July, 1976

Vol. Nine No. Three

FLUORIDE

OFFICIAL QUARTERLY JOURNAL

OF

INTERNATIONAL

SOCIETY for

FLUORIDE

RESEARCH

INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH

KAJ ROHOLM

Vol. Nine No. Three July, 1976 Pages 123 - 169

FLUORIDE

Quarterly Reports

Issued by

THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH

CONTENTS

The International Society for Fluoride Research will hold its Eighth Conference in London, England, May 29-31, 1977. Further details will appear in subsequent issues. The Program Committee is soliciting abstracts (up to 300 words) of papers to be presented at the conference dealing with any phase of fluoride research. Kindly send abstracts to the Society's office, P. O. Box 692, Warren, Michigan 48090. The deadline for the abstracts will be January 1, 1977.

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

SUBSCRIPTION RATES - Price per annum in advance including postage $18.00; Single copies $5.00.

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.

Contributors will receive copies of the issue of FLUORIDE containing their paper, free of charge.

FLUORIDE is listed in
Current Contents Agricultural
Food and Veterinary Sciences

INSTRUCTIONS TO AUTHORS

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

Preparation of Papers

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

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

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

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

5. Material and Methods - should be condensed; however if the methodology is new or developed by the author(s) it might be more detailed.

6. Results - should contain the direct conclusions of the experimental work.

7. Discussion should deal with the general conclusions. Reference should be made to other work on the subject with an indication whether the experimental results are in agreement or in disagreement with previous work. In short papers, results and discussion can be stated together.

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

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

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

Contributors will receive 10 reprints of their paper free of charge.

EDITORIAL

FLUORIDE CONTENT OF TEETH AND DRINKING WATER IN RELATION TO DENTAL CARIES

In 1948 McClure suggested that the ingestion of fluoridated water might increase the fluoride content of the dentition with a consequent reduction in caries experience (1). Subsequently, several studies demonstrated a positive relationship between the fluoride content of tooth enamel and the fluoride level of the water supplies (2, 3, 4, 5, 6). However, no quantitative estimate of the relationship between tooth decay and the fluoride content of the tooth enamel surface has been made.

A recent study by De Paola et al. (7) pertaining to this question is of considerable significance, since it is the first attempt to evaluate effectively the action of fluoridation by means of enamel biopsies by a seemingly objective method. The technique of doing enamel biopsies was developed by Brudevold et al. (8) in 1968.

De Paola et al. compared the mean weights of the biopsied enamel samples in fluoridated and unfluoridated communities. For the appraisal of tooth decay, they utilized the DMFS score (decayed, missing, and filled surfaces) in the high and low fluoride communities applying up-to-date statistical methods.

This approach is undoubtedly superior to any previous methods. However, like in most other similar epidemiological surveys it is difficult to control the many variables involved. For instance, it is doubtful that an examination of teeth by mirror and explorer can eliminate personal bias even though only two examiners were involved in the study. Furthermore, this study covered only persons up to age 16 who were lifetime residents in their respective communities.

The authors themselves raised two other questions: (a) Does the biopsy of a single tooth surface furnish a clue concerning the amount of fluoride in the entire dentition of the person from whom it was taken and (b) does a biopsy taken on the sound surface of a tooth permit conclusions concerning the fluoride content of a tooth which is susceptible to caries. The authors believed that neither point would materially affect their statistical results.

The study revealed clearly a correlation of the fluoride content of the right maxillary central incisor tooth (the sampling area was 20.1 mm^2) with the fluoride levels of the water supplies, but wide variations from one person to another of the caries preventive effect of fluoride were noted. Consequently, whereas in general caries tended to decrease with increasing fluoride, the dispersion of the data was considerable. In other words, within each of the study groups there were subjects for whom fluoride was a weak or non-existent caries determinant. This fact has also been evident in other studies as for instance in the early surveys by Dean, Arnold, and Elvove (9) who observed in four fluoridated and five non-fluoridated communities near Chicago inter-community differences of up to 20% in the fluoridated, and up to 46% in the non-fluoridated areas. Such variability in caries experience in a given community points, according to the authors, to "the powerful role of caries determinants other than fluoride".

This conclusion is further supported by the clinical results of topical fluoride application to teeth. The authors stated that clinicians (dentists) observe a wide response to topical fluoride treatments, and recognize the possibility of a high decay rate even for a regularly treated patient. They suggest that topical agents be developed which raise the fluoride content of the enamel significantly since, according to their data, "only at the highest fluoride levels does the worst caries experience become relatively moderate."

Thus, in spite of fluoride in water and of topical fluoride applications to the teeth, the caries problem is far from solved. In future research the diversity of cariogenic factors must be given attention and the relative role of each established.

Bibliography

1. McClure, F.J.: Fluorine in Dentin and Enamel of Sound and Carious Human Teeth. J. Dent. Res. 27: 287-298, 1948.

2. McClure F.J. and Likins R.C.: Fluorine in Human Teeth Studied in Relation to Fluorine in the Drinking Water. J. Dent. Res. 30: 172-176, 1951.

3. Isaac, S., Brudewald F., Smith F.A., Garner, D.E.: Relation of Fluoride in the Drinking Water to the Distribution of Fluoride in the Enamel. J. Dent. Res. 37: 318-325, 1958.

4. Yoon, S.H., Brudevold, F., Garner, D.E., Smith, F.A.: Distribution of Fluoride in Teeth From Areas With Different Levels of Fluoride in the Water Supply. J. Dent. Res. 39: 845-856, 1960.

5. Hargreaves, J.A.: Enamel Wear in Deciduous Teeth With Age, Related to Surface Fluoride Content. Caries Res. 1: 32-41, 1967.

6. Aasenden, R., Allukian, M., Brudevold, F., Wellock, W.D.: An In Vivo Study on Enamel Fluoride in Children Living in a Fluoridated and in a Non-Fluoridated Area. Archs. Oral Biol. 16: 1399-1411, 1971.

7. Bookstein, F., De Paola, P. F. and Warram, J.: Regression Based Techniques for the Estimation of Surface Enamel Fluoride in large Study Groups. J. Dent. Res. In Press, 1975.

8. Brudevold, F., McCann, H. G. and Gron, P.: An Enamel Biopsy Method for Determination of Fluoride in Human Teeth. Archs. Oral Biol. 13: 877-885, 1968.

9. Dean, H. T., Arnold, F.A. Jr. and Elvove E.: Domestic Water and Dental Caries. V. Additional Studies of the Relation of Fluoride Domestic Waters to Dental Caries Experience in 4,423 White Children, Aged 12 to 14 Years, of 13 Cities in 4 States. Publ. Hlth. Rep. (U.S.) 57: 1155-1165, 1942.

* * * * * * * * * * * * * * * * * *

SCANNING ELECTRON MICROSCOPIC STUDIES IN HUMAN INDUSTRIAL FLUOROSIS

by

J. Franke, Halle (Saale), D.D.R. and V. Horn
Brno, C.S.S.R.

SUMMARY: Scanning electron microscopic (SEM) studies were carried out on the periosteal and fractured surfaces of rib, tibia, and skull cap in three persons at different stages of industrial fluorosis. In the incipient stage of fluorosis, slight edema and impregnations with globular and crystalline material at the periosteal collagenous fibres were found. These impregnations and the edema become more extensive as the disease advances. In severe fluorosis a completely irregular orientation of abnormal thin fibres and thick coverings on the bone surface occurs. The fibres of the muscular and tendinous attachments to the bone are obviously the first to be mineralized. We observed atypical changes of the whole bone collagen in the actual collagenous fibres, the matrix, the mineralization of the bone and the ossification process itself. The SEM studies are compared with the normal histological picture of fluorosis and the findings are discussed.

During our studies of industrial fluorosis (1-9) we became aware of the lack of scanning electron microscopic studies of human fluorosis in the literature accessible to us. This fact and the extensive changes in the histology of fluorosed bones compared with normal bone instigated the following study:

Material and Methods

For the examination, we used bone material taken from three autopsies of workers in an aluminum smelter with skeletal fluorosis. The first case was a 56 year old man with severe (stage 3) fluorosis which developed during 14 years' exposure to fluoride. Chemical analysis of ash from a rib yielded a value of 1.15% (11500 ppm) fluoride. The periostal tissue and the surface of a fractured rib were examined. The second patient (case 2) suffered from moderate fluorosis after 10.5 years of fluoride exposure (stage 1 and 2). The fluoride content of ash from the iliac crest yielded a value of 0.74%. The fractured and the periosteal surface

---

From the Orthopaedic Clinic Martin-Luther-University Halle-Wittenberg, D.D.R. and J. E. Purkyne-University, Brno, C.S.S.R.

of the tibia were examined. The third sample was taken from a 67 year old man (case 3), who developed fluorosis (stage 2-3) after having worked 33 years in an aluminum smelter. During the last three years without exposure to fluoride, the degree of fluorosis decreased. Histologically we found only slight changes. The fluoride content 0.43% of the ash of the iliac crest was indicative of only slight fluorosis. We examined the fractured and periosteal surfaces of the skull cap and of a rib.

The bone samples were fixed in formalin and dehydrated in acetone of increasing concentrations. The bone samples which measured 10x10x15mm were fitted on a preparation stage and coated with gold. The examinations were carried out with the scanning electron microscope "Stereoscan" S 4-10 of the firm Cambridge at the Orthopedic University Hospital of Brno, Czechoslovakia.

Results

1. Slight Fluorosis (case 3)

On the surface of the skull, beside normally arranged structures of collagenous fibres (Fig. 1a) and nearly normal fettering of aponeurosis (Fig. 1b) areas, we found with distinct mineralization of collagenous fibres protruding into the bone (Fig. 1c). On the cross-section and the endosteal surface of the rib, we noted cell processes and fibres impregnated with globular and crystalline material (Fig. 2a)

The surface of the rib shows the same changes. In addition to normal areas, we noted zones with edema of fibres (Fig. 2b). Under higher magnification, impregnation of collagenous fibres and bundles can be seen more clearly (Fig. 2c). These structures represent a stage between normal collagenous connective tissue and immature, slightly mineralized bone tissue.

2. Moderate Fluorosis

On the periosteal surface of the tibia we observed frequently a more pronounced edema of the collagenous fibres (Fig. 3a). Also, areas with a well defined network of collagenous fibres, without mineralization but with deficient matrix formation (Fig. 3b). were seen. The highly mineralized, broken off insertion of tendons into the bone were impressive (Fig. 3c).

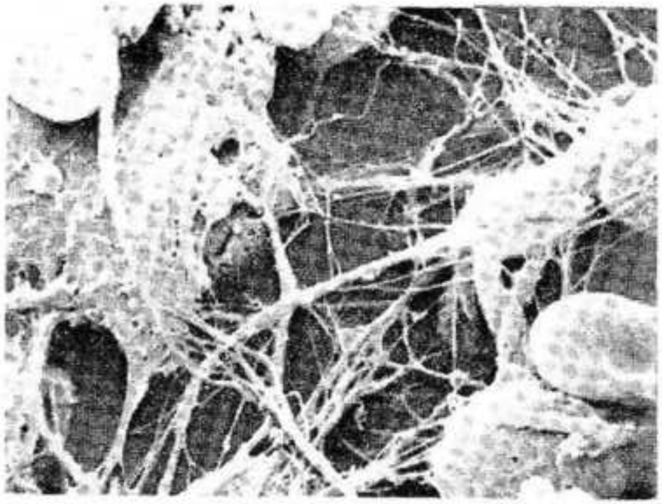
3. Severe Fluorosis (case 1)

The fractured surface of the rib is covered with homogenous or granular substances; at some places, heavy collagenous fibres (Fig. 4) can still be seen. On the surface of the rib, the collagenous fibres are abnormally thin, irregularly oriented and partially covered by atypical matrix (Fig. 5a). Under high magnification, single fibres as well as the whole bone surface (Fig. 5b) have a granular-like covering (Fig. 5c).

Fig. 1

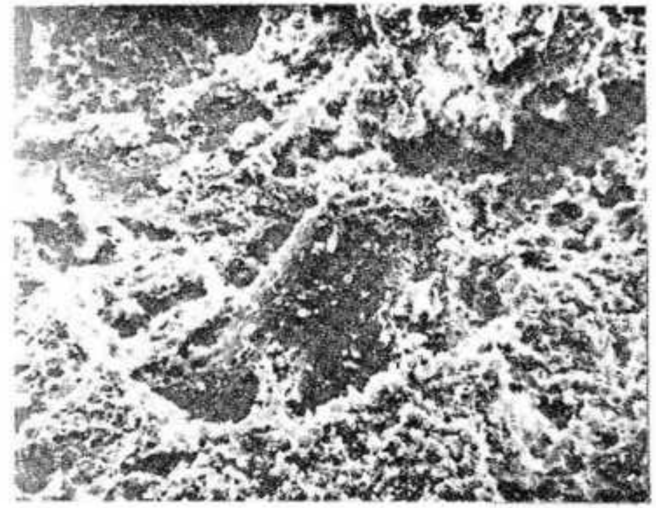
Periosteal Surface of Skull in Early Fluorosis

(a)



Normally Arranged Collagenous Fibres. X4700

(b)



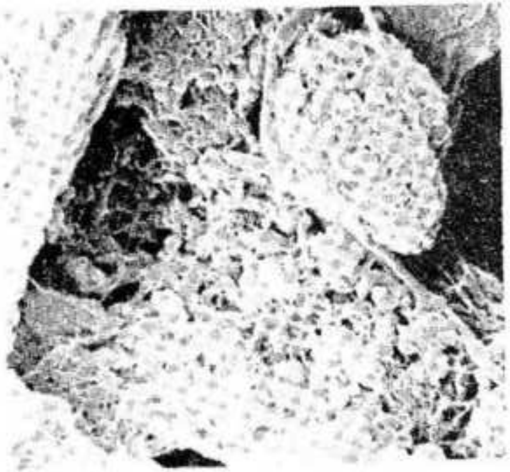
Almost Normally Appearing Aponeurosis. X95

(c) Areas with Distinct Mineralization of Collagenous Fibres Inserting into the Bone. X1850.

Fig. 2

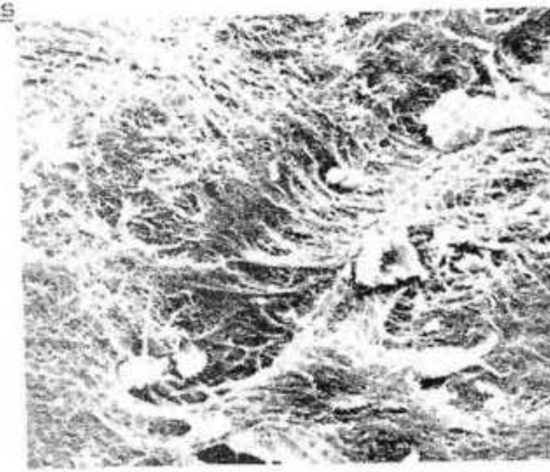
Fractured Surface of Rib in Early Fluorosis

(a)



Cell Processes and Fibres Impregnated with Globular and Crystalline Material. X5150.

(b)



Edema of Collagenous Fibres. X510.

(c)

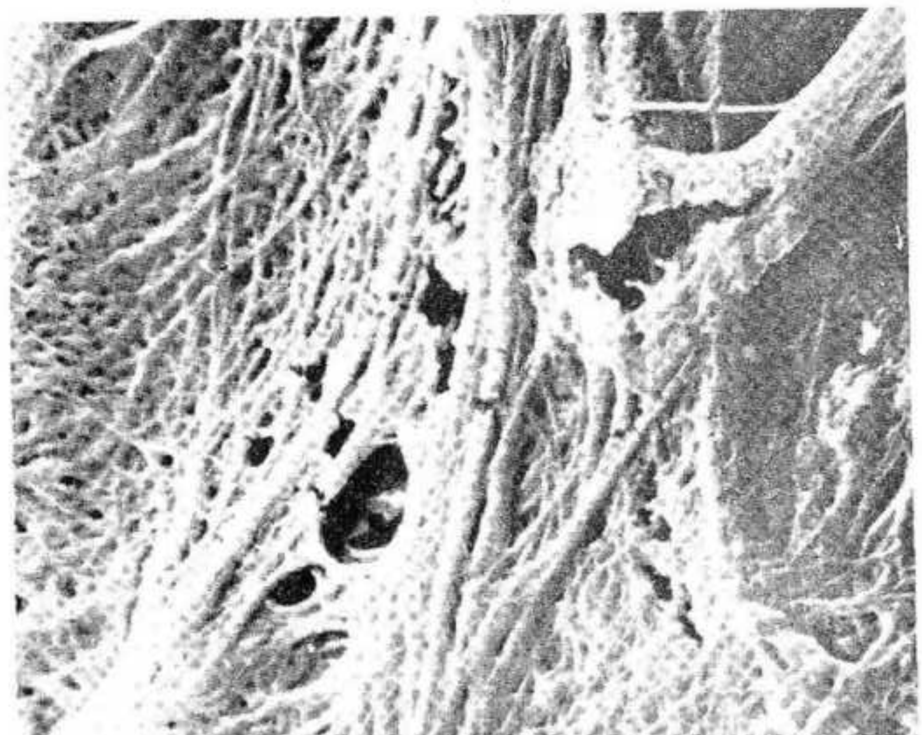
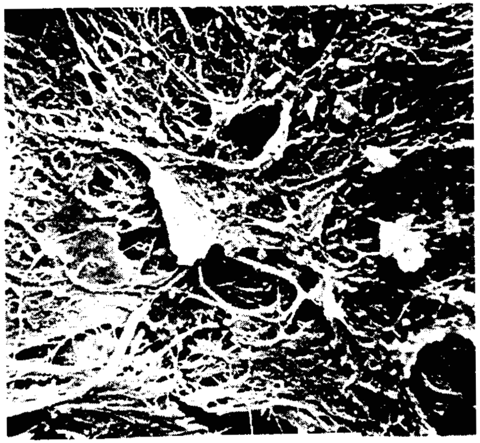


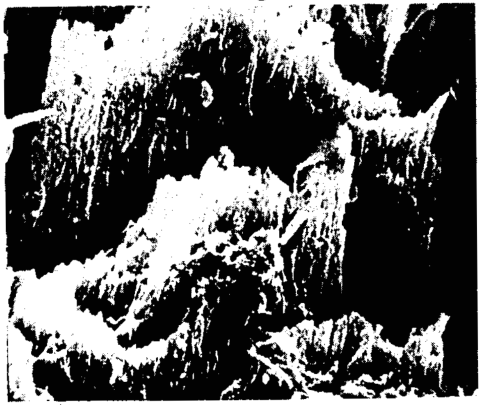
Fig. 3

Periosteal Surface of Tibia in Moderately Advanced Fluorosis

(a)



Distinct Edema of Collagenous Fibres. X2100.



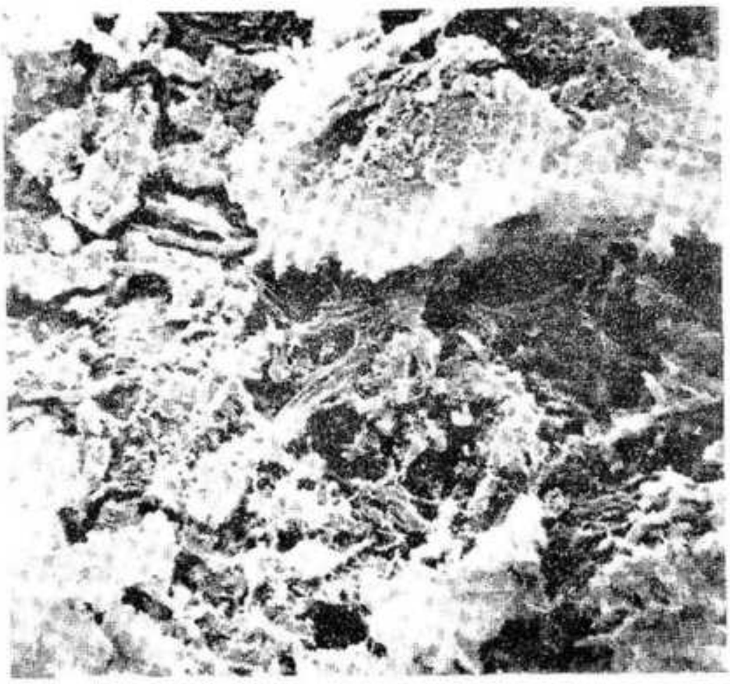
(c) Highly mineralized,
Broken Off Insertion
of a Tendon. X2250.

(b)

Areas with Clearly Distinguished Network of Collagenous Fibres and Deficient Matrix Formation. X5300.

Fig.

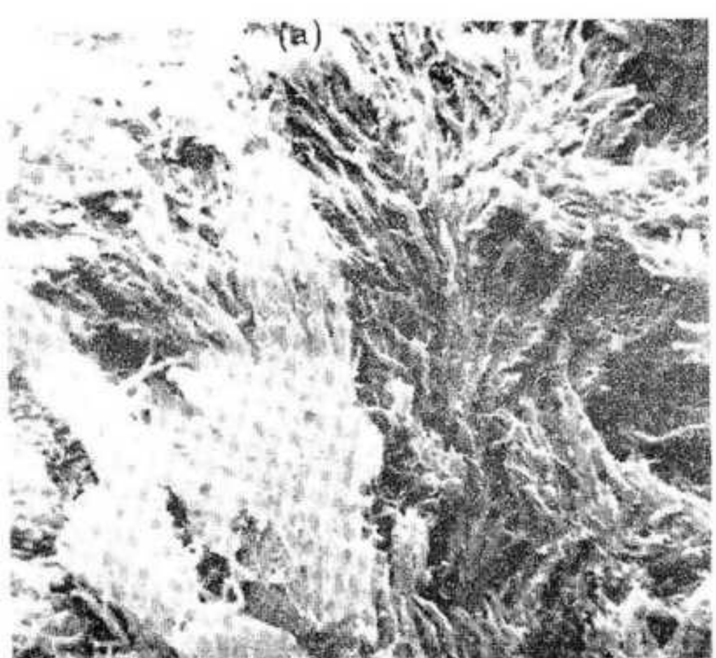
Fractured Surface of Rib in Severe Fluorosis



Homogenous or Granular Substances and Heavy Collagenous Fibres. X2000.

Fig. 5

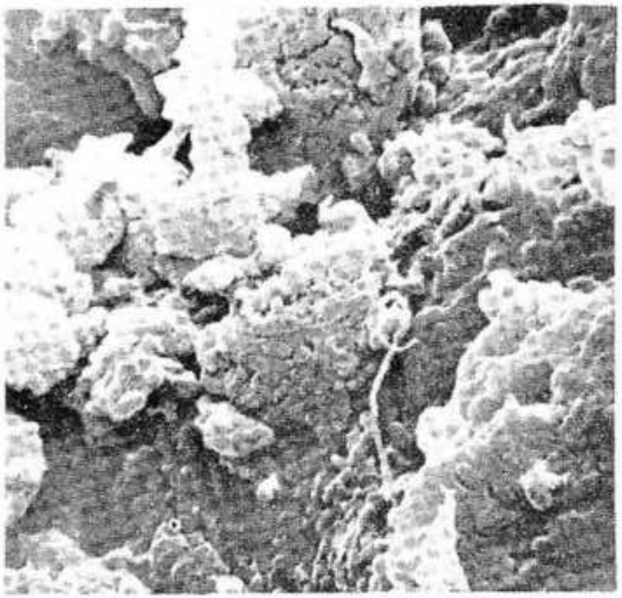
Periosteal Surface of Rib in Severe Fluorosis



Abnormally Thin, Irregular Oriented Collagenous Fibres. X4750.

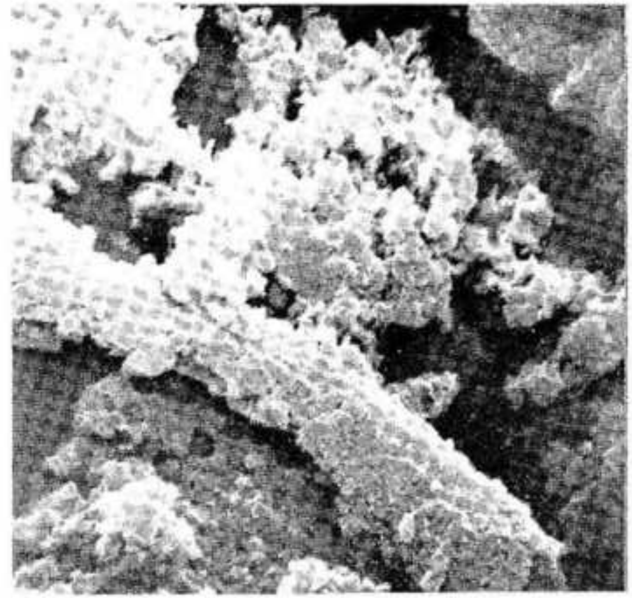
Periosteal Surface of Rib in Severe Fluorosis

(b)



Granular Covering of Bone Surface. X5300.

(c)

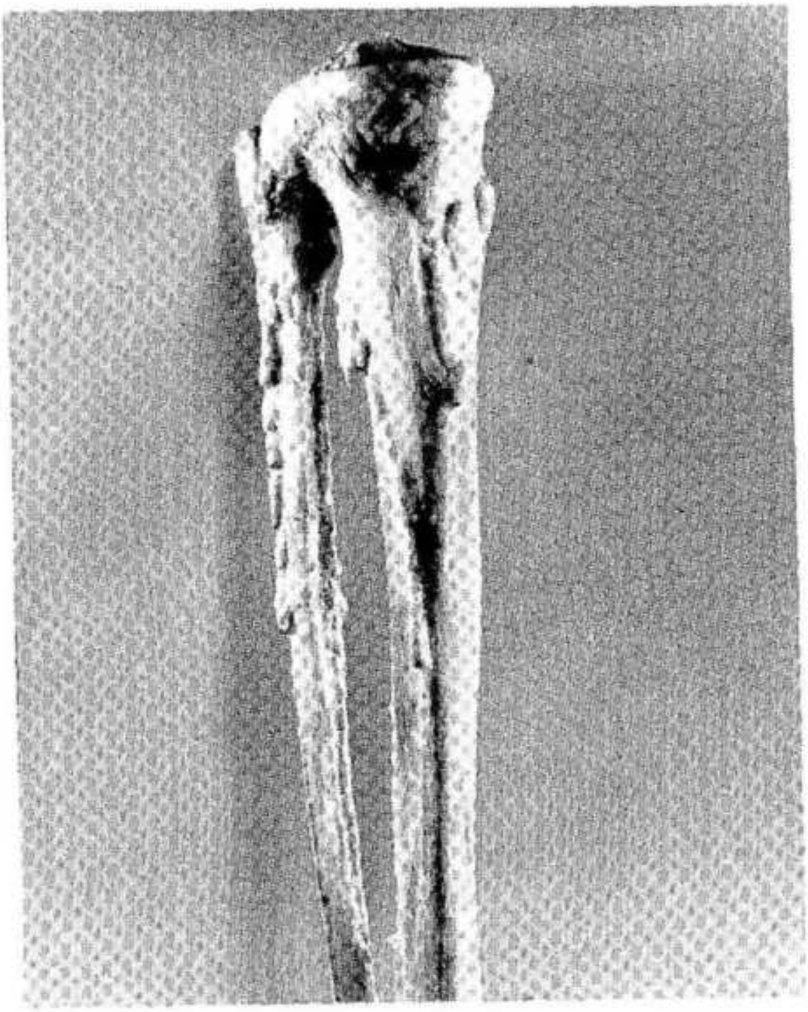


Granular Covering of Single Fibre. X5300.

(d). Transition from a Slightly Abnormal Surface (left side) to the Area of Bone Apposition with Impregnation and Surface Disintegration. X530.

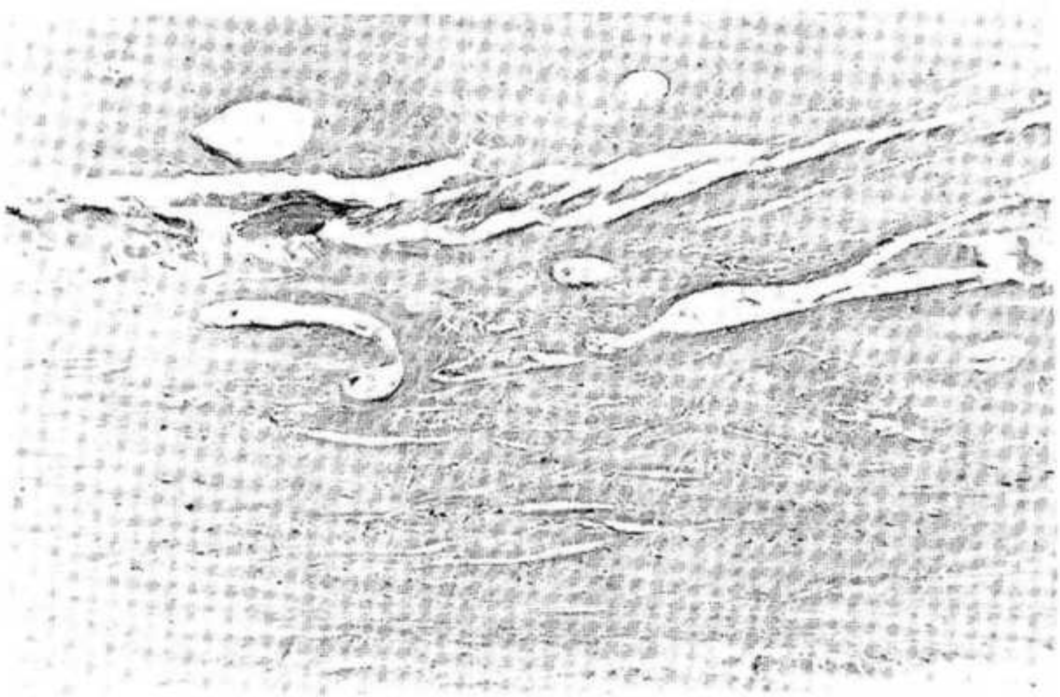
Fig. 6

Fluorosis III



Protruding Muscular and Tendinous Insertions

Fig. 7



New Bone Formation at the Periosteal Surface and in the Periosteum. X80.

Another picture shows the transition from a nearly normal surface (on the left side) to the area of bone apposition with impregnation and surface disintegration (right side) (5d).

Discussion

In the incipient stage of fluorosis we observed slight edema and impregnation with globular and crystalline material in the periosteal collagenous fibres. The edema and the impregnations increase as the disease advances and in severe fluorosis a completely irregular orientation of abnormal thin fibres and thick crystalline coverings on the bone surface occurs. The fibres of the muscular attachments and the tendons inserting into the bone were obviously mineralized at first. Macroscopically this results in the groin-like protruding muscular and tendinous insertions (Fig. 6).

In our histological studies of 25 iliac crest biopsies, we observed the following:

1. coarsening and condensation of spongy bone;

2. subperiosteal formation of fibrous bone with transformation into lamellar bone leading to the formation of exophytes;

3. thickening and spongiozation of the corticalis;

4. irregular matrix formation with a high trunover rate, mosaic structure of the cement-lines, formation of chalky granulae, different stainability, irregular arrangement of lamellae and osteons, and enlarged osteocyte cavities;

5. typical foci incrusted with calcium in the subperiosteal area of corticalis (residue of connective tissue of former periosteum which were enclosed in the newly formed bone and then encrusted with minerals).

Initially, we had found only periosteal formation of new bone, "incrustation foci", and thickening of spongy bone. The formation of irregular matrix and irregular lamellar structure as well as distinct formation of exophytes are phenomena of a severe long-lasting fluoride intoxication.

Some SEM-findings are closely comparable with the normal histology of fluorosis:

The appositional periosteal ossification, observed in beginning fluorosis (Fig. 7) corresponds to the impregnation of soft tissue in the SEM-picture which is an intermediate stage between connective tissue and immature bone. It corresponds to the formation of immature periosteal fibrous bone in the normal histological picture, which afterwards will be transformed into lamellar bone. In this process the tendinous and muscular insertions are affected most severely. In the normal histological picture, we also observed areas with pure incrustation of connective tissue without transformation into bone tissue, the so-called incrustation foci (1, 5, 9). However, this phenomenon can be observed more often in the SEM-picture.

The completely atypical structure and arrangement of collagenous fibres on the periosteal bone surface in severe fluorosis in the SEM-picture are in accordance with the irregular matrix in the normal histological picture.

The partly massive incrustation of intercellular substances is confirmed by the following chemical analyses;

1. A nearly 12% higher ash content of fluorotic bones of 22 iliac crest biopsies (ash content of fluorotic bones: 49.1% $\pm$6.8% compared with control bones: 37.3% $\pm$ 6.5%;

2. Twofold to threefold increased values of hydroxyapatite in the neck of the femur in two patients with moderate and severe fluorosis.

Consequently hyperossification takes place in addition to hypermineralization in human fluorosis.

Thus, the SEM-studies have enlarged our histological knowledge about bone fluorosis. They show that both the process of bone formation and the newly formed structures differ from normal bone: The mineralization affects the intercellular matrix of periosteal connective tissue; the newly formed bone consists of rather thin, irregularly-arranged fibres covered by atypical matrix. The collagenous fibres of periosteal connective tissue show edema and other atypical symptoms. The pathological changes are identifiable on the endosteum and in the bone itself. Thus we have here atypical symptoms of the whole bone collagen involving the actual collagenous fibres, the matrix, its mineralization, and the ossification process itself.

LITERATURE

1. Franke, J.: Chronische Knochenfluorose, Beitr. Orthop. Traum. 15: 680-684, 1968.
2. Franke, J.: Histological Changes of Human Fluorosis, Experimental Fluorosis in Animals and Osteoporosis Following Sodium Fluoride Therapy, Fluoride 5: 182-198, 1972.
3. Franke, J.: Die Knochenfluorose, Therapiewoche 23: 3954-3957, 1973.
4. Franke, J.: Wirkungen von Fluoriden auf das Skelettsystem unter besonderer Berucksichtigung der Industriefluorose und der Natriumfluoridbehandlung der Osteoporose, Halle (Saale), Med. Diss. (B) 1976.
5. Franke, J. u. E. Auermann: Die Bedeutung der Beckenhammpunktion mit histologischer and mikroanalytischer Untersuchung des gewonnenen Knochenmaterials bei der Diagnostik der Fluorose. Int. Arch. Arbeitsmed. 29: 85-94, 1972.
6. Franke, J., G. Drese u. P. Grau: Klinische, gerichtsmedizinische und physikalische Untersuchungen eines Falles von schwerer Fluorose, Kriminalistik u. Forens. Wiss. 7: 107-122, 1972.
7. Franke, J., H. Runge, F. Fengler u. H. Rempel: Erganzung zum Thema "Fluorosis" im Beitrag "Knochenstoffwechselstorungen" von A. J. Fischer Z. Orthop. 110: 280, 1972, Z. Orthop. 111: 221-224, 1973a.
8. Franke, J., R. Lahl, F. Fengler u. H.D. Hempel: Beitrag zur neurologischen Symptomatik der Fluorose - Zufallsbefund oder Ausdruck einer Organmanifestation. Dtsch. Gesundh. Wes. 28: 120-124, 1973b.
9. Franke, J., F. Rath, H. Runge, F. Fengler, E. Auermann u. G. Lenart: Industrial Fluorosis. Fluoride, 8: 61-83, 1975.
10. Horn, V. u. J. Franke: Rastelektronenmikroskopische Untersuchungen bei menschlicher Industriefluorose. Z. Orthop. in press.

Discussion

Dr. Moolenburgh: Can you say something about the ingestion of fluoride in industrial fluorosis?

Dr. Franke: It would be in the range of 10-20 mg of fluoride per day. Last year I reported that the duration of exposure of industrial workers to fluoride is about 15 to 25 years before fluorosis occurs.

Dr. Waldbott: To my knowledge, this is the first time that work of this kind on fluorosed bone has been reported. Studies with the scanning electron microscope have been made on teeth but not on the skeleton. Do workers in industrial plants inhale much fluoride and do you have any information on changes in their lungs?

Dr. Fanke: Whereas substantial amounts are being inhaled it is difficult to determine the amount that is inhaled or ingested because it varies from hour to hour. Workers complain of bronchitis, problems of the throat and lung complications. There is no way of knowing how much fluoride is inhaled through the lungs and how much enters through the gastrointestinal tract.

Dr. Cooke: In what form is the fluoride in the factories?

Dr. Franke: Gaseous and particulate.

* * * * * * * * * *

FLUORIDE BALANCE STUDIES IN ENDEMIC FLUOROSIS

by

S. S. Jolly
Patiala (Punjab) India

SUMMARY: Fluoride balance studies were undertaken in 20 cases of endemic fluorosis. Ten cases were studied on low fluoride intake in the hospitals (Group A) and ten in their villages (Group B). In non-fluorotic controls, total daily fluoride ingestion was 3.74 mg fluoride; in fluorotic subjects, 3.44 mg in Group A, 9.88 mg $\pm$ 3.94 mg in Group B. The major source of fluoride ingestion was drinking water and tea whereas other foods provided only insignificant amounts. Group A subjects showed a negative balance while in the hospital; in Group B the fluoride balance varied but was mostly positive. The urine was the main route of fluoride excretion.

It has long been recognized that in fluoride toxicity the total fluoride ingestion through various dietary sources must be taken into account although the fluoride concentration of drinking water is the major determining factor. Fluoride in water supplies at the recommended 1 ppm concentration was estimated to provide 1 to 2 mg fluoride daily,

---

From the Department of Medicine, Medical College, Patiala, India

an amount considered innocuous to human tissues. Dental mottling was thought to occur with ingestion of water containing more than 2 ppm (1) of fluoride whereas skeletal fluorosis was not believed to occur even at 8 ppm in the western countries. In India and Japan, however, dental fluorosis at less than 1 ppm and crippling skeletal fluorosis at 2 to 4 ppm fluoride in water have been well documented (2, 3, 4). It is stated that in addition to drinking water, food may provide a sizable proportion of the daily fluoride intake (5). Drinking water requirements and dietary habits also differ in different populations and may profoundly influence the daily fluoride intake. Tea has been shown to be exceptionally high in fluoride but data available regarding fluoride ingestion through different dietary media is scanty (6, 7) and we are equally ignorant regarding fluoride excretion through various routes.

Chronic fluoride intoxication is endemic in some parts of India. In Punjab, about one third of the general population exhibits varied degrees of dental and skeletal fluorosis, the effect of a high level of fluoride in water. Whereas excess fluoride ingestion in drinking water has been implicated as the major cause, the amount of fluoride being consumed from various other sources and its metabolism in chronic fluorotic patients has not been adequately studied. Little is known about the daily excretion of fluoride in these patients.

Material and Methods

Fluoride balance studies were carried out in 20 cases of endemic fluorosis, which were divided into Group A and B of ten subjects each. The patients in Group A were transported from the place of their residence in the endemic areas to a teaching hospital in Patiala. Fluoride balance studies in Group B were carried out in their respective villages during surveys of the endemic areas. A third group of ten consisting of patients who had never resided in any of the fluorotic areas were similarly studied in the hospital and served as controls.

The total dietary samples of all edibles including food, milk, tea and water ingested by the patient during a 24 hour period were collected. The fluoride content of a representative sample was estimated by the micro-diffusion method by means of a fluoride ion electrode. The fluoride content of tea was measured separately in hospitalized subjects whereas it was mixed with other foods in case of Group B subjects. Total amounts of urine and feces passed by the patient during the same 24 hour period were collected and their fluoride content estimated. The fluoride concentration of water was measured by the Scott-Sanchis method.

All studies were carried out over three consecutive 24 hour periods in Groups A and control, whereas in Group B a single 24 hour study period was undertaken.

All patients were examined clinically for dental mottling, bony exostoses, spinal deformities and neurological complications of fluorosis. The diagnosis was confirmed in each case by radiological studies of bones (2, 3).

Of the 20 patients 16 were male, 4 were females; their ages ranged from 25 to 70 years (mean 49.8 years). All had been residing in the endemic areas for at least 20 years and showed radiological evidence of fluorosis (Fig. 1 a, b, c). The fluoride concentration of water consumed by the hospitalized patients (Group A) was 0.6 ppm and up to 7.5 ppm in Group B patients.

Fluoride Ingestion

Details on the daily fluoride intake and output on each patient are given in Table 1. Average amounts of fluoride ingested daily by the control group and Group A patients were 0.62 and 0.65 mg in the hospital diet, 1.52 and 1.45 mg in tea and 1.60 and 1.34 mg in water respectively. The mean daily fluoride ingestion through diet plus tea in these two groups was 2.12 and 2.11 mg respectively. In Group B subjects, average fluoride ingestion in food (including tea) was 3.26 mg whereas that in water was 6.62 mg.

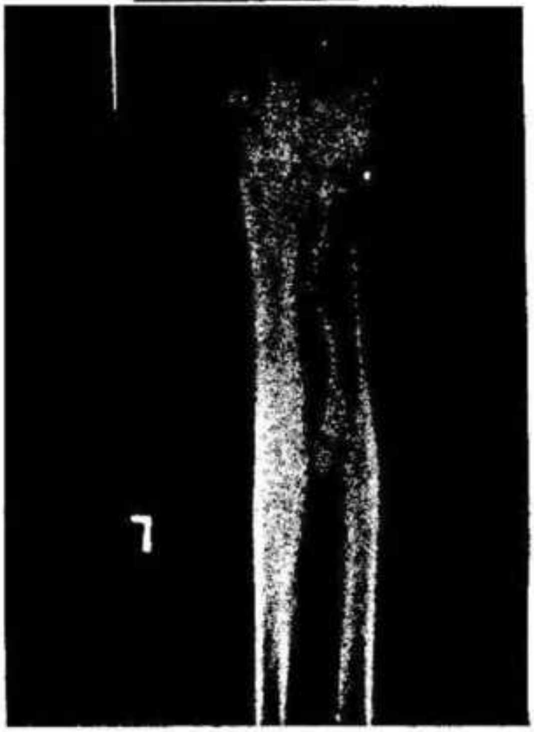
Total fluoride ingestion from all dietary sources was established at 3.74 mg in controls and 3.44 mg in Group A; 9.88 $\pm$ 3.94 mg in Group B. In the third group the markedly higher figures reflected the fluoride concentrations of the water source utilized by the patient (Fig. 2). It needs to be emphasized that these balance studies were carried out in winter when fluoride ingestion through water is minimal. During the summer months, fluoride intake through water increases 5 to 6 times and, therefore, the total fluoride ingestion would be correspondingly higher. The difference in fluoride ingestion between the two hospitalized groups was not statistically significant.

Thus it can be seen that the contribution toward daily fluoride ingestion in controls and in Group A was 18.9% and 16.6% by food, 42% and 40.6% by tea and 39% and 42.8% by water respectively. In Group B, the non-hospitalized patients water was responsible for 71.3 of the daily fluoride intake where as all foods (including tea) contributed the remaining 28.7%. The portion of waterborne fluoride intake was highest (82%) in a patient who consumed water containing 7.50 ppm fluoride.

Fig. 1

Radiographs of Fluorotic Bones

(a)
Forearm Bones

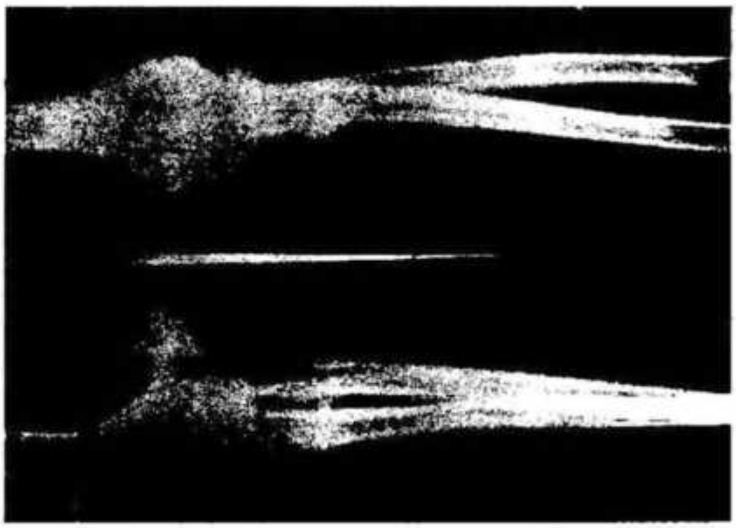


Osteosclerosis and Extensive Calcification of Interrosseous Membrane.

(b)
Lumbosacral Spine and Pelvis

Osteosclerosis, Osteophytosis and Calcification of Ligaments.

(c)
Forearm and Elbow



Huge Exostotic Deposit Over the Olecranon.

TABLE 1

(a) Fluoride Ingestion and Excretion

S. No.	Age & Sex	Fluoride Ingestion (mg/day)	Fluoride Excretion (mg/day)	Balance mg/day	Percent
1.	60 M	3.68	3.43	- 0.25	6.89
2.	40 M	3.76	3.45	0.31	8.25
3.	33 M	4.18	3.21	0.97	23.2
4.	25 M	3.94	3.81	0.13	3.3
5.	50 M	3.10	3.46	- 0.36	-11.6
6.	30 M	3.77	3.20	0.57	15.1
7.	45 M	3.98	3.36	0.62	15.5
8.	50 M	3.32	2.92	0.40	12.04
9.	50 M	3.55	3.12	0.43	12.1
10.	35 M	4.20	3.41	0.79	18.8

(b) In Hospitalized Fluorotic Patients (Group A)

S. No.	Age & Sex	Fluoride Ingestion (mg/day)	Fluoride Excretion (mg/day)	Balance mg/day	Percent
1.	30 M	3.17	5.40	-2.23	-70.34
2.	60 M	3.27	4.67	-1.50	-45.87
3.	45 M	3.35	7.87	-4.52	-130.5
4.	50 M	3.52	7.06	-3.54	-100.6
5.	25 M	3.86	9.10	-5.24	-135.7
6.	60 M	3.32	4.19	-0.87	-26.2
7.	50 M	3.73	5.74	-2.01	-53.9
8.	40 M	3.59	7.13	-3.54	-98.46
9.	45 M	3.17	7.36	-4.18	-131.86
10.	50 M	3.32	7.05	-3.73	-112.32

(c) In Non-hospitalized Fluorotic Patients (Group B)

S. No.	Age & Sex	Fluoride Ingestion (mg/day)	Fluoride Excretion (mg/day)	Balance mg/day	Percent
1.	52 M	8.23	5.07	3.16	38.39
2.	40 M	18.84	17.34	1.52	8.08
3.	62 M	8.55	6.19	2.36	27.6
4.	45 M	8.28	6.33	1.95	23.55
5.	50 M	9.98	9.51	0.47	4.7
6.	70 M	4.16	5.47	-1.31	-31.49
7.	42 F	11.76	7.54	4.22	35.89
8.	56 M	12.59	8.42	4.17	33.12
9.	45 M	7.86	8.29	0.43	5.47
10.	58 M	8.57	5.17	3.40	39.67

Fig. 2

Total Fluoride Ingestion

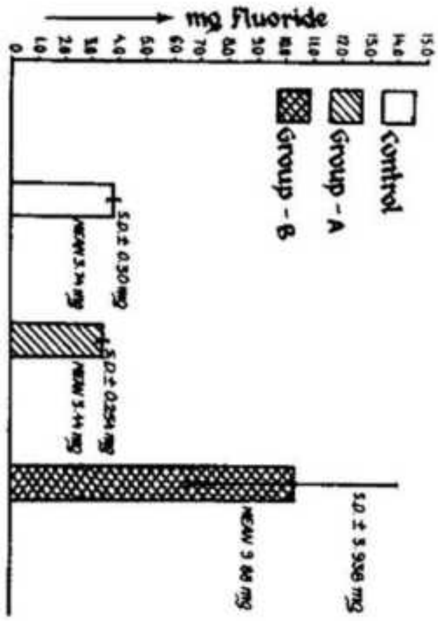


Fig. 3

Daily Total F Excretion Through Urine and Feces

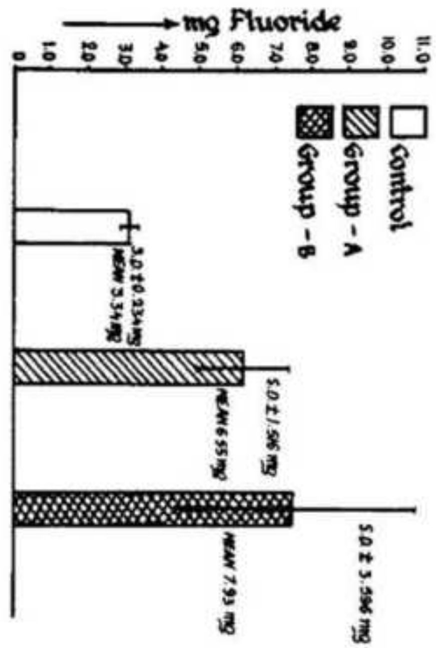
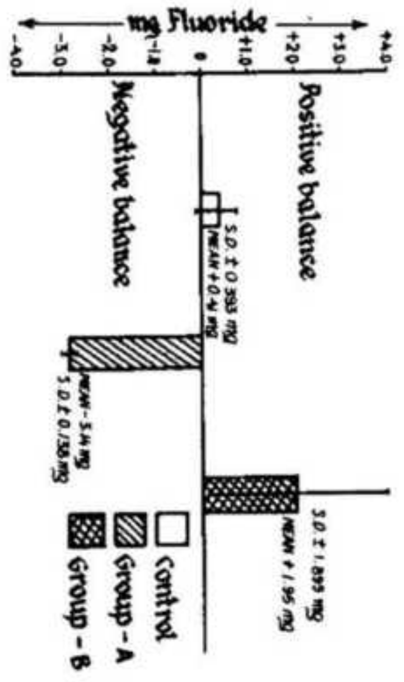


Fig. 4

Fluoride Balance



Fluoride Excretion

Average urinary fluoride excretion per 24 hours was 2.86 mg in the control group and 6.16 mg in patients with fluorosis in Group A whereas patients residing in their villages (Group B) excreted an average of 7.08 mg ± 2.15 mg fluoride in urine. Fecal fluoride excretion averaged 0.48 mg in controls, 0.40 mg in Group A and 0.85 mg in Group B. Since only 8 to 12% of the daily fluoride intake is excreted through the feces, this route is not an important one.

Total fluoride excretion through urine and feces was 3.34 mg in controls, 6.55 mg in Group A (which was far in excess of the current daily intake of 3.44 mg) and 7.93 ± 3.60 mg in Group B (Fig.3).

Fluoride Balance

The balance of ingestion and excretion was expressed as positive or negative depending upon whether excretion was lower or higher than intake (Fig. 4). In the control group there was an average positive balance of 0.41 mg which is not very significant. In Group A, all patients showed a varying negative balance ranging from 0.87 to 5.24 mg of fluoride daily. Obviously this much fluoride was being eliminated daily from the fluoride which had accumulated in the system during residence in endemic areas. Most of this loss occurred via urine. Patients belonging to Group B of the study were in a varying state of balance. Eight of the ten subjects showed a balance of ± 0.47 mg to + 4.22 mg; a slightly negative balance of - 0.42 mg and - 1.31 mg was observed in the other two subjects. This finding could only be explained by the possibility that there might have been a change in the water source utilized by the patient which could have led to excretion in excess of current ingestion and thus to a negative balance.

Discussion

The only available references regarding fluoride balance studies (8, 9) were carried out under experimental conditions. No worker has, as yet, carried out complete fluoride balance studies in endemic fluorosis. Fluoride intake in certain other countries through diet has been estimated to be in the range of 0.2 to 0.7 mg/day (10, 13). Total fluoride ingestion was reported to be 2 to 5 mg/day in fluoridated cities of Canada (11, 12). These figures agree with those of the hospitalized groups in this series.

It is well known that urine forms the main route for excretion of fluoride. In the current study, also, it was responsible for up to 92% of the daily output. Fecal fluoride probably represents mainly the unabsorbed fraction.

A state of equilibrium between ingestion and excretion was visualized at constant levels of fluoride intake by Machle et al. (14). This can be seen in patients of the control group who showed a mild positive balance. Unexpectedly, patients in Group B who continued to consume high fluoride water showed a positive balance. In view of their persistent fluoride intake, a state of equilibrium would be expected. One factor, which was not evaluated, namely, the excretion of fluoride through sweat, may explain the discrepancy (9).

In Group A, disruption of the state of equilibrium between intake and excretion was created by suddenly lowering the water fluoride level. This reduced the total fluoride intake to about 3.74 mg, whereas their counterparts in the endemic area consumed more than 9 mg a day, and led to mobilization of fluoride from skeletal stores and its excretion from the body through the urine. Largent and his co-workers observed that the excretion of the accumulated fluoride follows an exponential curve and gradually decreases until a new state of equilibrium at a lower level becomes established (8).

Conclusions

1. Fluoride ingestion is predominantly derived from drinking water. The contribution of fluoride from various food items in chronic fluoride toxicity was not very significant.

2. In the current study tea, with a fairly high fluoride content, constituted about 40% of the daily fluoride ingestion in subjects residing in low fluoride areas. However, the fluoride fraction from tea does not rise proportionately to the fluoride concentration of water.

3. The pattern of fluoride intake and excretion at different fluoride levels in drinking water confirms the earlier observations of others regarding a state of equilibrium at constant intake, and its disruption with change in water fluoride concentration which results in mobilization of stored fluoride.

Bibliography

1. Dean, H. T., Arnold, F.A., Jr., and Elvove, E.: Domestic Water and Dental Caries V. Additional Studies of the Relation of Fluoride Domestic Waters to Dental Caries Experience in 4,425 White Children aged 12 to 14 Years, of 13 Cities in 4 States. Public Health Rep. 57: 1155, 1942.

2. Jolly, S.S., Singh, B.M., Mathur, O.C., and Malhotra, K.C., Epidemiological, Clinical and Biochemical Study of Endemic Fluorosis With Special Reference to Factors Causing Toxicity. Brit. Med. J. 4: 427, 1968.

3. Jolly, S.S., Singh, B. M. and Mathur, O.C., Endemic Fluorosis in Punjab (India). Am. J. Med. 47: 553-563, 1969.

4. Minoguchi, G., Fluorides and General Health, Chapter 8 in Fluorides and Human Health. WHO Monograph Series No. 59, Geneva, 1970, p. 294.

5. Jackson, D. and Weidman, S. M., Fluorine in Human Bone Related to Age and the Water Supply of Different Regions. J. Path. Bact. 76: 451-459, 1958.

6. Lawrenz, M. and Mitchell, H. H., The Relative Assimilation of Fluorine from Fluorine Bearing Minerals and Food (Tea) and from Water and Food. J. Nutr. 22: 621-631, 1941.

7. Jenkins, G. N., Fluoride and Tea. Lancet 2: 960, 1969.

8. Largent, E. J., Fluorosis: The Health Aspects of Fluorine Compounds. Ohio State University Press, Columbus, 1961.

9. McClure, F. J., Mitchell, H. H., Hamilton, T.S and Kinser, C.A., Balances of Fluorine Ingested from Various Sources in Food and Water by 5 Young Men, Excretion of Fluorine Through the Skin. J. Ind. Hygiene and Toxicol. 27: 159, 1945.

10. Cholak, J., Current Information on the Quantities of Fluoride Found in Air, Food and Water, A.M.A. Arch. Ind. Health 21: 312, 1960.

11. Ham, M. P. and Smith, M. D., Fluorine Balance Studies in 4 Infants. J. Nutr. 53: 215-223, 1954.

12. Ham, M. P. and Smith, M. D., Fluorine Balance Studies in Three Women. J. Nutr. 53: 225-232, 1954.

13. Marier, J.R. and Rose, D., The Fluoride Content Some Foods and Beverages - A Brief Survey Using a Modified Zr-SPADNS Method. J. Food Sci. 31: 941, 1966.

14. Machle, W., Scott, E. W., and Largent, E. J., The Absorption and Excretion of Fluorides, Part I, The Normal Fluoride Balance. J. Ind. Hygiene and Toxicol 24: 199, 1942.

Discussion

Dr. Cooke: You say that the skeletal fluorosis in your region is predominantly male-oriented.

Dr. Jolly: Yes, in dental fluorosis the incidence in males and females is the same but that of crippling fluorosis is higher in males than in females.

Dr. Cooke: Could this be related to estrogen, since it promotes the absorption of Ca?

Dr. Jolly: To date the basis for it has not been scientifically evaluated. Estrogen may be one of the factors. We have tried to explain it on the basis of the heavy loads which male farmers carry on their heads leading to abnormalities.

Dr. Franke: There are several studies on the influence of pregnancy and bone mass. These studies show that pregnancy has no effect on the bone mass in the normal female.

Dr. Cooke: I must disagree with that because personally I have seen women who have virtually lost their teeth during pregnancy. There must be an effect on the bone.

Dr. Franke: Dr. Jolly, in endemic fluorosis you found high levels of alkaline phosphatase. In industrial fluorosis our levels were only slightly higher than normal.

Dr. Jolly: In our studies alkaline phosphatase levels were only slightly higher than normal. Other studies in India (Dr. Teotia) show higher levels. I agree our studies show values not higher than the upper levels of normal which is explained by the fact that fluorosis develops gradually over several years. On the other hand if administered therapeutically, the alkaline phosphatase goes very high.

Dr. Franke: Did you find osteoporotic changes in severe cases of fluorosis. I noted such changes in only a few cases of fluorosis.

Dr. Jolly: It depends upon your definition of osteoporosis. If you evaluate bone mass exclusively, then you may not find it.

TRANSCUTICULAR MOVEMENT OF FLUORIDE: ITS RELATION WITH LEACHING OF FLUORIDE FROM LEAVES

by

J. P. Garrec, A. Chamel and A. M. Lhoste
Grenoble, France

SUMMARY: Isolated cuticles, with neither stomata nor perforations show a very weak permeability to fluoride, from the inner to the outer surface, and the reverse. Under natural conditions, it was shown that the major portion of fluoride inside of polluted leaves can be easily removed (through the cuticle) by washing procedures. "In situ" the presence of stomata and many perforations, plus a strong cuticular transpiration due to fluoride could explain the important movement of fluoride through this cuticle.

Introduction

On the leaves, epicuticular waxes and cuticles have a great importance as mechanisms of defense against outside attack, such as atmospheric pollution. If waxes play an important role in the protection, the main barrier constitutes the cuticle of the leaf.

Nevertheless, the cuticle is a discontinuous layer. It has many natural (micropores, cracks) or fortuitous perforations (insect pricks, impacts of dust) as well as apertures of stomata. However, stomata are not true apertures in the cuticle, because they reach into the substomatal chamber (1).

Gas exchange between the interior of the leaf and the atmosphere occurs through stomata. By this route gaseous air pollutants such as HF may penetrate leaves. Nevertheless, in atmospheric pollution by fluoride, particulate matter which collects on the surface of leaves also contains fluoride. Characteristically particulate fluorides are low in phytotoxicity but due to rain or dew they may hydrolyze and produce HF in solution which is able to enter the leaves through the cuticle.

Fluoride which enters the leaf directly through the cuticle may be min-

---

From the Department de Recherche Fondamentale - Laboratoire de Biologie Végétale - Centre d'Etudes Nucléaires de Grenoble, France.

imal compared to that which reaches the leaf through the stomata, but in the case of heavy pollution by particulate fluorides it could be an important source of phytotoxicity. The use of isolated cuticles and radioactive F^{18} allows us to study transcuticular movements of fluoride.

Material and Method

Cuticle Isolation: The cuticles from the upper and lower surfaces of fully expanded pear leaves (Pyrus communis L. cv William), were enzymatically isolated from the underlying tissues using 2% pectinase and 0.2 % cellulase buffered at pH 3.8 (sodium acetate/acetic acid) as described by Schönherr et al. (2). Discs of fresh leaves (10 mm in diameter) were infiltrated under vacuum with the enzymatic solution; after about four weeks of incubation at 35° C the cuticles were washed thoroughly with distilled water and stored at 4° C with thimerosal (0.01 %) as an antiseptic. Before use, cuticles were dried at room temperature, after which they were carefully inspected under a light microscope.

Preparation of Radioactive tracer F^{18}: F^{18} (half-life 112 mn) is obtained by radioactivation, under a beam of 14 Mev fast neutrons, of ammonium fluoride (NH_4F), following the nuclear reaction F^{19} (n, 2 n) F^{18} (3). Under these conditions, for a solution of NH_4F containing 1000 µg/g of fluoride, we obtain an initial specific activity of approximately 10^{-2} µ Ci per gram of solution. The radioactivity is measured by gamma spectrometry in a well type scintillator NaI (TI) crystal 4 x 4 inches.

Penetration Determination: Penetration was determined as described (4) : an astomatous upper cuticle was fixed with silicone adhesive (Rhône-Poulenc-Paris) to the end of a thick-walled glass tube (7 mm inside diameter) with 0.5 ml radioactive fluoride solution (1000 ppm) inside and with 15 ml deionized water in a glass vial on the outside as a receiver. The liquid levels were equalized to eliminate hydrostatic pressure. At predetermined times, the vials were exchanged and the radioactivity of the receiver solution measured.

Results

Penetration of fluoride through isolated cuticles is very low (table 1): in spite of a high concentration gradient, the greatest value obtained after 201 minutes represented only 0.06 % of the donor (5). Thus, it was very difficult to have reliable precision for determining the radioactivity, taking into account the short half-life of F^{18}; with such small values there were important fluctuations between cuticles. The penetration from the outer to the inner surface was higher than from the inner to outer one, but the difference was not significant.

Table 1

Penetration of Fluoride Through Discs of Astomatous Isolated Upper Cuticle of Pear Leaf (temperature: 22° C)

Time (minute)	1	6	21	81	201
Outer to inner surface (mean of 8 replications)					
nmoles/cuticle disc	0	5.84	6.79	16,05	16.47
Percent of the donor	0	0.02	0.03	0,06	0.06
Inner to outer surface (mean of 4 replications)					
nmoles/cuticle disc	0.11	2.11	2.47	2.95	4.95
Percent of the donor	0.0	0.01	0.01	0.01	0.02

Discussion

Data reported herein suggest that penetration of fluoride through astomatous cuticles is very slow. Consequently, if fluoride is in the form of a soluble salt, cuticle and epicuticular waxes seem to constitute an initial barrier to foliar absorption of this element which is difficult to overcome.

Nevertheless in some cases, in contrast to our results when polluted leaves were washed, a large part of fluoride inside the leaves is easily removed through the cuticle. In fact, either after fumigation with HF which prevents particulate fluorides from collecting on the leaf, or after soil-contamination by fluoride which induces localization of fluoride inside the leaf exclusively, 20 to 80 per cent of the total fluoride accumulated in the leaves can be removed by washing procedures.

These observations were made by Thomas (6) on apricot leaves, by Jacobson et al. (7) on tomato, cotton plants and gladiolus leaves, by Benedict et al. (8) on alfalfa plants and by Ledbetter et al. (9) on tomato leaves by means of a fluoride radioisotope. Eddins (10) noticed that with bean leaves, the largest quantity of fluoride can be removed by washing and this release decreases with time as was shown by Ledbetter (9). Possibly necrosis of tissues can account for some of the decrease in fluoride penetration. Brewer et al. (11) show experimentally that sprinkled orange trees were found to accumulate much less fluoride than unsprinkled ones.

In our experiments showing a slow movement of fluoride through astomatous cuticle, only unperforated cuticle discs were submitted for the permeability assay. We must realize that most isolated cuticles present numerous perforations when examined microscopically.

Cuticle perforations are not due to the isolation procedure, but exist in intact leaves. The enzymatic isolation process does not physically alter the structure of leaf cuticle (12). It seems that normally the existence of numerous stomata and many perforations explain the important movement of fluoride in solution through the cuticle.

It is to be considered also that our results concern cuticles separated from the underlying epidermal cells. Perhaps they may be different "in situ" of intact leaves. Eddins (10) indicates that in an atmosphere of relatively high humidity plants secrete more fluorides to the leaf surface than do those in an atmosphere of low humidity. This implies an inverse correlation with transpiration under these conditions. Generally, the secretion of ions is directly correlated with the transpiration of the plants, but maybe fluoride is secreted through the epidermis and the cuticle of the leaf and not through the stomata and, therefore, fluoride excretion is not a function of transpiration. In the same way, Navara (13) has shown that when fluoride reaches plants through the atmosphere by experimental fumigation, a characteristic effect of fluoride is a gradual increase of the cuticular and decrease of the stomatar transpiration. Within 90 hours after fumigation, cuticular transpiration increases up to 93% of the total transpiration. It seems that, in situ, the important increase of the cuticular transpiration due to fluoride accelerates excretion of fluoride through the cuticle.

Conclusion

We demonstrated that isolated cuticles show a weak permeability to fluoride. But in situ, the many cracks of the cuticle, the presence of stomata and an important cuticular transpiration strongly modify movement of fluoride in solution through the cuticle.

Acknowledgments

The authors wish to thank Mr. G. Mourioux for his assistance in this work.

Bibliography

1. Martin, J.T., Juniper, B.E.: The Cuticle of Plants. Edward Arnold Ltd., London, 1970.

2. Schönherr, J., Bukovac, M.J.: Ion Echange Properties of Isolated Tomato Fruit Cuticular Membrane: Exchange Capacity, Nature of Fixed Charge and Cation Selectivity. Planta, 109: 73-93, 1973.

3. Bligny, R., Garrec, J. P., Fourcy, A.: Migration et accumulation du flour chez zea mais. C. R. Acad. Sci. Paris, 275 (D): 755-758, 1972.

4. Norris, R. F., Bukovac, M.J.: Some Physical Kinetic Considerations in Penetration of Naphthaleneacetic Acid Through Isolated Pear Leaf Cuticle. Physiol. Plant. 22: 701-702, 1969.

5. Chamel, A., Garrec, J. P.: Penetration of Radioactive Fluorine Through Isolated Pear Leaf Cuticle. (Unpublished)

6. Thomas, M. D.: "La pollution de l'air". Organisation Mondiale de la Sante, Geneve, serie de monographie, 46: 237-286, 1963.

7. Jacobson, J.S., Weinstein, L.H., McCune, D.C., Hitchcock, A.E.: The Accumulation of Fluorine by Plants. JAPCA, 16: 412-417, 1966.

8. Benedict, H.M., Ross, J.M., Wade, R.H.: Some Response of Vegetation to Atmospheric Fluorides. JAPCA, 15: 253-255, 1965.

9. Ledbetter, M.C., Mavrodineanu, R., Weiss, A.J.: Distribution Studies of Radioactive Fluorine 18 and Stable Fluorine 19 in Tomato Plants. Contributions from Boyce Thompson Institute, 20: 331-348, 1960.

10. Eddins, O.N.: Fluoride Retention in Leaf Tissue. Thesis for the M.S. Degree, University of Utah, Department of Botany, 1959.

11. Brewer, R. F., Sutherland, F.H., Perez, R.O.: The Effects of Simulated Rain and Dew on Fluoride Accumulation by Citrus Foliage. J. Amer. Soc. Horticultural Sci., 94: 284-286, 1969.

12. Norris, R. F., Bukovac, M.J.: Structure of the Pear Leaf Cuticle with Special Reference to Cuticular Penetration. Amer. J. Bot., 55: 975-983, 1968.

13. Navara, J., Holub, A.: The Effect of Fluoride upon Plants. Fluoride 1: 38-40, 1968.

* * * * * * * * * * * * * * * *

NATURAL COLONIZATION AND REINSTATEMENT OF MINERAL WASTE CONTAINING HEAVY METALS AND FLUORIDE

by

M.S. Johnson*
Liverpool, England

SUMMARY: Waste products discharged during processing of mineral ores containing fluorspar are deposited in tailings dams where they are unstable and subject to erosion by wind and water. Vegetative stabilization is restricted by the toxic metal content and low inherent fertility of the wastes which also contain high levels of fluoride. Plants colonizing tailings and metalliferous spoil containing fluorspar accumulate lead, zinc and fluoride. Fluoride levels in vegetation vary according to the species and physical/chemical characteristics of the substrate. Foliar symptoms of fluoride phytotoxicity are not apparent despite high leaf concentrations. Reinstatement of disused tailings dams is for amenity or recreational purposes because the agricultural value of the vegetation is restricted by the heavy metals and fluoride.

In the United Kingdom, the principal deposits of the important industrial mineral, fluorspar, occur in the Pennines of northern England (West Yorkshire and Durham) and in the Southern Pennine orefield (Derbyshire) within the Peak District National Park. Deposits are associated with mineralized Carboniferous Limestone in which fluorite (calcium fluoride, CaF_2) occurs together with barytes (barium sulphate, $BaSO_4$), galena (lead sulphide, PbS), sphalerite (zinc sulphide, ZnS) and calcite (crystalline calcium carbonate, $CaCO_3$).

In the Peak District, crude fluorspar ore is obtained mainly from deep-mining and opencast excavations, but also from extensive spoil heaps associated with abandoned lead/zinc mines which rejected fluorspar as a gangue mineral of no economic importance.

Fluorspar and associated minerals of commercial value are recovered by gravity concentration or flotation processes but large quantities of mineral waste (tailings) are produced during benefication of the ore. Tailings are discharged from processing mills into settlement lagoons in the form of a slurry of low solids content. Progressive de-watering of the effluent by profile drainage and surface evaporation effectively consolidates the material once a site has ceased to function as an active disposal area.

* Department of Botany, University of Liverpool, England.

Tailings produced by modern processing techniques consist mainly of very fine material (Table 1) and are subject to erosion by wind and water unless they are stabilized effectively. This is particularly important where a mining complex is adjacent to agricultural land because toxic mineral residues in tailings are a source of enviromental pollution and a potential hazard to livestock and crop production.

Physical or chemical stabilization of mine tailings is effective (1) but neither provides the permanent solution and esthetic improvement which can be achieved by establishing a vegetation cover. However, the physical and chemical characteristics of fluorspar tailings provide limitations to plant growth and development. Tailings are devoid organic matter, deficient in essential plant macronutrients and they contain elevated levels of phytotoxic heavy metals and fluorspar.

Physical and Chemical Characteristics

Five 100g samples were collected from the surface 10cm of mine waste at twelve abandoned metalliferous workings or disused fluorspar tailings dams in the Peak District (Table 1). In order to reduce heterogeneity, samples from each site were separately combined.

Particle size distribution was determined by wet-sieving 250g subsamples of spoil through 2mm and .2mm nylon screens (B.S. 8- and 72-mesh. The physical composition of the fine fraction (<.2mm) was determined by the hydrometer method (2).

Further samples were oven-dried for 10 days at 55^{o}C, sieved to B.S. 4-mesh (4mm) to remove organic debris and large pieces of fluorspar and limestone, and ground to B.S. 100-mesh (150 μ) in a stainless-steel, micro-hammer mill. The fine material was used for chemical analysis; 2g samples were pre-digested with 48% hydrofluoric acid before wet oxidation with nitric/perchloric acids at 140^{o}C. The diluted, filtered digests were analyzed by atomic absorption spectrophotometry (Pb, Zn) or emission spectroscopy (K). Phosphorus was measured spectrophotometrically (3) in perchloric acid digests (4); interferences from high fluoride concentrations were overcome by adding boric acid (5). Nitrogen was determined by a modified semi-micro Kjeldahl procedure (6). Total fluoride was determined by an ashing/alkali fusion technique (7, 8) modified to determine fluoride potentiometrically using an ion-selective electrode. Fusion melts were dissolved in 20% $^{v}/v$ perchloric acid and buffered to pH 5.5 with sodium acetate containing 1, 2 cyclohexylene diaminetetracetic acid (CDTA). Water soluble fluoride was measured potentiometrically in buffered saturated paste extracts of mine waste (9).

Table 1

CHEMICAL AND PHYSICAL CHARACTERISTICS

SITE	O/S GRID REF.	PARTICLE SIZE ANALYSIS (% w/w)*						CHEMICAL COMPOSITION (μg/gm oven-dried material)**						
Tailings dams		>2mm	2 - .2	.2 - .02	.02 - .002	<.002	pH	Pb	Zn	F	N●	P	K	F▲
BLEAKLOW	SK 233734	0	4	38	33	25	7.2	13000	5400	139000	87	58	1260	20.5
CAVENDISH MILL	SK 208753	0	1	52	31	16	7.6	10000	2190	124700	31	67	1130	6.1
GLEBE	SK 219764	0	2	30	35	33	7.7	27300	18500	117000	25	12	1360	8.8
HARBORO	SK 240550	0	4	30	29	37	7.3	8800	10100	184000	28	37	1440	26.8
MILL CLOSE	SK 263623	10	13	39	17	21	7.5	5950	38400	88500	37	88	1490	12.7
QUEEN	SK 288592	4	12	46	14	24	7.5	13800	7000	147900	60	62	2100	14.2
Coarse metalliferous mine waste														
CALVER	SK 242741	29	26	15	16	14	7.1	22000	7900	151500	72	45	1970	2.7
MASSON	SK 285591	27	32	19	10	12	7.4	13900	14700	93400	33	52	2230	2.9
NEW ENGINE	SK 224774	40	27	21	5	7	7.3	14200	24000	104000	20	49	1670	1.3
ODIN	SK 135835	36	42	9	11	2	7.5	18600	23700	170600	108	177	2400	3.2
SHAW ENGINE	SK 222771	46	33	14	3	4	7.3	22800	26700	121000	24	20	1620	1.1
WATERGROVE	SK 190758	55	25	7	9	4	7.2	26400	30600	110700	51	103	1270	1.2
UNCONTAMINATED PASTURE	SK 075743	ND	ND	ND	ND	ND	7.2	73	140	215	1475	2000	1750	<0.05

* International Soil Science Society Classification. Gravel (>2mm), coarse sand (2 - .2), fine sand (.2 -.02), silt (.02 - .002) and clay (<.002)

** Mean of 6 replicates. All spoils also contained baryte (3-24% w/w), calcite (21-56% w/w) and complex aluminosilicates (17-34% w/w)

● Air-dried material

▲ μg/ml. saturated paste extract

ND Not determined

All the mine wastes contain high levels of lead, zinc and fluoride and have a very low plant nutrient status with respect to nitrogen and phosphorus (Table 1). Concentrations of fluoride vary between 88500 and 184000 μgF/g. This compares with a range of 20 to 500 μgF/g in normal uncontaminated soils (10). Water soluble fluoride ranges from 1.1 to 26.8 μgF/ml compared to .01 to .2 μgF/ml in most natural waters and 1 to 4 μgF/ml where soils are naturally rich in fluorine-bearing minerals.

Metal concentrations in the older spoils are within the range reported by Smith and Bradshaw (11) for mine wastes where natural colonization by plants is restricted to a characteristic range of species (12) which have evolved populations tolerant of the toxic metals and low inherent fertility.

The vegetation cover is sparse on older spoil heaps where seedling establishment is effected by surface drought caused by the coarse texture and free-draining properties of the spoil. Few species establish naturally on these mineral wastes (Table 2) because of the adverse physical environment and the high metal content compared to recently produced fluorspar tailings. Concentrations in the latter wastes reflect improvements in mineral processing technology.

Natural Colonization and Accumulation of Fluoride by Plants

The natural flora at most sites is dominated by red fescue (*Festuca rubra*). This species is known to have evolved populations tolerant of heavy metals (11). Tolerance of the indigenous populations of *Festuca rubra* to lead and zinc was determined using the method described by Jowett (13).

Indices of tolerance (Table 2) suggest that zinc is the major factor determining the phytotoxicity of the substrate. Few populations are tolerant of lead and the species diversity of the flora is inversely related to the concentration of zinc in the spoil. There is no clear relationship between lead and fluoride in the substrates and the number of colonizing species. Where levels of zinc are high, the flora consists only of species which are known to have evolved tolerant populations (e.g. *Agrostic tenuis*, *Rumex acetosa*, *Plantago lanceolata*).

Plants were collected from each site for analysis. Roots were separated and discarded, and shoots were surface washed with .2% ($^{v}/v$) 'Teepol' followed by de-ionized water, in order to remove external particulate matter. This displacement was confirmed by examining washed plant material with a scanning electron microscope. Samples were dried at 50-60°C for 48 hours; higher temperatures were avoided because of possible losses of volatile fluorine compounds. The vegetation was ground (<200μ) in a micro-hammer mill to

Table 2

LEAD AND ZINC TOLERANCE IN INDIGENOUS POPULATIONS OF *Festuca rubra**

Site	Index of tolerance (%) (Pb)	(Zn)	No. of higher plant species present
BLEAKLOW	7.4	3.7	21
CAVENDISH MILL	3.4	3.2	23
GLEBE	9.0	16.2	13
HARBORO	2.7	2.9	13
MILL CLOSE	4.8	20.2	4
QUEEN	5.5	2.6	15
CALVER	3.7	4.2	12
MASSON	4.6	13.8	7
NEW ENGINE	8.2	23.8	5
ODIN	12.7	30.3	6
SHAW ENGINE	7.5	28.6	4
WATERGROVE	4.0	18.8	9
UNCONTAMINATED PASTURE	1.0	2.9	ND

* Indices of tolerance calculated from the rooting of tillers in solution according to the method of Jowett (13).

$$\text{Index of tolerance} = \frac{\text{length of longest root in solution containing lead (12}\mu\text{g/ml) or zinc (7.5}\mu\text{g/ml)}}{\text{length of longest root in solution of calcium nitrate (500mg/l)}} \times 100$$

ND - No data

FLUORIDE

obtain a very fine, homogeneous product. Lead, zinc and fluoride were determined using a non-fusion, wet oxidation procedure. 500mg samples were digested at 100°C in a mixture of concentrated nitric and perchloric acids (4:1 $^{v}/v$). Digests were cooled, diluted, filtered and analyzed by atomic absorption spectrophotometry (Pb, Zn) or by direct potentiometry (F) after buffering.

Lead and zinc concentrations range from 162 to 425 µg/g and 265 to 1085 µg/g respectively and are in close agreement with values reported in tolerant plants established on mine waste elsewhere in the United Kingdom (11). Concentrations of fluoride in vegetation are between 52 and 4700 µgF/g (Table 3) compared with a normal range of 1 to 15 µgF/g (14). Values greater than 100 to 150 µgF/g are unusual where fluoride is derived from the rooting substrate, even if the fluoride content of the soil is naturally high or is artificially increased by the addition of fluoride-bearing minerals (15).

The total fluoride content of the mine spoils is poorly correlated with that of the associated flora but water-soluble fluoride shows some agreement with plant concentrations. Fluorspar is a relatively insoluble fluoromineral (16 mg/l at 18°C) but high concentrations of water-soluble fluoride (6.1 to 26.8 µgF/ml) were recorded for finely-ground fluorspar tailings composed of particles of fine sand, silt and clay dimensions. Water-soluble fluoride ranged between 1.1 and 3.2 µgF/ml for the relatively coarse mine spoils.

Accumulation of fluoride is greater where plants are established on fine tailings. The intensity of grinding of the crude ore is an important factor determining fluoride uptake from mine waste because this process affects the specific surface area and solubility of the constituent minerals. The increased specific surface area of the fluoromineral may enhance plant uptake by accelerating the rate of replenishment of fluoride in the soil solution when the concentration is depleted. Soluble fluoride concentrations in excess of 30 µgF/ml have been reported in super-saturated solutions in contact with fluorspar tailings (16). This compares with concentrations of .8 to 8 µgF/ml when fluorspar is present in its natural physical state as a massive ore.

Established plants show no evidence of fluoride injury (necrosis or chlorosis) even where concentrations exceed 4000 µgF/g in leaf tissue. This suggests that fluoride is in a relatively inactive form and therefore not phytotoxic, or that the colonizing species are resistant. Species vary greatly in their susceptibility to this pollutant (15). Natural colonization of fluorspar tailings is unlikely to reflect any specific adaptation of the

Table 3

FLUORIDE CONCENTRATIONS IN PLANTS NATURALLY ESTABLISHED ON FLUORSPAR TAILINGS AND MINE SPOIL *

	Common bentgrass (Agrostis tenuis)	Red fescue (Festuca rubra)	Yorkshire fog (Holcus lanatus)	Sorrel (Rumex acetosa)	Birdsfoot trefoil (Lotus corniculatus)
BLEAKLOW	1625	1480	303	1025	4700
CAVENDISH MILL	NP	1135	215	NP	NP
GLEBE	1296	2280	265	583	3625
HARBORO	1780	1670	NP	375	NP
MILL CLOSE	1040	1810	137	225	NP
QUEEN	969	596	79	180	2455
CALVER	470	518	69	105	1045
MASSON	524	265	78	93	1295
NEW ENGINE	395	428	114	100	NP
ODIN	402	897	97	210	NP
SHAW ENGINE	380	334	52	67	NP
WATERGROVE	167	329	105	87	785
UNCONTAMINATED PASTURE	2.7	10.6	9.8	4.7	14.8

*Determined in buffered, acid digests by direct potentiometry;
**Mean F content (n=6) in µg/g dry weight; NP = Not present

Table 4

ANALYSIS OF FLUORIDE IN VEGETATION

SPECIES	SITE	ASHING/ FUSION ▲	ACID † EXTRACTION	DE-IONISED WATER	2M SODIUM ACETATE
		Fluoride recovered (µg/g. dry weight) ●			
Festuca rubra	Cavendish Mill	1105 (52)	1135 (38)	36	102
Festuca rubra	Glebe	2175 (59)	2280 (53)	59	97
Festuca rubra	Masson	276 (23)	265 (31)	27	29
Festuca rubra	Odin	920 (37)	897 (34)	34	67
Holcus lanatus	Bleaklow	278 (17)	303 (22)	26	49
Holcus lanatus	New Engine	106 (12)	114 (16)	23	37
Lolium perenne	Cavendish Mill	1672 (39)	1637 (46)	40	110
Lotus corniculatus	Glebe	3700 (87)	3625 (104)	116	305
Lotus corniculatus	Masson	1276 (29)	1295 (44)	26	48
Plantago lanceolata	Calver	387 (10)	401 (27)	34	46
Plantago lanceolata	Shaw Engine	349 (19)	346 (21)	32	37
Ranunculus repens	Queen	1140 (58)	1065 (44)	40	70
Rumex acetosa	Harboro	344 (26)	375 (30)	27	46
Rumex acetosa	Masson	84 (8)	93 (12)	20	28
Taraxacum officinale	Calver	1005 (30)	988 (24)	39	61
Trifolium repens	Cavendish Mill	4245 (69)	4290 (88)	155	274
Trifolium repens	Queen	2880 (49)	2935 (59)	108	162

● Mean values (n=6). 95% confidence intervals in parentheses;
▲ According to Hall (7, 8); † Concentrated nitric/perchloric acids

indigenous populations to elevated levels of fluoride. Commercial varieties of grasses and legumes were introduced onto some of these substrates from which they accumulated fluoride to very high concentrations in the shoots (1070 to 4250 μg/Fg) without visible symptoms of injury.

Total fluoride recovered from plant tissue after acid digestion is in close agreement with that determined after ashing and alkali fusion (Table 4). This indicates that non-acid labile inorganic and organic fluorine compounds were absent from the vegetation. Extraction of finely-ground ($<200\mu$) plant material with de-ionized water or 2M sodium acetate removes only 2 to 32% of the acid-extractable fluoride (Table 4). Thus, the greater proportion is present as acid-labile inorganic compounds which are not readily ionized. In this relatively innocuous form, fluoride is biochemically less toxic than more soluble inorganic fluorides (e.g. HF, NaF) and organofluorides which are recognized as potential metabolic inhibitors.

Reinstatement and Use of Inactive Tailings Dams

A vegetation cover is necessary to reduce the risk of pollution and to improve the esthetic value of recently disused tailings dams and abandoned mine workings. Natural colonization may take several years, and be ineffective ultimately. Therefore reclamation programs are undertaken to reinstate these areas.

Because of the high levels of phytotoxic metals, only populations tolerant of the adverse conditions will establish successfully on older spoil heaps unless an innocuous amendment (e.g. topsoil, sewage sludge) is applied as a surface treatment. Even then root penetration and upward movement of heavy metal ions may ultimately cause a deterioration of the sward unless metal tolerant varieties are sown.

On recently disused sites, limitations to plant growth may be less severe because the improved recovery of heavy metals from the crude ore has decreased their concentration in the discarded tailings. The principal limitation to the development of a vegetation cover is the low inherent fertility of the substrate. In the short term, this can be corrected by applying appropriate plant nutrients (normally nitrogen and phosphorus) either as organic manures or as inorganic, chemical fertilizers.

In order to prevent any deterioration of the developing sward, nitrogen-fixing legumes must be included in seed mixtures to counteract leaching of soluble nitrogen compounds in drainage waters. Reinstatement is usually for amenity/recreational purposes where a permanent, low-maintenance sward is required and where legumes are essential if repeated applications of nitrogen fertilizers are to be avoided.

The agricultural potential of reinstated tailings dams is restricted by the fluoride and heavy metal content of the vegetation, and by particulate contamination which may account for an important part of the total dietary intake of lead and fluoride by livestock. Lead concentrations are higher than 100 μg/g which has been reported as lethal to cattle (17) although values as high as 300 μg/g may be acceptable for short-term, intermittent grazing depending upon the species, age and condition of the livestock and the proportion of the diet which consists of contaminated vegetation. The toxicity of ingested fluorides varies with the solubility of the fluoride compound, the rate of absorption and the extent to which fluoride is retained in skeletal tissue. The maximum acceptable fluoride content of vegetation for cattle is 30 to 50 μgF/g and for relatively tolerant animals, including sheep, 70 to 100 μgF/g (18, 19). These currently adopted standards are in respect of soluble - rather than total-fluoride but they are still exceeded by concentrations of water-soluble and ionizable fluoride (20 to 305 μgF/g) in plants naturally established or introduced onto fluorspar tailings.

Bibliography

1. Dean, K.C., Havens, R and Valdez, E.G.: USBM Finds Many Routes to Stabilizing Mineral Wastes. Min. Engng. Dec. 1971, 61-63.
2. Bouyoucos, G.J.: A Recalibration of the Hydrometer Method for Making Mechanical Analysis of Soils. Agron. J., 43: 434-438, 1951.
3. Murphy, J. and Riley, J.P.: A Modified Single Solution Method for the Determination of Phosphate in Natural Waters. Analyt. Chim. Acta, 27: 31-36, 1962.
4. Sommers, L.E. and Nelson, D.W.: Determination of Total Phosphorus in Soils: A Rapid Perchloric Acid Digestion Procedure. Soil Sci. Soc. Amer. Proc., 36: 902-904, 1972.
5. John, M.K.: Colorimetric Determination of Phosphorus in Soil and Plant Material with Ascorbic Acid. Soil Sci., 109: 214-220, 1970.
6. Bremner, J.M.: Total Nitrogen. In Methods of Soil Analysis, Black C.A. (ed), Monograph of Am. Soc. Agron., 9: 1149-1178, 1965.
7. Hall, R.J.: Observations on the Distribution and Determination of Fluorine Compounds in Biological Materials, Including Soils. Analyst, 93: 461-468, 1968.
8. Hall, R.J.: The Distribution of Organic Fluorine in Some Toxic Tropical Plants. New Phytol., 71: 855-871, 1972.
9. Richards. L.A.: Diagnosis and Improvement of Saline and Alkaline Soils. U.S. Dept. Agr. Handbook, 60. Govt. Printing Office, Washington, D.C., 1954.
10. Robinson, W.O. and Edgington, G.: Fluorine in Soils. Soil Sci., 61: 341-353, 1946.
11. Smith, R.A.H. and Bradshaw, A.D.: Stabilization of Toxic Mine Wastes by the Use of Tolerant Plant Populations. Trans. Instn. Min. Metall. 81 (A): 230-238, 1972.

12. Antonovics, J., Bradshaw A.D. and Turner, R.G.: Heavy Metal Tolerance in Plants. Adv. Ecol. Res., 7: 1-85, 1971.
13. Jowett, D.: Population Studies on Lead Tolerant Agrostis tenuis. Evolution, 18: 70-80, 1964.
14. Thomas, M.D. and Alther, E.W.: The Effects of Fluoride on Plants. In Handbuch der Experimentellen Pharmakologie. Eichler, O., Farah, A., Harben, H. and Welch, A.D. (eds), Springer-Verlag, Berlin, 21, 231-306, 1966.
15. NAS.: Biological Effects of Atmospheric Pollutants: Fluorides, National Academy of Sciences, Washington, D.C., 1971.
16. Rudd, R.T.: The Impact of Slimes-Dam Formation on Water Quality and Pollution. J.S. Afr. Instn. Min. Metall., 74: 184-192, 1973.
17. Goodman, G.T. and Roberts, T.M.: Plants and Soils as Indicators of Metals in the Air. Nature (London), 231: 287-292, 1971.
18. Phillips, P.H. Greenwood, D.A., Hobbs, C.S. and Huffman, C.F. The Fluorosis Problem in Livestock Production. Rep. Comm. Animal Nutrition. NAS-NRC publ. No. 824, Washington, D.C., 1960, pp 29.
19. Oelschläger, W.: Fluoride-Containing Mineral Supplements in Agriculture. Fluoride, 7: 84-88, 1974.

Discussion

Dr. Waldbott: How much fluoride was found in the soil compared to the plant?

Dr. Johnson: The soil contained 88,000-184,000 ppm primarily in the form of CaF_2. Total fluoride in the soil is not related to that in the plant, but there is a direct correlation between the level of fluoride in the plant and the water soluble fluoride in the soil. Where water soluble fluoride is very high, elevated values of fluoride occur in the plant with fine materials.

Prof. Miller: Have you noticed any difference in exclusion of fluoride by plant species? There are certainly species differences. Red fescue and some other grasses accumulate 1000-2000 ppm on a dry weight basis. Other species accumulate much lower levels of fluoride. These values are consistent between sites.

Mr. Roelofs: Could plants such as Viola lutea, variety Calaminare and Thlaspi alpestre be used to beautify the area?

Dr. Johnson: Such attempts have been made but seed is difficult to obtain.

Dr. Waldbott: How close to these areas are people residing?

Dr. Johnson: Within about one-half mile. The water may contain about 15 ppm.

A PILOT STUDY OF THE RELATIONSHIP BETWEEN CARIES EXPERIENCE AND SURFACE ENAMEL FLUORIDE IN MAN

by

P. F. DePaola, F. Brudevold, R. Aasenden, E. C. Moreno, H. Englander
Y. Bakhos, F. Bookstein and J. Warram
Boston, Massachusetts

(Abstracted from Arch. Oral Biol. 20: 859-864, Dec. 1975)

The caries experience and the fluoride values of enamel (by biopsy) of 1447 children, 12-16 years old, residing in selected fluoridated and non-fluoridated communities were assessed to explore a possible relationship between dental caries and enamel fluoride. Fluoride was measured as log, mass F corrected to a standardized depth. A square root transformation of decayed, missing and filled surfaces (DMFS) was carried out in order to achieve homogeneity of variance for this variable across all levels of fluoride.

In the fluoridated water supplies, the concentration of fluoride in samples obtained at the time of the clinical examinations deviated from the expected 1 ppm in two cities (Danvers, Mass. 1.2 ppm, Kalamazoo, Mich. 0.67 ppm). The Midland, Texas water contained a fluoride level of 5-7 ppm naturally which was defluoridated down to 0.32 ppm 10 months prior to the study. In Charlotte, N.C. about 2/3 of the subjects were drinking fluoridated water and 1/3 non-fluoridated water. The number of subjects from each community was about 300. The examination of the teeth was carried out by mirror and explorer, and the conventional DMFS index was utilized. Fluoride of the enamel surface was determined by the biopsy procedure of Brudevold et al., 1968, and a standardized sampling area was utilized.

The mean weights of the biopsy samples showed significant differences which ranged from 0.090 mg in the non-fluoridated section of Charlotte to 0.131 mg in non-fluoridated Boston. The lowest enamel fluoride values (unadjusted) were found in the non-fluoridated areas, and the highest one in excessively fluoridated (5-7 ppm) Midland, with a range of intermediate values in the artificially fluoridated communities. The highest DMFS values were found in non-fluoridated Boston, the lowest ones in excessively fluoridated Midland. A regression analysis confirmed this impression. The Pearsonian correlation was $r = 0.38$ ($p < 0.001$).

This study was based upon the premise that the fluoride level of the biopsied tooth surface represents an index of fluoride for the entire dentition from which it was taken. Furthermore, the biopsies were taken on a sound surface whereas the enamel which supports an incipient caries lesion becomes enriched with fluoride during the earliest stages of carious attack. In spite of a general tendency for caries to decrease with increasing fluoride content there was considerable dispersion of the data. The authors found in each of the study groups individuals for whom fluoride was a "weak or non-existent caries determinant". However, in general, they noted a distinct association between the caries experience of the subjects and fluoride values of the surface enamel which was "the one controlled variable".

The authors refer to the 1942 survey by Dean, Arnold and Elvove which showed that in general caries was 2.6 times greater in children in five non-fluoridated communities than in four fluoridated ones. However, inter-community differences up to 20% were recorded in fluoridated areas and up to 46% in non-fluoridated ones. This fluctuation in caries within fluoridated and non-fluoridated communities indicates an important role of caries determinants other than fluoride. Similarly, in their own study, there was a marked variability in caries experience in a given community which the authors attribute to many uncontrolled causes such as diet, microbial status, etc.... "A statistical relationship between caries and fluoride can be demonstrated, but a wide range of fluoride values is required". They suggest that "regular repeated treatments over time with the currently available topical agents could effect an alteration in enamel fluoride with the corresponding shift in caries activity."

Again emphasizing the great individual variability in caries experience for each fluoride level including some rather high caries scores even in optimal fluoride areas, they state: "Unfortunately, then, while the effect of elevated enamel fluoride may be predictable for a group of patients, it is uncertain for any individual within the group".

Therefore, the authors recommend a "vigorous effort to bring under control the other determinants of the disease process. A preventive program based wholly or primarily upon fluoride enrichment of the enamel may produce inconsistent results. On the other hand, an eclectic approach aimed at the diversity of cariogenic factors will serve to amplify the fluoride effect suggested by the present data".

* * * * * * * * * * * * * * *

LONG-TERM OBSERVATIONS IN EXPOSURE TO FLUORIDES

by

K. de Vries, A. Löwenberg, H. E. V. Coster van Hoorhout, and J. H. Ebels
Groningen, Niederlande

(Abstracted from Pneumonologie 150: 149-54, 1974)

Approximately 20 to 30 percent of workers of an aluminum smelter developed dyspnea within the first three years following its opening. At first, the episodes of dyspnea were relatively rare but after several weeks and months they occurred more frequently, both inside of the smelter and for several hours after the workers were at home. In several individuals, the dyspnea was persistent but cleared up completely following abandonment of their work. The major contaminant emanating from the electrolysis pots was fluoride compounds; minor ones were carbon dioxide and sulfur dioxide. The pollutants escaped mainly from breaking the crusts which formed on the surface of the electrolysis bath. In the smoke, the fluoride concentrations ranged from 0.5 and 90.4 mg/m^3 for gaseous and between 0.7 and 25.4/m^3 for particulate fluoride.

In addition to dyspnea, 30% of the workers in the plant had asthmatic wheezing, 28% expectorated mucus and 31% had a chronic cough. These percentages were considerably higher than those of the control group. Pulmonary function studies showed only minor obstruction of air passages in 6 to 13% of the workers.

The authors also established that the workers had a higher reactivity to histamine than the nonworking population. They considered this parameter as an indication of greater irritability of the bronchial mucous membranes. Twenty eight percent of the patients gave a history of having had pulmonary disease prior to their employment at the smelter.

Seventy five percent of the patients with dyspnea gave positive reactions when skin tested for allergy; 41% had blood eosinopenia, 67% eosinophilia of the sputum and 91% a decreased histamine threshold. X-rays of the lungs revealed no noticeable change.

The symptoms cleared completely upon change of occupation. In several instances they recurred when the patient returned to work after sick leave. Prophylactically, the authors suggest a careful medical examination of applicants for their work, improved ventilation at the place of work and confinement of work at the electrolytic baths to closed cabins.

FLUORIDE LEVELS IN THE SURFACE ENAMEL OF DIFFERENT TYPES OF HUMAN TEETH

by

R. Aasenden, E.C. Moreno, and F. Brudevold
Boston, Massachusetts

(Abstracted from Archs. Oral Biol., 18: 1403-1410, 1973)

Little information is available on the fluoride content of the enamel of the different types of permanent teeth. Fluoride uptake in the enamel is expected to occur prior to, rather than following, the eruption of teeth since the rate of deposition of fluoride in the surface of the enamel is much slower before than after their eruption.

The authors compared the fluoride content of the upper anterior teeth in low fluoride Boston (less than 0.1 ppm fluoride) with the fluoride levels of the corresponding teeth in Danvers, Massachusetts (1 ppm) and Midland, Texas (5 to 7 ppm). In eight groups of 12 to 16 year-old school children who were lifelong residents of their respective communities two or three upper anterior teeth on each individual were biopsied. They employed a method recently described by Aasenden et al. which makes it possible to compare fluoride levels in teeth in vivo.

In all three areas the central incisors showed significantly less fluoride than the lateral incisors and the canines. In Boston, the low fluoride area, this difference amounted to 28% for the canines and 19% for the lateral incisors. This pattern was similar in Danvers, the fluoridated area, and in Midland, the natural fluoride area. There were also definite but less significant differences between the lateral incisors and the canines in Boston and Danvers. In all three areas, the differences in the mean fluoride concentrations were statistically significant between the compared teeth, except for those between the canines and the lateral incisors in the Midland children.

The observed differences in the fluoride concentration of the enamel in the three areas persisted up to a depth on the enamel surface of at least 3 to 4 µm. The authors concluded that the observed differences in the concentration of enamel fluoride developed prior to the eruption of the teeth. Their studies suggested that the rate of incorporation of fluoride into the tooth enamel increases with decreasing fluoride present in teeth.

* * * * * * * * * * * *

LACK OF EFFECT OF MASSIVE DOSE OF VITAMIN C ON FLUORIDE EXCRETION IN FLUOROSIS DURING A SHORT CLINICAL TRIAL

by

K.A.V.R. Krishnamachari and N. Laxmaiah
Hyderabad, India

(Abstracted from Am. J. Clin. Nutr. 28: 1234-36, 1975)

The authors studied the urinary excretion of fluoride in fluorotic patients following daily administration of 2 gm of vitamin C. They selected four individuals with endemic skeletal fluorosis who had been residing since birth in natural fluoride communities (between 5.6 and 8.4 ppm). They were maintained on a standard diet of 2,800 calories and about 40 to 50 g of protein of vegetable origin. The daily intake of fluoride through water and food was of the order of 1.0 to 2.0 mg. Twenty-four hour urine output was collected daily in polythene containers throughout the study period. Three of the four subjects received 2 g of vitamin C (Redoxon - 500 mg, Roche) in four divided doses for a period of 2 weeks from the 13th day of the study. The fourth - not supplemented by vitamin C - served as control. Marked variations in the urinary fluoride levels were found from one subject to another, and to a lesser extent in the same subject from day to day. In the three experimental subjects, the mean urinary fluoride excretions prior to supplementation by vitamin C were 5.3 ± 0.43, 8.5 ± 0.45, and 9.7 ± 0.57 mg in 24 hours; 4.8 ± 0.23, 8.5 ± 0.33, and 10.8 ± 0.33 mg in 24 hours after vitamin C was supplemented. For the control subject, the mean values were 10.6 ± 0.38 mg in 24 hours and 10.1 ± 0.49 mg in 24 hours, respectively, during the two periods. The authors concluded that administration of ascorbic acid does not, in any way, modify urinary fluoride excretion in fluorosis.

* * * * * * * * * * *

CHRONIC FLUOROSIS IN LABORATORY GUINEA PIGS

by

I.M. Parsonson, P. D. Carter, and J. Cruickshanks

(Abstracted from Australian Vet. J. 51: 362-363, 1975)

The authors observed a marked deterioration of health in guinea pigs in a colony of laboratory animals. Young stock less than 5 months of age appeared to be unaffected. They exhibited considerable weight loss leading to emaciation, a staring hair coat, and a reluctance to move. The food intake of the affected animals was reduced and their intake of water greatly increased. There was a 25% incidence of abortions and stillbirths in the breeding females. Autopsies failed to reveal any gross changes except for overgrown incisor and molar teeth, suggestive of chronic fluoride toxicosis.

The diet consisted of commercial rabbit and guinea pig pellets and fair quality pasture hay. When green clover was added to the diet, it failed to improve their condition.

Investigation of the commercial rabbit and guinea pig pellets revealed a fluoride concentration in excess of 300 µg/g whereas acceptable levels should be less than 20 µg/g. Teeth and bones from guinea pigs of varying ages representative of both stock males and breeding females were analyzed for fluoride. In teeth, stored fluoride ranged from 2000 µg/g in younger animals (8 months) to 9000 µg/g in the stock males (12 months). In bones the levels were up to 6000 µg/g and 11000 µg/g respectively. Calcium and phosphorous levels were within the normal range. In teeth and bones of normal guinea pigs that had not received pelleted fee, fluoride levels were 150 µg and 400 µg/g respectively.

* * * * * * * * * * *

FLUOROTIC MYELOPATHY, A RARE CASE, WITH A REVIEW OF THE LITERATURE

by

F. T. Lester
Addis Ababa

(Abstracted from Ethiop. Med. J. 12: 39-49, 1974.)

Lester described the case of a 42 year old worker with skeletal fluorosis and evidence of myelopathy encountered in Abyssinia. The illness developed insidiously about 10 months prior to his hospitalization with vague abdominal pains, increasing constipation, paresthesias and stiffness in both legs, and inability to walk. He also complained of stiffness and numbness in both hands. Eventually, the patient was completely bedridden and required daily enemas.

The examination showed restricted movements of legs and knees and reduced dorsal and plantar-flexion of the right foot. The

THE INTERNATIONAL SOCIETY for FLUORIDE RESEARCH

P.O. BOX 692 WARREN, MICHIGAN 48090

SPECIAL 4th CLASS RATE: BOOKS

RETURN POSTAGE GUARANTEED