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Korpiha, Finland

K.A.V.R. Krishnamachari, M.D.
National Institute of Nutrition
Hyderabad, India
EDITORIAL
Reliability of the Double-Blind Test in Fluoride Poisoning ........................................ 43

ORIGINAL ARTICLES
Morphometric Measurements in the Diagnosis of Fluorotic Changes in the Long Bones, Part I. The Forearm - by E. Czerwinski, Cracow, Poland ................ 46

Morphometric Measurements in the Diagnosis of Fluorotic Changes in the Long Bones. Part II. The Lower Leg - by E. Czerwinski, Cracow, Poland .... 51

Comparison of the Biochemical Mechanisms of Growth Retardation Caused by Fluoride and Ozone - by C. W. Chang, Phoenix, Arizona ................. 55

The Effects of Fluoride on the Growth and L-Ascorbic Acid Levels of Tissues from the Domestic Chicken (Gallus Domesticus) - by M. H. Yu and C. J. Driver, Bellingham, Washington ................... 60

The Effect of Fluoride on the Mineral Composition of Polluted Fir Needles (Abies alba Mill.) - by J. P. Garrec, R. Plebin and A. M. Lhoste, Grenoble, France. 68


ABSTRACTS

Reported Adverse Effects in Caries Preventive Use of Fluorides in Norway - by P. Lokken and C. F. Borchgrevink, Oslo, Norway .......................... 100


Magnesium-Fluoride Interrelationships in Man I. Effect of Fluoride on Magnesium Metabolism - by H. Spencer, L. Kramer, E. Wiatrowski, and D. Osis, Hines, Illinois .... 104

Changes in Urinary Ion Excretion and Related Renal Exposure Activities in Fluoride-Treated Rats - by Y. Suketa and E. Mikami, Shizuoka, Japan .......... 106

Incidence of Periodontal Diseases in Subjects with Various Degrees of Exposure to Fluorides - by E. Domzańska, Szczecin, Poland .......................... 107

BOOK REVIEW

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.

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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.

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   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.
The International Society for Fluoride Research will
hold its Ninth Conference in Fribourg, Switzerland,
July 23-25, 1978. Abstracts (up to 300 words) of
papers on any phase of fluoride research should be
submitted before May 15 to the I.S.F.R., Program
Committee, Box 692, Warren, Michigan 48090 U.S.A.
For hotel reservations notify Dr. Ben van Toledo,
Université de Fribourg, Institut de Biologie Animale,
Fribourg, Switzerland, indicating how many are coming,
whether a single bed or two beds are needed and for
how many nights.
EDITORIAL

RELIABILITY OF THE DOUBLE-BLIND TEST IN FLUORIDE POISONING

Acute fluoride intoxication can be readily documented by the presence of fluoride in the gastrointestinal tract, by assays of blood and urine, and by a low serum calcium level. To substantiate the diagnosis of chronic fluoride poisoning, especially prior to the onset of the typical bone and dental changes, is a much more difficult task, because no definite diagnostic criteria are available. An elevation of the 24-hour urinary fluoride content suggests a high fluoride uptake but does not prove fluoride toxicity except when extremely high. Serum fluoride is even less reliable since the body's tendency to homeostasis promptly levels off any sudden elevation of fluoride in the blood. Serum calcium, phosphorus, and alkaline phosphatase, although occasionally indicative of chronic fluoride intoxication, are usually erratic and do not provide a definite clue concerning the degree of chronic poisoning. An iliac crest biopsy which shows an elevated level of fluoride is somewhat more conclusive. However, most patients refuse to undergo this procedure for diagnostic purposes.

Waldbott (1) has made use of a double-blind test originally recommended by the Journal of the American Medical Association as follows: After recovery due to avoidance of F⁻ water and food high in F⁻, the patient is given 3 identical bottles of water labelled #1, 2, 3; two contain plain distilled water, the third 1 mg of F⁻ (2.2 mg NaF) per tablespoon, the daily dose recommended for prevention of tooth decay. He is instructed to take one tablespoon before breakfast from bottle No. 1 for one week, from bottle No. 2 the second week, and from bottle No. 3 the third week. Neither the physiciant nor the patient, only the pharmacist, knows which bottle contains fluoride.

This test was modified by Grimbergen and Moolenburgh (2). The pharmacist who prepared the test solution and the placebo was required to state their contents in a sealed envelope to a notary public, so that neither the patient nor the physician had any way of knowing which solution contained the fluoride. Nevertheless, only 50 percent of the suspected cases responded positively to the tests.

At a Norwegian Poisoning Center P. Løkken and C.P. Borchgrevink devised another double-blind test (3). After hospitalization they placed the patients on tablets without fluoride for two days; then for two days each on tablets without taste and without color, and on fluoride-containing tablets. When they found that a 7-year-old child failed to react to the fluoride-containing tablets they concluded that the child's disease was unrelated to fluoride. Minor lesions of stomatitis appeared in the mouth of a second child after taking placebo tablets which again suggested to the authors that fluoride was not involved in the child's illness. Indeed, in the 34 cases brought to the attention of the Poisoning
Center because of suspected poisoning this diagnosis was dismissed. Only six were subjected to further studies and in the two who underwent the double-blind test the results were inconclusive. This failure to recognize poisoning among persons who were obviously made ill by fluoride makes it necessary to scrutinize the reliability of the double-blind test.

Fluoride intake from sources other than the test dose, i.e. from food, water, and through inhalation is unpredictable and uncontrollable. There are wide variations in the fluoride content of food. The patient's resistance to a toxic agent varies from day to day, a fact which has been repeatedly demonstrated in other kinds of chronic poisoning such as intoxication by nitrogen oxides and sulfur dioxide. The amount of fluoride present in the body prior to the tests is another factor which undoubtedly enhances or detracts from the responsiveness to a fluoride test dose. Furthermore, in our daily diet we are ingesting numerous food additives which may interfere either synergistically or antagonistically with the test, in view of the great reactivity of the fluoride ion. Even removal of a patient to a hospital changes his environmental milieu to such an extent that his response to fluoride might be considerably altered.

In other words, the double-blind test like most other diagnostic tests, is helpful in documenting a case of fluoride poisoning, but it cannot be considered a sine qua non.

That this is true is clearly demonstrated in the article by Løkken and Borchgrevink who deny a relationship to fluoride intake in 33 out of 34 patients who were made ill following consumption of fluoride tablets and after mouth rinses with fluoride solutions. As is often the case when physicians are unable to diagnose an illness they seek alternate explanations for it. The Norwegian investigators attributed the illness to a psychosomatic cause or to the presence of otherwise harmless substances in the tablets.

The fallacy of relying solely on sophisticated methods of diagnosis rather than on a careful clinical appraisal of a case is clear. Dentists and physicians in general believe that fluoride is a harmless agent and, therefore, become complacent about its toxic action. An example of this attitude is the death of a 3-year-old Brooklyn, New York child who was asked to rinse his mouth with a 4% NaF solution (4), or the poisoning followed by an 8-day hospitalization of a dental health officer in New Zealand who, in order to demonstrate its harmlessness (5), swallowed an undisclosed amount of fluoride, at a political gathering.

G.L.W.
Bibliography

MORPHOMETRIC MEASUREMENTS IN THE DIAGNOSIS
OF FLUOROTIC CHANGES IN THE LONG BONES
PART I. THE FOREARM

by

E. Czerwinski
Cracow, Poland

SUMMARY: The fluorotic changes in the long bones of
95 aluminum workers were calculated by morphometric
measurements. The workers' age averaged 51.2 years
and their exposure to atmospheric fluorides 18.1
years. The forearms were X-rayed in supination and
compared with measurements of a control group of 30
non-exposed workers. The maximum cortical thickness
of the radius and ulna was determined by the author's
method. The cortical indices were then calculated.

The mean values of the indices of the radius and
ulna in the group of aluminum workers were significa-
cantly higher than those in the control groups. Mor-
phometric measurements of the bones of the forearm may
facilitate the diagnosis of osteofluorosis.

Skeletal changes are one of the basic criteria of chronic in-
toxication from fluoride compounds. These changes are established by
radiograms of the axial skeleton and the limbs (1,3,5,8,10). Some au-
thors emphasize the great value of changes in the long bones (1,5,8).
Since radiograms serve as an indicator of the bone structure, they may
find application in screening tests of workers exposed to fluorides.

Fluorotic changes, especially if they are not advanced are of-
ten difficult to diagnose on the basis of routine evaluation of radi-
ograms, which often vary due to technical conditions related to exposure
and development of the films. Therefore, in order to arrive at an ob-
jective evaluation and to eliminate divergences in the subjective vis-
ual evaluation of radiograms, we attempted to apply morphometric me-
ths.

The purpose of the current study was to assess the usefulness
of morphometric measurements of the bones of the forearm in the diag-
nosis of osteofluorosis.

From the Orthopedic Department, Cracow Academy of Medicine, Poland.
Presented at the 8th Conference of the International Society for Fluor-
Material and Method

Studies were carried out on a group of 95 aluminum factory workers, ranging in age from 37 to 69 (average 51.2), with a period of exposure ranging from 8 to 24 (average 18.1) years. Of these, 88 or 92.6 percent worked in the electrolysis department. A control group chosen according to age consisted of 30 manual workers not exposed to fluoride compounds, who had been X-rayed because of trauma to the upper extremities. These patients had not previously shown any evidence of bone and joint disease or any essential disturbances in other organs.

Radiograms of the forearms in supination, including the elbow and the radiocarpal joint, were made in all cases of both groups. On the assumption that osteogenetic processes predominate in osteofluorosis (3,7), in contrast to the progressive bone loss of osteoporosis (2,6), a suitable method of evaluating fluorotic changes was worked out. In numerous reports on osteoporosis the high sensitivity of the cortical indices of the long bones has been demonstrated on the basis of the measurement of the minimal thickness of the cortical bone (2,6,9). In evaluating fluorotic changes, therefore, the maximal thickness of the cortical bone was measured, and from this the corresponding cortical indices were calculated. The measurements were made on radiograms of right forearms by means of a typical sliding calliper with a possible accuracy of measurement up to 0.2 mm. The maximal thickness of the shaft (AB) and the minimal width of the medullary cavity (CD) were measured halfway along the radius and ulna over a range of ±5 cm (Fig. 1). The cort-

Figure 1
Measurement of the Maximum Cortical Thickness

![Diagram of the measurement of the radius and ulna]

\[ R \text{ - length of the radius} \]
\[ CIR \text{ - cortical index of the radius} \]
\[ CIU \text{ - cortical index of the ulna} \]

\[ \frac{1}{2}R \text{ - length of the radius} \]
\[ CIR \text{ - cortical index of the radius} \]
\[ CIU \text{ - cortical index of the ulna} \]

\[ AB-CD = \text{cortical index} \]
tical indices (CI) were then calculated according to the formula:
\[ \frac{AB - CD}{AB} = CD \]
By this method of measurement the cortical indices of
the forearms were determined in the group being studied
and in a control group.

Results

The mean values of the cortical indices for the radius and ulna
in the group under study were significantly higher than in the control
group (Fig. 2). Cortical index values of the radius (CIR) were 0.6963
\( \pm 0.0729 \) in the study group and 0.6467 \( \pm 0.0692 \) in the control group;
cortical index of the ulna (CIU), in the study group 0.7294 \( \pm 0.0858 \);
in the control group 0.6576 \( \pm 0.1007 \). The higher values of the cortical
indices in the group of aluminum workers provide evidence of an increase
in the mass of the bones examined. Because mechanical factors such as
overstrain may also lead to hypertrophy of the diaphysis in the long
bones (11), the relation between the cortical indices and the type of
work was also analyzed. We distinguished between the various groups of
workers, i.e. electrolyzer operators (who are under the greatest strain),
anode operators, other workers in the electrolysis department, and those
employed in other departments. No significant differences were found
between these groups of workers (4). Therefore, the mechanical factor
seems to have played no important role in the causation of the changes
observed; nor was there any relation between the cortical indices and
the length of exposure in selected groups exposed for 10 - 15 years, 15
- 20 years, and over 20 years (4). Therefore, factors other than fluo-
ride compounds may have influenced the occurrence of changes. It seems
probable that fluoride compounds and mechanical factors have a syner-
gistic action.

Figure 2

Mean Values for the Cortical Indices of the Radius
and Ulna in Investigated and Control Groups

\[ p < 0.01 \]

\[ p < 0.01 \]

Volume 11 Number 2
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The finding of significant relations between the indices and the ages of those examined in the age groups < 45, 46-55, and > 55 years is noteworthy (4). In patients 46-55 years of age the value of the cortical indices of the radius and ulna is significantly lower as compared with the other age groups. In this group typical fluorotic changes such as osteosclerosis and alterations in bone structure also appeared with significantly lower frequency (4). Figure 3 presents the cortical indices in the forearm of an aluminum worker (B) and of a control case (A).

Conclusions

In a group of aluminum factory workers the cortical indices of the radius and ulna were found to be significantly higher than in controls. Morphometric measurements of the bones of the forearm may be of diagnostic value in evaluating fluorotic changes (Fig. 3).

Figure 3
Radiograms of Forearm of Patient from Control Group (A) and the Forearm of an Aluminum Worker After 20 Years' Exposure

(A) = normal
(B) = fluorotic

Their values of cortical indices are: (A) - CIR = 0.5932, CIU = 0.6408;
(B) - CIR = 0.7819, CIU = 0.8282.
Bibliography


Discussion

Dr. Jenkins: Do you think fluoride is acting on fibroblasts or acting chemically on collagen fibers?

Dr. Czerwinski: Whether fluoride is acting on fibroblasts or on collagen fibers remains to be established.

***************
MORPHOMETRIC MEASUREMENTS IN THE DIAGNOSIS
OF FLUOROTIC CHANGES IN THE LONG BONES
PART II. THE LOWER LEG

by

E. Czerwinski
Cracow, Poland

SUMMARY: Radiograms of the legs, including the knee joint, were made of 50 aluminum workers (average age 50.1 years) whose average duration of exposure to fluoride was 18.2 years. A control group consisted of 40 workers not exposed to fluoride. The maximum cortical thickness in selected sites of the metaphysis and diaphysis of the tibia was determined. The cortical indices of the metaphysis and diaphysis were calculated. The measurements of the fibula were not taken into consideration on account of the great variability in the diameters.

The mean values of the cortical indices of the metaphysis and diaphysis of the tibia were significantly higher in the aluminum workers compared with the control group. The diagnostic value of measurements of the tibia in evaluating fluorotic changes in the long bones is emphasized.

In view of the usefulness of radiograms of the long bones in the diagnosis of fluorosis (1-5), we desired to devise a method for an objective evaluation of the findings. For this purpose we utilized morphometric measurements, which have been applied to osteoporotic changes in the long bones (3,6-8). The problem and a method of measuring the bones of the forearm were outlined in Part I. In the current paper we discuss the results of studies on the application of morphometric measurements in the evaluation of fluorotic changes in the lower legs.

Material and Method

A group of 50 aluminum factory workers aged from 42 to 63 (average 50.1 years) was studied. They had worked in an aluminum factory for 10 to 27 years (average 18.2 years). A control group consisted of 40 subjects selected from manual workers similar in age to those studied but not exposed to fluoride compounds. Radiograms of the patients

From the Orthopedic Department, Cracow Academy of Medicine, Poland.

were made because of injuries to the lower limbs. Previously they had had no complaints referable to the bones or joints or significant disturbances in other organs. Radiograms of the lower leg in the A-P position, including the knee joint, were made in all cases in both the studied and the control groups. On the right tibia, separate measurements were made of the metaphysis and diaphysis. We determined the indices on two levels because radiograms of the lower legs in adults do not usually include the entire lower leg because of the size, and also because in everyday practice radiograms of the knee joint are more often taken from above the metaphysis of the tibia.

Measurements at the metaphysis were made at a distance from the spaces of the knee joint equal to the width of the metaphysis (E), while the measurements at the diaphysis were made halfway down at the site where the cortical bone was thickest (Fig. 1). In these places, the maximal thickness of the bone (AB) and the minimal width of the medullary cavity (CD) were determined with a typical sliding caliper, similar to the measurements in the region of the forearm (Part I). The corresponding cortical indices were calculated by means of the formula: \[
\frac{AB - CD}{AB} = CD
\]

We made no morphometric measurements of the fibula because of the great variability in its parameters, both in the group studied and in the controls.

Figure 1
Measurements of the Maximum Cortical Thickness

The cortical indices for the metaphysis (CIM) and diaphysis (CID) of the tibia in the group investigated were significantly higher than those in the control group. These values were for the index of metaphysis of the tibia: group studied = 0.2902 \pm 0.1107 and, control group = 0.2020 \pm 0.0471. The cortical index of the diaphysis of the tibia: group studied = 0.6181 \pm 0.1143 and, control group = 0.5500 \pm 0.0877.
Many authors report thickening of the cortical bone of the long bone shafts in osteofluorosis (1, 5, 7), but no mention is made in the available literature of morphometric evaluation of radiograms of these bones, whereas morphometric measurements in the evaluation of microscopic changes in osteofluorosis have been reported (2, 9).

We also tried to determine what part mechanical factors may have played in hypertrophy of the diaphysis of the long bones. All the
material was divided according to the extent of exposure to overstrain, i.e. electrolysis operators and other workers. Furthermore, the values of the cortical indices in groups with different periods of exposure have been compared, namely those below and above 18 years, as well as those for younger and older subjects, namely below or above 55 years. No significant relations were found by statistical methods between the cortical indices and the nature of the work, the duration of exposure, or the age of those examined (4).

The absence of any linear relationship between the values of the cortical indices and the duration of exposure may be due to the part played by additional factors influencing conditioning the toxic action of fluoride, such as the importance of personal immunity, the state of nutrition, etc. (5,7).

Conclusions

Significantly higher values of the metaphysial and diaphysial cortical indices of the tibia were found in a group of aluminum factory workers as compared with a control group. Morphometric measurements of the bones of the lower leg may be of use in the diagnosis of fluorotic changes in the bones.

Bibliography

1. Andreyeva, T.D. and Girskaya, E.Y.: K rientgienodiagnostike kostnoy izmienieniy pri fluorozye u rabochih kriolitovyh i alumi-
niewih zawodov. Voprosy Gigieny i Profissyonalnoy Patologii v Tsvietnoy i Chernoy Metallurgii, Sverdlovsk, 1971, pp. 52-54.
3. Barnet, E. and Nordin, B.: The Radiological Diagnosis of Osteo-
Environment on the Occurrence of Changes in the Long Bones. Doc-
7. Jolly, S.S.: Hydric Fluorosis in Punjab. Fluoride in Medicine, 
8. Meema, H.E.: The Combined Use of Morphometric and Microradio-
scopic Methods in the Diagnosis of Metabolic Bone Diseases. 
9. Schenk, R.K., Mertz, W.A. and Reutter, F.W.: Fluoride in Osteo-
porosis. Quantitative Histological Studies on Bone Structure and 
Bone Remodelling in Serial Biopsies of the Iliac Crest. Fluoride 
COMPARISON OF THE BIOCHEMICAL MECHANISMS OF GROWTH RETARDATION CAUSED BY FLUORIDE AND OZONE

by

C.W. Chang
Phoenix, Arizona

SUMMARY: The biochemical alterations induced by fluoride are the disintegration of polysomes into smaller particles. This results in the formation of heterogeneous ribosomal components including a decrease in ribosomal RNA particles. These changes are caused by the enhanced specific activity of ribosomal ribonuclease. The findings supply evidence for the destruction of messenger RNA as one of the factors responsible for the loss of polysomes, the site of protein synthesis.

In contrast, ozone specifically decreases the population of chloroplast ribosomes including polysomes and the level of 23S ribosomal RNA. The reduction in chloroplast ribosomes is caused by the reaction of ozone with sulfhydryl groups of the ribosomal proteins.

Both fluoride and ozone cause growth retardation by influencing the site of protein synthesis and the amount of ribosomal RNA. However, the mode by which each of these air pollutants modifies these metabolic constituents at the site of action differs.

The biochemical mechanisms of growth retardation by fluoride and ozone have never been discussed in air pollution studies. This review article is based upon my publications up to 1975. The mechanisms are explained by presenting the experimental facts obtained from study-

From the United States Department of Agriculture, Western Cotton Research Laboratory, Phoenix, Arizona 85040.
ing the effects of these two air pollutants on the site of protein synthesis and its related metabolities. Growth rate is known to be controlled by the rate of protein synthesis (1), which is closely associated with the content of RNA (2).

The chloroplasts were found to be the site of fluoride accumulation (3). This finding was obtained by fractionating the fluoride-polluted navel orange leaves into their subcellular components. Hexane-carbon tetrachloride mixtures of various densities were used for media. The chloroplasts also are the target which ozone attacks preferentially in pinto bean leaves (4). However, each air pollutant alters differently the site of protein synthesis associated with the growth complex.

Fluoride inhibits root growth (5). Chang (6) investigated the amount and particle distribution of ribosomes and the alterations associated with ribosomal components in fluoride-treated corn roots. Increasing fluoride concentrations caused reduction of the sum of the RNA and protein content of ribosomes to about two-thirds the control level. Also, sucrose gradient sedimentation profiles of ribosomes were determined. The data showed a progressive reduction in the level of ribosomal particles heavier than the 80S monosome with increasing fluoride concentrations. However, the relative number and concentration of monosomes, subunit A and subunit B combined, increased correspondingly. These findings indicated that the sum of monosomes and breakdown products changed at the expense of polysomes, the site of protein synthesis. Reduction of polysome level also reflected absolute decrease in polysome material, because fluoride decreased total RNA and protein of ribosomes per unit fresh weight of roots.

It was also reported that fluoride modified specific activities of subcellular ribonuclease (6). Assays of subcellular distributions of ribonuclease activity in fluoride-treated materials showed that all subcellular components, except the plastid fraction, showed progressive increase in activity with increasing fluoride concentrations. However, ribonuclease specific activity of the microsomal component was increased most by fluoride. Activity of plastid components was not compatible with the fluoride concentrations. The level of this enzyme activity also was not related to that of the soluble fraction. The data indicated a negligible amount of enzyme contamination between the plastid component (presumably also other particulates) and the soluble fraction.

Ribonuclease activity in vivo is known to be directly controlled by the ratio of potassium, calcium, and magnesium ions (7). It is also found that fluoride forms a complex with magnesium ions in inhibition of enolase activity (8). Therefore, fluoride possibly influences ribonuclease activity by altering the ratio of these free ions required for the normal level of this enzyme activity in vivo. The enhanced ribonuclease specific activity would be due to enzyme activation by fluoride. The findings supply evidence for the destruction of messenger RNA as one of the factors responsible for the loss of polysomes.
Using sucrose density gradient, Chang (6) analyzed the size distribution of ribosomal RNA from fluoride-treated corn roots. The control ribosomal RNA had two major peaks, the first peak of heavier ribosomal RNA particles, 23S, and the second peak of lighter ribosomal particles, 16S. Fluoride caused a fall in the first peak and a rise in the second one, which was spread over a wide range beyond the 16S position. Such results indicated that the increase in ribonuclease activity by fluoride degraded the first peak and induced the second peak of heterogeneous ribosomal RNA particles. Detailed results from research on the effects of fluoride on growth have appeared elsewhere (9,10).

Ozone is a major phytotoxicant which acts as an oxidant in the photochemical smog complex. A study was made of cytoplasmic and chloroplast ribosomes in the primary leaves of pinto bean plants exposed to ozone (3). The isolated ribosomes were analyzed by sucrose density gradient. Ozone at the levels of 0.35 ppm for 20 to 35 min did not change the concentrations of various sedimenting particles of the cytoplasmic ribosomes. Ozone at similar levels, however, specifically decreased the population of chloroplast ribosomes per unit fresh weight of leaves. The distribution pattern of these chloroplast ribosomes was characterized by the low concentration of the fast-sedimenting polysome particles concomitant with the low magnitude of other slow-sedimenting components. The kinetics of ribosome populations during leaf growth demonstrated that ozone did not influence the daily levels of the different ribosomal components of cytoplasmic ribosomes. However, ozone prematurely decreased the concentrations of polysomes and other components of chloroplast ribosomes below control level at the early stage of leaf development.

Ozone (an oxidant with a redox potential of +2.07V) is reported to oxidize a number of amino acid residues (11), including the sulfhydryl group (12) of proteins. Cysteine is most susceptible to ozone in comparison with other amino acids in aqueous solution (11). Ozone may destroy the integrity of polysome particles in pinto bean leaves by the reaction with the sulfhydryl group of ribosomal proteins. This possibility was successfully demonstrated in later studies (13).

Chloroplast ribosomes and cytoplasmic ribosomes were isolated from the primary leaves of control and ozone-treated pinto bean plants. On the basis of a unit of ribosomal protein, the levels of ribosomal sulfhydryl groups and their kinetic responses to 5,5'-dithiobis(2-nitrobenzoic acid) were assayed by Ellman's procedure in the presence of sodium dodecyl sulfate. The sedimentation profile of ribosomes was determined by sucrose density gradient. Ozone, 0.30 ppm for 20 to 50 min, decreases the levels of sulfhydryl groups in chloroplast ribosomes far more than in cytoplasmic ribosomes. Both ozone and p-chloromercuribenzoate directly dissociate polysomes and monosomes of isolated chloroplast ribosomes into smaller particles in vitro. The levels of sulfhydryl groups which react with 5,5'-dithiobis(2-nitrobenzoic acid) are higher in chloroplast ribosomes than in cytoplasmic ribosomes. The reac-
tion with 5,5'-dithiobis(2-nitrobenzoic acid) of this higher level of sulfhydryl groups in chloroplast ribosomes goes to completion much faster than the reaction of the lower level of sulfhydryl groups in cytoplasmic ribosomes (13). Such different observations in the presence of sodium dodecyl sulfate indicate that the different responses of the two classes of ribosomes to ozone may be attributed to the different amino acid residues of their ribosomal proteins and/or their different geometric configurations.

The maintenance of the polysome complex is reported to require energy (14). The influence of ozone on the polysome population of chloroplast ribosomes could also be mediated through a nonribosomal factor of impaired energy production. This assumption was supported by the study of effects of ozone on photosystem II in chloroplasts (15).

To determine the influence of ozone on the photochemical activity of photosystem II, spinach plants were exposed to 0.35 ppm ozone for 50, 60, 70, and 80 min. The leaves were harvested immediately after treatment in an effort to determine the reaction rate before visible leaf symptoms developed. Ozone curtailed the activity of the Hill reaction, but the rate was never inhibited more than about 45% of the control level, regardless of the length of treatment. Inhibition was maximal within about 10 min after ozone began to affect the activity (after 60 min). A similar response was also observed in the chloroplast ribosomes of pinto bean leaves exposed to ozone (4). These results indicate that the restricted supply of energy could be one of the factors for disintegration of chloroplast polysomes, since photosystem II is coupled with photophosphorylation responsible for ATP production.

A study was made of the relationship between the level of ribosomal RNA and the rate of growth in pinto bean plants treated with 0.35 ppm ozone for 20-40 min (16). Cytoplasmic ribosomes and a fraction containing both cytoplasmic and chloroplast ribosomes were isolated from primary leaves. The 80S monomers from pinto bean cytoplasm are made up of 25S and 16S ribosomal RNA components, whereas the corresponding values for ribosomal RNA of 70S chloroplast ribosomes are 23S and 16S. However, the ribosomal RNA sample prepared from chloroplast ribosomes contained three peaks at 25S, 23S, and 16S. The 16S peak was assumed to contain both cytoplasmic and chloroplast ribosomal RNA fractions. Ozone decreased the relative peak height of 23S ribosomal RNA as compared with the constant level of 25S ribosomal RNA. The decrease in 23S particles was related to the rate of growth inhibition which occurred in ozone-treated pinto bean leaves.

Bibliography


Discussion

Dr. Susheela: Fluoride inhibits protein synthesis and polyribosomes are decreased.

Dr. Lovelace: It is possible that the effect is due to fluoride pulling out magnesium ions.
THE EFFECTS OF FLUORIDE ON THE GROWTH AND L-ASCORBIC ACID LEVELS OF TISSUES FROM THE DOMESTIC CHICKEN (GALLUS DOMESTICUS)

by

M.H. Yu and C.J. Driver
Bellingham, Washington

SUMMARY: The growth of organs and the content and distribution of ascorbic acid in tissues have been studied in growing chicks fed a diet supplemented with 150 ppm of fluoride (as NaF). No differences in the body weight were observed between the control and the fluoride-treated birds at the end of 4 weeks, but the experimental birds showed a sharp increase in the weight of tibia and pectoralis and a decrease in the size of the comb. A slight weight gain was also shown in the gizzard, heart, kidney, and liver in these birds. The fluoride treatment caused a marked decrease in the ascorbic acid concentration in the heart, spleen, brain, gizzard, pancreas, and pectoralis, while the level was elevated in the lung and kidney. The experimental data suggest that supplementary fluoride intake by growing chicks even at the 150 ppm safe level can cause marked physiological and biochemical changes including changes in tissue ascorbic acid metabolism.

Introduction

Ascorbic acid is widely distributed in the tissues of most animals and plants. Its ability to participate in many biochemical reactions is well known. It is an antioxidant and the ascorbic-dehydroascorbic system is involved in biochemical oxidation/reduction reactions. It has been well established that the vitamin participates in the hydroxylation reactions of lysine and proline into collagen (1). Recent work by several research workers has related the activity of ascorbic acid to the enhancement of the formation of cyclic-AMP and corresponding physiological effects (2,3).

It has been known for a number of years that a similarity exists between the symptoms of fluorosis and those of avitaminosis C. Among children living in an industrially polluted area, vascular fragility, hemorrhage, and dental fluorosis were found to be most pronounced in those suffering a vitamin C deficiency (4). Phillips and Chang (5) reported the

From the Huxley College of Environmental Studies, Western Washington University, Bellingham, Washington 98225.

beneficial effect of vitamin C supplements on the survival of rats kept on high intakes of fluoride.

Poultry have been known to be much more resistant to the adverse effect of fluoride than cattle and sheep. Toxic levels of fluoride for chicken have been reported to be at 500 ppm (6) and 1000 ppm (7) with observed reduced growth. Phillips et al. (8) reported that the safe levels of fluoride in the total ration of chicken are between 150 and 300 ppm as NaF. The physiological changes that may occur through fluoride intake within the safe levels have not been thoroughly investigated. In view of the diverse and significant role that ascorbic acid plays in animal tissues, we sought to determine the effect, if any, of a low dose of fluoride on the organ growth and the content and distribution of the vitamin in various tissues of growing chicks. This paper is concerned with some preliminary data obtained in the study.

Materials and Methods

One-day-old White Leghorn cockerels (Gallus domesticus) were used in these experiments. The chicks were divided into two groups. One group, which served as controls, was given the basal diet only, obtained locally from a commercial source. The chicks in another group were fed the same diet supplemented with 150 ppm of fluoride, as NaF. The feed and water were given ad libitum. The birds were maintained in brooder cages in an air-conditioned room on a 12:12 light cycle. At the end of 4 weeks five birds from each group were sacrificed, and the soft tissues were removed, weighed, and kept at -10°C until use. The organs were pooled in order to obtain an adequate amount of tissue for analysis.

For the ascorbic acid determination, the organs were ground with a volume of the extraction medium consisting of acetic acid-meta-phosphoric acid (9). The extracts were strained through layers of cheesecloth and centrifuged. The concentration of ascorbic acid in the supernatant was then determined by titration with 2,6-dichlorophenolindophenol. The ascorbic acid contents were expressed as mg per 100g tissue weight.

Results

Under the experimental conditions, the weight gains of both the controls and the fluoride-treated birds were essentially linear following the first week of relatively slow growth period. Treatment with fluoride at the concentration used (150 ppm) did not appear to alter the total body weight of the birds compared to those fed a normal diet (Fig. 1). However, a retarded growth of the combs and tails was exhibited by the fluoride-treated birds. When 17-day-old chicks were examined, 70% of the controls had combs whose size was at or above 9 mm x 20 mm, whereas only 30% of the combs of the experimental birds attained this size. Their tails also showed a 25% reduction in length.
(Table 1).

**Figure 1**
Body Weight of Control and NaF Treated Chickens

![Graph showing body weight comparison between control and NaF treated chickens.]

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>NaF</th>
<th>Percent of Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>154&lt;sup&gt;b&lt;/sup&gt;</td>
<td>158</td>
<td>102.6</td>
</tr>
<tr>
<td>Tail length, mm</td>
<td>20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15</td>
<td>75.0</td>
</tr>
<tr>
<td>Percent of chicks with combs ≥ 9 mm x 20 mm</td>
<td>70</td>
<td>30</td>
<td>42.8</td>
</tr>
</tbody>
</table>

<sup>a</sup>The 17-day-old chicks were used in this experiment.
<sup>b</sup>Values are the average of 10 birds.

Fluoride treatment did not affect the weight of the tissues of the lung, bursa, pancreas, and spleen, whereas those of the gizzard,
heart, kidney, and liver showed a slight increase. There was a marked gain in the weights of the tibia and pectoralis, the increases over the controls being 40% and 23%, respectively (Table 2). The effect of fluoride on the weight of various tissues was further demonstrated by the data showing the ratio of tissue weight to body weight. Figure 2 presents the weight of tissue as percent of body weight.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Weight, g</th>
<th>Percent of Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>1.94a</td>
<td>1.91</td>
</tr>
<tr>
<td>Bursa</td>
<td>1.69</td>
<td>1.76</td>
</tr>
<tr>
<td>Gizzard</td>
<td>10.41</td>
<td>11.32</td>
</tr>
<tr>
<td>Heart</td>
<td>2.33</td>
<td>2.50</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.71</td>
<td>3.09</td>
</tr>
<tr>
<td>Liver</td>
<td>8.23</td>
<td>9.01</td>
</tr>
<tr>
<td>Lung</td>
<td>2.23</td>
<td>2.31</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.88</td>
<td>0.96</td>
</tr>
<tr>
<td>Pectoralis</td>
<td>8.30</td>
<td>10.23</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.49</td>
<td>0.51</td>
</tr>
<tr>
<td>Tibia</td>
<td>2.51</td>
<td>3.51</td>
</tr>
</tbody>
</table>

aValues are average of 5 organs.

Figure 2

Ratio of Tissue Weight to Body Weight of Control and NaF-Treated Chickens
The variation in the ascorbic acid concentrations of various tissues is marked. Ascorbic acid levels in the growing chicks were high in the bursa, liver, and pancreas, followed by the spleen, brain, and kidney but they were low in the lung, heart, and pectoralis. The birds treated with fluoride showed a sharp decrease in the ascorbic acid levels in the brain, gizzard, heart, spleen, pancreas, and pectoralis, ranging from 12% to 42%. In contrast, the vitamin levels of the lung and kidney were elevated 66% and 17%, respectively (Fig. 3).

**Figure 3**
Comparison of Ascorbic Acid in Tissues of Control and NaF-Treated Chickens

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Ascorbic acid levels of tissues in fluoride-treated chickens.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td></td>
</tr>
<tr>
<td>Bursa</td>
<td></td>
</tr>
<tr>
<td>Gizzard</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td></td>
</tr>
<tr>
<td>Pectoralis</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
</tr>
</tbody>
</table>

(%) of control 0 50 100 150 200

**Discussion**

Our data indicate that at the 150 ppm concentration used, fluoride caused significant changes in the growth of several organs in the growing chicks, despite the fact that virtually no differences occurred in the total body weight between the normal and fluoride-fed birds (Fig. 1). Absolute weight gains were shown in the gizzard, kidney, liver, tibia, and pectoralis (Fig. 2). Ramseyer et al. (10) reported the presence of hypertrophy and hyperplasia in the renal tubules of rats receiving low dosages of fluoride in the drinking water. Such lesions as these may account for the increased weight in the kidney and other tissues found in the experimental birds. In contrast, a 25% reduction in the tail length and a marked reduction or delayed growth in the comb were noted (Table 1). It appears that the weight gains and losses occurring in several tissues may somehow compensate each other with the result that no net differences in the body weight could be observed be-
between the normal and the experimental birds. It should be emphasized that the concentration of fluoride used in this study is within the "safe" level for the chicken, as reported by other researchers (8).

In addition to the tissue weight changes, several kinds of tissues in the fluoride-fed birds showed a sharp decrease in the ascorbic acid concentration. The three muscle tissues, the heart, gizzard, and pectoralis, all increased in fresh weight, but their ascorbic acid contents were markedly lowered (Table 2 and Fig. 3). Depletion of ascorbic acid such as this could be important in view of the possible role that the vitamin plays in the metabolism of avian muscle. Chinoy (11) suggested that ascorbic acid might act as a participant in the energy transfer mechanisms in the muscle fibers of birds.

It has been shown that the ascorbic acid concentration in various parts of the brain is quite high. Ascorbic acid is required in the synthesis of neurotransmitters noradrenaline and serotonin, as well as in the protective association with noradrenaline (12). There seems a general agreement that the greater the mental stress conditions to which individuals are subjected the greater is the requirement for the vitamins (13,14,15). The sharp decrease in the vitamin level shown in the brain of the experimental birds could indicate depletion of the vitamin arising from stresses caused by fluoride treatment. The growth of the comb has been shown to be hormonally controlled (16,17). Since ascorbic acid appears to play a role in the growth of the comb (16), the retarded growth of the latter and the ascorbic acid depletion in the experimental birds may be due, in part, to the changes in the endocrine glands caused by fluoride. That the synthesis of ascorbic acid is affected in the fluoride-treated chicks is suggested by the increased concentration of the vitamin shown in the kidney of the fluoride-fed birds (Fig. 3). Since in chickens the kidney is the organ responsible for the synthesis of ascorbic acid (18), an elevated rate of production by this organ may be necessary in order to meet the increased requirement not only by the kidney itself but also by other tissues that showed a sharp decrease of the vitamin (Fig. 3).

It is of interest to note that both fluoride and ascorbic acid are involved in carbohydrate metabolism. Whereas fluoride has been known to adversely affect several enzymes involved in carbohydrate metabolism, ascorbic acid appears to regulate it (19), although the synthesis of ascorbic acid itself is dependent on the availability of carbohydrates. The marked decrease in the vitamin level in several tissues studied (Fig. 3) could be due to the enhanced requirement for the vitamin caused by fluoride injury at the cellular level.

It should be noted that most of the tissues found to have lowered ascorbic acid level are metabolically highly active ones. Since ascorbic acid appears to be always present in large quantities in tissues of high metabolic activity (20) a depletion of tissue ascorbic

FLUORIDE
acid will no doubt have an adverse effect on a number of cellular processes. A rapid depletion of tissue ascorbic acid may, therefore, play an important part in fluoride toxicity.

Bibliography


**Discussion**

Dr. Waldbott: Regarding gain in weight in fluoride-treated animals, have you considered that fluoride causes an increase in fluid retention?

Dr. Yu: We noted increased weight on the 36th day, and later a 6% increase. Fluid accumulation because of fluoride, could be the reason.

Dr. Runge: Did you notice any changes in behavior of the animals? In Halle a striking feature was that chickens were less active.

Dr. Yu: Indeed, yes we observed behavior differences in those treated with fluoride and those with fluoride and Vitamin C. Those treated with fluoride seemed to be more dull. Fluoride and Vitamin C chickens seem to be more aggressive.

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FLUORIDE
THE EFFECT OF FLUORIDE ON THE MINERAL COMPOSITION OF POLLUTED FIR NEEDLES (ABIES ALBA MILL.)

by

J.P. Garrec, R. Plebin and A.M. Lhoste
Grenoble, France

SUMMARY: The chemical composition of fir needles is altered by the accumulation of fluoride. Our data indicate that fluoride pollution tends to reduce the concentration of Mg and Mn and to increase the concentration of Ca and of F. They also indicate that the fluoride level has no consistent effect on levels of other major nutrient elements, such as N, P and K. Nevertheless, when the content of fluoride in needles reaches 400 ppm, no further depletion of Mg and Mn occur. Above 400 ppm fluoride has no significant effect on the levels of the remaining elements. Moreover, a similar depletion of Mg and Mn in relation to fluoride accumulation is observed and a distinct correlation may be found between these two elements.

Introduction

Many studies have been conducted on how the accumulation of fluoride affects the mineral nutrition of plants. P or K deficiency is believed to be related to elevated fluoride levels (1,2), whereas Mg deficiency tends to reduce such accumulation (2). Deficiencies of Ca and N have no effect (1-4). Brennan et al. (5) believe that an optimal level of Ca, P and N favors fluoride accumulation, whereas a deficiency or imbalance of one of these elements inhibits the uptake of fluoride. McCune et al. (6), on the other hand, have found that mineral nutrition has no effect whatsoever on F accumulation in plants while Holub et al. (7) observed that an increase of Ca, Mg and P favors accumulation of fluoride in pea seeds.

Comparatively little work has been done on the influence of fluoride on the composition of leaves (8-10). Since most of these studies deal exclusively with vegetation subjected to low level fluoride pollution, fluoride produced only minor variations in the different inorganic plant constituents.

From the Laboratoire de Biologie Végétale, Département de Recherche Fondamentale, Centre d'Études Nucléaires, Grenoble, France.

Materials and Methods

We used branches of fir (*Abies alba*, Mill.) taken from the same slope of a valley but at different altitudes, in the vicinity of an aluminum factory which was causing atmospheric fluoride pollution. Both, in these branches and in the atmosphere, the rate of fluoride pollution decreases with increasing altitude. If we assume that the composition of the soil is relatively uniform in this area of the slope, the fluoride concentration in the needles must be considered to be the chief parameter that influences their mineral composition. From these branches, we selected needles of the same age and for the analysis we used solely 3-year-old needles.

In these needles, potassium was determined by flame spectrometry, and magnesium was measured by atomic absorption spectrometry. Phosphorus and nitrogen were determined by means of a Technicon Auto-Analyzer as recommended by Varley (11). Manganese and fluorine were measured by neutron radio activation - manganese by means of thermal neutrons (12) and fluorine by means of neutrons of 14 Mev (13).

Results

Mineral Composition of Soil: At the different sites from which plant material was obtained, variations in soil composition are shown in Table 1.

<table>
<thead>
<tr>
<th>From Soil Extract in Sodium-Acetate pH 4.8 (in mg/l)</th>
<th>From Raw Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>potassium</td>
<td>Organic matter (in %) 15 ± 5</td>
</tr>
<tr>
<td>magnesium</td>
<td>manganese (in µg/g) 859 ± 227</td>
</tr>
<tr>
<td>calcium</td>
<td>pH (H2O) 6.25 ± 0.62</td>
</tr>
<tr>
<td>phosphorus</td>
<td></td>
</tr>
</tbody>
</table>

These data indicate that the composition of the soil may be considered identical for the part of the slope used in our study.

Mineral Composition of the Needles

Magnesium: Figure 1 shows Mg content as a ratio of fluoride concentration in the needles. We find a rapid decline in the Mg content up to a fluoride pollution rate of about 400 µg/g which is followed by relative stability despite the marked increase in fluoride concentration which reached 2,000 µg/g. In areas of high pollution, the magnesium content (800 µg/g) was about half of that of healthy plants (1600 µg/g).
Manganese: Figure 2 represents the manganese content of the needles in relation to their fluoride content. As in the case of magnesium, the manganese content decreases significantly up to a fluorine value of about 400 μg/g. Furthermore, the manganese concentrations also remain constant despite the large increase in fluoride in the needles. The decrease of manganese due to the action of fluorine is about fourfold.

Nitrogen, Phosphorus and Potassium: Figures 4, 5 and 6 represent the fluoride-induced variations in nitrogen, phosphorus and potassium. The concentrations of these three elements undergo only slight variations in spite of the marked increase in the ratio of fluoride in the needles.
Figure 4
Effect of Fluoride on Nitrogen Concentration

Figure 5
Effect of Fluoride on Phosphorus Concentration

Figure 6
Effect of Fluoride on Potassium Concentration
Discussion

In plants, fluoride interferes with many basic physiological processes, probably by inhibiting the respective enzymes involved. These enzymes function with cofactors consisting of bivalent metals which would complex with fluoride. The chief elements studied by us which act as cofactors are magnesium, manganese and calcium. All these elements form more or less insoluble precipitates with fluoride. We have shown that fluoride produces a decrease in magnesium in the needles. McNulty et al. (14) state that fluoride reduces the fraction of ether-soluble magnesium proportionately to the decrease in chlorophylls A and B. Brewer et al. (10) also found in leaves of orange trees a reduction of their magnesium content resulting from fluoride. This decrease in magnesium appears to be related to the degradation of chloroplast structure by fluoride (15); in fact, at cell level, fluoride accumulates mainly in the chloroplasts (16).

With respect to the decrease of manganese resulting from fluoride, Brewer et al. (10) have also recorded a decrease of this element in fluoridated plants. Leonard et al. (8) observed in leaves of lemon trees, that the effect of fluoride was identical to that caused by manganese deficiency. As in the case of magnesium, the decrease in the ratio of manganese, a minor element involved in the functioning of the photosynthesis process (17) could also be related to alteration of the chloroplasts by fluoride. Our results show that, in plants, Mg and Mn concentrations underwent identical changes. In fact, there is a correlation in the concentrations of magnesium and manganese for each level of fluoride ratio. Statistical analysis shows that this correlation is a linear regression (Fig. 3) at the 5 percent level of significance (correlation coefficient 0.873) and thus the equation is as follows: \( Y = -3.83 + 0.760 \times X \). This parallelism may arise from the

Figure 3
Correlation Between Magnesium and Manganese Concentrations

\( y = 0.760x - 383 \)
\( r = 0.873 \)
similar properties of Mg and Mn at enzymatic level. At the beginning and up to an accumulation of about 400 μg/g, fluoride appears to act directly or indirectly on Mg or Mn in proportion to its concentration. Beyond that, when F concentrations in the needles continue to rise, the remaining Mg and Mn would appear to be in forms in which they are not affected by fluoride.

In a previous study (18) we showed that the concentration of calcium doubled chiefly in its oxalate form in highly polluted needles. This phenomenon was observed by Walter-Levy et al. (19) in Chara fragilis Devaux. Abutalybov et al. (20) reported a 6.5-fold acceleration of calcium penetration in wheat roots as an effect of fluoride. Interestingly, changes in fluoride-induced calcium composition are similar to those induced by aging, i.e. an increase in total calcium and in calcium oxalate. This fact had already been demonstrated by Penot et al. (21). However, Brewer et al. (10) in their pollution scale found no effect of fluoride on the concentration of calcium. On the other hand, according to Pack (22), fluoride has the same effect on plants as a calcium deficiency but this might be the result of precipitation of certain essential forms of calcium as CaF₂ (23) rather than that of a decrease in total calcium.

At the level of the three chief constituents of vegetable matter, i.e. nitrogen, phosphorus and potassium, we have shown that fluoride does not appear to have an effect on their respective concentrations. Fluoride does not appear to affect absorption of N, P and K into plants (24). Brewer et al. (10) found only minor fluctuations of N and P caused by fluoride but, on the other hand, noted a marked increase in K. According to McNulty (25) as well as Pack et al. (22), fluoride does not affect the inorganic phosphate content and acid-soluble phosphated compounds in plant extracts. Lamprecht (26) observed no detectable effect of fluoride on the total nitrogen, proteinic or soluble, whereas Chollet (27) found a decrease in soluble nitrogen compensated by an increase in proteinic nitrogen; but he stated, nevertheless, that fluoride did not affect the total nitrogen content. Penot et al. (21) demonstrated that under certain conditions fluoride stimulated the absorption of anions such as P but had no effect on the absorption of cations such as K and Ca.

Conclusion

In plants, heavy fluoride pollution produces a change in mineral composition: concentrations of magnesium and manganese decrease in an identical manner whereas the calcium ratio increases. On the other hand, the principal constituents of vegetable matter, nitrogen, phosphorus, and potassium, do not undergo appreciable variations. Moreover, we have shown that a certain fraction of magnesium and manganese due to its form remains totally unaffected by fluoride.
This effect of fluoride at the level of the mineral composition of plants appears to be due largely to the inactivation of many enzymes through the formation of more or less insoluble complexes with their different cofactors.

Acknowledgement

The authors wish to thank Mrs. A.R. Cooper, for English translation.

Bibliography


Discussion

Dr. Oelschlager: How do you explain the extraordinary loss of magnesium and manganese? Is it accomplished through the destruction of chlorophyll and chloroplast?
Dr. Garrec: I think this is the most likely hypothesis.

Dr. Fluhmer: The fluoride concentrations are high. Did you take samples close to the plant and did you wash your sample?

Dr. Garrec: The samples were taken near the plants and in France vegetable matter is never washed. It could be a source of error, but we were afraid of removing internal minerals.

Dr. Miller: Did you see visible evidence of decrease in chlorophyll?

Dr. Garrec: We did not study this aspect.

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**UPTAKE AND TRANSLOCATION OF FLUORIDE IN HELIANTHUS ANNUUS L. GROWN IN SAND CULTURE**

by

J.A. Cooke, M.S. Johnson and A.W. Davison
Sunderland, Liverpool and Newcastle, U.K.

**SUMMARY:** In Helianthus annuus seedlings grown in sand culture for five weeks the concentration of fluoride in the root and shoot was generally proportional to the concentration in the substrate. Highest concentrations were present in the roots and the concentrations in the leaves decreased acropetally. In a more detailed six week study the dynamics of uptake and translocation differed markedly. Whereas the total fluoride in the plant increased steadily in proportion to increased root dry weight, the amount translocated to the shoot each week was reduced to almost zero after four weeks. The fluoride in the shoot accumulated initially in the most physiologically active leaves. It appears, therefore, that the acropetal pattern of leaf accumulation is a function of leaf age rather than of position on

From the Biology Department, Sunderland Polytechnic, Sunderland, U.K., Botany Department, The University, Liverpool, U.K., and the Department of Plant Biology, The University, Newcastle, U.K.

the stem. The dynamics of accumulation in leaves suggested that a portion of the fluoride in a senescing leaf was retranslocated to younger leaves. Thus although there was an increase in the immobility of the fluoride in the root over the study period, a portion of the fluoride in the shoot could be translocated from older to younger leaves.

**Introduction**

The uptake of fluoride from soils by plants is regarded as a function of the available fluoride in the soil solution (1), of the capacity of the soil to replenish the fluoride in the soil solution when it is depleted (2), and of many other factors including pH, and the amounts of clay and organic matter (3). In normal soils, the total fluoride is usually in the range 20–500 µg/g dry weight (4), with the water-soluble fluoride being 0.1 µg/cm$^3$ or less. Generally, such levels of soil fluoride availability do not lead to fluoride concentrations in plant leaves greater than 2–20 µg/g dry weight (5). Recently leaf concentrations between 200 and 5000 µg/g dry weight have been reported in plants colonizing fly-spar wastes (6) but at these sites the water-soluble soil fluoride was elevated to between 2.4–9.6 µg/cm$^3$ and may reach as high as 26.8 µg/cm$^3$ (7).

The sites of accumulation of fluoride within dicotyledonous plants are generally regarded as decreasing in importance in the order: Root > First leaves > Second leaves > Third leaves (8,9). The higher concentrations in the root relative to the shoot may be used to distinguish between the soil and air as the source of the plant fluoride (10).

Although the distribution of fluoride in field-collected material can be measured (e.g. 6) experimental data concerning the dynamics of fluoride uptake and translocation to the shoot are limited. Interpretation of the dynamics of fluoride uptake is made difficult in studies where there has been a possibility of reduced fluoride availability or changes in its molecular form, external to the root surface (e.g. 11,12). Other experiments using the isotope F$^{18}$ have yielded useful information but are limited to periods of a few hours. Accordingly, experiments have been designed which attempt to follow the dynamics of uptake and translocation during seedling growth of Helianthus annuus.

**Materials and Methods**

Helianthus annuus L. cv. 'Pole Star' fruits were germinated in Petri dishes on filter paper moistened with deionized water and the seedlings transplanted into sand after 5 days. Long Ashton culture solution (14) was given on alternate days to the range of fluoride solutions and the sand was washed through with deionized water between...
treatments to prevent interaction between fluoride and the constituents of the culture solution. Polystyrene containers (Mono Ltd.) were used as pots in all experiments. The treatments were set out in random design and conducted in a growth cabinet. At each harvest shoots and roots were separated and each washed with two or three changes of deionized water. Prior to drying at 60°C the shoots were separated into stems and leaves.

Fluoride was analysed by direct acid extraction of dry, ground plant material and the fluoride determined potentiometrically (15,16).

**Results**

*Helianthus annuus* is a fast growing species and after germination the cotyledons become the first functional photosynthetic organs. The first two or three ranks of true leaves are produced in pairs and thereafter in sets of three. The appearance of a 6 week old *H. annuus* seedling is shown in Figure 1. In this plant the cotyledons are no longer present and the first rank of leaves i.e. the oldest, are senescing. In the following description the leaves are numbered acropetally (i.e. from oldest to youngest) as shown in Figure 1.

**Figure 1**

*Six Week Old Helianthus Annuus Seedling*
The first experiment was conducted to establish the relationship between the concentration of fluoride in the substrate and the concentration of fluoride in the various organs of H. annuus seedlings. Sodium fluoride solutions containing 10, 50 or 100 µgF/cm³ were given twice a week for 5 weeks; the controls received deionized water. There were ten replicates of each treatment and each replicate was harvested and analyzed separately for fluoride.

There were no significant differences in the dry weights or the appearance of the plants with the different treatments. The concentrations in the various tissues are shown in Table 1.

<table>
<thead>
<tr>
<th>Treatment (F in µg/cm³)</th>
<th>Roots</th>
<th>1st leaves</th>
<th>2nd leaves</th>
<th>3rd leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.3 (2.8)</td>
<td>3.8 (3.0)</td>
<td>4.0 (1.4)</td>
<td>3.1 (1.0)</td>
</tr>
<tr>
<td>10</td>
<td>24.4 (4.7)</td>
<td>23.7 (8.0)</td>
<td>16.3 (4.4)</td>
<td>12.1 (3.1)</td>
</tr>
<tr>
<td>50</td>
<td>274 (141)</td>
<td>47.4 (7.1)</td>
<td>29.8 (4.5)</td>
<td>15.0 (1.6)</td>
</tr>
<tr>
<td>100</td>
<td>507 (136)</td>
<td>104 (14.6)</td>
<td>45.5 (5.8)</td>
<td>28.3 (3.9)</td>
</tr>
</tbody>
</table>

The amount of F taken up by the plants was generally proportional to that in the substrate. Thus a doubling of the treatment solution concentrations from 50 to 100 µgF/cm³ approximately doubled the concentrations in the root and in each rank of leaves. In the controls and 10 µgF/cm³ treatment there were similar concentrations in the roots and first leaves. However, the general pattern of uptake, with the concentration decreasing in the following order—root > first leaf > second leaf > third leaf—was similar to earlier work with tomato (9).

Figure 2 shows the concentrations of fluoride in each leaf of a pair of another five replicate plants treated with 100 µgF/cm³ for five weeks. The close correlation between the fluoride concentration in each leaf of a pair emphasizes the importance of leaf position or age in the amount of fluoride which accumulates.

A subsequent experiment with H. annuus was designed to study the dynamics of accumulation of fluoride during early seedling growth. Only one fluoride concentration, 200 µgF/cm³, was used. The controls received deionized water. Five replicates of each treatment were harvested every week for six weeks.

There were no significant differences in the mean dry weights of the controls and the fluoride-treated plants as previously reported.
There were also no significant differences in stem, cotyledon or root growth or in root/shoot ratio. However, there were significant differences in leaf growth (Fig. 3). 200 μgF/cm³ caused premature senescence of the leaves and equivalent leaves did not attain the same dry weight as the controls.

Figure 4 shows the changes in concentration of fluoride (μg/g dry weight) in the various organs during the course of the experiment. The concentration in the root increased until week three when a tendency to a maximum was approached. The concentration in the leaves also reached a steady level, this time after the fourth week. The concentration in the stem dropped markedly after the third week.

Changes in dry weight during growth often can cause wrong interpretation in the changing patterns of accumulation when measured on a dry weight basis. Therefore Figure 5 shows the total amount of fluoride (in μg) in the different organs. Here the pattern is somewhat different. The amounts of fluoride increased steadily in the roots and leaves parallel to growth. The steady increase in total root fluoride is significantly correlated \( r = +.97; p < .01 \) to the increase in root dry weight over the six week period. Because only a relatively small percentage of the fluoride was present in the shoot there was also a
Figure 3
Dry Weight (mg/leaf) of Successive Leaves of Helianthus Annuus
Against Time, With and Without Fluoride (200 µM/cm²)
Treatment.
(Vertical Bars are 95% Confidence Limits)

1st leaf
CONTROL
TREATED

2nd leaf

3rd leaf

Time (weeks)

Dry weight (mg)
Figure 4
Concentration of Fluoride (µg/g dry weight) in the Different Organs of Helianthus Annuus with Fluoride (200 µg/cm³) Treatment

COTYLEDONS

LEAVES

ROOTS

STEM

Volume 11 Number 2
April, 1978
Figure 5
Fluoride (μg) in the Different Organs of Helianthus Annuus with Fluoride (200 μg/cm²) Treatment

- Cotyledons
- Leaves
- Roots
- Stem
significant correlation between root dry weight and the total amount of fluoride in the plant ($r = + .97; p < .01$). Figure 6 shows very little net translocation of fluoride from the roots per week, after week three. The increase in total leaf fluoride after week 3, therefore, is proba-
bly due to the movement of fluoride from the stem which showed a sharp
decrease at this time (Fig. 5), and not translocation from the root.

Figure 7 shows changes in the distribution of fluoride in the
different ranks of leaves during the experiment. The total amount of
fluoride in the first and second leaves decreased after week 5, when
these leaves were losing dry weight (Fig. 3). This suggests that some
fluoride was being retranslocated from the older to younger leaves be-
cause the latter showed an increase in fluoride content with age yet
the total amount of fluoride translocated to the shoot greatly de-
creased (Fig. 6).

In a further experiment the fluoride solution was given for a
two week period, at three different stages of growth. These were: 1)
200 \( \mu g F/cm^3 \) from week 0 to 2, thereafter flushed with deionized water.
2) 200 \( \mu g F/cm^3 \) from week 2 to 4, thereafter flushed with deionized wa-
ter. 3) 200 \( \mu g F/cm^3 \) from week 4 to 6, thereafter flushed with deion-
ized water. These plants were all harvested after six weeks.

Table 2 shows the distribution of fluoride (\( \mu g \)) in the plant
when fluoride was given for two weeks at different times during the ex-
periment.

<table>
<thead>
<tr>
<th>Table 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoride (( \mu g )) in Root and Shoot of H. Annuus</td>
</tr>
<tr>
<td>Treatment Period (weeks)</td>
</tr>
<tr>
<td>Root</td>
</tr>
<tr>
<td>Shoot</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>% in Shoot</td>
</tr>
</tbody>
</table>

200 \( \mu g F/cm^3 \) given for different 2-week periods. All
plants were harvested after the sixth week.

The amounts of fluoride in the root increased as the fluoride
was given at later periods. This tends to confirm that the uptake of
fluoride is proportional to the total root weight or surface area of
the root system. The percentage of fluoride translocated to the shoot
decreased in relation to the delay in giving it. This trend again sug-
gests, as earlier findings (Fig. 6), that a decrease in the rate of
translocation may occur at high root fluoride levels.

The fluoride in the cotyledons and leaves (Table 3) seemed to
reflect their stage of growth and development in that those which were
most rapidly expanding at the time of the fluoride application had the
highest total fluoride.
Table 3
Fluoride (µg) in Cotyledons and Leaves of H. Annuus

<table>
<thead>
<tr>
<th>Treatment Period (weeks)</th>
<th>0-2</th>
<th>2-4</th>
<th>4-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotyledons</td>
<td>3.40</td>
<td>2.06</td>
<td>0.03</td>
</tr>
<tr>
<td>1st Leaf</td>
<td>3.64</td>
<td>7.50</td>
<td>1.63</td>
</tr>
<tr>
<td>2nd Leaf</td>
<td>4.93</td>
<td>7.16</td>
<td>11.22</td>
</tr>
<tr>
<td>3rd Leaf</td>
<td>1.83</td>
<td>2.41</td>
<td>8.19</td>
</tr>
<tr>
<td>4th Leaf</td>
<td>0</td>
<td>.44</td>
<td>.76</td>
</tr>
<tr>
<td>5th Leaf</td>
<td>0</td>
<td>.09</td>
<td>.27</td>
</tr>
</tbody>
</table>

Fluoride (200 µg/cm³) given for different 2 week periods. All plants were harvested after the sixth week.

Discussion

The uptake of salts by roots can be regarded as a chain with four links: the movement of salts in the soil to the root surface; uptake into the root; transport across the root to the xylem; and the movement in the xylem to the shoot (18). This paper presents results of experiments concerned with the last three links in the chain with respect to fluoride.

The uptake into the H. annuus root was proportional to the concentration of ionized fluoride in the substrate (Table 1) and the length of exposure to one particular concentration (Fig. 5). However, there was a tendency for a maximum root concentration to be reached at around 4000 µF/g dry weight when exposed to 200 µF/cm³ fluoride solution. These data are generally consistent with previous work with barley (19) which showed that fluoride uptake was passive in response to a concentration gradient and was not affected by anaerobic conditions. Venkateswarlu, Singer and Armstrong (19) also indicated that 98.6% of the fluoride taken up was in the water-extractable fraction, 1.1% in the exchangeable fraction and 0.3% in the non-exchangeable fraction. This was compared with chloride where the non-exchangeable fraction was 80%. These results suggest that the majority of the fluoride was restricted to free diffusion into cell walls and intercellular spaces (i.e. the free spaces or apoplas) and little fluoride passed into the cytoplasm through the plasmalemma (i.e. into the symplasm). The centripetal transport of fluoride across the root is probably, therefore, in the apoplas. However, some symplasmic transport, at least across the endodermis, is regarded as necessary for translocation to the shoot (20). Thus there may be a regulatory mechanism at the endodermis restricting the entry of fluoride into the cytoplasm. This would explain the different dynamics of root uptake and translocation reported here. However, this regulated transfer of fluoride from the apoplas to the symplasm must explain not only the generally low percentage of fluoride translocated to the shoot (e.g. Table 2) but also the seemingly decreasing rate of translocation shown in Figure 6.
Once the fluoride has reached the xylem it is readily translocated to the leaves although in the very young seedling there may be a temporary 'pool' of fluoride in the stem which is depleted with the formation of the leaves (see Fig. 5). The pattern of fluoride distribution in the leaves suggested that it was accumulated in the most physiologically active leaves (Table 3). Therefore the acropetal pattern of decreasing leaf accumulation found in Table 1 and reported elsewhere (8,9) is due to differences in the stages of development. It is likely that the movement to the leaves is passive and in the transpiration stream. If fluoride is present in this stream it could move in the cell walls, which are probably the main pathways of water movement in the leaves (21), without absorption into cells until it reaches the edges of the leaf lamina—the main sites of accumulation of fluoride (9). This accumulation of fluoride in the leaves decreased the maximum dry weight attained before senescence (Fig. 3).

The dynamic pattern of accumulation in the leaves shown in Fig. 7 suggests that not all the fluoride is immobile and permanently fixed in the older leaves but some can be retranslocated to the younger leaves. This is consistent with other work (9,13) which showed that fluoride, when taken up by the leaves as gaseous HF, was translocated to the roots, suggesting movement in the phloem.

Acknowledgements

J.A.C. is grateful for the financial support of the Wellcome Foundation and a grant-in-aid from the Royal Society during the course of this work.

Bibliography

8. Brennan, E.G., Leone, I.A. and Daines, R.H.: Fluorine Toxicity in

FLUORIDE


Discussion

Dr. Carlson: In what way do you believe that fluoride is translocated? Is it connected with or influenced by growth hormones? Or carbohydrates?

Dr. Cooke: I do not know but a portion of it is mobile. I do not know whether it is bound or free. It would be interesting to work on this problem with radioactive fluoride.

Dr. Welsh: Was this a drip culture?

Dr. Cooke: Plants were established in sand, and solutions were applied to the sand in two or three applications of fluoride solution per week.

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Volume 11 Number 2
April, 1978
SOME PHYSIOLOGICAL AND ULTRASTRUCTURAL CHANGES
OF VICIA FABA L. AFTER FUMIGATION WITH HYDROGEN FLUORIDE

by

I. Horvath, A. Klasova and J. Navara
Bratislava, Czechoslovakia

SUMMARY: Fourteen day old plants of Vicia faba L. cultivar Inovec were fumigated by 50 ppb HF concentrations and the effects of HF on photosynthesis, respiration and growth were observed during continual fumigation for the whole testing period (7 days). Preliminary observations on the dynamics of changes in the chloroplast ultrastructure were made as well.

Continuous fumigation of plants by HF resulted in strong inhibition of the photosynthetic activity, and of the growth processes during the whole testing period, even though no visible symptoms of injury were observed on the plants. The ultrastructural observations showed that the inhibition of the photosynthetic activity was accompanied by a step by step destruction of the granal and lamellar membrane system of the chloroplasts.

Observations made 24 hours after stopping the HF fumigation (50 ppb) showed a dynamic repairing of the processes studied. Based on their repair processes, respiration was found to be relatively the most stable process. An average increase of 7.1% in the respiration rate over the whole test period together with a 8.7% decrease in the growth processes, indicated that treated plants compensated in such a way for the increased energy and material demands necessary for the repair processes, within the frame of autoregulation.

Introduction

Published reports on the physiological changes of plants under the influence of fluorides were initiated, first of all, by visually observing injury on plants, growing in the surroundings of industrial enterprises, which emit pollutants of this type. It is well known to biologists that visible symptoms of damage to plants are the consequences of biochemically-physiological injuries already realized, such as struc-

From the Institute of Experimental Biology and Ecology, SAS, Bratislava, Mlynske Nivy 59, Czechoslovakia.
tural changes at the ultrastructural level. A study of these primary changes not only gives information important for the complex evaluation of the role of fluorine within the plant organism, but it can contribute considerably to the knowledge of plant ecology in contaminated regions.

In the current work, the authors studied the dynamics of photosynthetic activity, changes of respiration and of growth processes during continuous fumigation of plants by hydrogen fluoride, and also the possibilities of repair of these processes, after the short-time fumigation had stopped. This information is considered important, especially data on the possibility of repairing physiological processes under conditions of discontinuous fumigation. Such a study can give a realistic picture to the reaction of plants under natural ecological conditions.

**Material and Methods**

*Vicia faba*, cultivar Inovec, was used as the experimental plant. The seeds were imbibed for 12 hours in distilled water, followed by germination on wet filter paper in photographic dishes at a temperature of 25°C for four days. The germinating seeds were selected visually according to the length of their primary roots and, thereafter, transported to the fumigation chambers, where they were further cultivated in half-concentrated Knop’s solution in plastic dishes, which had been adapted to enable continuous circulation of the nutrient solution. The fumigation chambers were air-conditioned for a constant temperature (20°C ± 0.5°C) and a relative air humidity (35 ± 4%). Constant illumination (Booolux) was ensured in the chambers by a panel of luminiscent tubes (80 W) combined with 25 W bulbs to enrich the spectra. Day and night periods changed after 12 hours respectively. A constant stream of fresh air was brought into the chambers ensuring total exchange of the air, 12 times per hour.

The plants were cultivated simultaneously in three fumigation chambers (with an equal number of plants in each of them). One chamber was used for the control plants, the second one for the fumigated plants for 24 hours and the third one for the plants fumigated continuously. For the fumigation of plants with 50 ppb concentrated HF, a fumigation system was used, constructed as described by Thompson and Ivie (1).

Fumigation began in every case 14 days after germination of plants. For the determination of photosynthetic intensity and the respirational rate representative samples of plants were taken always at the end of the night period with an equal number of individual plants (3 to 10) in every case, according to their degree of development. The overground parts of the plants were separated from the roots and placed in the fumigation chamber. The intensity of photosynthesis and respi-
ration, respectively, was determined in a closed circle by an infrared-analyzer (INFRALYT III), according to the methods described in detail by Jesko (2). The assimilating chamber was placed in a fourth fumigation chamber, ensuring conditions for measuring the photosynthesis rate practically equal to those of plants growing in the fumigation chambers.

The activity of the growth processes was evaluated by determining the increase in dry weight of the overground parts. Regular, representative sampling during the whole testing period (7 days) enabled us to evaluate the relative growth rate (RGR₆) according to the equation:

\[
RGR₆ = \frac{\ln W₂ - \ln W₁}{t₂ - t₁} \quad [g \cdot g^{-1} \cdot day^{-1}]
\]

where \( W₁ \) = dry substance of the overground parts at the beginning of the testing interval, \( W₂ \) = dry substance of the overground parts at the end of the testing interval, \( t₂ - t₁ \) = the time interval between two samplings of dry substance (24 hours).

The chemical analyses of the fluorine content in the plants were made by the method of Nicholson (3) modified by Holub (4). Small segments of the leaf tissue were fixed in glutaraldehyde and \( K_{2}MnO₄ \), embedded in low-viscosity epoxy resin (5) and examined with a Tesla BS 500 electron microscope.

### Results and Discussion

The fumigation of the horse-bean plants by HF at a concentration of 50 ppb for 24 hours caused a considerable depression of the photosynthesis rate (Fig. 1). However, as early as 24 hours after discontinuing the fumigation, a tendency was apparent for repairing the photosynthetic activity of the fluoride-treated plants. This phenomenon has been observed by Hill and Bennet (6,7). The authors stressed the possibility of total repair of this process by some plants after short-time fumigation with high concentrations of HF. However, the manner of photosynthetic repair, in our experiments, is very interesting. After the initial decrease of photosynthetic activity, a relatively dynamic restoration of this process occurred. The total process is characterized by a change from initial inhibition to stimulation. An increase in photosynthetic activity with the fumigated plants at the end of the testing period gave an average photosynthetic rate comparable to the controls over the whole testing period.

The authors are of the opinion that the question of inhibition and stimulation of the photosynthetic activity is related to the developmental changes of the affected plants (Fig. 2). Fumigation of the horse-bean by HF for 24 hours caused a very strong decrease of growth rate of the above ground parts. The inhibition of the photosynthetic activity, observed at this time, accounted to a large extent for this phenomenon. Forty-eight hours after stopping fumigation, RGR₆ increased considerably, even above the level of the control plants. The increased
Figure 1
Relative Values of the Photosynthesis Rate of Vicia faba L. at Continuous Fumigation (50 ppb) and after Discontinuing the 24 Hours' Fumigation

Caption to the curves:  = controls
= plants fumigated for 24 hours
= plants fumigated continually

Figure 2
Changes of the $RGR_w$ of Vicia faba L. at Continuous Fumigation and after Stopping of the 24 Hours' Fumigation
activity of the growth processes was maintained to the end of the test period, giving more tissue for the assimilates consumption "sink" (2) which secondarily would give favorable conditions for an increase in photosynthetic activity. In spite of the fact that photosynthetic activity of the fumigated plants approached average values over the entire test period compared to the control plants, the rate of the growth processes of the fumigated plants was 8.7% lower than the untreated controls. The inhibiting effect of fluoride compounds on plant growth has been observed by many authors (8-11).

A diminution of the growth processes due to unfavorable environmental factors, may be influenced by increased demands by plants on energetic requirements of the repair processes (12,13). The toxic action of fluorides on the plant organism may also be considered one of the unfavorable factors (14). The energetic contributions to the repair processes is reflected in affected plants by increased respiration within the autoregulating process. This phenomenon was observed also in plants influenced by fluoride compounds (14,15,17). Our current results, concerning changes in the respiration rate also show this phenomenon (Fig. 3). After discontinuing 24 hour fumigation with HF the respiration rate of the plants showed two maxima, representing strong stimulation of this process. In spite of a slight depression immediately after stopping fumigation, 48 hours after stopping fumigation, and at the end of the test period, the average respiration rate of the fluoride-treated plants was 7.1% higher than that of the controls, which is a symptom of mobilization of energy for insuring the repair processes.

When horse-bean plants were fumigated continuously, a 19% decrease in the photosynthetic rate was found after 24 hours (Fig. 1). This decrease was interrupted by a temporary increase on the third day but the decrease was intensified until at the end of the test period, the fumigated plants showed a photosynthesis rate of only 56% of that of the controls. It is noteworthy that, leaf necrosis was not observed in any case in the fluoride-fumigation chamber, up to the fifth day of fumigation. Only some weakly-developed individual plants (Figs. 3-4) were observed to be necrotic; however, they were not used in taking representative samples for measuring photosynthesis, respiration or the rate of the growth processes. At the end of the testing period, necrosis was only evident in about 2% of the total leaf area.

The inhibition of the photosynthetic rate of the continuously fumigated plants for the whole testing period (Fig. 1) was reflected also in strong inhibition of the growth processes of the above ground parts of the fumigated plants (Fig. 2). The RGR_w of the fumigated plants was 41.7% lower than in the controls, averaged over the whole testing period. It is, however, important to emphasize that the fumigated plants preserved their potential ability of respiration, in spite of the strong inhibition of the physiological processes mentioned above. This is shown by the increased respiratory activity at the period be-
Relative Values of the Respiration Rate of Vicia faba L. at Continuous Fumigation and after Stopping the 24 Hours' Fumigation

Dynamics of Fluorine Accumulation in the Overground Parts of Vicia faba L. at Continuous Fumigation and after Stopping the 24 Hours' Fumigation

between 48 - 120 hours of fumigation. At the time when the macroscopic injury of the plants was generally evident, a definitive depression of this physiological process occurred as well.

The observed inhibition of the photosynthetic activity and of the growth processes of the plants fumigated continuously with HF was probably a direct consequence of considerable fluoride accumulation in the aboveground parts, and also in the roots (Figs. 4-5). Since only
Figure 5
Dynamics of Fluorine Accumulation in the Roots of Vicia faba L. at Continuous Fumigation and after Stopping the 24 Hours' Fumigation

The aboveground parts of the plants were fumigated with HF; the increased fluorine content in the roots was probably a consequence of very intensive translocation, a possibility mentioned also by Romney et al. (18). However, the general appearance of macroscopic injury was observed only after 144 hours of continuous fumigation, at a time when fluoride accumulation reached 24.5 mg F/100 g of dry matter in the aboveground parts of the plants. In previous work (19), horse-bean plants were fumigated at a concentration of 100 ppb HF under approximately the same conditions as reported here. In this case, the macroscopical injury of the plants manifested themselves as early as 24 hours after the initiation of fumigation, together with a considerably lower fluoride accumulation in aboveground parts (10 mg F/100 g dry substance). From this it can be concluded that the total fluoride accumulated in the whole plant is not directly related to the macroscopic injury of cells or the meristematic region. It seems that the quantity and rapidity of the fluoride accumulation directly in the cells or the tissues affected, without the possibility of its dynamic translocation, is of general importance for manifesting the macroscopical injury.

It was found that the photosynthetic activity of the continuously fumigated plants was depressed even though macroscopical injury on the assimilating organs was absent. This indicates the effect of HF on photosynthetic and related processes (negative changes in the wa-
Destruction of the photosynthetic apparatus was apparent as early as 24 hours after fumigation was initiated. The chloroplasts of the mesophyll cells of the treated plants (Fig. 6) showed lack of grana and dilation of the thylacoids, when compared with the control (Fig. 7). It is interesting that the grana and the lamellar system of the chloroplasts reappeared after 48 hours of fumigation (Fig. 8). Because of the limited observations made with a limited number of samples, it cannot be stated unequivocally, whether this was a restoring process, or a biological variation in the samples studied. The increased activity of the Golgi apparatus, however, indicates that it is possible to anticipate a certain degree of reparation.

After 72 hours of fumigation (Fig. 9) increased destruction of the chloroplasts was observed. There was strong reduction of the grana and of the lamellar structure of the membrane system, together with the occurrence of reticulate formations at the periphery of the chloroplasts. The high activity of the Golgi apparatus was again remarkable.

In spite of the preliminary nature of our electron microscopic observations of the chloroplasts in the mesophyll cells, there was an obvious destruction of the photosynthetic apparatus by fluoride followed by the decrease in photosynthetic activity.

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Figure 6
Control Plants, Chloroplasts of Mesophyll Cells in Vicia faba L. with Normally Developed Structure (enlarged 14 000 x)

G - grana, T - thylacoids

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Volume 11 Number 2
April, 1978
**Figure 7**
Chloroplasts of Mesophyll Cells in Vicia faba L. after the 24 Hours' Fumigation with 50 PPB HF (enlarged x 25 000)

T - thylacoids, S - starch

**Figure 8**
Chloroplasts of Mesophyll Cells in Vicia faba L. after the 48 Hours' Fumigation (enlarged x 32 000)

G - grana, T - thylacoids, GA - Golgi apparatus

**Figure 9**
Chloroplasts of Mesophyll Cells in Vicia faba L. after 72 Hours' Continuous Fumigation (enlarged 44 000 x)

G - grana, T - thylacoids, S - starch, GA - Golgi apparatus
Bibliography


REPORTED ADVERSE EFFECTS IN CARIES
PREVENTIVE USE OF FLUORIDES IN NORWAY

by

P. Løkken and C.F. Borchgrevink
Oslo, Norway

(Abstracted from Nor. Tannlægeforen Tid, 87:248–254, 1978)

Thirty-four cases of fluoride intoxication including "sores" in
the mouth, perioral dermatitis, atopic eczema, urticaria, irritation of
eyes, abdominal pains, vomiting, and diarrhea, were reported to the Poisoning Center in Oslo, Norway. Twenty-six of these patients attributed
their illness to ingestion of fluoride tablets; eight of them to brushing the teeth with fluoride toothpaste, painting them and rinsing their
mouths with fluoride compounds.

In Norway, 24 million tablets containing 0.25 mg fluoride are
sold annually. In 1974, some 80% of children between the ages of 7 and
15 received topical fluoride treatments. Rinsing, brushing, and painting the teeth with fluoride-containing agents is also a common practice. Sixty percent of commercial tooth pastes in Norway contain fluoride.

The above reports to the Center occurred between 1970 and 1977
but since 1976 adverse effects have been reported less frequently than
in previous years. In all cases, the symptoms cleared up following avoidance of fluoride. Only 6 of the 34 patients could be examined; the others refused to submit to clinical studies. Of the 6, only 4 underwent provocative tests.

On the basis of these tests and of a review of the histories, the authors were not convinced that the difficulty was related to fluo-
ride in 23 of the 24 cases. In the remaining case, they termed the re-
lationship questionable. In some, the authors believed that the re-
creations were caused by agents other than fluoride present in the tablets, such as sorbitol and mannitol which pass through the intestinal tract and occasionally may account for diarrhea and stomach upsets. In most
cases, the illness was attributed to "the emotionally charged trend of
debate." Some patients, they stated, vomited when their teeth were
rinsed with drinking water without fluoride. In most instances, the
authors called the condition coincidental and due to some other illness.

The provocative test is described as follows: Two children who
were hospitalized received no tablets for two days; on subsequent two day periods tablets without taste, without color and without fluoride; and fluoride-containing tablets. One of the two patients, age four, after
taking fluoride tablets for eight days had "blemishes in and around the
mouth." At age 5 painting of his teeth three times, and at age 6 once,
precipitated abdominal pains, loss of appetite, and "other discomfort." Following the last treatment "an external rash and sores in the mouth" appeared. At age 7, the double-blind test, carried out in the above-described manner, produced no reaction. On the basis of this test, the authors concluded that the child's disease was not related to fluoride.

Another 7-year-old child had "sores in the mouth up to 2 mm in diameter" which occurred within 7 to 8 hours after the mouth was rinsed with a fluoride solution in school. However, the same condition was also observed at other times when the patient did not use mouth rinses. This child was hospitalized at the allergy service of a hospital but, after four weeks, the tests were not completed. During the hospitalization, minor vesicles appeared in the mouth after placebo tablets. Since the test was interrupted no conclusion was reached, but the authors felt it unlikely that the condition was related to fluoride. (For further comments, see editorial on page 43.)

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EFFETS DE L'INTOXICATION FLUORÉE SABAIGUÉ DU LAPIN SUR LES MÉTABOLISMES FLUORÉ ET PHOSPHOCALCIQUE ET SUR LA RADIOGRAPHIE DU SQUELETTE

by

J. Elsair, J. Poey, M. Reggabi, F. Hattab, N. Benouniche and C. Spinner
Alger, Algeria


The authors encountered endemic fluorosis in Algeria due to drinking water containing 3 to 5 ppm which provided a daily intake of 6 to 40 mg of fluoride or 0.1 to 0.7 mg/kg/day for a person weighing 60 kg. This induced them to carry out experiments on the effect of long-term daily intake of fluoride by studying balances of calcium, phosphates, and fluoride, and the X-ray appearance of the skeleton.

Albino rabbits of both sexes weighing 2,660 kg, received in their drinking water 100 ppm of fluoride in the form of sodium fluoride for 10 months which provided a daily intake of 21.4 mg during that period. In addition, the animals and the controls received in their drinking water between 0.32 to 0.60 mg/l of fluoride naturally. A synthetic diet with a known content of minerals was used.
The following parameters were studied: blood and urinary levels and the content of calcium, phosphorus and fluoride in drinking water, food, and feces. The determinations were made for four days at the beginning and again at the termination of the 10 month period. During the four day study periods, the authors determined the coefficient of digestive utilization (CUD) expressed in percentage. The bone changes were recorded according to the classification of Pinet.

Results: During the course of the study, several animals died of an intercurrent infection which was not considered related to fluoride intoxication. However, one rabbit died following paralysis of both posterior legs and sphincter insufficiency which was believed to be fluoride induced.

The animals showed no changes in weight after 10 months compared with their weight prior to the experiments; food intake decreased only slightly at the end of the experimental period.

Serum fluoride increased significantly during the 10 months but the fluoride excretion in feces and urine rose only slightly. Intestinal absorption of fluoride increased significantly. The coefficient of digestive utilization also rose in spite of relative hyperfluoruria.

The calcium level in the blood decreased markedly during the 10 month study; the phosphatemia rose very slightly. The alkaline phosphatase of the blood did not vary. However, toward the end of the experiment, this trend tended to change due to diminution of intestinal absorption of phosphorus and inversion of the coefficient of utilization of calcium (fecal calcium exceeded calcium uptake). Also renal reabsorption of phosphorus declined significantly, a parameter which, in the authors' belief, is more reliable than the determination of phosphaturia.

In one of the rabbits, the X-rays showed changes in the trabeculation and the bony contour of the dorsal and lumbar spine and the pelvic bones but, otherwise, the skeleton appeared to be normal. In another animal, the periosteum of the right femur was thickened and abnormal trabeculation as well as a ragged bony structure with osteophyte formation were noted.

The authors stressed the nonhomogeneity of their findings. Whereas the fluoride intake through food did not vary throughout the experiments, there was considerable variation in the consumption of waterborne fluoride compared with the pre-experimental uptake, because of initial polyuria and polydipsia which was followed by a return to normal. In direct relation with varying amounts of water consumed during the experimental period, more fluoride was retained at first and less toward the end.
The authors relate the degree of chronic intoxication in the animals following uptake of fluoride in water of 21.4 mg per day for 10 months to their experience with humans. They found radiological changes slightly less or equal to those which are induced in adult men 40 to 60 years of age, chronically poisoned by fluoride in water at 3-5 ppm. This concentration is equivalent to a daily fluoride intake of 10 times less per body weight, namely 0.1 to 0.7 mg/kg, based on a duration of several decades.

In the human subjects residing in the endemic fluoride area the authors had observed beginning tubular involvement with polyuria, increased urea clearance prior to the appearance of radiological changes, and involvement of the glomeruli in the later stage with diminution of creatinine clearance. This sequence has also been described by others.

In discussing their results the authors point to higher fluorsemia, strongly positive fluoride balance during the developing fluoride intoxication which they attribute to an increase in the coefficient of the digestive utilization, i.e. intestinal absorption of fluoride.

A decrease in blood calcium leads to a disturbance in the calcium phosphorus metabolism but little or no change was observed in the phosphate and alkaline phosphatase levels. In the literature, calcemia has been reported low, normal, and elevated whereas phosphatemia either remains within the normal range or decreases.

The negative calcium and phosphorus balance is due 1) to a reduction in absorption of intestinal calcium and phosphorus and therefore greater loss of calcium through the feces and 2) to a loss of urinary calcium and phosphorus because of reduced renal reabsorption of phosphorus. Hypocalcemia induces secondary hyperparathyroidism which leads to bony destruction with histologically demonstratable demineralization, negative calcium balance by mobilizing bone calcium and, furthermore, by direct inhibition of calcium absorption by fluoride. This in turn leads to hypercitratemia, slight increase of calcium and phosphorus and diminution in the renal absorption of phosphorus.

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FLUORIDE
MAGNESIUM–FLUORIDE INTERRELATIONSHIPS IN MAN
I. EFFECT OF FLUORIDE ON MAGNESIUM METABOLISM

by

H. Spencer, L. Kramer, E. Wiatrowski, and D. Osis
Hines, Illinois


The following facts are known concerning the magnesium–fluoride relationship in animals:

Magnesium intake decreases intestinal absorption of fluoride in the rat. Less fluoride is deposited in the skeleton in relation to increasing magnesium levels and when magnesium is combined with calcium. On the other hand, elevated bone fluoride levels are associated with higher concentrations of magnesium in bones. Fluoride prevents magnesium-induced calcifications of soft tissue in animals. In man endemic fluorosis is less frequent where the water supply is high in magnesium and calcium.

The authors investigated the effect of fluoride on the magnesium metabolism in man during varied intake of calcium and phosphorus:

Thirty-three fluoride and magnesium balance studies were carried out on 13 normal, fully ambulatory male individuals whose diets were strictly controlled. For several weeks prior to the beginning of the study, they consumed a basal diet containing on the average 230 mg calcium, 800 mg phosphorus, 250 mg magnesium, and 1.8 mg fluoride/day. However, due to use of 1 ppm artificially fluoridated water the total fluoride intake per day averaged 3.9 mg and was kept constant throughout the study period. The diet was analyzed for fluoride, magnesium, calcium, phosphorus, and nitrogen in each 6-day metabolic study period. Fluoride and magnesium balances were determined in controls and in experimental subjects. The constant diet was supplemented by an additional 10 mg fluoride (as NaF) during the various intakes of calcium and phosphorus.

For the magnesium and fluoride balance studies, the levels of calcium intake were 200, 1,400, and 2,200 mg/day. During each of the three periods of calcium intake, two levels of phosphorus, namely 800 and 1,400 mg per day, respectively, were administered. Complete 24-hour collections of urine and of stool were obtained.

Of the 13 patients, three with osteoporosis received approximately 45 mg fluoride per day. As control, they first received an average of 3.1 mg fluoride per day for an average of 29 days and then an additional 42.8 mg fluoride for 90 days.
Results: During the three levels of calcium intake and during relatively low or high phosphorus intakes the fluoride balances were similar in the controls to those during fluoride supplementation. The increase of fluoride intake from an average of 4.1 mg F/day to 13.6 mg F/day resulted in a corresponding increase of fluoride in the urine and feces with highly positive fluoride balances. The magnesium balances were also similar in the various control studies during low, and during various calcium and phosphorus intakes. Addition of an average of 9.5 mg fluoride did not change the urinary or fecal magnesium excretions or the magnesium balances during the differing calcium intakes. Nor was there any significant difference during low and high phosphorus intake. The fluoride balances, however, differed significantly during the low and high fluoride intakes.

In the three osteoporotic patients who received an addition of 40-45 mg fluoride, the urinary fluoride increased more than 10-fold, the fecal fluoride approximately 50-fold, and the positive fluoride balances increased markedly. An average magnesium intake of 242 mg/day produced an equilibrium of the magnesium balance at +10 mg/day. Fluoride supplementation did not change the serum magnesium balance during the 200 to 2200 mg/day calcium intakes and the 800 to 1400 mg/day phosphorus intakes. However, increased magnesium excretion in the feces was directly related to increased calcium intake and caused a decline in the magnesium balance.

In summary, an approximate threefold increase in fluoride intake, from about 4 to 14 mg per day, did not affect urinary and fecal magnesium excretions or the magnesium balances. When phosphorus intake was increased by 75% during the various calcium intakes, the urinary and fecal magnesium excretions and the magnesium balances during both low and high fluoride intake remained constant. However, high calcium intake, regardless of the phosphorus or fluoride intake, resulted in a significant increase in the fecal magnesium.

The authors commented on fluoride's differing effects according to the species of animals. In magnesium-deficient dogs, fluoride appears to prevent soft tissue calcifications but it failed to affect pre-formed calcified lesions. On the other hand, in magnesium-deficient and magnesium-adequate rats increased fluoride intake led to calcifications of the kidneys.
Changes in Urinary Ion Excretion and Related Renal Exposure Activities in Fluoride-Treated Rats

by

Y. Suketa and E. Mikami
Shizuoka, Japan


The authors studied the mechanism of polyuria which occurs after administration of fluoride to animals and man. Specifically they investigated the changes in urinary ion excretions and related renal enzyme activities such as Na\(^{+}\) + K\(^{+}\)-stimulated adenosine triphosphatase [(Na\(^{+}\) + K\(^{+}\))-ATPase], Mg\(^{2+}\) and Ca\(^{2+}\)-stimulated adenosine triphosphatase [(Mg\(^{2+}\) + Ca\(^{2+}\))-ATPase], acid phosphatase, and alkaline phosphatase.

Male Wistar albino rats were placed for one week on a basal diet and then given a fluoride supplement (NaF, 50 mg/kg) as a single oral dose and continued on the same diet accompanied by distilled water ad libitum. The ion determinations were carried out by means of an atomic absorption spectrophotometer; inorganic phosphate was determined according to the method of Tausky and Shorr, fluoride in urine and serum by the method of Hall et al.; microsome and mitochondria were prepared according to the method of Jørgensen, (Na\(^{+}\) + K\(^{+}\))-ATPase activity according to Post and Sen, (Mg\(^{2+}\) + Ca\(^{2+}\))-ATPase activity according to Bond and Clough; for acid and alkaline phosphatase activities, the method of Linhardt and Walter was used with p-nitrophenyl phosphate as the substrate.

Urine volume was highest during the first day following the administration of the single dose of NaF. Subsequently it gradually decreased to near control values. The urinary fluoride concentration reached a maximum at 6-12 hours after fluoride administration with excretion of only 13.9% of the total dose of fluoride within 1 week after the treatment. The serum fluoride reached its maximum 30 minutes after consumption of the dose and by the 10th hour it had declined to near control values.

Urinary calcium excretion and urinary inorganic phosphate excretion was markedly increased on the first day following administration of the single dose of fluoride. Urinary sodium, potassium, and magnesium also were significantly elevated. The serum Na\(^{+}\) concentration fell in relation to decreased renal (Na\(^{+}\) + K\(^{+}\))-ATPase activities. The serum K\(^{+}\) concentration increased slightly during the period from 0 to 12 hours after which it declined rapidly.

Serum magnesium increased significantly in the treated rats, whereas calcium and inorganic phosphate declined. The renal mitocho-
drial (Ca^{2+} + Mg^{2+})-ATPase activity and acid phosphatase activity decreased significantly compared to control values. Twelve hours after the fluoride administration the renal cellular fluoride content increased about sevenfold.

The authors previously reported that phosphaturia associated with polyuria, is characteristic of fluorosis. The current study shows that this condition is also associated with significant increases in urinary K^+, Na^+, Mg^{2+}, Ca^{2+}, and inorganic phosphate. The decrease in mitochondrial ATPase activity in kidneys is believed to be responsible for the urinary sodium loss and the decrease in serum sodium. The significant increase in urinary calcium may be attributed to the decrease in (Ca^{2+} + Mg^{2+})-ATPase activity. The decrease in alkaline and acid phosphatase activities observed in these studies is probably responsible for the phosphaturia in fluorosis.

The figures were averages of 6 rats each.

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INCIDENCE OF PERIODONTAL DISEASES IN SUBJECTS WITH VARIOUS DEGREES OF EXPOSURE TO FLUORIDES

by

E. Domzalska
Szczecin, Poland.

(Abstracted from Paradontologia Polska, 4:1005-1011, 1972)

The author studied the incidence of periodontal diseases in 81 subjects with various degrees of fluoride intake: 1) The inhabitants of Malbork consumed water with 2.8 - 3.2 ppm fluoride, 2) Workers of the Szczecin phosphate fertilizer plant were exposed to particulate and gaseous fluorides, and 3) The drinking water in Szczecin contained 0.05 - 0.25 ppm fluoride.

Periodontal diseases were evaluated by means of the index of Kotzschke. Fluoride levels in urine, saliva and blood were determined by the Bumsted and Wells modified alizarin-circonium method. The highest values of fluoride in the urine, saliva and blood were found in the workers at the phosphate fertilizer plant, followed by the Malbork inhabitants; the level in the Szczecin population was lowest.

FLUORIDE
A correlation was observed between the periodontopathy index of Kotzschke and fluoride levels in urine, saliva and blood calculated by means of the Pearson correlation coefficient. The periodontopathy index of Kotzschke in these subjects was low in all groups. In the subjects with periodontal changes, the mean value of the index of Kotzschke was highest in the population of Szczecin, lower in Malbork and lowest in plant workers.
BOOK REVIEW

PROCEEDINGS OF THE SYMPOSIUM ON FLUOROSIS - OCTOBER 1974
INDIAN ACADEMY OF GEO SCIENCE, HYDERABAD, INDIA, 1977

In India approximately 1/2 million people are suffering from fluorosis. It is, therefore, not surprising that Indian scientists have carried out extensive investigations on this serious problem, which have brought forth a vast amount of information on fluoride and fluorosis.

In October 1974 the Indian Academy of Geoscience in collaboration with several other scientific and governmental agencies organized a symposium in Hyderabad which brought together scientists from several disciplines for the purpose of alleviating the serious threat to the country's health. The 534 page proceedings of the symposium issued in 1977 is an excellent source of information on the subject.

The book is divided into five sections. The first 13 chapters outline all important natural fluoride areas of India, their geological structures, and the distribution of fluoride-bearing minerals. Several papers describe improved methods of fluoride analysis. Section 2 deals with the role of fluoride in agriculture, i.e. fluoride uptake by plants and its effect on the growth of wheat and peas. Of special interest is paper 15 which presents a review of the fluoride content of Indian food showing that, in the natural fluoride Podoli area, rice heads the list with the value of 11.3 ppm, whereas tomatoes and bananas contain least fluoride, namely 0.33 and 0.84 ppm.

Section 3 (papers 17-33) gives a detailed account of natural fluoride levels in drinking water in various areas throughout the country. In semi-arid regions, much higher levels were found than in the ground waters of humid areas. In this section, the search for agents to defluoridate water supplies is described and the possible use of magnesium, aluminum, and calcium compounds is discussed.

Sections 4 and 5 deal with the clinical aspects of fluorosis in animals (papers 34-39). They include an excellent review of treatment of fluorosis and preventive measures in cattle.

On the subject of human fluorosis in Section 5 (papers 40-55) articles by Teotia, Jolly, Susheela, Krishnamachari, and Venkateshwalu, among others, provide information on the biochemical and clinical features of the disease, on the effect of serpentine, and numerous data on the skeletal and dental aspect. A report by Reddy on surgery to relieve spinal compression in skeletal fluorosis and another concerned with treatment of the disease by intravenous injections of magnesium hydroxide (0.2% or 2-5 mg per day) are of special interest.

The book is a virtual treasure chest for those interested in
the subject. Whereas a combination of numerous papers by many authors cannot help but contain some repetitious data they, in no way, detract from the value of this book. Its major weakness is the absence of an index. This deficiency, however, is counterbalanced by the table of contents, which includes 55 titles and their authors.

G.L.W.

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