliva relationship. The values obtained by us corresponded to the first specimen of the morning because we noted a close relationship between this sample and 24-hour specimens (Table 6). As in the case of saliva, it was impossible to distinguish between ionic and total fluoride due to the low protein content of normal urine.

The method used was an adaptation of that proposed by Neefus et al. (28), Cernik et al. (29), Tusl et al. (30), Singer et al. (31), and Mu-Wan Sun (32).

Reagents and Instruments: See Serum Fluoride.

Determination: In a polyethylene tray, mix 3-5 ml of urine with an equal amount of TISAB, and proceed as with the previous determinations. The values obtained are shown in Table 5. The fluoride concentration in water was established by direct measurement with the selective electrode.

Table 5

Buena Esperanza		San Luis		Buenos Aires	
Fluoride Content (ppm)					
Water	Urine	Water	Urine	Water	Urine
5.00	3.30	Home Water Supply 1 ppm	2.20	Home Water Supply 0.1 ppm	1.70
2.60	3.70		1.60		1.50
3.00	2.20		1.90		0.90
4.50	5.80		2.00		0.85
6.20	5.20		2.10		0.84
4.00	4.80		1.20		0.50
5.20	5.80		1.50		1.30
6.40	7.20		2.10		0.60
4.80	5.60		1.80		1.20
-	-		1.10		0.90
-	-		-		0.32
-	-		-		0.68
-	-		-		0.42
$\bar{X}$ = 4.63	$\bar{X}$ = 4.84	$\bar{X}$ = 1	$\bar{X}$ = 1.75	$\bar{X}$ = 0.1	$\bar{X}$ = 0.90

IV. Mathematic Interrelations

From the statistical study of the values given in the tables, based on the method of minimum squares, we were able to draw up the formula given for each particular case:

IONIC F^- /TOTAL F^- RELATION IN SERUM (TABLES 1 AND 2)

In areas of high (2.6-8 ppm) concentration in water:

Total F^- = 0.0232 + 1.70 x ionic F^- ± 0.0112 (S_{yx}); Correlation coefficient (r) = 0.98; Significant at: $p << 0.01$

In areas of medium (1 ppm) concentration:

Total F^- = 0.022 + 1.92 x ionic F^- ± 0.078 (S_{yx}); Correlation coefficient (r) = 0.89; Significant at: $p << 0.01$.

In areas of low (0.1 ppm) concentration:

Total F^- = 0.04 + 1.64 x ionic F^- ± 0.02 (S_{yx}); Correlation coefficient (r) = 0.98; Significant at: $p << 0.01$

Likewise a mathematic formula can be deduced from the extreme values (0.1-8 ppm) which makes an acceptable calculation possible ($p << 0.01$) of the concentration of total F^- from the ionic F^-.

Total F^- = 0.019 + 1.74 x ionic F^- ± 0.02 (S_{yx}); Correlation coefficient (r) = 0.99; Significant at: p <<0.01.

A study of the values obtained reveals a close relationship between ionic fluoride and total fluoride in serum, represented by a mathematical equation. Therefore, if the ionic fluoride values are known, it is possible to calculate the amount of total fluoride with a highly significant p value in each of the three groups included in our investigation. Depending upon the fluoride content in drinking water, one need only to apply the corresponding formula, although the following general one makes a close evaluation of the concentration of total fluoride in serum possible.

SERUM F^-/SALIVA F^- RELATION (TABLES 1,2,3)

IONIC SERUM F^-/SALIVA F^-

In areas with a high (2.6-8 ppm) concentration in water:
Ionic F^- = 0.022 + 0.944 x saliva F^- ± 0.026 (S_{yx}); Correlation coefficient (r) = 0.65; Significant at p = 0.05

In areas of medium (1 ppm) concentration in water:
Ionic F^- = 0.019 + 0.859 x saliva F^- ± 0.003 (S_{yx}); Correlation coefficient (r) = 0.91; Significant at p <<0.01

In areas of low (0.1 ppm) concentration:
Ionic F^- = 0.995 x saliva F^- ±0.023 (S_{yx}); Correlation coefficient (r) = 0.806; Significant at p <0.025.

SERUM F^-/SALIVA F^-

Areas with high (2.6-8 ppm) concentration:
Total F^- = 0.05 + 1.67 x saliva F^- ± 0.04 (S_{yx}); Correlation coefficient (r) = 0.66; Significant at p <0.05.

Areas with medium (1 ppm) concentration:
Total F^- = 0.03 + 1.67 x saliva F^- ± 0.01 (S_{yx}); Correlation coefficient (r) = 0.70; Significant at p <0.025

Areas with low (0.1 ppm) concentration:
Total F^- = 0.06 + 1.41 x saliva F^- ± 0.05 (S_{yx}); Correlation coefficient (r) = 0.63; Significant at p <0.025

Evidently it is possible to calculate with a high degree of significance the value of ionic and/or total fluoride in blood serum if one knows the saliva fluoride concentration.

SERUM F^-/URINE F^- (TABLES 1,2,5)

The statistical study is not indicative of a relation between the levels of serum and urinary fluoride in any of the six possibilities under analysis. Consequently, it is not possible to calculate the amount of fluoride in serum from the amount in urine or vice versa.

SERUM F^-/DRINKING WATER F^- (TABLES 1,2)

We have calculated a general formula with respect to 0.1-8ppm fluoride in drinking water.

IONIC SERUM F^-/DRINKING WATER F^-

Ionic F^- = 0.072 + 0.0134 x water F^- $\pm$ 0.024 (S_{yx}); Correlation coefficient (r) = 0.58; Significant at $p < 0.001$

TOTAL SERUM F^-/DRINKING WATER F^-

Total serum F^- = 0.1509 + 0.0211 x water F^- $\pm$0.075 (S_{yx}); Correlation coefficient (r) = 0.54; Significant at $p < 0.01$

It is thus possible to calculate the concentration of ionic or total fluoride in serum when their values in drinking water are known, even in the presence of variations such as those ranging between 0.1 and 8 ppm in the drinking water. Higher concentrations were not investigated.

SALIVA F^-/URINE F^- (TABLES 3,5)

No mathematical relation was found for the values under study.

SALIVA F^-/DRINKING WATER F^- (TABLE 3)

The values of the three areas are likewise condensed in a single formula. Saliva F^- = 0.0607 + 0.0130 x water F^- $\pm$ 0.025 (S_{yx}); Correlation coefficient (r) = 0.76; Significant at $p << 0.001$

A close relation was found between the fluoride consumed with drinking water and that eliminated with saliva. Thus, saliva fluoride can be quantified with reasonable certainty when the fluoride content of the drinking water of a given individual is known.

URINE F^-/DRINKING WATER F^- (TABLE 5)

In this case as well, the three relations studied are considered in a single formula:

Urine F^- = 0.84 + 0.865 x water F^- $\pm$1.00 (S_{yx}); Correlation coefficient (r) = 0.94; Significant at $p << 0.01$

The above statistical study makes it possible to present a formula by means of which the amount of fluoride in the urine based on the fluoride content of drinking water can be determined with a $p << 0.01$ accuracy.

RELATION BETWEEN 24-HR. URINE AND THE FIRST MORNING SPECIMEN(FVM) (TABLE 4)

Urinary F^- (24-hr) = 0.026 + 0.8655 x FVM $\pm$ 0.173 (S_{yx}): Correlation coefficient (r) = 0.96; Significant at $p < 0.001$

A highly significant correlation exists between the 24-hr. urinary fluoride content and that of the first morning micturition. Therefore we conclude that the relationship is sufficient to determine the amount of fluoride in the latter.

Conclusions

Fluoride levels in drinking water are closely related to those in blood serum, urine and saliva. There is also a correlation between the fluoride content of the different biological fluids with the exception of urinary fluoride which is not related. In the serum a very close correlation between ionic and total fluoride was found.

The above correlations are so close that a mathematical formula can be worked out to enable the calculation of fluoride in urine, saliva and blood of a given individual provided the fluoride content of drinking water is known. Inversely, it is possible to calculate fluoride levels in drinking water or in the body fluids other than urine, when the levels of any of the others are known. As an alternative, samples of saliva may be used instead of blood for the quantification of serum fluoride. The first morning urinary specimen can be used instead of the total 24-hr. specimen.

Quantification of total fluoride in serum is possible with a sample of only 1 ml upon direct electrode evaluation of ionic fluoride, and the application of the corresponding formula , with an accuracy of $p << 0.01$.

Whereas, in general, the above-outlined interrelations exist because of individual differences from person to person, discretion in the application of these formulas should be used when dealing with individual patients.

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EFFECT OF NaF ON TISSUE VITAMIN C OF GROWING COCKERELS (Gallus domesticus)

by

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SUMMARY: Supplementation of fluoride in the diet of growing cockerels resulted in significantly altered tissue ascorbic acid and dehydroascorbic acid levels. At the end of four weeks of treatment ascorbic acid content had decreased in the liver, adrenal gland, testes, heart, gizzard, kidneys, and pectoralis, whereas the dehydroascorbic acid level was lower in the heart, gizzard, pectoralis and kidneys. At the end of eight weeks, a marked depletion in both forms of ascorbic acid was manifest in many tissues studied. A striking increase in dehydroascorbic acid concentrations of the kidneys may be an important phenomenon and response in the fluoride-treated birds.

Introduction

The possible interaction between fluoride and dietary vitamin C was first reported by Phillips in 1933 (1). Phillips and Stare (2) showed that exposure to elevated levels of fluoride resulted in increased vitamin C levels in several tissues of dairy cattle. Subsequently, several workers observed the ameliorating effect of vitamin C on fluoride toxicity (3-6). Reports on such effect of vitamin C are, however, contradictory. Thus Phillips and Chang (3) observed that in rats fed high fluoride diets their survival time increased when they were provided with vitamin C. Wadhwani (5) reported that ascorbic acid administered to fluorotic monkeys had an alleviating effect. A beneficial effect of the vitamin supplements on human subjects suffering from fluorosis was also reported (6). Venkateswarlu and Rao (7) observed, however, that the administration of vitamin C had no beneficial effect on fluorotic guinea pigs, Neither urinary nor fecal excretion of fluoride, nor skeletal retention of it were modified.

We reported previously that the ascorbic acid levels of several tissues from growing cockerels fed diets supplemented with 150 ppm F^- were altered (8). A follow-up study has now been conducted to determine the effects of various levels of fluoride on the content of both ascorbic and dehydroascorbic acids in various tissues.

Materials and Methods

Forty-eight one-day-old Single Comb White Leghorn cockerels (Gallus domesticus) were obtained from a local hatchery and raised in an animal

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room on a basal diet for seven days. Following the stabilization period, the birds were randomly divided into four groups composed of 16, 16, 8, and 8 birds each. Birds in the first group continued to receive a basal diet of commercial starter mash throughout the experimental period and were used as controls. Those in the second, third, and fourth groups were fed the basal diet supplemented with 150, 300, and 500 ppm F^-, as NaF, respectively. Other growing conditions were the same as described previously (8).

One-half of the birds from each group were sacrificed at the end of 4 and 8 weeks, respectively. They were dissected and the soft tissues removed. The excised tissues were cleared of any excess connective tissue, blotted free of blood, and weighed, after which they were placed in a container containing a cold solution of metaphosphoric-acetic acid, and stored at -10°C until use. The excised tissues included the brain, heart, lungs, liver, spleen, pancreas, gizzard, adrenal gland, testes, kidneys, bursa of Fabricius, and the right pectoral muscle. The ascorbic acid content of each tissue sample was determined by the method described previously (8). For total ascorbic acid determination, the method described by Hughes (9) using homocysteine and potassium phosphate was followed. The difference between total ascorbic acid and ascorbic acid (reduced form) was considered as dehydroascorbic acid.

Blood samples used for ascorbic acid analysis were collected from the jugular veins of the birds directly into a 50 ml centrifuge tube containing a mixture of cold 2% oxalic acid and 4% metaphosphoric acid. Plasma samples for analysis were then obtained by centrifugation. The vitamin C level of the samples was determined by the method used for the analysis of soft tissues described previously.

For fluoride determination of the bone tissue, the right tibia of each bird was removed, weighed, and dried in a forced-air oven at 103°C for 48 hours. The dried bone samples were then ground in a mortar, and the ground samples defatted with anhydrous ether in a Soxhlet extraction apparatus. The fat-free bone samples were ashed, and the ashes subjected to fluoride determination by the Singer and Armstrong method (10) using an Orion Model 94-09A fluoride ion electrode.

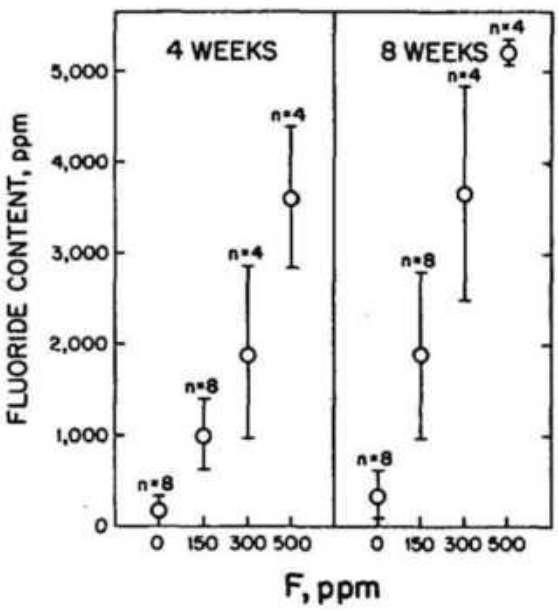
Results

In the growing chick, large quantities of fluoride were deposited in the bones from diets containing added fluoride. As shown in Fig. 1, the amount of fluoride deposited in the tibia increased in direct relation to the increase in dietary fluoride, and the length of the treatment period. The deposition nearly doubled between 4 and 8 weeks of treatment in all groups of birds.

Of the twelve different tissues studied, eight showed significantly altered ascorbic acid (AA) and/or dehydroascorbic acid (DHA) levels. These include the liver, adrenal gland, testes, hearts, gizzard, pectoralis, spleen, and kidneys. Other tissues also showed numerical variations compared to the controls. However, because they were not statistically significant, they were not included in this report.

Figure 1

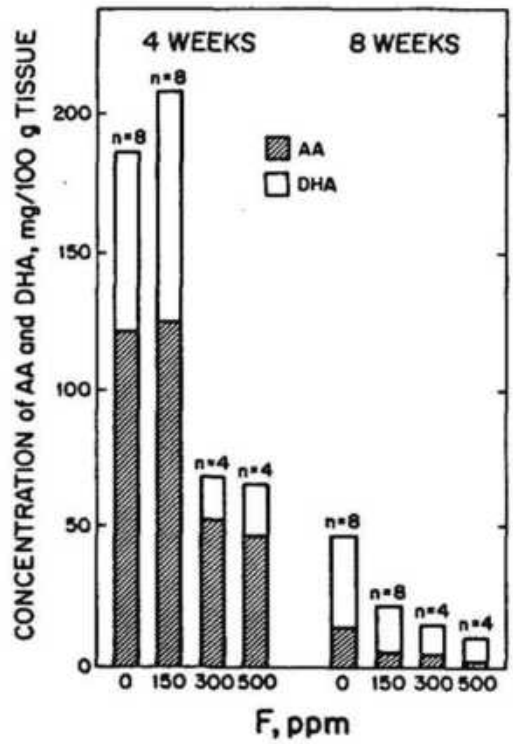
F^- Content of Tibias



Chicks fed supplemental fluoride.

Figure 2

AA and DHA Levels of Adrenal Glands



The adrenal gland of the birds treated for 4 weeks (5 week old) were found to have the highest concentrations of AA. The total ascorbic acid (TAA) content of the controls was 190 mg per 100 g fresh tissue (Fig. 2). Nearly two-thirds of this amount was found to be in the reduced form (AA). The TAA content showed a marked drop at the end of 8 weeks of treatment, to 50 mg per 100 g fresh tissue. Less than half of this value was in the reduced form. Except for those from the birds of the 150 ppm F^- group treated for 4 weeks, the adrenal glands of all the experimental birds showed a significant depletion in both forms of ascorbic acid. In addition, it was found that the higher the level of supplemental fluoride used, the greater the depletion. At 4 weeks, the liver AA showed a reduction of 57% for the 300 ppm F^- group and 62% for the 500 ppm F^- group, respectively (Fig. 3). A progressive decline in both AA and DHA was again observed in all the experimental birds after 8 weeks of treatment. The differences were statistically significant ($p < 0.05$).

While AA of the spleen of the experimental birds appeared unaffected throughout the experiment, the DHA contents of the birds treated with 300 and 500 ppm F^- for 4 weeks showed marked increases over the controls (Fig. 4). Such increases were diminished at 8 weeks, but they remained significant.

Figure 3

AA and DHA Levels of Liver

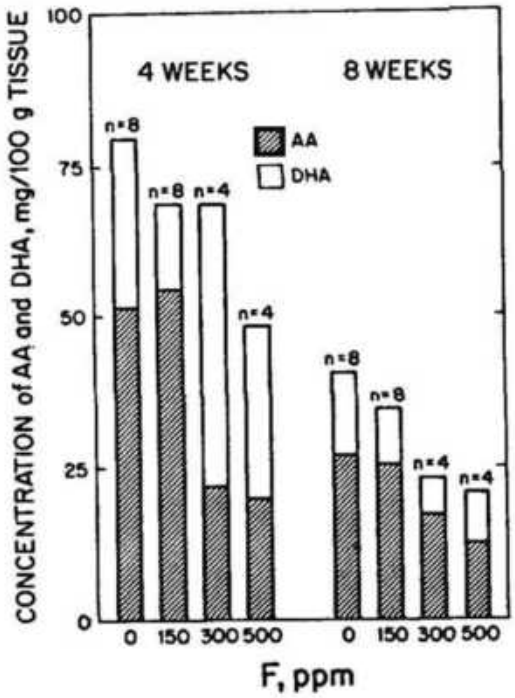


Figure 4

AA and DHA Levels of Spleen

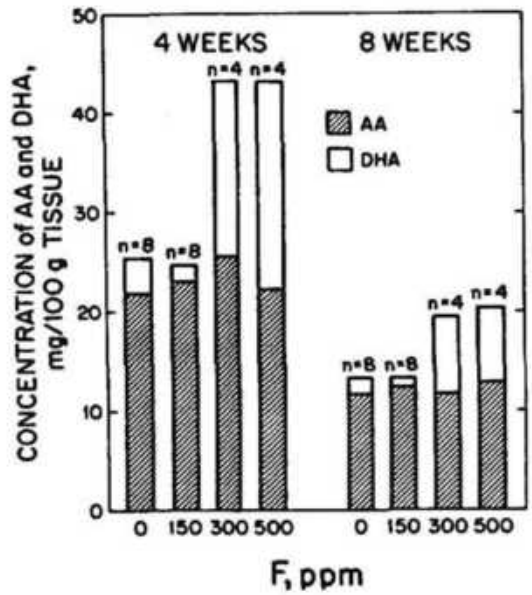
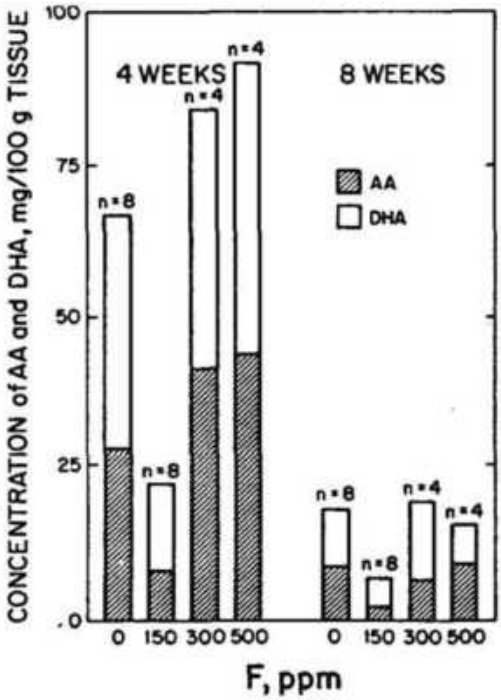


Figure 5

AA and DHA Leves of Testes



Both AA and DHA of the testes of the experimental birds treated with 300 and 500 ppm F^- for 4 weeks showed significantly higher levels, whereas by 8 weeks no such increases were observed (Fig. 5). On the other hand, the testes from those birds of the 150 ppm F^- group showed a decrease of more than 50% in both AA and DHA in comparison with the controls.

The experimental chicks fed diets containing 300 and 500 ppm of supplemental F^- for 4 weeks showed significant ($p < 0.05$) decreases in AA content of the heart, gizzard, and pectoralis (Figs. 6-8). In contrast, the DHA levels were enhanced in all these muscle tissues. A general depletion of both AA and DHA was apparent in all these tissues at the end of 8 weeks of treatment.

Figure 6

AA and DHA Levels of Heart

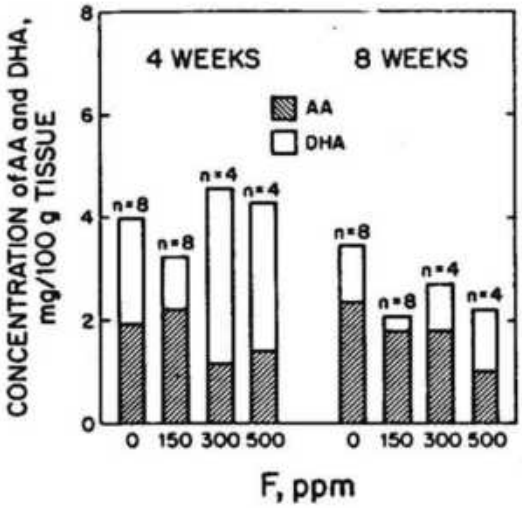


Figure 7

AA and DHA Levels of Gizzard

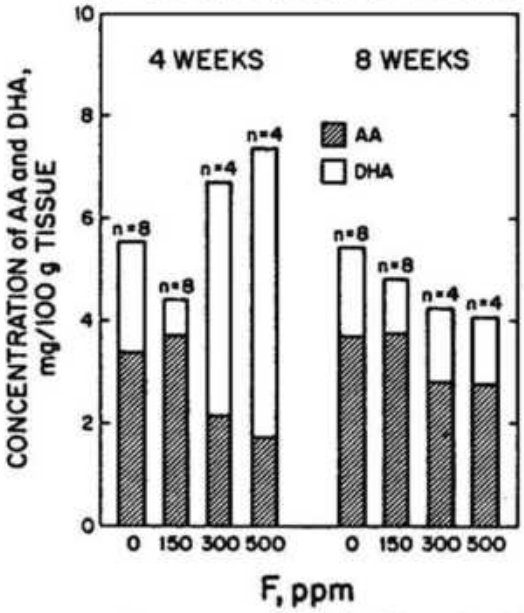
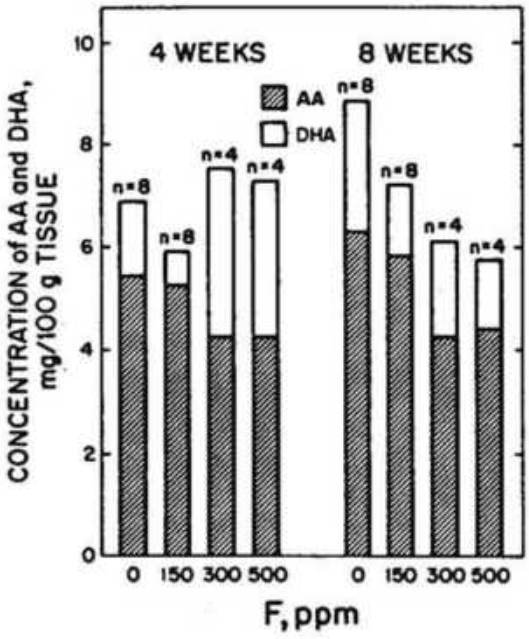


Figure 8

AA and DHA Levels of Pectoralis



A progressive decline in AA was observed in the plasma of the experimental birds treated for 4 weeks (Fig. 9). The samples from birds of the 500 ppm F^- group also showed significantly lowered levels of DHA. The decline in the AA levels of the plasma samples from the experimental birds persisted throughout the experimental periods.

Whereas the AA levels of the kidneys of the experimental birds were slightly lowered, the DHA levels were significantly enhanced in all those samples (Fig. 10). The increases were dependent on the levels of F^- added in the diet and, with the 500 ppm F^- group, an increase over the controls was approximately fivefold. It is seen that the rise in the DHA content continued until the 8 weeks, at which time the increase was much diminished.

Discussion

The results obtained from this study which confirm our earlier preliminary work (8), suggest a strong association between fluoride adminis-

Figure 9

Plasma AA and DHA Levels

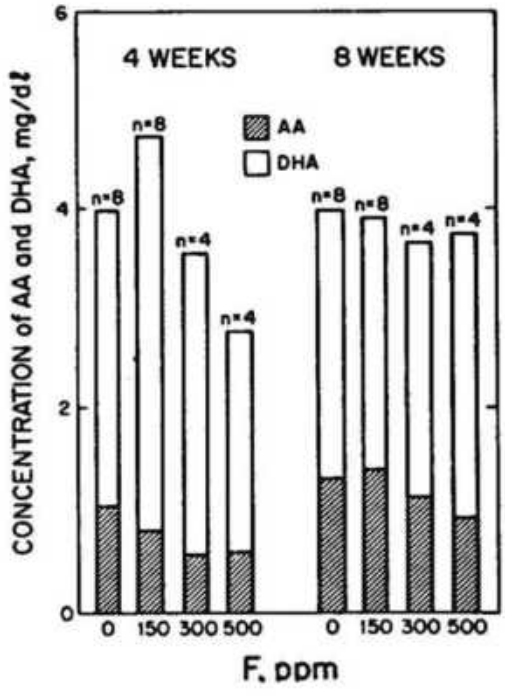
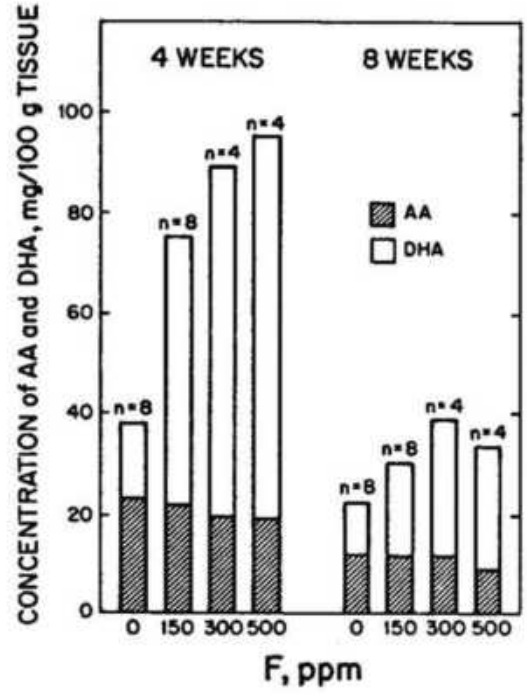


Figure 10

AA and DHA Levels of Kidneys



tration and altered tissue vitamin C distribution in the growing Gallus domesticus. Similar experimental data have been shown with other species of animals by several workers (2, 3, 11). In addition, our data showed significant changes in the ratio of AA and DHA in the tissues of the experimental birds. These observations suggest the hypothesis that fluoride could induce changes in vitamin C metabolism in chickens.

It is clear that supplementation of fluoride in the diet of growing cockerels profoundly enhanced vitamin C synthesis in the kidneys (Fig.10). As noted earlier (8), the kidneys are the site of vitamin C synthesis in chickens. It is of interest that a major portion of the total ascorbic acid in the kidneys of the experimental birds was found in the oxidized form (DHA). The kidneys have also been shown to have a potent ability to oxidize ascorbic acid in both in vivo and in vitro studies (12,13). According to Martin (13), the kidneys facilitate the transport of ascorbic acid by oxidizing the vitamin to a non-ionic, more lipoid-soluble form which can more rapidly cross cellular membranes. The increased levels of DHA in the kidneys of the experimental birds noted in this study may suggest that the synthesized ascorbic acid is rapidly oxidized for transport to various organs under fluoride-induced stresses. On the other hand such increases may in part represent a biochemical response occurring in the kidneys themselves, since the kidneys can be subjected to high concentrations of fluoride under the experimental conditions. Suketa and Terui (14) recently reported a reduction of renal $(Na^+ + K^+)$- ATPase ac-

tivity by administration of fluoride.

Increased levels of vitamin C have been observed in the liver of dairy cattle (2) and in the adrenal gland of rats (3,7,11) treated with fluoride. Although our data showed a slightly increased vitamin C content in those tissues from birds treated with 150 ppm F^- for 4 weeks, higher fluoride levels, i.e., 300 and 500 ppm, all resulted in significant decreases in ascorbic acid content (Figs. 2 and 3). No increases in either AA or DHA were observed in tissues of experimental birds treated for 8 weeks. A similar depletion of the vitamin was seen in the muscle tissues as well (Figs. 6-8).

A progressive decline in both TAA and AA in the plasma of the experimental birds is shown in this study (Fig. 9). Whereas AA content continued to show the same trend in samples from the 8 week groups, the differences in the TAA levels between F^--treated and control birds were insignificant. Depletion of plasma ascorbic acid levels resulting from stresses in chickens has been reported (15).

As shown in Fig. 2, fluoride treatment generally resulted in drastic drop of adrenal AA and DHA levels in the growing chick. Depletion of adrenal ascorbic acid has been considered a common measurement of stress in the fowl (15-17). Our data suggest that fluoride induced a stress in the birds and that depletion of adrenal ascorbic acid is more rapid than replenishment, despite an enhanced synthesis of the vitamin by the kidneys of the experimental birds (Figs. 2 and 10). The importance of maintaining sufficient amounts of ascorbic acid in the adrenal gland has been emphasized by several workers. According to Lewin (18), maintenance of proper levels of ascorbate is essential for preventing the conversion of adrenalin and noradrenalin to adrenochromes, highly toxic compounds acting as potent inhibitors in many enzymatic reactions. Adrenochromes have been shown to uncouple oxidative phosphorylation in liver mitochondria (19), and inhibit ATPase activities (20). Disturbance of adrenal activity by fluoride has also been shown (21).

As mentioned previously, the elevated DHA level in the kidneys of the fluoride-treated birds strongly suggests an enhanced renal ascorbic acid biosynthesis in response to fluoride toxicosis. It should be noted that changes in the distribution of tissue ascorbic acid had occurred in birds exposed to dietary fluoride even at a level as low as 150 ppm. The generally recognized safe level of fluoride as NaF for the chicken is 150-300 ppm (22). It should be pointed out that little differences in body weight were observed between the fluoride-treated birds and the controls throughout these experimental periods. However, because significant alterations in vitamin C metabolism clearly have taken place, other serious physiological, biochemical, and possibly, behavioral disturbances might occur in the cockerel exposed to relatively low levels of fluoride.

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Omission: In the article by Geeraerts, F., Fluoride 14:155-160, 1981 the following acknowledgement should have been added:

Acknowledgement

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We are much indebted to M. VanDeputte (Laboratory of Pharmaceutical Chemistry, Farmaceutisch Instituut, Vrije Universiteit Brussel) for the determinations of the fluoride concentrations in the water and the food administered to the animals.

Abstract

OSTEOMALACIA DEVELOPING DURING TREATMENT OF OSTEOPOROSIS WITH SODIUM FLUORIDE AND VITAMIN D

by

J.E. Compston, S. Chadha, and A.L. Merrett
London, England

(Abstracted from Br. Med. J., 281:910-911, 1980)

A 61-year old woman with back pain and history of vertebral compression fractures was treated with 50 mg NaF daily, 50,000 units Vitamin D weekly, and 600 mg calcium gluconate twice daily. After two months, the calcium supplements were discontinued because the patient was unable to tolerate them. Dietary calcium intake was 851 mg per day.

During treatment, the pain increased and three additional vertebral compression fractures occurred. Plasma calcium, phosphate and alkaline phosphatase concentrations remained normal throughout treatment. The iliac bone biopsy showed development of osteomalacia in spite of high plasma 25-OHD concentrations.

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CHLOROPLAST ELECTRON TRANSPORT, PROTEIN AND RNA IN FLUORIDE-TREATED PEA SHOOTS

by

D.J. Ballantyne, and B.L. Glover
Victoria, B.D., Canada

(Abstracted from Environmental Experimental Botany 21:83-88, 1981)

Shoots of 14-day-old peas (Pisum sativum cv Tall Telephone)were placed in solutions of KF, KCl or water. Chloroplasts prepared from lm M KF-treated shoots had a reduced rate of total electron transport. Probably this effect was mostly on Photosystem II of photosynthesis. Photosystem I of photosyn thesis was not influenced by the KF-treatment. The chloroplast fraction prepared from KF-treated shoots had a decreased level of chlorophyll but an increase in protein. KF-treated shoots had an increase in RNA.

Author's Abstract

(Reprints: Department of Biology, University of Victoria, Victoria, B.C., Canada V8W 2Y2).

CLINICAL APPEARANCE OF DENTAL FLUOROSIS IN PERMANENT TEETH IN RELATION TO HISTOLOGIC CHANGES

by

A. Thylstrup, and O. Fejerskov
Aarhus, Denmark

(Abstracted from Community Dent. Oral Epidemiol. 6:315-328, 1978)

The authors have proposed a new classification of dental fluorosis on the basis of a dental health survey which they conducted on 122 children of Northern Tanzania residing in 3 different areas where water supplies contained 3.5 (Arusha), 6.0 (Kisongo) and 21.0 ppm (Maji ya Chai) of fluoride respectively. The classification, which includes 9 scores and a control, describes the histological features behind the individual scores of dental fluorosis.

To correlate the degree of fluorosis with the histopathology of teeth, Thylstrup et al. recorded the degree of dental fluorosis on the buccal, occlusal and lingual surface of each tooth. Scores 1-5 pertain to the degree of opacity whereas scores 6-9 involve defective enamel (pits). In group 9 "loss of (the) main part of enamel with change in (the) atomic appearance of surface" was characteristic.

The increasing degree of dental fluorosis was directly related to the degree of subsurface porosity. In general, the posterior buccal and lingual surfaces were mainly affected. The maxillary molars and premolars were more involved on the lingual surfaces whereas, among the mandibular teeth, the buccal surfaces were more affected. The maxillary incisors appear to be most susceptible to mottling.

In contrast to formerly accepted views, the distribution of dental fluorosis within the individual followed the same pattern irrespective of the level of fluoride in drinking water. The authors present evidence that the enamel thickness rather than the length of exposure to body fluids determine the degree of dental fluorosis.

They point to the limitation of the Dean index which is "unable to distinguish between children having only two teeth with pitting and those having all their teeth pitted to a varying degree." They also questioned whether Dean's index could provide sufficient information regarding effect of fluoride on the dental hard tissues.

"When discussing so-called optimal doses the dental profession usually considers dental fluorosis only from a cosmetic point of view whereby the gradient of early changes indicative of a biologic effect of fluoride are ignored."

****

Abstract

MICRORADIOGRAPHY OF THE EFFECT OF ACUTE AND CHRONIC ADMINISTRATION OF FLUORIDE ON HUMAN AND RAT DENTINE AND ENAMEL

by

Fejerskov, O., Yaeger, J.A., and Thylstrup, A.
Aarhus, Denmark and Framington, Connecticut

(Abstracted from Arch. Oral. Biol. 24:123-130, 1979)

The authors studied by microradiography the effect of prolonged water-borne fluoride and of single injections on the developing enamel and dentine in human teeth and on the continuously growing incisors of rats. A single injection of 10-20 mgF/kg produced hypermineralized zones which were followed by hypomineralization in enamel as well as dentine, both in humans and rats. The authors concluded that dental fluorosis is an abnormality of enamel and dentine. In the acute response, an almost simultaneous development of hyper-and hypomineralization zones of enamel and dentine occurs which the authors explain on the basis of a hastening of crystal growth simultaneously with inhibition of fluoroapatite formation. They believe that the lesions associated with continuous administration of fluoride are due to an inhibition of mineralization.

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RADIOLOGICAL CRITERIA OF INDUSTRIAL FLUOROSIS

by

Boillat, M.A., Garcia, J., and Velebit, L.
Lausanne, Switzerland

(Abstracted from Skeletal Radiol., 5:161-165, 1980)

Bone radiographs of 43 potroom workers in two aluminum factories were compared with radiographs of 18 controls on whom the diagnosis of industrial fluorosis had been confirmed by bone biopsy. The mean age of the workers was 62 years; the mean occupational fluoride exposure was 27 years. The time which had elapsed between the end of exposure and the examination varied from zero to twenty years.

The eighteen male workers who served as controls were described as having "no significant occupational exposure to fluoride." Nine of them however, were foundry workers "of the same area as the exposed subjects."

The workers were divided into 4 groups based on the bone fluoride levels namely: I. control, II. bone fluoride between 2100 and 4000 ppm, III. bone fluoride level of 4000 to 6000 ppm, IV. bone fluoride level of 6000 to 11,000 ppm. However, no bone biopsies were available for the control group.

The fluoride workers had a higher frequency of joint pain and stiffness than the controls. Some had hypocalcemia but the parathyroid hormone levels were normal. The mean bone fluoride content was 5617 ppm ± 2143 ppm. The mean urinary fluoride level was 1.95 mg/g, creatinine SK = 0.89 compared to 0.80 mgF/g creatinine SK = 0.38. The bone density, particularly in the spine and pelvis, was slightly increased among the potroom workers. In both groups, the trabeculae were blurred at several sites and periosteal bone formation was rare. Ossification of tendons, ligaments and muscles (hyperostosis) were predominant in the potroom workers. Arthrosis was frequent in the cervical and lumbar spine. Hyperostosis, which was significantly more frequent among the potroom workers increased with the bone fluoride content.

Hyperostosis of fluorosis cannot be differentiated from Forestier's syndrome which may also have extra-spinal symptoms. Other conditions to be considered in differential diagnosis are ankylosing spondylitis, acromegaly, pachydermoperiostosis and hypertrophic pneumic osteoarthropathy.

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