FLUORIDE

OFFICIAL QUARTERLY JOURNAL

OF

INTERNATIONAL

SOCIETY FOR

FLUORIDE

RESEARCH

INTRODUCTION TO FLUORIDE JOURNAL OF THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH

Fluoride, the official journal of the International Society for Fluoride Research, has been, since 1968, the only international scientific journal devoted to uncensored publication of legitimate research concerning all aspects of the biological effects of fluoride.

The formation of the International Society for Fluoride Research (ISFR) and the birth of the Fluoride journal are largely due to the efforts of Dr. George L. Waldbott. Dr. Waldbott was born in Speyer, Germany in 1898. He specialized in allergic and respiratory diseases. Beginning in the 1950'5, he turned his attention, increasingly, to adverse health effects of environmental pollutants, especially fluoride. His work in this area continued until his death on July 17, 1982.

Dr. Waldbott undertook a comprehensive survey of the biomedical literature on fluoride taking his cue from the pioneering research of the Danish physician and health officer, Kaj Roholm (1902-1948) on the symptoms of the incipient stages of skeletal fluorosis. As a result of his studies, Waldbott made contact with leading fluoride investigators world-wide and soon recognized that, in spite of publishing his reports in highly respected peer reviewed medical journals, mostly in Europe, the clinical details of his investigations were blocked from appearing in leading U.S. medical journals.

In an effort to lift this blanket of apparent censorship, Waldbott organized the first international symposium on the toxicology of fluorine compounds held in Berne, Switzerland on October 15-16, 1962. This successful effort, attended by over 30 researchers from 11 countries, led to a similar conference in Detroit, Michigan in 1966. This sponsored by the newly formed American Society for Fluoride Research that became the International Society for Fluoride Research (ISFR) that held its first meeting in 1967 in Frankfurt, Germany. During the past 3 decades, 21 additional conferences of the ISFR have been held in over 10 countries throughout the world. Since 1990, conferences have been held in the U.S.A., Japan, China and Hungary.

Fluoride, the official journal of the ISFR, first appeared in July 1968 under the title Fluoride Quarterly Report and received its shorter title in 1970 even though its publication has continued on a quarterly basis.

Since 1968, the picture of Dr. Kaj Roholm has appeared on the cover of Fluoride as a tribute to this scientist who became, what many consider, "the greatest authority of all time on the biological effects of fluoride". Dr. Roholm's book, Fluorine Intoxication, a Clinical-Hygienic Study, published in 1937, remains one of the most sought after reference texts on fluoride and is cited frequently today in the literature. Many decades have passed since Roholm's book first appeared, but, most of the data that he presented are as new to most scientists today as they were then. Great advances have been made in fluoride research since that time and these have been recorded, for more than 3 decades, in the pages of the journal, Fluoride.

Study of the contents of the journal over the more than 30 years since its first publication is evidence of this. Within its covers is a wealth of original peer-reviewed studies on all aspects of fluoride that have been prepared by researchers from every quarter of the globe. Past issues also contain abstracts from the international literature, reviews of major studies and reports on subjects that are relevant to fluoride. As is the case in other periodicals, past issues contain letters to the editor expressing, at times, viewpoints that are highly provocative.

The strength of the ISFR and the authors of publications to be found in Fluoride are the many scientific disciplines to be found in its executive, advisory and editorial hoards and membership. These include physicians, dentists, orthopedic surgeons, veterinarians, biologists, chemists, biochemists, geologists, plant physiologists, toxicologists and others. The thread of fluoride binds these together in a united and coherent forum. The journal, Fluoride permits uncensored publication of research concerning all aspects of the biological effects of fluoride.

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From articles published in Fluoride 25 (2) 53-54.1992 and in the Waldbott Commemorative Issue Fluoride 31 (1) 1-12, 1998.

THIRTY-FIVE YEARS OF *FLUORIDE*

This two-part issue of *Fluoride* contains cumulative author and subject indexes covering the entire span of publication to date, from 1968 through 2002.

THE BEGINNING[1,2]

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Beginning in the 1950s, he turned his attention, increasingly, to adverse health effects of environmental pollutants, especially fluoride. His work in this area continued until his death on July 17, 1982.

Dr Waldbott undertook a comprehensive survey of the biomedical literature on fluoride, taking his cue from the pioneering research of the Danish physician and health officer, Kaj Roholm (1902-1948), on the symptoms of the incipient stages of skeletal fluorosis.

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During the past 4 decades, 24 additional conferences of the ISFR have been held in over 10 countries throughout the world. Since 1990, conferences have been held in Japan, China, Hungary, USA, and Poland.

Fluoride, the official journal of the ISFR, first appeared in July 1968 under the title *Fluoride Quarterly Reports* and received its shorter title in 1970 even though its publication has continued on a quarterly basis.

Since 1968, the picture of Dr Kaj Roholm has appeared on the cover of *Fluoride* as a tribute to this scientist who many consider, "the greatest authority of all time on the biological effects of fluoride". Dr Roholm's book, *Fluorine Intoxication, a Clinical-Hygienic Study*, published in 1937,[3]

remains one of the most sought after reference texts on fluoride and is cited frequently today in the literature. Many years have passed since Roholm's book first appeared, but most of the data that he presented are as new to most scientists today as they were then. Great advances have been made in fluoride research since that time, and these have been recorded, for more than 3 decades, in the pages of *Fluoride*.

The first editor of the journal was Dr Waldbott, who continued in this capacity until his death in 1982. His widow, Edith M. Waldbott, even though she was advanced in years, served as "interim editor" until 1991 when John Colquhoun, BDS, PhD, was appointed as editor.

From 1955 to 1962, Edith Waldbott inaugurated, edited, and published *National Fluoridation News*. This experience was undoubtedly of value in support of her husband's efforts during the early years of the journal.

Mrs. Waldbott died on January 14, 1997, aged 93 years. The third issue of *Fluoride* for 1991 identified Dr Colquhoun as "editor nominee" and announced that she was leaving her position as "interim editor" and Elizabeth Ramsay, one of the Waldbott daughters, became "interim business manager".

John Colquhoun achieved splendid results during his tenure that ended in November 1998 shortly before his death on March 23, 1999. Prof Albert W Burgstahler succeeded him as editor, and Bruce Spittle, MB, DPM was elected managing editor and treasurer, positions which they still hold.

From the publication of the first issue of *Fluoride* in July 1968 to the present, the name of Professor Burgstahler has appeared on the masthead under various titles. He is identified as "coeditor" with Dr Waldbott; "acting editor" with Edith Waldbott; and, "coeditor" and, later, "scientific editor" with Drs Colquhoun and Spittle. There have also been a number of coeditors over the years. These include K Jankauskas, J Yiamouyiannis, GW Miller, KAVR Krishnamachari, M-H Yu, JR Lee, and B Spittle.

CONTENTS

Perusal of the contents of the journal over the 35 years since its first publication reveals that within its covers is a wealth of original peer-reviewed studies on a wide range of topics relevant to fluoride that have been prepared by researchers from every quarter of the globe. Past issues also contain abstracts from the international literature, reviews of major studies and reports on subjects that are relevant to fluoride. As is the case in other periodicals, past issues contain *letters to the editor* expressing, at times, viewpoints that are highly provocative.

The strength of the ISFR and the authors of publications to be found in *Fluoride* are the many scientific disciplines that are represented in its executive, advisory, and editorial boards and membership. These include physi-

cians, dentists, orthopedic surgeons, veterinarians, biologists, chemists, biochemists, geologists, plant physiologists, toxicologists, and others. Their interest in fluoride has created a united and coherent forum. *Fluoride* permits uncensored publication of research concerning all aspects of the biological effects of fluoride.

The October 1982 issue contains a 15-year cumulative index for 1968-1982. It also marks the passing of Dr Waldbott. A February 1998 Commemorative Issue celebrated the 100th anniversary of Waldbott's birth.

Woven into the fabric of the journal are threads too numerous to present in total in this editorial. There are some that can be identified and presented as examples of what may be found by delving into the back issues.

ENVIRONMENTAL TOXICOLOGY

From the earliest issues, *Fluoride* has published a wide variety of research papers, abstracts, special articles and editorials on the environmental issues concerning fluoride.

In 1969, for example, *Fluoride* published H E MacDonald's comprehensive study of sources, problems, disposal of waste fluoride and the effects of fluoride pollution on crops and livestock.[4] Fluoride emissions from phosphate processing plants were discussed by FL Cross and RW Ross.[5] A lengthy abstract of Roholm's report on the fog disaster in the Meuse Valley in 1930 is an outstanding presentation of the problem of air pollution involving fluoride.[6]

Later issues of the journal published special reports by JR Marier in 1972[7] and E Groth in 1975[8] on the ecological effect of fluoride. Both present a review of the literature and deal with the adverse effects on wildlife of low level environmental pollution by fluoride. Marier presented a case for consideration of synergistic effects of the multiple pollutants in water. Does this foreshadow the work showing the action of fluoride and aluminum reported by Varner, Jensen and others in 1998, 26 years later?

Groth lamented the fact that data from field studies, especially on the marine and fresh water ecosystems were virtually non-existent.

C van Hook was the author of a paper published in 1974 that stressed the importance of biological monitoring of airborne fluoride emissions.[9] He used the Silverbow area of Montana as an example.

Over the years, the basic concepts laid down in the early volumes have been elaborated upon. The effects on cattle were described in 1981 by L Krook and GA Maylin.[10] Others reported effects on other domestic animals such as sheep and camels and wild animals including many species of deer as well as voles and wild pigs. GW Miller[11] and M-H Yu[12] have continued to show the way in which fluoride operates to alter the physiological functions of plants. In 1995, the journal published a research report by NP Grit-

san and others that showed that among environmental pollutants studied for their effect on plants, fluoride was the most damaging.[13]

Of major interest is the effect on humans of fluoride contamination from numerous sources such as air, water and soil pollution from fluoride emitting industries, burning of coal, volcanic fog (Vog), excessive fluoride in brick tea (China), and drinking water contaminated either by nature or the hand of man.

CRIPPLING SKELETAL FLUOROSIS

In 1937 adverse non-dental effects of fluoride on humans were discovered. It was in this year that Roholm published his report on the illness afflicting Danish cryolite workers[3] and his study of the disastrous fog in the Meuse Valley in 1930.[6] It was in 1937 that HE Shortt, CG Pandit and TNS Raghavachari brought to world attention the condition of endemic fluorosis in India.[14]

In 1968 *Fluoride* published an exhaustive study of all aspects of endemic fluorosis in the Punjab (India) prepared by SS Jolly.[15] This was complete with illustrations of clinical features and radiographs. The journal published an up-date by Jolly in 1973 and other subsequent papers. In 1969 the journal published a research paper by SPS Teotia and others from India of metabolic studies of skeletal fluorosis and an approach to treatment.[16] In later volumes Teotia and his co-workers presented further research papers as follow-up.

In 1976, the journal published a paper by KAVR Krishnamachari that described the widespread occurrence in some areas of India of genu valgum associated with endemic fluorosis.[17]

In recent years the journal has published research papers on the prevalence of fluorosis in China and elsewhere. An editorial by J Li and S Cao in 1994 summarized the extent of the problem in China, the efforts to study fluorosis, the implementation of preventive measures, and early diagnosis and treatment.[18] The editorial pointed out that a 1990 survey revealed that, in China, 300 million people lived in endemic fluorosis areas. Of these, 3 million had skeletal fluorosis and 40 million, dental fluorosis. The journal has, especially since 1994, published useful research reports and abstracts that illustrate the far-reaching aspects of Chinese research and activities in this area of concern.

DENTAL FLUOROSIS

Skeletal fluorosis is the end result of long-term exposure to chronic fluoride intoxication. Dental fluorosis occurs as a result of fluoride exposure during tooth development. Skeletal fluorosis is graded according to the appearance of bone on X-ray. Dental fluorosis is visible to the naked eye and has over the years presented a problem in classification. In 1934 H Trendley

Dean developed an "index" to classify this physical sign but this was seen by many to be limited in its usefulness.

In 1971 *Fluoride* published an elaborate classification developed by T Takumori of Japan.[19] This consists in 4 systems that were subdivided into 5 degrees. For anyone interested in dental fluorosis this paper is well worth revisiting even though it has now, since 1978, been replaced by the Thylstrup-Fejerskov Index (TFI). The latter is considered to be superior to Dean's Index and is less complex than that of Takumori but lacks some of its precision.

Recent reports published in the UK, Canada, and the USA have shown concern that the prevalence of fluorosis is high as a consequence of increased fluoride intake from toothpaste, fluoridated vitamin supplements, a number of dietary sources including brick tea in China, and the practice of water fluoridation. *Fluoride* has, over the years, published studies and editorials concerning dietary fluoride.

R Kinter presented an editorial on dietary fluoride in the USA in 1971.[20] He re-visited the subject in a 2-part editorial in 1991.[21] Other early papers on dietary fluoride were published that originated from authors in countries such as Germany, Czechoslovakia and Canada. Studies have been published in the journal that reported on the amount of fluoride in specific food products such as tea, fruit juices, wines, and pharmaceutical products. These must be taken into account in calculations of total fluoride burden and its acute as well as long-term effects.

In 1997 K Akiniwa reviewed the acute toxic dose of fluoride and demonstrated that poisoning is caused by exposure to lower doses than commonly suggested.[22] Akiniwa recommended that the acute toxic dose should be re-examined. This emphasizes the need to be aware of total fluoride ingested from all sources on a daily basis.

NON-SKELETAL AND NON-DENTAL PROBLEMS

A large number of published studies deal with the non-skeletal and non-dental problems associated with fluoride exposure from a variety of sources. The non-skeletal effects of chronic fluoride intoxication were of special interest to GL Waldbott, the founder of the journal. As a result, the early issues contain informative editorials on the subject.

In 1976, papers were published that were presented to a "Symposium on the Non-skeletal Phase of Chronic Fluorosis" with an introduction by Waldbott.[23] The reports that follow discuss the effects of fluoride on muscles, joints, arteries, thyroid, spinal cord, and kidneys and the subject of allergic reactions to fluoride.

Many of the vague symptoms encountered during the early stages of fluorosis resemble those of hypothyroidism, especially fatigue. A review by JR

McLaren was published in 1976 as a special article.[24] In it, McLaren referred to the work of P Gallerti who, in 1958, reported on the use of fluoride to treat the overactive thyroid.

A common symptom, gastric irritation, is described by AK Susheela as "an early warning sign" in an abstract published in a 1989 issue.[25] Studies carried out by Susheela and her colleagues that were published in the journal in 1992 show the detrimental effect of fluoride on the gastric mucosa.[26]

NJ Chinoy and her colleagues have, over the years, made many contributions to the journal that show the adverse effects of fluoride on fertility, especially in males. Most of the experimental work of this group that has been published has been on animals but observations have been made regarding low human fertility in the endemic fluorosis areas of India.

During the past decade, the journal has published research papers on the relationship between fluoride ingestion and intelligence. Studies from China published in 1995 and 1996 show a decrease in IQ of children exposed to fluoride in soot and gases from coal combustion and in drinking water.[27, 28]

P Mullenix and her colleagues in the USA demonstrated these adverse neurological effects of fluoride in animal experiments. An abstract of their paper was published in the journal in 1995.[29]

INDUSTRIAL (OCCUPATIONAL) FLUOROSIS

Many of the research papers, abstracts and editorials dealing with occupational fluoride exposure expand on the work of Kaj Roholm with cryolite workers.

From 1975 to 1981, the journal published research reports from H Runge, J Franke and their colleagues from Germany[30,31,32] and E Czerwinski and his colleagues from Poland[33,34] along with some others that dealt with many aspects of industrial (primarily, aluminum smelter) fluorosis.

Runge and Franke present Fritz's expansion, in 1958, of Roholm's classification of skeletal fluorosis to include clinical manifestations that precede the discovery of x-ray findings.[35]

In addition, these two groups of researchers present details of skeletal changes found on x-ray and bone biopsy as well as clinical and laboratory clues required for early detection and monitoring. Along with others, such as Palzic in 1993,[36] Runge and Franke present evidence both genetic and physical that could be used for pre-employment screening of those workers most likely to be candidates for severe disability.

The similarity between the skeletal changes in occupational fluorosis and endemic fluorosis has not gone unnoticed as is attested in a 1978 report by Czerwinski.[37]

A number of reports have been published in the journal over the years that present assessments of techniques that may be used to determine and moni-

tor body fluoride burden from urine, hair and nails and from chemical constituents of blood such as alkaline phosphatase, sialic acid (SA) and glycosaminoglycans (GAS). Susheela and her colleagues show the use of the SA/GAS ratio to differentiate between skeletal fluorosis and ankylosing spondylitis that it resembles in an abstract published in 1989.[38]

In 1997, the journal published a paper by E Czerwinski on the use of computer enhancement of x-rays to provide earlier diagnosis.[39] PZ Chen and XC Meng, in 1996, described the use of computerized tomography (CT), a method of using x-ray transmissions and a minicomputer to reconstruct a graphic image of a "slice" of body area, to illustrate how calcification of ligaments within the spinal canal can provide a mechanism to explain spinal nerve paralysis in skeletal fluorosis.[40]

FLUORIDE AS TREATMENT FOR OSTEOPOROSIS

The use of fluoride compounds to treat osteoporosis is controversial. Both sides of the argument are presented in the back issues. There is agreement on the positive relationship between fluoride ingestion and increase in bone density as shown on x-ray. However, there are differences of opinion as to whether or not this can lead to prevention of fractures of the vertebrae and the possibility that this treatment may lead to increased fractures in areas such as the proximal femur (hip).

The journal, in 1997, published an overview by J Franke of 35 years of his research on the use of fluoride in the treatment of osteoporosis.[41] He concluded that fluoride therapy, carefully monitored, is beneficial and safe. The journal had previously, in 1994 and 1996, published abstracts of the research of CYC Pak supporting the use of slow acting fluoride preparations in the treatment of osteoporosis. A critical review of the use of slow release fluoride in osteoporosis by J Lee was published in 1996.[42]

CH Søgaard, using a rat model, is quoted in an abstract published in a 1997 issue of the journal as stating that her studies showed a "detrimental effect on bone quality". [43] In 1999 the abstract of the study by PJ Meunier of random controlled studies concluded with the statement that the data for fluoride in the treatment of osteoporosis was either lacking or inconclusive.[44] In 2001, the journal published the abstract of a meta analysis by DH Haguenauer and others who concluded that although the treatment increased bone density, it did not reduce vertebral fractures but does lead to an increase in non-vertebral fractures and gastrointestinal side effects.[45]

This is in agreement with Waldbott's view as long ago as 1973. In one of his many unsigned editorials, Waldbott reviewed the literature and stated that "…fluoride treatment of osteoporosis … should be viewed with skepticism because of questionable efficacy and the possibility of serious side effects".[46]

There have been a number of papers and abstracts published in the journal over the years on this subject; those referred to above give some idea of the continuing debate.

FLUORIDATION OF DRINKING WATER

Many studies, abstracts, and editorials are to be found over the years that deal with the process of water fluoridation; that is, the deliberate addition of fluoride compounds to drinking water in an effort to improve oral health. These studies address the issues of safety and efficacy of this process that is said by its supporters to reduce the incidence of dental caries.

The ISFR, officially, takes no stand either for or against fluoridation. As a result, the journal publishes studies from both sides of this scientific and political issue.

CONTROVERSIAL INTERPRETATION

There are a number of contradictions. Many of the journal's authors who deal with fluoride intoxication from industry, coal burning and contaminated water supplies see a major public health *problem*. The promoters of water fluoridation to improve oral health see a public health *triumph*.

Increased bone mineral density (BMD) in the workplace or endemic area is an early sign of fluoride accumulation, a prelude to possible crippling disease. The same finding in a patient under treatment for osteoporosis is a signal of therapeutic success. An increase in BMD observed in young women residing in a fluoridated community compared to a similar sample residing in a non-fluoridated community is interpreted by one team of researchers as a finding of preventive value for future osteoporosis.

Dental fluorosis in reports from endemic areas and those inflicted by neighborhood pollution is a *visible sign* of chronic fluoride intoxication during tooth-forming years. The same disease is understood by advocates of water fluoridation; but is referred to as a *cosmetic* effect.

NON-SKELETAL SYMPTOMS

The myriad of vague symptoms such as fatigue, gastrointestinal upset, muscular pain and weakness described by Fritz as preceding industrial fluorosis[35] are also encountered by those sensitive individuals that are exposed to fluoridated drinking water.

Waldbott described his early clinical work on fluoride intoxication (1955-1956) mainly in European journals and later in the early volumes of *Fluoride*. A posthumous contribution on the same topic was published in the 1998 Commemorative Issue of *Fluoride*.[47] Waldbott's observation that the symptoms could be cleared by replacing fluoridated water with distilled water was confirmed by a double blind study by GW Grimbergen published in the journal in 1974.[48]

FLUORIDATION AND DOWN'S SYNDROME

In 1957 and 1959, I Rapaport published reports showing that there was a significant relationship between residence in a fluoridated community and an increased prevalence of Down's Syndrome in younger mothers. The connection between fluoridation and Down's syndrome was supported by AW Burgstahler in an editorial in 1975[49] and a paper published in abstract form in the journal in 1997.[50] A review by K Takahashi presenting evidence that fluoridation is associated with a higher incidence of Down's syndrome is to be found in the journal in 1998.[51]

FLUORIDATION AND CANCER

In 1977 the journal published a research paper by J Yiamouyiannis and D Burk on the subject of age-dependence of cancer mortality related to artificial fluoridation.[52] These researchers compared the official US mortality figures in the 10 largest fluoridated US cities with those of the 10 largest US non-fluoridated cities. They reported that the average mortality for cancer increased faster in the fluoridated cities. In the same issue, an editorial by GL Waldbott reviewed the circumstantial, experimental, clinical, and statistical evidence prior to 1977 that indicated a positive relationship between fluoride and cancer.[53] Much of this evidence had been published in earlier issues of the journal.

The Yiamouyiannis and Burk study led to US Congressional hearings in 1977 that forced the National Cancer Institute (NCI), the Environmental Protection Agency (EPA), and the National Institute for Dental Research (NIDR) to nominate fluoride for study by the National Toxicology Program (NTP).

The report of the study carried out by Battelle Laboratories was released by the NTP in 1990. An editorial written by R Carton for the journal and published in 1991 criticized the published finding.[54] Carton, an employee of the EPA at the time, alleged that the conclusion that there was "equivocal evidence of osteosarcoma" in male rats had been subject to misinterpretation and possible downgrading of results.

Subsequent to the release of the NTP report, the journal published abstracts of the studies of RN Hoover and PD Cohn that were supportive of a relationship between osteosarcoma in humans and residence in a fluoridated community. J Lee reviewed this evidence, along with the NTP findings, in an editorial published in 1993.[55]

Recently, in 2001, K Takahashi, K Akinawa and their colleagues presented a statistical review using US data that gave further support for a connection between fluoridation and cancer.[56]

FLUORIDATION AND HIP FRACTURES

The treatment of osteoporosis with fluoride led to the reporting in the journal from 1986 to the present of the complication of increased incidence of hip fractures. Probably, as a consequence of this complication, a number of studies were carried out on the possibility of a relationship between long-term residence in a fluoridated community and fractures of the hip. In the majority of published studies, a positive correlation was reported. These papers are to be found in abstract form in the journal under such authorship as MFR Sowers, SJ Jacobsen, C Danielson, C Cooper, and others.

In a 1993 issue, an editorial by J Lee reviewed these studies and the interpretation placed on them by the US National Research Council report *Health Effects of Ingested Fluoride.*[57] Lee revisited this "continuing debate" in 2000. In an editorial, Lee pointed out additional support from a large 1995 study in France that had found the same positive correlation between water fluoride exposure and hip fracture increase.[58]

Lee pointed to the evidence that fluoride may increase bone *quantity* but also decreases bone *quality* and bone *strength*. He referred to the study of L Krook published in a 1998 issue of *Fluoride* that showed that the fluoride-induced increase in serum alkaline phosphatase, interpreted by conventional medicine as a sign of osteoblast activity, is actually a reflection of increased mortality of osteocytes in bone.[59] This process, according to Krook, releases the enzyme when the cells are killed by fluoride.

FLUORIDE AND ALUMINUM

In 1998, the journal published 2 abstracts of the work carried out by J A Varner, RL Isaccson and others that documented the development of significant pathological changes in the brains and kidneys of rats produced by a combination of fluoride at 1 ppm and aluminum.[60,61] These experiments replicated the situation commonly occurring in the treatment of water with both fluoride and alum.

A comprehensive review of the interactions of fluoride and aluminum by A Lubkowska, B Zyluk, and D Chublek was published as an editorial in *Fluoride* in 2002.[62]

SILICOFLUORIDES

In 2000 *Fluoride* published an abstract of the research of RD Masters and MJ Coplan on the association of silicofluorides treated water and elevated blood lead levels.[63] These researchers reported on the findings from a lead screening of over 280,000 children in Massachusetts. In 2001 another abstract of a report prepared by Masters, Coplan, and others showed similar findings from a study of 151,225 venous blood level tests from children ages 0-6 inclusive collected by the New York State Department of Children's Health.[64]

In a review editorial,[65] Masters and Coplan addressed the observation that silicofluoride and sodium fluoride behave differently in the body. The silicofluorides do not undergo complete dissociation and that 3 times the amount of fluoride crosses the gut/blood barrier than is the case with sodium fluoride. Two disturbing conclusions are brought forward. First, the commercial grade of silicofluorides used in water fluoridation since 1947 have never properly (or officially) been tested for safety in water fluoridation. Second, there is a statically significant association between silicofluorides and elevated blood lead levels. The risk to children of this finding alone is (or should be) obvious.

EFFICACY OF FLUORIDATION

From the outset, the journal has presented research papers, abstracts and editorials dealing with both the support and condemnation of the "benefits" to be achieved from water fluoridation as a way of preventing dental caries.

In 1981, the journal published a paper by R Ziegelbecker.[66] This researcher processed through his computer the results of all published studies to that date dealing with the relationship between fluoride in water and dental caries. These data included those of H Trendley Dean that formed the foundation of the concept. Ziegelbecker found no relationship. Later, in 1993, using the World Health Organization oral health data bank collected in 1987, he again showed that there was no inverse relationship between dental caries incidence and water fluoride levels.[67]

In 1990, the journal published the analysis by J Yiamouyiannis of the results (obtained through the Freedom of Information Act) of the 1986-1987 oral health survey of 39,207 US schoolchildren ages 5-17 that had been carried out by the National Institute for Dental Research (NIDR).[68] Yiamouyiannis showed that there were no significant differences in decay rates of permanent teeth or the percentages of decay-free children in fluoridated, partially fluoridated or non-fluoridated areas. The "official" report by JA Brunelle and JP Carlos of the NIDR showed a "benefit" of 17.7% (DMFS). This represents a difference of less than one tooth surface.

J Colquhoun, in an article published in *Fluoride* in 1993, showed that in New Zealand there had been a decline in caries prevalence that commenced *before* fluoridation was started.[69]

In recent years, *Fluoride* has published papers and abstracts that show prevalent opinion that fluoride does not have a systemic anti-caries effect but works topically. Studies have been published that show that when fluoridation is stopped, there is no increase but a continued decline in tooth decay, except as refined sugary foods and beverages reverse the trend.

FINAL REMARKS

Has the journal *Fluoride* fulfilled its mandate? It has been a forum for those who have expanded on the work of Roholm. It has provided a medium for research papers that may have been declined by the establishment-oriented traditional journals. At the same time, the journal has provided easy access to abstracts of many studies published elsewhere. It has also published special articles and editorials that have served as a review mechanism.

Those members of the ISFR whose names have appeared on the masthead of the journal during the past 35 years have served well and deserve our gratitude.

Fluoride has become a veritable encyclopedia covering all biomedical and related aspects of fluoride research. A glance at the 35-year cumulative index provides proof of this. Nevertheless, the reasons for its origin thirty-five years ago remain. *Index Medicus*, published by the US Public Health Service's National Library of Medicine, a major source of references to fluoride-related health topics, continues to decline the inclusion of *Fluoride* in its list of journals indexed.

The foremost effect of this ban is to deprive physicians, dentists, and other interested groups of a source of information concerning the effect of fluorides on humans, animals, and plants. This action appears to be motivated by the Public Health Service's desire to protect its stand on fluoridation in spite of the fact that the ISFR has at no time been involved in the politics of this program. This strongly suggests that those who do not want the truth to be known put the exclusion of *Fluoride* from *Index Medicus* in place to protect the image of fluoride and fluoridation.

The modern-day would-be-censors of scientific information should heed the warning that, eventually, the truth will come out and be heard. A step in this direction is the search engine *SciFinder Scholar* that *Chemical Abstracts* has introduced and that permits retrieval of papers in journals such as *Fluoride* that *Index Medicus/PubMed* do not cover. This could mean that justice will finally prevail.

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Editorial Office: 727 Brighton Road, Ocean View, Dunedin 9051, New Zealand

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After the appropriate revisions have been made, authors should submit the revised version either electronically as an attachment to an E-mail or by mail or courier as a hard copy in duplicate, together with a PC-formatted (not Macintosh) diskette that *includes* the *revised* text, references, tables, charts and figures (Word or WordPerfect is acceptable). Wherever possible, figures created on a computer should also be included on diskette in their native format (e.g., figure1.xls -Microsoft Excel; figure 2.cdr - CorelDraw; figure3.jpg, gif, tif, etc.-Adobe PhotoShop, Picture Publisher, etc).

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JULY 1968 VOL 1.

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SUBSCRIPTION RATES - Price per annum in advance including postage $12.00; Single copies $3.00. Cost for I.S.F.R. members $4.00 annually. Contributors will receive FLUORIDE - QUARTERLY REPORTS plus 20 reprints of their own paper free of charge.

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VOL 1. JULY 1968 PAGES 1 - 53

FLUORIDE

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Issued by

THE INTERNATIONAL SOCIETY for FLUORIDE RESEARCH

2930 W. Grand Blvd. Detroit, Michigan 48202 U.S.A.

Editor:
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Detroit, Michigan

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CONTENTS

INTRODUCTION

A group of scientists engaged in fluoride research in one department of a U.S. university was completely unaware of studies on fluoride underway in another department of the same university. In Italy, a well known scientist carrying out research on fluoride had no knowledge of fluoride research which had emanated from another university only a few miles distant. This lack of communication in fluoride research prompted the establishment of the International Society for Fluoride Research and of Fluoride - Quarterly Reports.

The Reports are issued in order to establish closer rapport between scientists of various disciplines in this and other countries, and to disseminate research findings on fluoride more effectively than in the past. They will feature, particularly, research data from non-English speaking countries which, in general, are not readily accessible to the scientific community in the U.S.A.

Many difficulties must be overcome in order to accomplish the Society's goal. For example, translation of papers from foreign languages into English, the official language, has already caused considerable delay in the appearance of The Reports.

No advertising is contemplated as a means of financing The Reports. Instead, an auxiliary of the I.S.F.R., consisting of lay persons, is being established. Its structure will be similar to that of the American Cancer Society, the Arthritis Foundation, The American Heart Association, etc. The Auxiliary will raise funds for research fellowships in conjunction with existing research institutions. Neither the I.S.F.R. nor the Auxiliary will engage at any time in political activities concerned with fluoridation or air pollution by fluoride.

An editorial committee of scientists, recognized as experts in fluoride research, from various countries and various disciplines is being formed. The format of The Reports will be improved as soon as funds permit. For the future an Editorial and Abstract Section featuring current research are planned. Except for occasional review articles, The Reports will be confined to papers featuring original research.

Most papers in the first volume of The Reports were presented at the First Conference of the International Society for Fluoride Research in Frankfurt, Germany, October, 1967. The October 1968 issue will feature papers dealing with chronic and acute intoxication in humans. The January 1969 issue will deal with fluoride as an air pollutant. The April issue will feature experiments bearing on fluoride metabolism.

ERRORS IN FLUORIDE ANALYSIS

by

W. Oelschläger
Hohenheim, W. Germany

Analysis for fluoride is subject to many errors. Some occur prior to the actual analysis, i.e., at sampling, grinding and ashing. Others arise if the samples contain much silica, if the distillation is not complete and if the distillate is not correctly concentrated. Before describing the method itself and explaining the semi-automatic distillation apparatus, the preparation of calcium oxide which has to be added to material of animal origin before it is ashed, will be discussed.

1. Errors upon Sampling

The procedure of sampling and preparation of the samples is often ignored. Even though the method of analysis may reach a high degree of accuracy, careless sampling will lead to inaccurate results. Recommendations for taking representative samples of forage can be found in the manual "Die Futtermittel" published by Woehlbier (1). Difficulties may arise with sampling of grassland crops. Since grain forage is the basis of the nutrition of ruminants, sampling of roughage requires much care. The composition of the sample with regard to the portions of grasses, herbs and legumes must correspond as closely as possible to the actual composition of the grassland since the F content of these individual components varies considerably.

Moreover, the results of F analysis of a sample will be substantially in error if it is contaminated by soil. F content of soil is usually higher than that of grass, hay, clover, etc. Our F balance studies in the ruminant revealed that the digestibility of F in vegetable matter is approximately the same as that of F in soil ingested by the animal. Therefore, substantial amounts of soil contained in roughage must be eliminated from the sample. Much of the soil drops off when the animal consumes its forage and is therefore not ingested. In order to obtain a measure of "cleanness" of a sample, the contaminating dust should be removed and its F content determined.

A high F content of leaves is not necessarily due to industrial emission. The source may be F in pesticides. In Western Germany a substance called "Werrenex" is used widely for the destruction of crickets. In Great Britain, U.S.A. and many other countries fluoroacetamide, a very powerful contact insecticide, is used for destruction of plant lice and termites. The German firm "Bayer" (Leverkusen) introduced a F containing spray called "Euparen" (dichlorfluoramide), which is powerful against scab. It is now widely used in fruit growing and horticulture. Upon inspection of apricot orchards in Switzerland in 1965 we found that in two of them, approximately ½ to 1 km apart and about 4 to 5 km distant from an aluminum reduction plant, the leaves were necrotic. They contained 140 to 240 ppm F (with an average 210 ppm) in the dry matter. On the other hand the leaves of trees from adjoining orchards appeared to be healthy; their F content was normal i.e. 5 to 8 ppm in dry matter.

2. Errors in Grinding the Samples

Only such types of mills are suitable which prevent loss of material at grinding. With most grinding mills small particles such as blooms and leaves are blown away. This results in loss of fluorine. An investigation of red clover revealed approximately equal amounts in leaves and stalk, namely about 5.2 ppm, whereas the remainder of the plant contained less than half this amount, i.e. 2.1 ppm on a dry basis.

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Furthermore, it is imperative to obtain at grinding a very fine product which assures a high degree of homogenicity. In order to determine errors in connection with grinding, the red clover was washed with doubly distilled water before drying in order to eliminate contamination by soil. It was ground rather coarsely (approx. 1mm screen). The grist thus obtained was shaken in a glass bottle by circular movements in order to separate the coarse particles from the fine. The top layer contained 6.1 ppm of F, the bottom 14.1. In ground barley, we found wide variations in F content. Of a batch prepared for a F balance trial 7 samples were taken with F values between 0.7 and 1.2 ppm (average 1.1 ppm) in the dry matter. This finding induced us to separate the pellicle from the grain. The pellicle contained 3.4 ppm F, the grain only about 1/5 this amount i.e. 0.7 ppm. Therefore grinding must be done very thoroughly.

For F determination in soil it is imperative to take a large sample and to ascertain that it is thoroughly ground because the quantity to be weighed for F determination has to be fairly small, i.e. approximately 0.1 gram. Greater quantities such as 0.5 grams or more would necessitate distilling more than 1000 ml. Furthermore, if soil contains a large amount of silica, F determinations are usually inaccurate.

The importance of thorough grinding of soil prior to analysis is illustrated in the following trial: About 400 kg of soil collected in the vicinity of an aluminum reduction plant were mixed as thoroughly as possible and strained repeatedly through a screen of a few millimeter mesh. From this material 21 samples of about 30 g each were taken. Soil thus sampled and treated was used in a balance trial to investigate the digestibility and retention of F derived from soil. All 21 soil samples were ground in a mortar and F contents of 590 up to 2290 ppm (average 840 ppm) were found. Double determinations for each of the 21 samples agreed closely. The greatest difference was 18%. Much better results were obtained with 5 very thoroughly ground samples of 200 g each. In these 5 samples the difference of the duplicate determinations was about 5% and in the remaining 4 samples less than 2%. Due to the solubility of the F in dilute acid it is likely that the top layer of this soil contained F of industrial origin.

For the determination of F in leaves a considerable quantity has to be ground. From this material a subsample has to be taken for analysis. This procedure is necessary because grinding of the leaves produces in addition to the grist some hairy material which contains more F than the remaining parts of the leaves. In the fine material we found 14 ppm (dry matter) and in the hairy bundle 49 ppm. Furthermore, in wine leaves collected near an F emitting industry 67 ppm were found in the fine material, 77 ppm in the hairy bundle. In order to obtain a homogeneous sample 5 to 10 grams must be taken and the whole material analyzed.

3. Errors in Ashing in the Muffle Furnace

Faulty results may be expected when the furnace is heated to high temperatures, because the muffle (lining of the furnace) made of clay always contains F. We found in it 350 ppm. F is likely to escape as gaseous silicon tetrafluoride (SiF_4). When a new furnace was heated at temperatures of 400°C, 600°C and 800°C and the air drawn off for two or more hours at a temperature of 450°C, no gaseous fluorine was detected in the furnace; at 600°C and 800°C, 70 µg and 320 µg respectively were found. When red clover was ashed overnight at temperatures of 450°C, 600°C and 800°C the average F values were at 450°, 1.7 ppm; at 600°, 2.7 ppm; and 800°, 3.2 ppm. In a simultaneous investigation of this source of error, Gisiger, director of the Agricultural Research Station, Liebefeld-Berne (Switzerland), obtained results that agreed with ours. Upon ashing samples of roughage in a furnace - which had been in operation for more than 10 years - for 2, 6, 12, 24 and 48 hours at 540°C, he found consistently increasing F values, namely 20, 21, 23, 28 and finally 37 ppm.

The above-mentioned reasons induced us to recommend, as long as 8 years ago, that ashing be done at 450° instead of 550° to 650°C. With material of animal origin, such as urine, milk, heart, liver, lung, bones etc., considerable time at 450°C is required for ashing. To prevent contamination, the ignition spaces of our furnaces including the doors are covered with gas-tight casings of nickel plate. During the last 4 to 5 years, this arrangement proved to be successful. Iron-chromium-nickel sheeting was not suitable.

Samples containing comparatively large amounts of silica should not be heated to more than 550°C as fluorine is likely to escape at higher temperatures. For this reason, the samples should be inserted as quickly as possible into the preheated furnace. Depending on the fineness of the sample, the furnace should be kept closed in order to avoid conflagration (temperature up to 800°C).

4. Samples Containing Slightly Elevated Amounts of Silica Fuse with Sodium Hydroxide

When ashing samples high in silica, probably silicium-oxy-fluorides are produced which bind fluorine tightly. Therefore, fusion with sodium hydroxide is absolutely necessary. On analyzing grass samples containing .5% silica without sodium hydroxide-fusion we found 2.5 ppm F, with fusion 3.2 ppm. In another grass sample containing 1.06% silica without fusion 1.3 ppm was found, with sodium hydroxide fusion the amount was twice as high, i.e. 2.6 ppm. With a grass sample containing 1.62% silica, the F content was 3 times higher when ashed with sodium hydroxide i.e. 0.9 ppm without, and 2.7 ppm with fusion. Fusion with sodium hydroxide provides an additional advantage since carbon particles, occasionally present in the ash, will be oxidized completely.

5. Errors during Distillation of Fluorine

Errors of considerable magnitudes may be due to insufficient distillation, i.e. too small a volume of distillate. When ashes are high in silica, it also makes a difference whether fluorides of high solubility or ashes of biological material are analyzed.

Tables 1 and 2 show the amounts of fluorine in 500, 1000, 1500, 2,000 and 2,500 ml distillate of various samples. We obtained 500 ml of distillate in approximately 35 to 40 minutes, and 1000 ml in about 60 - 65 minutes.

TABLE 1

F Content in 500 and 1000 ml Distillate upon Distillation of Different Amounts of F (as NaF)

F added	Number of Samples	Fluorine found in 500 ml Distillate: µg	Average µg F	Minus Blank Test Result µg F	%	in 1000 ml: µg F	Minus Blank Test Result µg F	%
0	17	0-0,7	0,3 (blank test)					
2,0	5	1,9-2,5	2,2	1,9	95			
5,0	5	4,7-5,5	5,1	4,8	96			
10,0	5	9,5-10,2	9,8	9,5	95			
20,0	5	18,0-19,6	18,8	18,5	93			
50,0	5	47,4-48,9	48,0	47,7	95			
100,0	1		95,0	94,7	95	98,4	98,1	98
500,0	1		490,0	489,7	98	496,0	495,7	99
1000,0	1		980,0	979,7	99	988,1	987,8	99

When there was less than 50 µg F in 500 ml of distillate an aliquot part of the distillate was evaporated to 50 ml before the colorimetric determination.

TABLE 2

Passing of F into Distillate during Ashing.
F in Distillate of Ashes of Different Substances.

Sample	Orig. Wght. g	Cont. of Si %	F found in Distillates 500 ml µg F	%	1000 ml µg F	%	1500 ml µg F	%	2000 ml µg F	%	2500 ml µg F	%	mgG/100g dry
Soybean Oil Meal	10	0,1	23,6	96	24,7	100	-	-	-	-	-	-	0,25
Dried Sugar Beet Pulp *	10	1,5	286,0	73	350,3	89	370,3	94	380,0	97	386,8	98	3,94
Ash of Bones	0,1		410,0	99	415,5	100	-	-	-	-	-	-	415
Fish Meal	10	0,2	1630	93	1703	97	1730	99	1744	99	1753	100	17,5
Heart	20		10,4	100	-	-	-	-	-	-	-	-	0,05
Liver	20		21,3	100	-	-	-	-	-	-	-	-	0,11
Urine	50		230,5	95	240,5	99	242,2	100	-	-	-	-	0,48
Faeces	5		104,2	71	127,1	87	140,3	96	144,4	99	146,0	100	2,92
Water	500		73,0	89	79,8	98	81,7	100	-	-	-	-	0,16/l
Flue Dust (Al-Factory)	0,1	0,1	13600	99	13700	99	13737	100	-	-	-	-	13737
Rock Phosph. (Curaphos)	0,1	0,3	465	91	510	100	-	-	-	-	-	-	510
Soil	0,1	18	70,0	91	73,3	95	75,8	98	77,0	100	-	-	77,0
Soil **	0,3	18	188,0	82	210,0	92	216,6	95	220,7	97	223,9	98	76,1
Soil **	0,5	18	263,0	72	311,0	86	323,5	90	333,5	92	340,9	94	72,2
Red Clover	10	0,03	60,8	96	63,3	100	-	-	-	-	-	-	0,63
Alfalfa	10	0,1	30,4	90	33,7	100	-	-	-	-	-	-	0,34
Hay	10	0,6	58,5	67	73,8	85	82,0	94	85,7	98	87,2	100	0,87
Grass	10	0,8	12,5	62	15,9	79	18,6	92	20,1	100	-	-	0,20
Leaf of Cherry Tree	10	0,3	56,5	97	58,5	100	-	-	-	-	-	-	0,59
" Apricot - "	10	0,3	47,0	91	51,5	100	-	-	-	-	-	-	0,52
" Apple - "	10	0,3	72,5	90	79,0	98	81,0	100	-	-	-	-	0,81
Ground Grain of Barley	10	0,3	7,0	82	8,6	100	-	-	-	-	-	-	0,09
" of Oat	10	0,5	12,3	83	13,8	93	14,9	100	-	-	-	-	0,15

*Fluorine distillation only quantitatively with a volume of 3500 ml distillate.
**3500 ml and 5000 ml distillate are required with soil samples weighing 0,3 g and 0,5 g.

6. Reducing the Distillates

When 50 ml of fluorine-containing solution are required for colorimetric determinations, it is advantageous, depending on the concentration, to reduce the whole distillate or an aliquot of it prior to photometric measuring in order to achieve analytical accuracy. Addition of a sodium hydroxide solution to the F containing solution, which has a pH of approximately 4.2 up to 7 or even more is undesirable. It leads to major errors, especially with samples of low F content. Toward the end of the evaporation the solution becomes rather alkaline and corrodes even quartz glass. Thus, not only fluorine of the glass (Table 3) goes into solution but aluminum, iron and silica, etc., as well.

TABLE 3

Fluorine in Glass and Quartz Glass

Quartz Glass	0,67 mg F/100 g
Pyrex Glass	0,91 mg F/100 g
Vicor Glass (English Quartz Glass)	1,17 mg F/100 g
Duran Glass	9,64 mg F/100 g
Jena Glass	162,60 mg F/100 g

These elements disturb to a varying degree the photometric detection of fluorine with Alizarinkomplexan and Lanthan-III-nitrate (reagent of Belcher) Table 4.

TABLE 4

Effect of Disturbing Elements of F Determination with Alizarinkomplexan and Belcher Reagent After Application of 10,0 µg F

Disturbing Element in µg:	0	10	30	50	100	200	300
	F found in µg						
Al	10,0	7,5	1,8	0	0	0	0
Fe	10,0	11,1	11,7	13,0	14,6	muddy	muddy
Cu	10,0	10,2	10,1	9,1	1,8	0	0
Zn	10,0	10,3	10,4	10,4	9,1	4,3	1,8
PO_4 *	10,0	10,0	9,6	9,4	8,0	8,2	7,2
Ni	10,0	10,0	10,0	10,0	10,4	10,8	11,2
Cr	10,0	10,0	10,0	9,9	9,8	9,6	9,4
As **	10,0	10,0	10,0	10,0	9,8	9,8	9,8

1 mg each Na, K, Ca, Mg, B, SO_4, NO_2, S_2O_5, 30 mg SiO_3 and 300 µg Cr do not interfere with F determination

* PO_4: 1 mg = 1,2 µg F; ** As: 1 mg = 8,7 µg F

Evaporation to reduce the distillate should not be carried out in open glass, quartz or platinum dishes. Gases present in the laboratory air such as So_3, No_2, Cl_2, and other foreign elements are likely to get into the solution. After evaporation of 500 ml double distilled water to a volume of 50 ml we found 2.5 µg copper and 12.3 µg iron. Therefore, we evaporate in a round quartz flask which is connected with a condenser. Half automatic operation is obtained by a liquid-switch relay, i.e. evaporation of the solution is automatically switched off when the desired volume is attained. Table 5 shows the amount of fluorine found in 50 ml solution after evaporation of 100, 200, and 500 ml double distilled water to which 0, 2.0, 5.0, 10.0 and 20.0 µg fluorine were previously added.

TABLE 5

Fluorine Found after Evaporation of 100 ml, 250 ml and 500 ml Aqua bidest. which Contained from 0 to 20 µg F

Volume Aqua bidest. ml	F added µg	F found µg		Volume Aqua bidest. ml	F added µg	F found µg	
100	0	0	0	100	10,0	10,0	10,0
250	0	0	0,2	250	10,0	10,2	10,1
500	0	0,2	0,3	500	10,0	10,3	10,3
100	2,0	2,0	2,1	100	20,0	20,0	19,8
250	2,0	2,1	2,1	250	20,0	20,1	20,2
500	2,0	2,3	2,2	500	20,0	20,4	20,3
100	5,0	4,9	5,0				
250	5,0	5,1	5,0				
500	5,0	5,2	5,3				

7. Preparation of Low Fluoride Calcium Oxide

During ashing of such substances as leaves, clover, alfalfa, hay, potatoes, forage and sugar beets, oats, barley, wheat and corn, milk, urine and faeces, fluorine does not escape. With other material it may do so in considerable amounts. Upon ashing seed rich in oil, such as hemp, papaver and flax, we found the values to be 20 to 30% deficient. Substantially lower values were found when we analyzed bees (70%), animal tissues such as muscle, heart, liver, lung, spleen (50 to 80%) and blood serum, plasma and erythrocytes (approx. 50%). Upon addition of magnesium and especially calcium salts, before ashing of such samples, fluorine will be quantitatively bound. Calcium oxide and other substances which may be used for this purpose, are usually contaminated by considerable amounts of F.

We tried to obtain calcium oxide low in fluorine by the von Fellenberg method: Dissolved bicarbonate of sodium is added to a saturated solution of calcium chloride. Three successive precipitations are discarded. Finally the bulk of the precipitated chalk is calcined in the muffel furnace at 1000°C. This method has the disadvantage that a considerable amount of fluorine escapes on calcination as silicon-tetrafluoride (SiF_4) from the chamotte lining of the furnace; we found 350 ppm. In precipitated chalk with a degree of purity "pro analysi" (Merck) we found 2.6 µg fluorine per gram and in calcium oxide prepared from this material (after heating in the muffel furnace without a gas-tight lining of nickel sheeting for 1 hour at 1000°C) 27 µg fluorine per gram.

A very low F content was found in calcium oxide produced as follows: to 500 grams of calcium chloride ($CaCl_2$, $2H_2O$ Merck "pro analysi") dissolved in 1000 ml water 80 g of sodium carbonate dissolved in 450 ml water was added; the precipitated chalk was discarded. After repetition of this procedure the bulk of chalk precipitated with sodium carbonate. The use of doubly-distilled water and polyethylene vessels is advisable.

The third precipitation was calcined in a crucible furnace, the inside of which was fitted with a gas-tight lining and the door coated with platinum foil. The fluorine content of calcium oxide prepared in accordance with the above-mentioned procedure was 0.3 µg per gram. This value corresponds to that of a blank run. The fluorine content of the second precipitation was twice as high and that of the first precipitation 25 times as high.

Summary

In fluoride analysis significant errors may occur on sampling, grinding and ashing of biological and other substances. They are most troublesome with samples of low-fluorine content and with samples of which only small quantities are available.

Special precautions are indicated if samples contain silicic acid. Losses of fluorine as well as contamination on distillation and evaporation in the case of low-fluorine content are discussed. Before ashing animal materials (i.e. bees, heart, liver, blood etc.), calcium oxide has to be added. Preparation of calcium oxide, containing a minimum amount of fluorine, is described.

Finally, the method of fluorine determination is outlined. Examples for plant and animal materials are given. Sources of error beginning with the preparation of the samples up to photometric determination with lanthanum-III-nitrate and alizarin-complexon, are described. As samples we used roughage, succulent food concentrates, oil-containing seeds, leaf-samples, bees, animal tissues, urine, faeces, and soil. The distillation is carried out with fluorine - and nitrate - free 24 H sulfuric acid and iron-II-sulfate as reducing agent. The volume of the distillate can be reduced with automatic devices made of a glass with a very low fluorine content.

The accuracy of the method and the reproducibility of the results are quite satisfactory (Table 6), even with samples of low F content.

TABLE 6

Exactness of F Determinations in Different Samples

Substance	n	mg F / 100 g dry Substance			Average Coefficient of Variation of 3 Parallels in each Case. %
Ground Grain of Barley	6	0,10	±	0,020	7,9
Dried Sugar Beet Pulp	6	2,53	±	0,296	2,5
Palm Kernel Oil Meal	6	0,24	±	0,034	8,2
Coconut Oil Meal	6	1,05	±	0,051	4,3
Concentrate	5	0,16	±	0,032	5,5
Hay	6	1,14	±	0,737	6,6
Soil *	6	79,74	±	6,753	1,4
Urine	18	0,28	±	0,051	6,3
Urine	15	0,71	±	0,131	3,4
Urine	15	1,66	±	0,155	2,3
Urine	12	2,12	±	0,124	2,4
Faeces	17	3,15	±	0,334	2,9
Faeces	5	7,85	±	0,835	0,6
Faeces	4	12,54	±	0,275	1,6
Faeces	8	16,41	±	1,441	1,7

*Samples ground as fine as possible.

THE ANALYTICAL PARTITION OF THE FLUORINE COMPOUNDS PRESENT IN SOME TROPICAL PLANTS AND SOILS

by

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For several years I have been interested in plants which are capable of synthesizing carbon-fluorine compounds. For a long while it was generally assumed that such compounds did not occur in nature, until in 1944 the South African biochemist Marais (1) proved that potassium fluoroacetate is the toxic principle of the tropical plant, Dichapetalum cymosum. This plant, commonly called gifblaar, meaning poison leaf, is indigenous to the veldt of Pretoria and is responsible for the deaths of many grazing animals because it is almost the first green vegetation to appear in spring. As little as 20 g of fresh leaves has been known to kill sheep. Since the original work of Marais, monofluorinated organic compounds have been isolated from at least four other plants, two more of the Dichapetalum species (2) and two which grow in the tropical regions of Australia. These are Acacia georginae called gidyea (3) and Gastrolobium grandi florum, known as heart poison bush (4).

In 1960, a small group of biochemists, working with Sir Rudolph Peters at Cambridge, succeeded in isolating and identifying the main toxic principle of another of the poisonous African plants, D. Toxicarium (5). In this case it is a long-chain carboxylic acid which was called fluoro-oleic acid. This compound, with other fluoro-intermediates, is present in the seed of the fruit and is even more toxic than fluoroacetate.

The pharmacological significance of the fluoroacetates and their related compounds and of their profound effects upon the heart and central nervous system is well established: They are among the most poisonous substances known. Sir Rudolph Peters (6) showed that animal tissues can metabolize fluoroacetate to fluorocitrate which blocks the Krebs tricarboxylic acid cycle by inhibiting the action of the enzyme aconitase. His classical postulation of this in vivo situation, which he called "a lethal synthesis," is the accepted explanation of the mode of poisoning.

In order to study the synthesis of carbon-fluorine compounds by these plants, I have had to examine very closely various analytical procedures for the accurate assessment of fluorine in plants and soils.

The accurate determination of fluorine in biological specimens is extremely difficult and presents a special set of problems. The procedure will necessarily vary according to the material. Techniques involving the isotope ^{18}F or pyrohydrolysis do not easily lend themselves to this kind of sample; one has to rely on more conventional chemical methods.

At the very beginning it had to be decided what forms of fluorine were to be measured. The total fluorine could be obtained by a suitable ashing procedure followed by the chemical determination of the fluoride. But fluorine exists in a number of forms in the plants under investigation, namely free inorganic fluoride, compounds containing fluorine which are acid and alkali-labile, and long-chain carbon compounds which need to be ashed with strong alkali or oxidising agents to free the fluorine. In some plants, fluorine is present with silica, and these also

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require special degradation treatment. In soil, most of the fluorine is combined in very insoluble fluoro-minerals containing calcium, magnesium, and silica. As far as I know, no naturally occurring organic compounds of fluorine have so far been isolated from soil.

Therefore the fluorine compounds were partitioned in the rather arbitrary manner shown in Table I.

TABLE I

Partition of Fluorine in Plants and Soils

	Soils	Plants
Inorganic	Water extractable - simple fluorides	Acid-extractable ($N.HClO_4$) - simple fluorides
	Perchloric acid diffusible fluorides and fluorophosphates of Ca, Mg	Perchloric acid diffusible - ? protein bound F
	Complex minerals with Al, Ca, Mg, Si, after alkaline fusion	Fluoride with silica after alkaline fusion
Organic	None so far identified	Alkali-labile - short chain CF compounds, i.e. fluoroacetate
		Long-chain CF compounds soluble in organic solvents

In soils the water-extractable fluorine is probably easily available to all plants. That they may absorb it selectively, is another matter for research. I have called the fluoride which is liberated by treatment with 60 percent perchloric acid at 60 ^{0}/C over 24 hours the "acid-labile" or "diffusible" fluoride. In soils this can be regarded as the potentially total available fluoride as far as the plant is concerned. It will include fluorapatite as well as simpler fluorides but probably not organically-bound fluorine nor some of the complex mineral and fluoro-silicates. To determine the total fluorine in soils the material must be fused with strong alkali.

As far as most plant tissues are concerned, the total fluorine equals the diffusible or acid-labile fluorine but in the species under investigation, in addition to inorganic fluoride, there are two groups of organically-bound fluorine which are not degraded by strong perchloric acid. Some are alkali-labile and others are soluble in organic solvents such as light petroleum or carbon tetra-chloride. The alkali-labile group consists of the shorter chain compounds such as fluoroacetate from which the fluorine can quite easily be removed by treatment with strong alkali (5). It is possible that some of the fluorine present in these plants is bound to protein.

Very little is known about the inorganic fluorine compounds in plant tissue. Some plants contain high levels which suggests that the fluorine is present in a comparatively inactive form, possibly combined with calcium or magnesium or perhaps as fluorophosphates and stored in the various tissue cells.

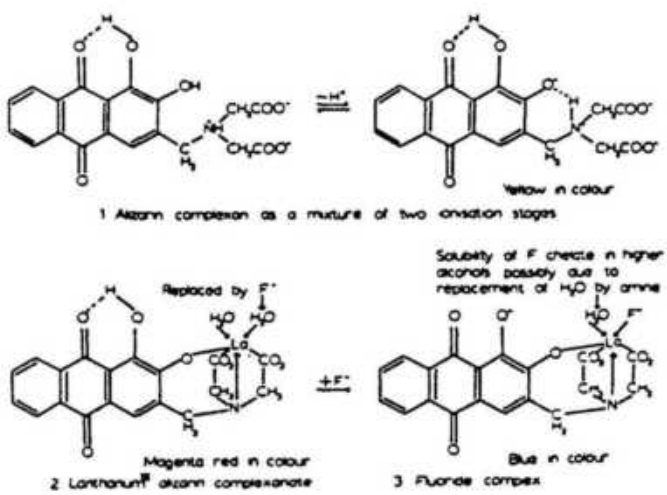
Having decided on this general pattern of partition, I next applied the method of analysis. Distillation procedures for the separation of small amounts of fluorine (as hydrofluoric acid) from interfering substances are quite unreliable. The separation of hydrofluoric acid by diffusion which was first proposed by Singer and Armstrong in 1954 (7), has, however, gained considerable popularity among biochemists, and several elegant variations have now been described. The method I use consists of absorbing the diffused HF, produced by the action of strong perchloric acid, onto filter paper treated with magnesium succinate. The separation is done in a small polythene bottle of 20 ml capacity (8, 9). The advantages of such a method are the quantitative separation of fluoride quite free from interference and the large numbers of determinations which can be handled.

For the actual estimation of the fluoride ion I have adopted the reaction with alizarin complexan which was first described by Belcher and his colleagues at Birmingham in 1958 (10).

Alizarin complexan, a comparatively new chemical, is a substituted anthraquinone forming red chelates with certain rare metals, notably cerium and lanthanum. These chelates are changed to blue by the action of the fluoride ion. In later work, Leonard and West (11) made a passing reference to the solubility of the blue fluorochelate in pentyl alcohol containing an amine, and this observation led me to investigate the reagent to determine fluorine at submicrogram levels (8). This reaction was found to be very sensitive in the presence of a succinate buffer of pH 4.6. The formation of the lanthanum fluorochelate is preferable to that of cerium. Solubility studies in organic solvents revealed that the blue fluorochelate is extracted quantitatively into isobutyl alcohol containing hydroxylammonium chloride, whilst the lanthanum chelate alone is only very sparingly soluble. The fluoro-complex is thus formed free from color interference from the reagent and a virtually colorless blank is obtained. The fluorochelate in isobutanol is measured at 570 mμ, and there is a straight-line relationship in absorbance for 0.1-1.0 μg of fluoride in 4 ml of solvent. The separation and measurement of as little as 0.1 μg of fluoride is quite feasible. The chemical reactions are shown in Figure 1.

Fig. 1

The Reaction of Alizarin Complexan with Inorganic Fluoride.



M.A. Leonard of Queens University, Belfast, has thoroughly investigated the chemistry of the reaction and believes that the fluorochelate is formed because fluorine replaces one of the water molecules of the metal chelate. Its solubility in the organic solvent is due to the replacement of the other water molecule by an amino group (11).

To return to the beginning of the analytical operation, in the preparation of the sample, it is often very difficult to know how to strike a balance between possible contamination and possible loss of fluorine. Some workers go to considerable trouble to wash their plants with dilute solutions of detergents to remove surface contamination but frequently this is neither practicable nor necessary.

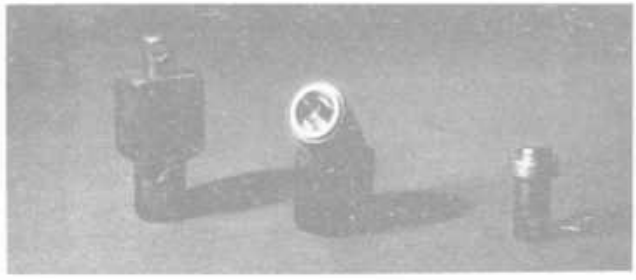
For most plant specimens, a quick distilled water rinse is quite adequate. Drying itself can be a source of considerable error. Most workers dry specimens at temperatures between 60 and 105°C for several hours. Although this is safe for many materials, I have measured losses in leaks of over 10 per cent of the total fluorine even when they were freeze-dried. From a practical point of view, drying at 25°C in a moving current of air is probably safest but drying at 60°C overnight is usually satisfactory. Soil is conveniently air-dried for two days at 20-25°C.

Grinding does not usually present problems but the fineness to which the sample is ground is important. For soils and some plant specimens a special ball mill is used, the interior of which is heavily chromium-plated with heaters of chromium-plated steel rod. To obtain a representative sample, the ground material is put through a nylon or stainless steel sieve with a mesh aperture of 50 microns. It must be remembered that the fluorine level varies considerably with the location of the tissue within the plant. Plants growing in fluorine-rich soils tend to have more fluorine in the roots than in the leaves or stems, but those growing in contaminated atmospheres have the highest levels in the leaves and especially in the tips.

Another important stage in the preparation of the sample is ashing to destroy the organic matter and to convert all fluorine to inorganic forms. Some fluoro-compounds are extremely stable even when ashed at 600°C with strong oxidizing agents. Combustion in a Parr-type bomb with sodium peroxide or metallic sodium is sometimes necessary. Anyone who has used this apparatus knows the difficulties of sealing it and of obtaining satisfactory recoveries of fluoride. For some of my work with long-chain fluoro-fatty acids, special bombs were made. The combustion chamber and caps were made of pure nickel and the interior surfaces kept highly polished to prevent absorption of fluoride on the metal. The jackets were made of vanadium/tungsten steel which made them virtually non-corrodable. Sealing was effected by means of a pure gold washer (Fig. 2).

Fig. 2

Special Parr-type Bomb Showing Polished Interior.



For most animal and plant tissues and soils, ashing in small platinum crucibles was found to be very useful but contamination is a major problem. It is surprising how much fluoride can be found in high-grade chemicals. Calcium, magnesium, lithium, and sodium salts contain the highest levels of fluorine. It is necessary to purify many of the reagents to obtain acceptably low blanks.

A suitable fixing agent for the fluorine during ashing is a mixture of lithium hydroxide and magnesium succinate. The most serious source of contamination is probably from the lining of the muffle furnace itself. Several micrograms of fluorine can often be washed from an empty platinum crucible after it has been in a muffle furnace for a few hours at an elevated temperature. In the presence of an alkali much larger quantities of fluorine can be absorbed and many of the published figures for animal and plant tissues may be too high because of such contamination.

I have tried, with variable success, a number of ways of dealing with this extremely difficult problem, including using a silica-lined muffle furnace. For most purposes, I now carefully heat individual platinum crucibles over a small spirit burner. This is not as tedious as may be thought, and it is reliable. It takes only a few minutes to reduce 100 mg of sample to an ash. The ashes of soils and silica rich plants are then fused with strong alkali to break down complex fluorides. Recoveries of up to 1 µg of added fluoride put through the whole procedure are consistently more than 90%, and usually more than 95%. The blanks are satisfactorily low with the outlined procedure.

A source of error which is not very obvious, but important in the direct diffusion of HF with the kind of materials which were analysed, is the actual sampling for determination. At first I weighed up to 100 mg of finely ground plant or soil directly into the diffusion bottle or platinum crucible, and I obtained very erratic results. When the specimens were ground to a particle size of 50 microns or less the results were more reproducible but still variable. With direct diffusion the trouble seems to be the inability of perchloric acid to disintegrate the sample which tends to form lumps. In the search for a solution to the problem I found that very finely ground plant and soil particles can be held in homogenous suspension for a long while with a solution of 0.1% agar, and the reproducibility of sample weights in successive aliquots is surprisingly good (Table 2). I now routinely prepare suspensions of all specimens in agar and obtain good replicate results.

TABLE 2

Weights of Plant and Soil Suspensions (mg/ml) in 0.1% Agar

Plant A		Plant B		Plant A		Plant B	
51.3		22.7		49.3		21.7 •	
51.6		22.7		49.3		21.7	
51.5		23.0		49.6		21.0	
51.1		23.0		49.2		21.2	
51.3		22.8		49.5		20.8	
51.9		22.6		49.0		20.6	
51.7		22.7		48.9		21.2	
51.5		22.8		49.7		21.0	
51.3		22.6		49.7		21.7	
51.0		22.7		49.4		22.2	
Mean 51.4	0.5	22.8	0.2	49.4	0.4	21.3	0.8

• Very sandy

TABLE 3

Fluorine Partition in Some Tropical Plants

Species (leaves)	Fluorine Total	µg/g Dry Wt. Diffusible	F. Acetate	F. Oleic
Acacia georginae (Toxic)	44	23	+	
Dichapetalum cymosum	88	14	+	+
Dichapetalum stuhlmanni	144	9	+	+
Dichapetalum toxicarium	84	61	?	?
Dichapetalum toxicarium (nut)	1443	610	+	+++

In Table 3 some indication is given of the partition of the fluorine compounds in a selection of the Tropical plants under study. It is hoped to publish these analyses in detail at a later date.

Obviously a wide variety of techniques such as infra-red spectroscopy and gas chromatography must be employed for the actual identification of specific components, but the final evaluation of any research findings must hinge on the ability to determine accurately the often extremely small amounts of fluorine which are present. For this kind of research the micro-analytical approach which combines a diffusion technique with the alizarin complexan reaction has so far produced the most reliable results.

ACKNOWLEDGEMENT: I wish to thank Prof. J.H. Burnett and Prof. S.L.R. Ranson of the Dept. of Botany, University of Newcastle upon Tyne, who have so kindly provided the facilities for much of this work.

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FLUORIDE METABOLISM IN ACACIA GEORGINAE

by

R. A. Peters and M. Shorthouse

Author's Abstract

We have shown that seedlings of A. georginae take up fluoride from solutions of NaF (15.75 mM). The uptake is much greater from a solution initially at pH 4.0 than from one at pH 6.6. Organically combined fluoride is present in greater amounts when the uptake of fluoride is greater, and tends to be larger in the roots than in the aereal parts. We do not think that the fluoride induces an inhibition of enolase in the plant, or that a chloro-compound is an intermediate. The possibility that fluorophosphate is an intermediate has been examined carefully. We can find none in the plants, though we think that both A. georginae homogenates and other extracts hydrolyse this slowly. Synthesis of organically combined fluoride has been observed in homogenates of seedlings of approximately 30 cm in height part of which has been identified as fluoroacetate. We are trying to improve the conditions for this synthesis.

From Department of Biochemistry, Cambridge, England.

THE DEGREE OF VARIATION IN URINARY FLUORIDE LEVELS IN SUBJECTS NOT UNDULY EXPOSED TO FLUORIDE

by

René Truhaut and Nguyen Phu Lich
Paris, France

The monitoring of the degree of exposure to various toxic compounds in man's environment, and the extent of consequent uptake, is one of the main preoccupations of toxicologists and hygienists. The aim of such studies is to implement required therapeutic or preventive measures. The "preventive" aspect is particularly important in cumulative or "hypersensitive" toxicities where, as a result of retention in the human organism, the long-term manifestations of toxicity are often without gross clinical symptoms, and are thus particularly insidious. This is a typical result where mineral fluoric derivatives are involved.

The fluoric component, absorbed in sustained small doses, is stored in the calcified and parenchymal tissues of the organism, and is thus able to provoke chronic intoxications, i.e., fluoroses, with diverse etiologies. The affliction is classified into two main groups: Fluorosis of a geological or telluric origin, and fluorosis of industrial origin (1). Such situations have prompted interest in fluoride determinations in soil, water, foods, industrial effluents, and biological fluids and tissues of exposed subjects, all in an effort to obtain precise data concerning the extent of exposure.

However, as is the case with other elements (As, B, Pb, Hg, etc.), fluorine is normally present in all living matter and, especially, in the human organism. This should not be surprising, because the widespread distribution of this halogen in geological formation, soil and water attests to its availability for incorporation by vegetation and by members of the animal kingdom. As a consequence of ingestion, fluoride is stored in human tissues. It is therefore important to distinguish between what might be termed "normal" physiological fluoride and that prevailing under toxic conditions, and to attempt to determine a maximum "normal" concentration.

The presence of fluoride in calcified tissues (bones and teeth) has been recognized for a long time. The levels found in these tissues can be influenced by type of nutrition (especially in areas using fluoridated water), by hygienic practices (use of fluoride-containing tooth pastes) and by the age of the subject. Data is usually reported on an ash-basis and, in the case of human adults, can vary from 0.50 to 1.50 g/kg (500 to 1500 ppm) in bone, and from 0.19 to 0.30 g/kg (190 to 300 ppm) in teeth (13).

Concentrations in blood have been determined by several authors. The values in the earlier reports range between 350 and 1450 µg/l (0.35-1.45 ppm) with most of them in the neighborhood of 800 µg/l. Levels found in plasma and serum are higher than those detected in erythrocytes (13). According to Albritton (2), the normal concentration of fluoride in blood varies between 100 and 450 µg/l (0.10-0.45 ppm). More recent data, obtained with more reliable methods, yield lower values. Largent (3) obtains values which do not exceed 90 µg/l, whereas Taves (4) reports an average value of 13 µg/l for "diffusable" fluoride in human serum. Singer and Armstrong (5,6) report average values of 130 µg/l for "total" fluoride in human serum.

From the Faculty of Pharmacy, University of Paris.

In the case of urine, the results obtained by Goldemberg and Schraiber (7), i.e., from 3 to 10 mg/l, are clearly too high. The same applies to the value of 1 mg/l, reported in Machle's first study (8) and the 0.92 mg/l reported by Brun et al (9). However, the levels of 0.40 mg/l reported in Machle et al.'s second study (10), the 0.30 to 0.50 mg/l range reported by McClure and Kinser (11) in humans on low fluoride intake, and the 0.33 to 0.50 mg/l values of Teichman et al (12) all suggest that "normal" urinary fluoride does not exceed 0.50 mg/l.

As concerns other biological material, fluoride is found in feces and in hair (13).

The determination of fluoride in biological organisms, as a means of assessing the degree of uptake, is of great interest. The ideal procedure would dictate emphasis on those tissues that most avidly accumulate fluoride, i.e., bone. Unfortunately, a diagnostic procedure that requires a bone biopsy is neither convenient nor practical, although it is sometimes resorted to in veterinary practice.

The biological material in which it is most feasible to analyze for toxic substances is urine. As concerns fluoride, numerous studies have shown that, despite its cumulative nature, there is a degree of proportionality between urinary fluoride levels and the ingested dosage (3, 8, 11, 14-16). This fact has prompted us to study the "normal" variation in urinary fluoride levels in the human organism.

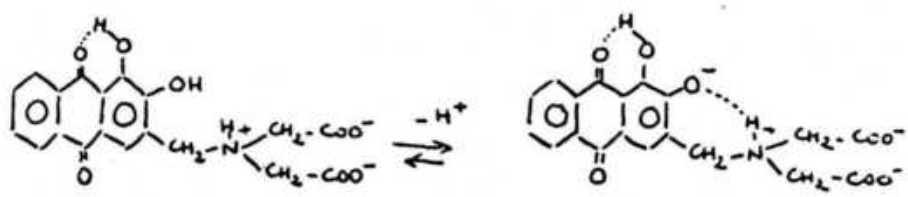
Interference by numerous anions and cations preclude the direct analysis of fluoride in urine. It is necessary to isolate the fluoride, whether by steam-distillation (1, 9, 11, 17-19), ion-exchange (20-23), or micro-diffusion techniques (12, 24-30).

The actual analysis depends on a volumetric (9, 17, 18, 31, 32) or spectrophotometric procedure (5-7, 12, 19, 21-30, 33-43). Most of the photocolorimetric techniques are based on the same general principle: Formation of a colored lake involving a dye (SPADNS, Sodium Alizarin-sulfonate, Erio-chrome Cyanine R, etc.) and a metallic ion (usually zirconium). The intensity of the pigment is bleached by fluoride ion, which complexes the metallic ion and thus reduces the extent of lake formation (5, 7, 20, 21, 24, 25, 28, 41, 42).

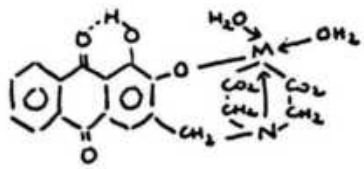
The first method for the direct determination of fluoride was proposed by Belcher et al. (33, 34) and was based on the reaction between Alizarin Complexone, Cerium and fluoride ion. However, this reaction required 60 minutes to attain equilibrium (35, 36). To overcome this disadvantage, several modifications were proposed (12, 26, 27, 38-40, 44). With the use of a water-soluble organic solvent (acetone, dioxane, methanol, or acetonitrile), equilibrium could be attained in 20 minutes and sensitivity was increased. Best results were obtained with acetone (44).

We believe that a detailed examination of this color-reaction is of interest.

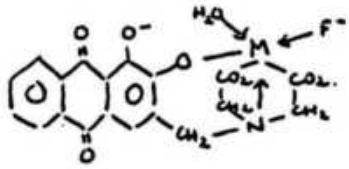
At pH 5.0-5.2, Alizarin Complexone exists in 2 ionic forms:



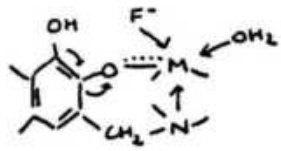
With the introduction of a rare-earth trivalent metal ion, (Ce, La, Pr, etc.), a red chromogen is produced with the following structure:



The presence of fluoride causes formation of a ternary complex with a blue color, when the more electronegative fluoride ion substitutes for one of the water molecules bound to the rare-earth metal:



The specificity of this reaction thus depends on the exceptional, electrophilic properties of the fluoride ion, which deprotonates the phenol group (45).



The influence of acetone on the sensitivity of the color-reaction is, according to Belcher and West (35,36), caused by its relatively high basicity and its weak dielectric constant, favoring formation of the ternary complex.

In our study, we have used the variant of Belcher and West's original method as proposed by Teichman et al (12), with prior isolation of the fluoride ion by microdiffusion. Perchloric acid was used as a generating agent and silver sulfate was introduced to prevent diffusion of chloric ions (which could interfere with the fixation of the fluoric compound by alkali).

Reagents and Apparatus

1. Polyethylene ("Millipore") microdiffusion cell; Millipore Filter Corp., Bedford, Mass., U.S.A.

2. Sodium Hydroxide, 0.5 M: Dissolve 20 g of sodium hydroxide pellets in 500 ml of redistilled water, then dilute to 1/1 with absolute ethanol.

3. Lanthanum-Alizarin reagent: Dissolve 8.2 g of A.R. sodium acetate in 20 ml of redistilled water, add 6.0 ml of glacial acetic acid, and transfer to a 200 ml capacity volumetric flask. In a separate container, dissolve 0.0479 g of Alizarin Complexone (Hopkins Williams Co., "Alizarin Fluorine Blue") in 1.0 ml of 20% ammonium acetate, add 5.0 ml of redistilled water and 0.1 ml of concentrated ammonium hydroxide, then filter the solution and add to the 200 ml volumetric flask, also adding 100 ml of

redistilled acetone. In yet another container, suspend 0.0612 g of lanthanum chloride in a few milliliters of redistilled water, add 2.5 ml of 2.0 N hydrochloric acid, then warm the solution until dissolution is complete. After cooling, add this solution to the 200 ml volumetric flask, mix the contents, then dilute to volume with redistilled water.

4. A.R. grade perchloric acid (d. = 1.615).

5. A.R. grade silver sulfate.

Procedure

With a micro-pipette, introduce 0.1 ml of 0.5 M NaOH drop-by-drop into the external compartment of a microdiffusion cell. Allow to dry. Then, into the internal compartment, add 1.0 ml of urine, 0.3 g of silver sulfate, and - rapidly - 2.0 ml of perchloric acid. Affix the cover and mix the contents of the diffusion cell by rotation. Allow diffusion to take place for 24 hours at 45°C.

Afterwards, allow the cell to cool before removing the cover. Add a few drops of redistilled water to the NaOH compartment and, as dissolution takes place, transfer to a 10 ml capacity volumetric flask; continue this process until a total of 5.0 ml of water has been so utilized. Then, add 3.0 ml of Lanthanum-Alizarin reagent to the transferred solution and dilute to 1 ml with redistilled water. Mix the contents thoroughly and read at 622 mu after a waiting-time of 20 minutes. The spectrophotometer is standardized with a solution containing 3.0 ml of Lanthanum-Alizarin reagent diluted to 10 ml with redistilled water and standards containing up to 10 ug fluoride are similarly prepared.

Results with Urine

Table I summarizes results obtained in a survey of 46 human-beings not unduly exposed to fluoride, whether from work-or home environment or consumption of fluoride-rich foodstuffs and/or water. In these cases, the fluoride content of the drinking-water averaged 0.3 mg/l (0.3 ppm).

All analyses represent duplicate determinations on pooled 24-hour samples of individual urines collected in polyethylene containers and analyzed within a week after collection.

TABLE I

Urinary Fluoride Determinations in 46 Cases

0 to 0.25 mg F/l of urine	16 cases
0.26 to 0.50 " " "	13 "
0.51 to 0.75 " " "	11 "
0.76 to 1.00 " " "	5 "
> 1.00 " " "	1 case

Total No. of cases:	46
No. of Analyses:	92
Average fluoride content of urine:	0.40 mg/l
Maximum " " " "	1.20 "
Minimum " " " "	0 "

For comparative purposes, Table 2 summarizes data obtained by several investigators.

TABLE 2

Urinary Fluoride Determinations Reported in Literature

F in Urine, mg/l . Average	Range	Procedure	No. of Cases Surveyed	Reference
-	3-10	Distillation	23	Goldemberg and Schraiber (7)
1.00	0.5-2.9	"	139	Machle (8)
0.40	-	-	-	Machle et al (10)
0.92	0.3-1.6	Distillation	30	Brun et al (9)
0.35*	-	"	611	McClure and Kinser (11)
0.30**	-	"	145	
0.40***	-	"	121	
0.50	-	Ion-exchange	15	Teichman et al (12)
0.34	0.-1.36	Microdiffusion	241	
0.40	-	"	20	
0.40 ****	0-1.20	"	46	Truhaut and Phu Lich

* Fluoride-free drinking water
** 0.1 mg F/l of drinking water
*** 0.2 " " " " "
**** 0.3 " " " " "

Conclusions

The combined procedure based on convenient isolation of fluoride (e.g., microdiffusion) and a spectrophotometric method that is sensitive, specific, and reliable, permits meaningful evaluation of the variability of urinary fluoride excretion in human-beings not unduly exposed to fluoride. Our survey of a relatively small group of (46) individuals has revealed a urinary fluoride concentration varying from 0 to 1.20 mg/l, averaging 0.40 mg/l (0.40 ppm).

We propose to increase the scope of these studies and to survey a greater number of individuals, so as to extend knowledge pertaining to urinary fluoride excretion, which we deem to be of indispensable value in the assessment of toxic exposure.

Acknowledgement

We wish to express sincere thanks to Dr. Monkman (Ottawa, Canada), who supplied the microdiffusion cells used in this study.

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THE SOLUBILITY OF VARIOUS FLUORINE COMPOUNDS IN SOIL

by

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In 1953 Nömmik (1) examined 31 samples of soil of varying clay content for fluorine and studied its solubility. Table 1 presents his results.

TABLE 1

F Content and F Solubility in Soils of Different Clay Content
(According to Nömmik)

Clay Content %	No. of Soils Tested	Soluble F Water mg/kg	%	Citric Acid mg/kg	%	HClO mg/kg	%
5	10	5.3	7	26.4	32	76	100
5-15	4	9.5	5	29.2	15	189	100
above 15	17	20.3	6	53.8	15.6	346	100

In "low-clay" soils fluorine is significantly more soluble in water and particularly in citric acid than in soils rich in clay. However, the content of soluble fluorine increases with higher clay content and particularly with its total fluorine content. If the total F level in soils were equal, fluorine would go into solution more readily in soils rich in clay than in low clay soils. Nömmik (1) found in growing forage plants the following mean values: In plants grown on 29 sandy soils with 710 ppm of fluorine: 21.6 ppm F (dry matter); in plants grown on 28 soils with 2640 ppm of F: 28.2 ppm F. In other words, whereas the "high-clay" soils contained nearly 4 times as much F as the sandy soils, there was only a slight difference in the F levels in plants grown in the two kinds of soil while they were in the growing stage.

In 1961 Alther (2) concluded on the basis of a limited number of tests that F levels of soils depend mainly on their content of calcium carbonate. By adding calcium carbonate to the soil F could be fixed as CaF_2 in soil and thus F would be made unavailable for uptake by plants. Therefore, he concluded that F of industrial origin accumulating in soil would be of little or no significance.

Own Studies

In soil tests on old natural meadows near the F emission area of Rheinfelden, no influence of carbonates upon the F levels took place (Table 2). The uppermost layers of the soil showed no increase in F. On the other hand, since near an industrial plant more than 50 kg of F/hectar (or 45 lbs./acre) are deposited yearly on soil through sedimentation and through rain and since no increase in F in the upper layers occurred, it must be concluded that there was a substantial transport of F into the deeper layers of the soil. Therefore, a significant solubility of airborne F (3) must be anticipated.

From the Eidenossische, Agrikulturchemische Anstalt, Liebefeld-Bern, Switzerland.

TABLE 2

F Content of Soil Samples from the Lower Frick Valley

No.	Origin of Specimens	Distance from Factory km	pH	$CaCO_3$	ppm F in Soil Depth of 0-2.5 cm	2.5-10 cm
1	b. Römerturm, W. Rd.	0.4	6.0	0	470	460
1a	b. Römerturm, E.	0.5	7.7	4.2	800*	
2	Heuhaus Salmen, N.	1.1	6.1	Trace	870	890
3	Linde E.	1.3	6.9	3.5	690	680
4	b. Jägerstübli, 70 m W.	2.0	5.8	Trace	500	520
5	Bäumlismatt, S.	2.5	5.9	Trace	850	1010
6	Hölzli, E.	3.0	6.9	Trace	760	780
7	Wolfshöhli W/E Rd.	4.0	7.4	16	500	520
8	Wolfshöhli W/W Rd.	4.0	5.7	0	550	630
9	Wolfshöhli I	4.5	6.0	Trace	270	320
10	Sunnenhof	4.3	6.3	Trace	280	290
11	Schupfart, Schönbühl	13.0	6.4	Trace	1330	1380
12	Schupfart, Brühl	13.5	6.7	Trace	1050	1000
13	Kaisten, Eigenmatt	18.0	6.2	Trace	670	730
14	Kaisten, Eigenmatt	18.0	7.4	21	800	870
15	Kaisten, Hundsbühl below	18.0	7.5	50	620	640
16	Kaisten, Hundsbühl above	18.0	7.0	8	1470	1490
17	Kloos W. Rheinfelden	4.0	6.5	Trace	340	310
18	Kloos W. Rheinfelden	4.0	6.5	Trace	500	490

* Corresponds to 0-10 cm

We tested F uptake of plants from soils with varying calcium saturation in acid and alkaline soils and in soils containing 260 ppm to more than 1,000 ppm. Pot experiments were carried out at first with Italian ryegrass and one year later with red clover on the same soil. The results with the first and second cutting are presented in Table 3, arranged according to increasing F content of the soils. These experiments prove that

TABLE 3

F Uptake by Italian Ryegrass and Clover from Soils with Different F Content

Origin	pH (H_2O)	$CaCo_3$ %	ppm Soil	Ryegrass Cutting 1.	2.	Clover 1962 Cutting 1.	2.
Kalkparz. Liebefeld	7,3	0,8	260	4,5	5,0	9,0	12,0
Waldhof, ca 500 m South	5,8	0	280	5,5	7,0	9,0	18,0
Eichhof, 600 m South	5,8	0	305	5,5	9,5	9,0	19,0
Garten Liebefeld	7,2	4,5	335	5,5	6,0	8,0	13,0
Gross-Grütt, Tower	6,6	trace	405	8,0	11,0	13,0	20,0
Tannenhof	7,0	trace	700	5,5	7,5	6,0	16,0
Neumatt	7,5	0,6	735	7,5	7,5	12,0	12,0
Kaisten, Valley	7,7	2,5	755	5,5	5,5	7,0	11,0
Schupfart, near Bridge	7,7	19,0	755	5,5	6,5	10,0	24,0
Schupfart, Highway to Frick	7,4	0,3	800	4,0	9,0	9,0	21,0
Neuhof Frick	7,8	12,0	860	6,0	6,0	11,0	17,0
Tannenhof Frick, East	7,9	21,0	1020	6,0	9,0	9,0	14,0

F levels in the experimental plants bear no relation to F in soil. However, the carbonate content of the soil seems to affect F uptake in plants. They take up less F from carbonate rich soils than from soils poor in $CaCO_3$, provided that the total F in the soil remains about equal (compare soil 1:2; 3:4; 5 and 6:7 and 8 in Table 3). However, the respective differences were not clear-cut.

In general the experimental forage samples showed high F levels compared with those from meadows. The second cuttings contained more F than the first ones, probably because the pots were regularly irrigated and the seepage water after passing through the soil was caught in the saucers and again returned into the soil. This may have been due to the fact that more evaporation takes place during midsummer causing the F to become more concentrated in soil.

F Fixation in the Soil

A 2 year experiment with red clover revealed a higher F uptake in plants in pots to which cryolite was added than in those with NaF and KF. (Table 4). Since the second

TABLE 4
F in Clover (Dry Weight) with Added F to Soil

F Added g per Pot	F Added mg% to Soil	Cutting	Added F Salt: Sodium Fluoride (1955,	Sodium Cryolite VR. 1622)	Potassium Fluoride (1956,	Potassium Cryolite VR. 1638)
0	0	first	11,0	11,0	8,0	8,0
0,5	10	first	9,0	12,0	10,0	8,0
1,0	20	first	9,0	22,0	13,0	17,0
3.0	60	first	17,0	31,0	22,0	33,0
9,0	180	first	62,0	77,0	70,0	110,0
0	0	second	13,0	13,0	8,0	8,0
0,5	10	second	8,0	12,0	12,0	17,0
1,0	20	second	14,0	13,0	17,0	25,0
3,0	60	second	20,0	53,0	30,0	43,0
9,0	180	second	85,0	233,0	71,0	104,0

cutting showed higher F levels than the first, one must assume that F fixation in the soil occurred slowly.

The following experiment was carried out: Sodium and potassium salts of F were added to soil in increasing amounts in pots with perforated bottoms. The pots contained no plants and were watered regularly. Water which was flowing through the soil was collected and used again for daily watering. After 3 weeks the water was measured and supplemented to a volume of 300 ml and again added to the soil. The 300 ml of water was now analyzed for its content of F, and Na and K. The pots remained untouched during the winter. In the spring they were again watered in the same manner as before and the seepage water collected as indicated above.

TABLE 5

Details about the Experimental Soil

Mechanical Analysis: Granules 0.002 mm: 17.3% clay;
0.002-0.02 mm: 17.2% silt
0.02-2.0 mm: 65.5% sand, 2.5% humous

Classification: Weak Humous Sandy Loam

Exchange capacity calculated - According to Kappen: 12.0 m Eq/100 gm;
According to Mehlich: 11.5 m Eq/100 gm.

p.H. in H_2O: 6.6;
Lactate soluble nutrients in mg%: 5.7 P_2O_5; 5.2 K_2O.

As noted in Table 6, the F content of the water used to flow through the soil increased distinctly during the 2 years, probably because the subsequent late water samples were taken during mid-summer when more water evaporated and therefore F became more concentrated than at other times. On the other hand, the higher temperatures may have induced a change in equilibrium in the distribution of F between soil and water.

TABLE 6

F in Seepage Water in mg F

Added to Soil	25.6. 1963 1	9.7 2	30.7. 3	27.8. 4	24.9 5	Washed Out Total mg F*	8.6. 1964 1	29.6. 2	20.7. 3	Washed Out Total mg F*
0	0,33	0,14	0,16	0,41	0,70	0,52	0,17	0,42	0,50	0,32
0	0,26	0,19	0,16	0,41	0,69	0,51	0,12	0,16	0,41	0,21
Mean	0,29	0,16	0,16	0,41	0,70	0,52	0,14	0,29	0,46	0,26
NaF										
0,5	1,2	0,9	1,0	1,3	1,3	1,8	1,0	1,3	4,2	2,0
1,0	4,2	1,3	2,9	2,4	2,4	4,0	2,6	3,1	9,0	4,4
3,0	19,1	17,9	18,3	21,2	22,7	29,8	12,7	15,8	40,7	20,8
6,0	65,0	55,8	63,6	58,2	67,6	93.1	52,5	63,9	191,2	92,3
9,0	94,4	67,5	74,9	74,2	74,4	115,6	107,7	109,0	301,6	155,5
Na-Kryol.										
0,5	1,6	1,1	1,1	1,2	1.1	1.8	2,0	3,6	9,3	4.5
1,0	2,0	1,8	2,0	2,2	2,9	3,3	2,3	3,9	8,5	4,4
3,0	11,0	10,6	10,3	14,1	16,0	18,6	12,9	10,9	28,7	15,8
6,0	68,2	64,3	64,7	65,4	68,4	99,3	62,4	81,5	83,1	68.1
9,0	96,4	73,5	96,7	97,4	93,2	137,2	82,1	110,3	110,8	91,0
KF										
0,5	1,5	0,9	1,2	0,5	0,7	1,4	1,0	2,5	2,7	1,8
1,0	1,8	1,4	2,1	1,8	1,8	2,7	2,1	1,8	5,4	2,8
3,0	10,0	7,7	8,0	8,5	9,8	13,2	10,3	7,7	16,7	10,4
6,0	17,8	27,4	22,7	22,7	22,7	33,9	21,4	18.0	31,7	21,3
9,0	36,5	44,6	45,2	35,1	44,6	61,8	30,5	39,6	70,7	42,2
K-Kryol.										
0,5	1,8	1,2	1,3	1,5	1,3	2,1	1,3	1,5	4,7	2,2
1,0	2,0	2,5	3,1	3,0	3,1	4,1	2,7	3,6	8,9	4,6
3,0	38,1	32,5	27,0	22,0	25,4	43,4	19,8	19,0	44,7	25,0
6,0	111,4	112,2	119,6	121,8	114,4	173,8	104,6	104,9	377,0	175,9
9,0	218,8	182,7	157,7	166,8	185,9	273,6	169,0	168,9	440,2	233,4

• 0,3 of the total 1 to 5 in 1963 and of 1 to 3 in 1964 because of removal of 300 ml.

In the water of the pots to which F was being added, the F concentration increased with the amounts of the added salts. However, the F level of the succeeding water samples of the same pot remained at constant levels. Therefore, only slightly more F remained fixed in the soil. The F values of the NaF series were significantly higher than those of the KF series. The reverse was true in the cryolite series. In both years the F content of the potassium cryolite series was higher than that of the sodium series. Whereas during the first year the F values of the water in the sodium cryolite series were only slightly higher than those of the NaF series, the potassium cryolite series showed a significantly greater solubility than that of the KF series. This difference was particularly conspicuous with high F doses.

During the second experimental year the water samples failed to show the same consistency of the F content as in the previous year. The F levels in the NaF series showed a striking increase. In contrast with the results of the first year, the sodium fluoride series showed higher values than the corresponding cryolite series. For both potassium salts the results were similar to those of the previous year. The water of the 3rd fraction of the K_3 Al F_6 series showed a surprisingly high increment in F concentration.

The above data indicate that even in a mineral soil with a high content in clay, F fixation takes place not as fast and not as intensively as one would assume on the basis of purely chemical considerations. With constant water saturation of soil an equilibrium between soil and water tends to occur. If the seepage water is repeatedly returned to the soil, this equilibrium tends to favor a higher F concentration in water.

Less explainable is the observation that following F addition to soil as sodium and potassium cryolite the water of the soil shows higher F concentration than upon addition of NaF and KF.

It can therefore be concluded that F blown in with the air does not remain solidly bound to the soil even after 2 years. On the other hand, higher F values derived from soil occur in clover only when 20 mg per 100 g of soil are added (20 mg/100 g correspond to 600 kg/ ha at a depth of 20 cm). The preservation of the relatively high solubility of fluoride is difficult to explain on the basis of the presence of sodium and potassium, since in both cryolite series higher F values were found in water than in water of the corresponding NaF and KF series with twice as high an alkali content (Table 7).

TABLE 7

Proportion of Alkali/F in 5th Seepage Water
(Summer 1963)

g F per Pot	Na F Series			Na_3AlF_6-Series			KF-Series			K_3AlF_6-Series		
	Na mg/l	F mg/l	Na:F	Na mg/l	F mg/l	Na:F	K mg/l	F mg/l	K:F	K mg/l	F mg/l	K:F
0.5	79	1.3	61	50	1.1	45	14	0.67	21	10	1.3	8
1.0	149	2.4	62	63	2.9	22	44	1.8	24	26	3.1	8
3.0	178	22.7	7.8	195	16.0	12	163	9.8	17	120	25.4	5
6.0	486	67.6	7.2	305	68.4	4.5	395	22.7	17	292	114.4	3
9.0	661	74.4	8.9	340	93.2	3.7	714	44.6	16	458	185.9	2.5

Exchange of Potassium and Sodium Ions

The potassium and sodium content of the seepage water will be discussed here only casually. In the NaF and the Na_3 Al F_6 series with increasing Na doses, increasing quantities of K were exchanged and appeared in the seepage water. However, in the K series doses up to 3gF/6kg of soil in KF as well as in K_3 Al F_6 an increase of the exchanged Na occurred which subsequently resulted in a progressive decrease with higher doses (4,5). This phenomenon can be explained on the basis that K ions when present in soil at low concentrations are being exchanged with Na ions which is retained between the layers of the clay minerals. With higher K concentrations, however, a narrowing of the layers and therefore a further decline of the Na exchange takes place.

Summary

In experimental plants F added as cryolite resulted in a higher fluoride uptake than when simple KF and NaF were added.

In pot experiments extending over 2 years, with a somewhat weak humous sandy loam, the solubility of fluorine in the form of NaF, KF and their corresponding cryolites was determined by analysis of the seepage water for F. The results were surprising in as much as with equal doses of F in the seepage water the cryolite pots showed consistently higher F levels than those with simple NaF and KF. When more sodium was added, an increase of exchange potassium occurred whereas with increasing potassium the exchangeable sodium showed a high level at first but subsequently a distinct decrease. This is explained on the basis of the reduction of the difference of the clay layers in the soil.

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FLUORIDE UPTAKE IN PLANTS

by

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In air-polluted areas fluoride is taken up in plants mainly through leaves, stalks and bark. From "normal fluoride" soils relatively little F reaches the plant.

TABLE 1
F Content of Plants in Uncontaminated Areas (mg% Dry Weight) (1)

Wahlsted (Holstein)			
Soil (Humus) 5.2			
Spruce	N	Young	0,43
Spruce	N	Old	0,81
Spruce	W	Young	0,42
Spruce	W	Old	0,78
Larch	N		1,03
Larch	W		0,49
Birch	L		0,70
Elder	L		1,10
Raspberry	L		1,06
Fern			0,89
Holzbach (Hessen)			
Soil (Clay) 8.3			
Spruce	N		0,67
Pine	N		0,59
Oak	L		0,59
Linden	L		1,25
Walnut	L		1,38
Elder	L		1,57
Beech	L		0,99
Mountain Ash	L		1,83
Apple	L		1,20
Plum	L		1,84
Cherry	L		1,11
Pear	L		2,39
Corn	L		0,70
Potatoe	L + S		1,30
Swede Turnip	L		0,98
Common Beet	L		1,08
Sunflower	L		1,99
Sorrel	L		2,93
Fern			1,07

Griebel (Holstein)		
Soil (Humus, Clay) 11.2		
Spruce	N	0,56
Pine	N	0,78
Oak	L	1,03
Chestnut	L	0,50
Elder	L	1,54
Maple	L	0,68
Linden	L	1,66
Raspberry	L	1,96
Bardowick (near Luneburg)		
Soil (Humus, Sandy) 26.5		
Spruce	N	0,79
Pine	N	0,53
Oak	L	0,54
Beech	L	0,89
Common Beet	L	0,52
Beet, White	L	0,21
Beet, Red	L	0,28
Carrot	R	0,22
Carrot	L	0,68
Oberdorf (Switzerland)		
Soil (Sandy Clay)		
Edge of forest		35,8
Meadow		37,0
Beech	L	0,43
Apple	L	0,32
Elder	L	0,57
Common Beet	L	0,48
Potatoe	L + S	1,06
Alfalfa		0,51

L = Leaves N = Needles R = Root W = Wood S = Stalks

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The F content of individual parts of a plant varies considerably: Leaves and stalks contain more F than fruit and seeds. Roots are rich in F whereas tubers contain less. In plants the F content ranges between 0,25 and 2,00 mg% (2,5 - 20 ppm). F uptake is independent of the magnitude of F in soils.

TABLE 2

F Content of Plants Grown in Different Soils (Pot Experiment) (2)
(mg F/100 g Dry Weight)

PLANT	SANDY	MARSHY SOIL	CLAY
Soil	14,50	48,50	29,60
Cherry Leaves	0,41	0,36	0,62
Summer Barley	0,16	0,11	0,38
Green Cabbage	0,42	0,54	----
Potatoe (Leaves and Stalks)	0,46	0,76	----
Red Currant Leaves	0,90	----	1,02
Radish Leaves	0,59	0,63	----
Spinach	0,37	0,44	1,18
Oat Plant	----	----	0,40
Summer Rape	0,53	0,27	0,53

In pot experiments with soils of varying F contents (Table 2), addition of about 5 mg% of such soluble F compounds as NaF and KF failed to increase the F levels of the plants. This observation conflicts with that of Gericke and Kurmies (3), who noted a slightly increased F uptake in plants when 7 to 8 mg% of F was added to the soil.

TABLE 3

F Content of Plants After Addition of 15 mg % F (as NaF)
(mg F/100 g Dry Weight)

PLANT	SANDY SOIL	MARSHY SOIL OR CLAY
Potato (Leaves and Stalks)	2,97	1,97
Spinach	1,73	1,20
Summer Rape	0,59	0,45
Oat	0,31	0,28
Summer Barley	0,39	0,33
Morello Cherry Leaves	0,54	0,53
Red Currant Leaves	0,94	0,80
Radish Leaves	0,75	0,55

Addition of 15 mg% F to the experimental soils (sand with 14,5 mg% F; marsh or clay with 29,6 to 48,5 mg% F) increased F in sand to 130% and in marshy or clay soil to 62% (Table 3). Larger doses of the order of 30 to 60 mg% added to the soils increased the fraction of F in spinach as NaF by 190 to 675% and as CaF_2 by 38 to 260% (Table 4).

TABLE 4

F Content of Spinach after Increasing Additions of F to Soil (mg %)

F added to Soil	F in Leaves
0,0 Control	0,53
28 mg% F as NaF	1,55
42 mg% F as NaF	2,55
56 mg% F as NaF	4,13
30 mg% F as CaF_2	0,83
45 mg% F as CaF_2	0,73
60 mg% F as CaF_2	1,91

Upon addition of 150 mg% of F to the soil the F levels of leaves and stalks of winter-rye increased 10 to 30 times, those of roots 5 to 25 times (Table 5).

TABLE 5

F in Winter-Rye after Addition of NaF and CaF_2 to Soil (pH 6,5)

SOIL	DRY WEIGHT OF LEAVES + STALKS g	DRY WT. ROOTS g	F IN LEAVES + STALKS mg%	F IN ROOTS mg%
00 (Control)	9,8	9,6	0,6	1,99
50 mg% F as NaF	10,0	7,7	6,7	11,6
100 mg% F as NaF	4,6	7,0	15,8	32,3
150 mg% F as NaF	2,5	9,7	20,0	52,0
50 mg% F as CaF_2	9,1	9,3	2,0	17,2
100 mg% F as CaF_2	9,0	8,8	3,3	(171,7)
150 mg% F as CaF_2	9,5	8,2	4,5	68,6

Following addition of 100 to 150 mg% of F to winter-wheat its yield was reduced to 40 to 65% when NaF was added, by 3% after addition of CaF_2. This reduction in yield was more pronounced with wheat than with rye (Fig. 1).

Fig. 1

Effect of F Supplements to Soil on Fresh Weight of Winter Wheat

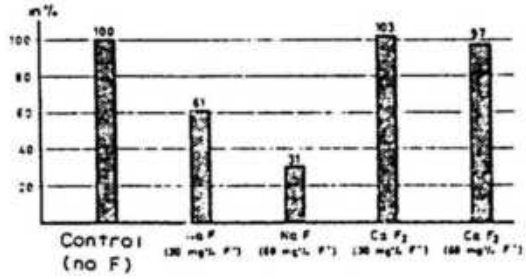
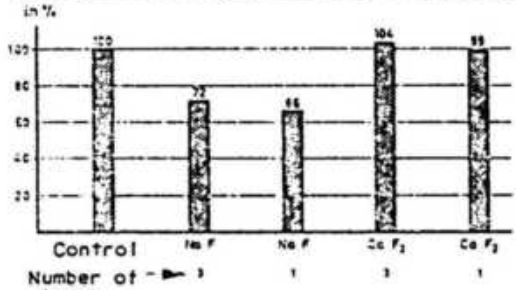


Fig. 2

Fresh Weight of Tradescantia after 40 mg% F in Single and in Divided Doses



The effect of F derived from NaF is fairly constant (Fig. 2), a graph which presents data on multiple doses. The fresh weight of a Tradescantia sprout was reduced by 28% after 40 mg% of F in three doses as compared to 34% following a single dose of 40 mg% F. Addition of CaF_2 had practically no effect.

TABLE 6

F Uptake by Winter Rye from Different Soils (1)

	50 mg% F as NaF (single dose)				50 mg% F as NaF (3 doses)				No NaF (Control)			
	Number of Plants	Weight of Grain	Weight of Straw	F Content of Straw mg%	Number of Plants	Weight of Grain	Weight of Straw	F Content of Straw mg%	Number of Plants	Weight of Grain	Weight of Straw	F Content of Straw mg%
Sandy loam	14	20.0	21.0	3.77	16	25.5	26.0	6.00	18	29.3	28.3	1.10
Compost	18	24.5	29.5	1.06	19	27.8	33.0	1.17	17	34.8	38.0	0.75
Marshy soil	19	29.5	29.5	0.76	22	33.5	31.8	0.59	19	28.8	28.5	0.57

Table 6 presents the results on yields of winter rye after addition of single and multiple doses of F to the soil. 50 mg% of F was given as a 2% NaF solution. A single dose reduced production more than threefold the individual doses. F fixation in straw showed a threefold increase with the single dose, a sixfold with three doses. Greater reduction in growth took place in sandy soil than in marshy soil.

Fig. 3 demonstrates the effect of F uptake on the weight of plant tissue according to Pavlik (4).

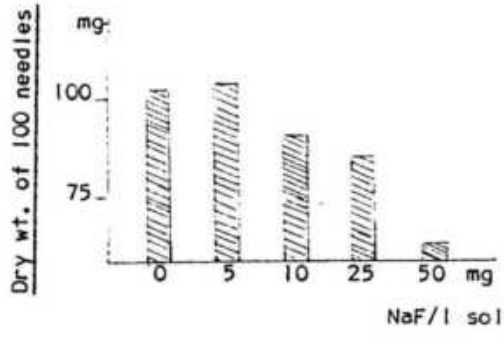


Fig. 3

Dry Weight of Spruce Needles Grown in Solution with 5, 10, 25 or 50 mg NaF/l.

These results demonstrate that F content of the plants increases when soluble F compounds are added to the soil. Therefore, plants in emission areas with soil enriched by F, take up increased amounts of F.

In a pot-experiment with soil from a polluted area containing 473 mg% of F, beans showed a F content of 8.51 mg% in the leaves, 192 mg% in the roots. With other soils, the beans showed a lesser increase in F content. These increases must be considered the

TABLE 7

F Uptake in Beans in Pot Experiments with F Polluted Soils (2)

Factory near	mg% F in Soil	mg% F in Roots	mg% F Leaves
Weser	22,5	10,5	1,28
Rhein	473,0	191,7	8,51
Elbe	65,5	11,7	1,43

result of F containing pollutants. However, in these experiments increments are relatively small compared with those which occur naturally near a factory as the result of F uptake through leaves. In general, the leaves take up much more F from the air than fruit.

Damage to fruit by F pollution is rarely described in the literature. Holte (5), Garber (6), Dassler and Grumbach (7) reported such damage from an area near a HF factory where CaF_2 was treated with sulfuric acid. The pears showed round or irregularly formed, leathery brown changes around the core. The meat of the fruit was dry and flour like. Their F contents are reported in Table 8 and the reduction of their yields by F emissions in Table 9.

TABLE 8

F Content of Different Parts of Pears

Kind and Origin	Special Portion		Central Portion		Portion of Stem	
	F mg% (dry)	Color of pulp of Necrosed Fruit	F mg% (dry)	Color of pulp of Necrosed Fruit	F mg% (dry)	Color of pulp of Necrosed Fruit
"Gute Luise" Polluted Area	0.492	brown heavy	0.394	brown with green spots	0.227	clear
"Gellerts Pear" Polluted Area	0.629	dark brown heavy	0.597	brown with green spots	0.236	clear
"Gute Luise" Uncontaminated Area	0.098	clear	clear	clear	clear	clear
"Gellerts Pear" Uncontaminated Area	0.120	clear	clear	clear	clear	clear

TABLE 9

Pear Production and Decrease after F Emission (1964)

Origin	Productive Trees	Bearing Trees Number	%	Production per Tree kg	Fruit Damaged by F %	kg
Street Section I	42	15	35	53	35	250
Street Section II	69	47	67	85	20	719
Street Section III	85	63	74	95	1	53

In heavily exposed areas the production of fruit decreased by 35% and the number of productive trees by 65%. Thus the total productivity declined sharply.

Benson (8) reported the bursting of peaches in F emission areas as a characteristic feature of F damage. This phenomenon can be duplicated by artificial exposure to F gases. An exposure from 14 to 58 ppb of HF for 14 hours, twice weekly for 3 months induced bursting in 50% of the peaches, 10 to 18 ppb in only 30% and 3 to 19 ppb in only 25%. Controls showed no damage.

Mohamed et al (9) showed cytological changes by HF upon the chromosomes of tomatoes. Seedlings of a high yielding species of tomato were exposed to 3 ug HF/m^3 for 2 weeks. There was no visible damage. Yet the histology of the plant showed fragmentation and bridge formations in the chromosomes. This pathology was observed during the mitosis and meiosis of the treated plants. The percentage of these histological changes was related to the duration of the exposure to gas. The authors concluded on the basis of the test that HF can produce mutations in plants.

Summary

Plants take up F compounds from the soil through the roots and from the air (as gas or in solution) through the leaves.

The natural fluoride content of plants derived from soil varies between 0.25 and 2.0 mg% (2.5 to 20 ppm). It is independent of the F content of the soil.

If soluble F compounds are added to soil at the order of magnitude of more than 15 mg%, F uptake in the plant increases. The uptake is dependent on the type of soil. In F polluted areas the F content of plants is markedly increased mainly through uptake by leaves and other supraterranian parts (stalks, bark).

Damage to fruit through F emissions has been described and there may be extensive reduction in productivity. Damage by F compounds to chromosomes of plants has recently been described.

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FACTORS DETERMINING THE SENSITIVITY TO HF IN PLANTS

by

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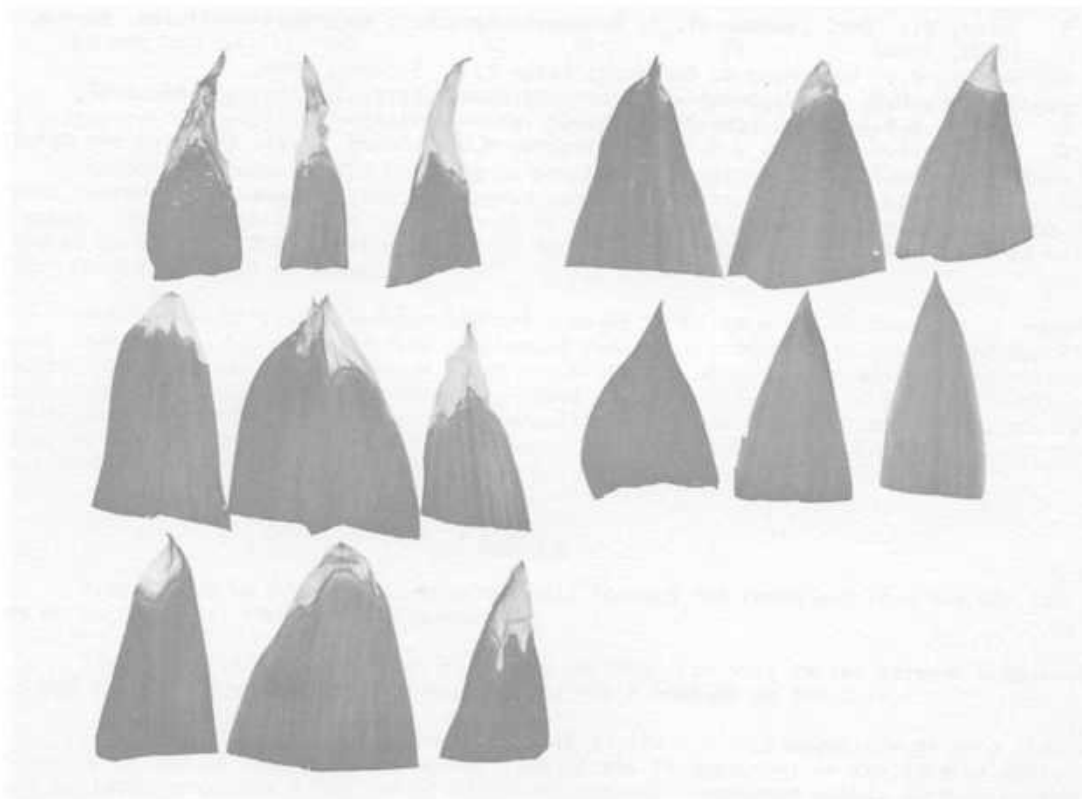
In order to investigate the difference in sensitivity between varieties of a plant species to a toxic gas, artificial fumigation is applied in small greenhouses.

The experiments in question were carried out with tulips because of the great difference in susceptibility to HF between different varieties (Fig. 1).



The highly sensitive variety "Blue Parrot" contains less fluoride in the injured parts of the leaves than "Preludium" the least sensitive one as shown on Table I.

From the Institute of Phytopathological Research

TABLE 1

F Content of "Blue Parrot" (Very Sensitive) and "Preludium" (Resistent).
(7.5 cm leaf tips were divided into 3 parts)

Variety	Fumigation (ppb HF)	F Content in ppm 0-2.5 cm	2.5-5 cm	5-7.5 cm	0-7.5 cm	Mean Damage at Leaf Tips Length in cm
Blue Parrot	4.2	15	10	7	10	2.32
Blue Parrot	-	18	13	6	11	2.94
Preludium	2.9	66	5	7	18	0.17
Preludium	2.4	92	12	10	27	0.22

Note: Accumulation of F in the Tips of the Leaves

Leaves were sampled at specific intervals after fumigation. A gradual increase in F content was observed. It indicates that a translocation of F occurs in the leaves (Table 2).

TABLE 2

F in Leaf-Tips (2.5 cm long) of "Preludium" Harvested at Different Times Following HF Fumigation for 6 Hours

Date	Fumigation (ppb HF)	Untreated*	Days After Fumigation 0	2	8
3-29-66	13.5	34	52	86	105
4-4-66	14.9	34	70	110	138

*Determined on 4-6-1966

When exposed to very low HF-concentrations, distinct leaf-tip injury and low F content in the injured leaf-tips occur in the "Blue Parrot" tulip. Preludium on the other hand shows hardly any damage but stores a considerable amount of F in leaf tips. These two facts are utilized in the experimental fields to trace very low F concentrations in the atmosphere by growing "Blue Parrot" and "Preludium" tulips together and determining at regular intervals the extent of leaf-tip injury of the "Blue Parrot" variety and the increase in F content of leaf tips of "Preludium" tulips 2.5 cm in length (Table 3).

TABLE 3

Injury (cm) and F Contents (ppm) of Leaf Tips 2.5 cm long, in Experimental Fields Near HF-Emitting Factory.

Harvest Date 1966	Blue Parrot (Sensitive) Average Length of Leaf Tip Injury (cm)			Preludium (Resistent) F Content of the 2.5 cm Leaf Tips (ppm)		
	I Field	II Field	Control Field	I Field	II Field	Control Field
4/12	0.27	0.19	0.0	60	13	9
4/26	0.47	0.21	0.0	67	19	6
5/2	0.82	0.44	0.34	140	29	14
5/9	1.31	0.72	0.58	211	79	17
5/16	1.92	1.09	0.66	222	148	28
5/23	2.18	1.82	1.05	372	194	33

These data indicate that F accumulates in the green leaf-tips even if very low concentrations of HF are present in the atmosphere. However, as soon as the tips become necrotic no further accumulation takes place. This observation is further supported by the fact that guttation occurs no longer at the most distal portion of the leaf and that water droplets appear at the line of demarcation between healthy and necrotic tissues.

Conclusion

1. The most susceptible tulip variety contains the smallest amount of F in the injured tissues as indicated by fumigation experiments with about the lowest concentration of HF (4.2 ppb) that produces injury.

2. The toxic action of HF is determined by migration to and accumulation of F in special parts of the plants (in particular the leaf tips).

3. During long-term exposure of leaves to extremely low atmospheric HF concentrations, HF is taken up through the leaf surface and subsequently migrates to the leaf tips, where it accumulates.

The above three factors may determine the extreme sensitivity to HF of certain varieties of susceptible plant species.

(Editor's note: The method described here may constitute a useful and sensitive tool with which to establish proof of air contamination by fluoride.)

TOLERANCE OF SEVERAL PLANTS TO FLUORIDE CONTAINING EFFLUENTS

by

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In our clearing pond containing effluent fluoride water we noted repeatedly plants which were growing uncultivated. We therefore examined 7 different plants and shrubs according to the experimental set-up illustrated in fig. 1. The newly potted plants developed normal, green leaves. The two daisies started to blossom. The effluent water from the air purification equipment of the aluminum plant showed an average F concentration of 20 mg/l; its pH was 5.9, the temperature 18°C.

Fig. 1

After 4 months the plants were taken out and F levels of the leaves determined as shown on table 1.

TABLE 1

F Content of Experimental Plants after 4 Months
(mg/100 g Dry Substance)

Reygrass	4 Tests	115, 155, 210, 235
Forsythia		180
Liguster	2 Tests	185, 435
Lilac		340
Ash	2 Tests	385, 410
Daisy	2 Tests	465, 530
Philadelphuns	2 Tests	1250, 1350

Surprisingly, the leaves showed very high F levels without dying off, an experience unknown until now. The clearing basin was situated next to the electrolysis hall. Therefore, in addition to F uptake from the clearing basin F was also taken up by the plants from the air.

From the Aluminum Plant Rheinfelden, Germany.

THE EFFECT OF FLUORIDE UPON PLANTS

by

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In the following we wish to review data on fluoride's effect upon plants with which our institute has been concerned during recent years.

F Effect on Germination of Seeds

Various concentrations of NaF were applied to the substrate of several kinds of seed under constant conditions. The result of this study enabled us to establish 4 groups of seeds according to their natural tolerance to fluoride:

a) very sensitive: peas, soy bean, sweet peas (wicke) and cabbage;
b) sensitive: radish, barley, broccoli;
c) less sensitive: corn, cauliflower, luzerne, mustard, oats, clover, turnips;
d) tolerant: poppy, carrots, tomatoes.

The seeds covered under d) were in no way affected even by F concentrations as high as 3-6 g/l (3000 to 6000 ppm). The degree of tolerance in a certain species was not dependent upon its F content. Immediately after application of F to the seeds, the sensitive plants contained considerably less F than the tolerant ones. The tolerant species showed a high ash content particularly in calcium. They also contained high F levels naturally. In the tolerant species F had stimulated germination when its concentration was low, namely 0.0001 - 0.03 g/l (0,1 to 30 ppm) (1,2).

Effect of Calcium Magnesium and Phosphorus Upon F Uptake of Pea Seeds

Addition of Ca, Mg and P immediately prior to application of fluoride had different effects upon the F uptake of pea seeds. Calcium salts reduced the inhibition of the growth due to F and increased the F concentration in the tissues of the seeds. Mg added to the substrate prior to application of F stimulated the development of the seeds more than equivalent doses of Ca. P was least effective.

The capacity to inactivate fluoride was determined by observing the percentage of germination of the treated seeds. Calcium appeared to be most effective, then magnesium, followed by phosphorus (3).

F in the Substrate Related to Growth and Productivity of Certain Plants

The reactions of several plants grown in nutrient solutions were observed after addition of F at different concentrations under constant conditions of temperature, light and moisture in the air. Assessment of the production of the 12 day old plants demonstrated that dicotyledons (sweet peas, field-pea, bush-bean and horse-bean) are more sensitive than the monocotyledons (sugar-zirok, besen-zirok, spring barley, rye and corn).

From the Institute for Horticultural Biology of the Slovakian Academy of Sciences
Bratislava, Czechoslovakia

Growth and productivity of the dicotyledons ceased with a single dose of 0.5 to 9.20 gm/l F, whereas the monocotyledons continued their growth with concentrations of 0.25 gm/l. Inhibition of growth and absorptive capacity of the root system was noticeable even with as low concentrations of F as 10^{-4} to 10^{-3} gm/l.

Plants grown under conditions of high F concentrations suffer visibly of deficiency in several nutrients. Uptake of calcium decreased considerably as the result of reduction of its soluble portion in the nutrient solution, in the root system and in the supra-terranian portion of the plant. There was also a reduction in uptake of Mg, P, and K.

F assays made simultaneously proved that addition of fluoride in the substrate leads to a maximum accumulation of fluoride in the root system. This fact confirms the view that fluoride compounds are translocated very slowly in the plant organism (4).

Fluoride and the Water Balance of Plants

A disturbance of the metabolism of plant tissue due to the presence of fluoride is associated with variations of their water balance.

If F is applied to the nutrient solution the supra-terranian portions of the plant become wilted. Our observations demonstrate that the unfavorable hydration of the affected plant is the result of changes in the uptake as well as in the balance of water.

We noted that the water output among plants which received varying doses of F, takes place in several phases. The first phase of the F action is an increase in intensity of transpiration. In the second phase, the water output declines gradually approximating that of the control plants. In the third phase the intensity of transpiration decreases sharply.

With respect to water uptake our observations demonstrate exactly the reverse: Already after a few hours the F effect in the substrate leads to a marked, progressive reduction of water uptake.

The above changes are undoubtedly an expression of high F accumulation in the roots as well as a disturbance of the metabolism in the root system.

When fluoride was applied through the atmosphere by experimental fumigation, we observed decreased intensity of the total transpiration and less water uptake, even before visible damage to the plant occurred. When the plants were fumigated with low concentrations of F (0.07 mg/m^3) three phases were observed in the total transpiration. First, the total transpiration decreased, it increased in the second phase and decreased again. The latter phase is associated with visible damage to the plant.

A gradual increase of the cuticular and a decrease of the stomatar transpiration is characteristic of the effect of F. Within 90 hours after the fumigation, the portion of cuticular transpiration increases up to 92.9% of the total transpiration.

The change in the quantitative distribution between the cuticular and the stomatar transpiration is, we believe, the decisive cause of the negative water balance in plants damaged by atmospheric F (5-7).

Fluoride and the Water Balance of Plants

(Species James Grieve)

Apple tree leaves damaged following application of sodium fluoride in various concentrations were studied. In the acute phase the leaves turned brown and wilted, but no changes were apparent upon visual examination. During long term applications the

color of the leaves changed to yellow. Both chlorophylls and carotinoids were examined after thin-layer chromatographical separation and spectrometric analyses:

Chlorophyll: Damage to chlorophyll was associated with sudden marked water loss, wilting and brownish discoloration of the leaves. It was accompanied by a relatively high content of green matter in the leaves. In contrast, leaves with yellow coloring were associated with a relatively high water content because of the strong catabolism of chlorophyll. Leaves which were visibly undamaged showed only an insignificant decrease of the chlorophyll content. Among the two components of chlorophyll, chlorophyll[6] appeared to be more stable. In disagreement with other authors we noted only little pheophytin in the damaged leaves. The metabolites which we recognized were hydrophilic. They were probably chlorophylline and pheophorbid.

Carotinoids: We observed also an interaction of oxidizing (violaxanthin and neoxanthin) and reducing carotinoid-forms (lutein and beta-carotin) at the time when changes in respiration of the trees occurred as the result of the intoxication. In the most vital leaves, especially those visually undamaged, this action was most pronounced. In brown leaves in which postmortal disintegration had already taken place, this phenomenon was not encountered.

In all instances there was no increase in pigment, while the trees were recovering.

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THE EFFECT OF FLUORIDE EMISSIONS NEAR A HYDROGEN FLUORIDE FACTORY

by

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Chronic fluoride poisoning of cattle is of the greatest economic significance. Many widely varying factors determine the effect of F emissions such as the physical and chemical form of the emitted compounds, geographic, climatic and meteorological conditions, management and feeding practices, and the animals' age and productivity. Therefore, observations concerning the toxicity and tolerance of F compounds in one country under different experimental conditions do not necessarily apply to a particular situation elsewhere.

In the following experiments we studied the occurrence, the extent and the development of fluorosis in 12 experimental cows which were brought up in an emission-free area and placed under controlled conditions upon a farm within the emission area of a hydrogen fluoride factory. The animals were observed for a 3½ year period near the factory and subsequently placed for 2 years into an emission-free area.

Method of Investigation

The farm yard and experimental barns of the 40 ha (hectar) farm were situated 750 meters east of the F emitting factory. The fields upon which the animals were feeding were 0.5 to 2 km north, northeast, east and south of the factory (Fig. 1).

Fig. 1

Experimental Area with Barn and Feeding Areas I-X, XII-XIV

From the Clinic for Cattle Diseases of the School of Veterinary Medicine, Hannover. (Dir. Prof. G. Rosenberger)

Management and Feeding Practices

The 12 Holstein cows were purchased in spring 1961 at emission-free areas in Oldenberg and Luneburg. They were brought into the experimental area on 5/10/61, and remained there until 11/5/64. The herd consisted of 4 old cows, 4 pregnant heifers aged 2½, and 4 heifers 3/4 to 1½ years old. They had been certified as being free of tuberculosis and brucillosis. During the summer months of 1961-1963 they grazed on 9 permanent pastures throughout the day. During the winters of 1961-62 and 1963-64, they were fed, in the barn, beets, leaf-silage of sugar beets, hay and feed-straw grown on the farm. Grain grown on the farm with a 1 to 3% mineral supplement was added to the feed rations. The feed rations were periodically weighed. On pasture the water was supplied by an automatic pump from a river; in the barn the cows received city water. They were artificially inseminated by the official insemination agency of Low Saxony. During the 3rd experimental summer (1964) animals in the barn were divided into two groups and fed forage with different F content. During the last two experimental years the cattle were kept in an emission-free area on the experimental station of the school of Veterinary Medicine of Hannover. Regularly, every 6 months 2 animals were slaughtered. By thus eliminating further fluoride intake, we attempted to observe any improvement of the clinical manifestations and to determine periodically the F content of bones, organs and excretions, as well as the effect upon general health and productivity.

Tests Performed

During the experimental period samples of all food rations and drinking water were analyzed for F (altogether 378 determinations). Some food samples were also analyzed for their nutrient content, mineral and trace elements. In addition, we determined the levels of F in the air, of precipitated dust, of blood, urine and milk (a total of about 1500 tests). We also examined the organs and bones of all animals and their offspring which were slaughtered or died spontaneously during the experimental period. Every ½ year bone biopsies from the tail vertebrae were studied for their F content (65 determinations).

Productivity and General Health

The milk production was tested in the usual manner by the official milk production control. The animals were periodically weighed, regularly examined and their nutritional state was carefully recorded, in order to detect any intercurrent illness and to study early manifestations of fluorosis. The incisor teeth of all cattle were photographed twice a year and the bones were palpated regularly and any changes recorded. Upon impairment in walking, the animals were temporarily housed in the clinic for exact studies of the cause and for X-ray examination.

Fluoride Emissions in the Experimental Area

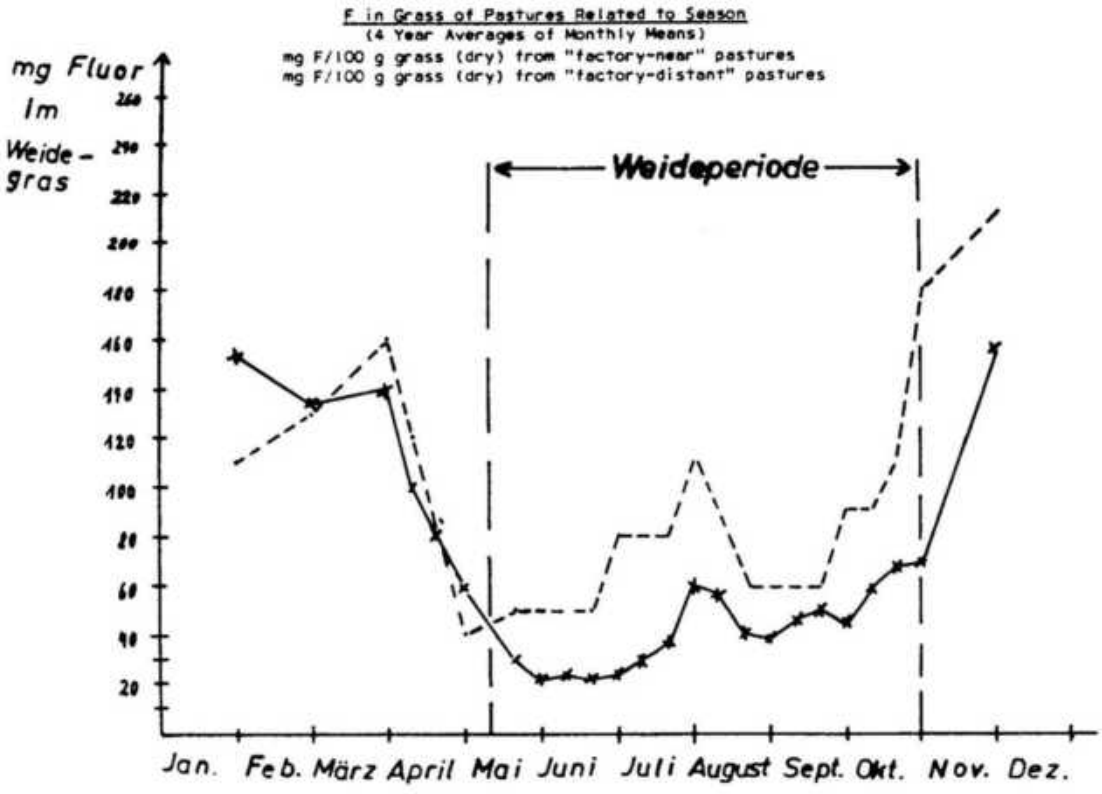
All F values recorded here refer to F in 100 gm of absolutely dry tissue. The factory emits F principally as SiF_4 which, in the presence of water, produces HF according to the formula: $SiF_4 + 2H_2O = SiO_2 + 4HF$. The extent of the emission area is not known.

The topography of the area is determined by a river valley. A canal with high banks is located north of the factory running in a north-easterly direction, a small town at the south-east and south. The prevailing winds come from the west and south-west. The principal effects, therefore, occur east and north of the factory.

According to Fig. 1 fields of permanent pasture (I-IV, VIII, IX) and three grain fields (X, XII, XIV) lie 400 to 800 m north, northeast and east of the factory. These "factory-near" areas of about 9.4 ha were exposed to heavy emissions. The 4 year average F content of the grass grown during the grazing period of May to Oct. was 43 mg%. The 5 other permanent pastures (V-VII, XI, XII) with a total area of 12.1 ha are located 800-1600 m east, southwest and northwest of the factory. These "factory-distant" areas were utilized for hay production. They received relatively little F emissions from May through October as indicated by a 4 year average of 8 mg%. This value representa a 12 times higher F content than the average 0.67 mg% determined by Wöhlbier et al. in emission-free forage.

The monthly averages of the F content of grass varied considerably, depending on the season. The variations were more pronounced in the "factory-near" than in the "factory-distant" fields (Fig. 2). Since the emission from the factory during the whole

Fig. 2



year remained fairly constant and since the effect of weather conditions was limited to only short periods and tended to average out during the course of a month, the different F levels in grass must be attributed to the growth of grass. The decisive factor in F content of grass is the duration of the growing time, i.e. the period between the development of plant parts and harvest or pasturing by the cows. As indicated in Fig. 2, the F levels in grass are lowest during the fastest growing period in May and June. They increase with declining growth from July to September. The highest values are reached during the period of rest in the winter months. Variations from this rule result from changes in weather conditions (low temperatures in spring and dry periods in the summer, etc.). F levels in grass are also affected by the management of the pasture.

Among the other kinds of forage grown on the farm, the F content of sugar beet leaves which are used as silage was distinctly influenced by emission. Sugar beet leaves harvested from "factory-near" field X showed an average of 20 mg% of F as compared to 4 mg% in the "factory-distant" fields where most F was bound to the portion of the leaf close to the soil.

The number of F determinations of drinking water was too small to estimate whether or not the river water was affected by the F emissions. Upstream of the factory, however, the river contained distinctly lower F levels than downstream, namely 0.3 mg/l versus 0.5 - 1.1 mg/l. Emission-free river water contains 0.25 mg/l F according to Gericke and Kurmies (2). Therefore an influence of the drinking water upon the health of the animals cannot be disregarded.

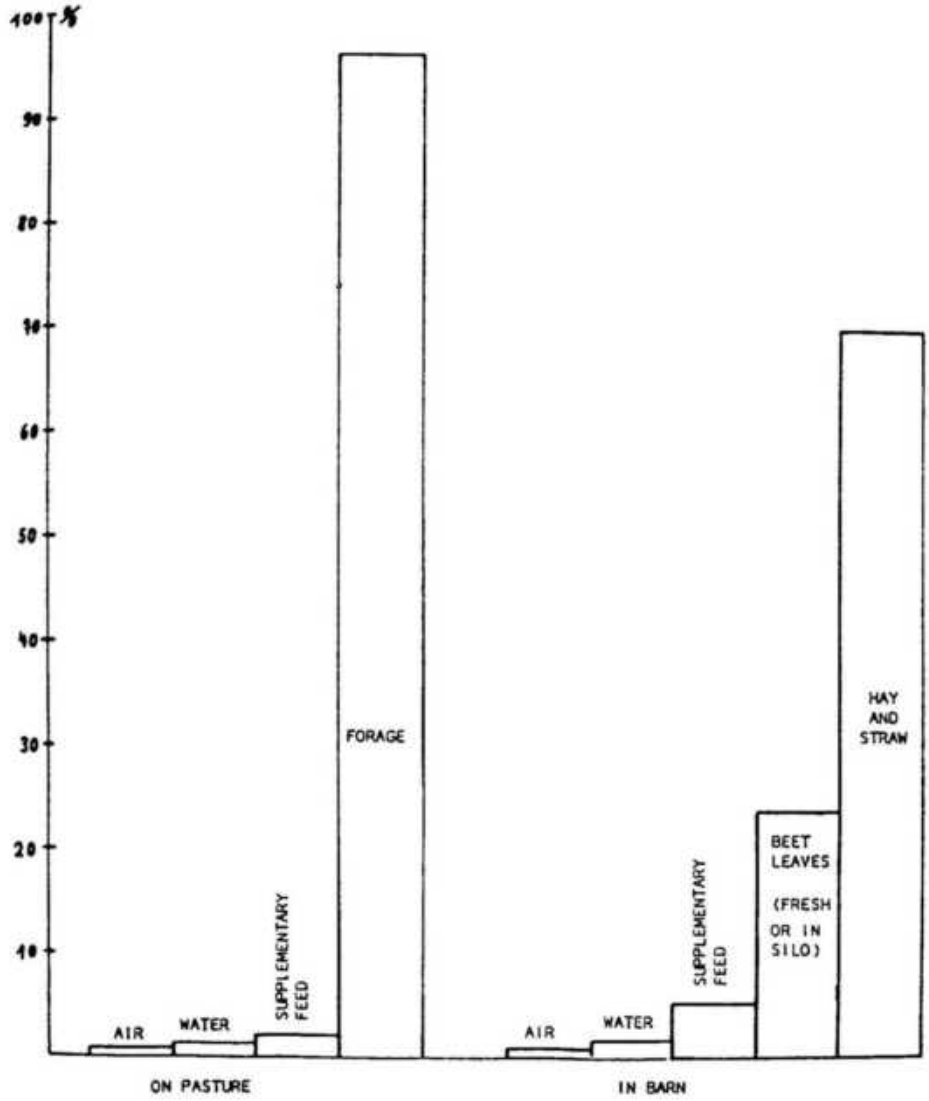
Fluoride Uptake by the Cattle

From Air: In fluorosis the effects of F pollution are determined by the amounts of F which the organism takes up within a certain period. The uptake depends upon F inhaled from the air, imbibed by water and ingested by forage. F uptake through the skin is insignificant.

In 23 samples of air derived from "factory-near" pasture, the F content ranged between 10 and 100 mcg/m^3, depending on climatic conditions. The respiratory volume of a grown-up cow amounts to 0.2 m^3 per minute. If all inhaled F is absorbed only 3 to 30 mg F per animal per day or 0.006 to 0.06 mg/kg body-weight is taken up. Thus the average uptake of 10 mg F per animal per day through the respiration is insignificant (Fig. 3).

Fig. 3

Average F Consumption Through Inhaled Air, Drinking Water and Forage on Pasture and in Barn

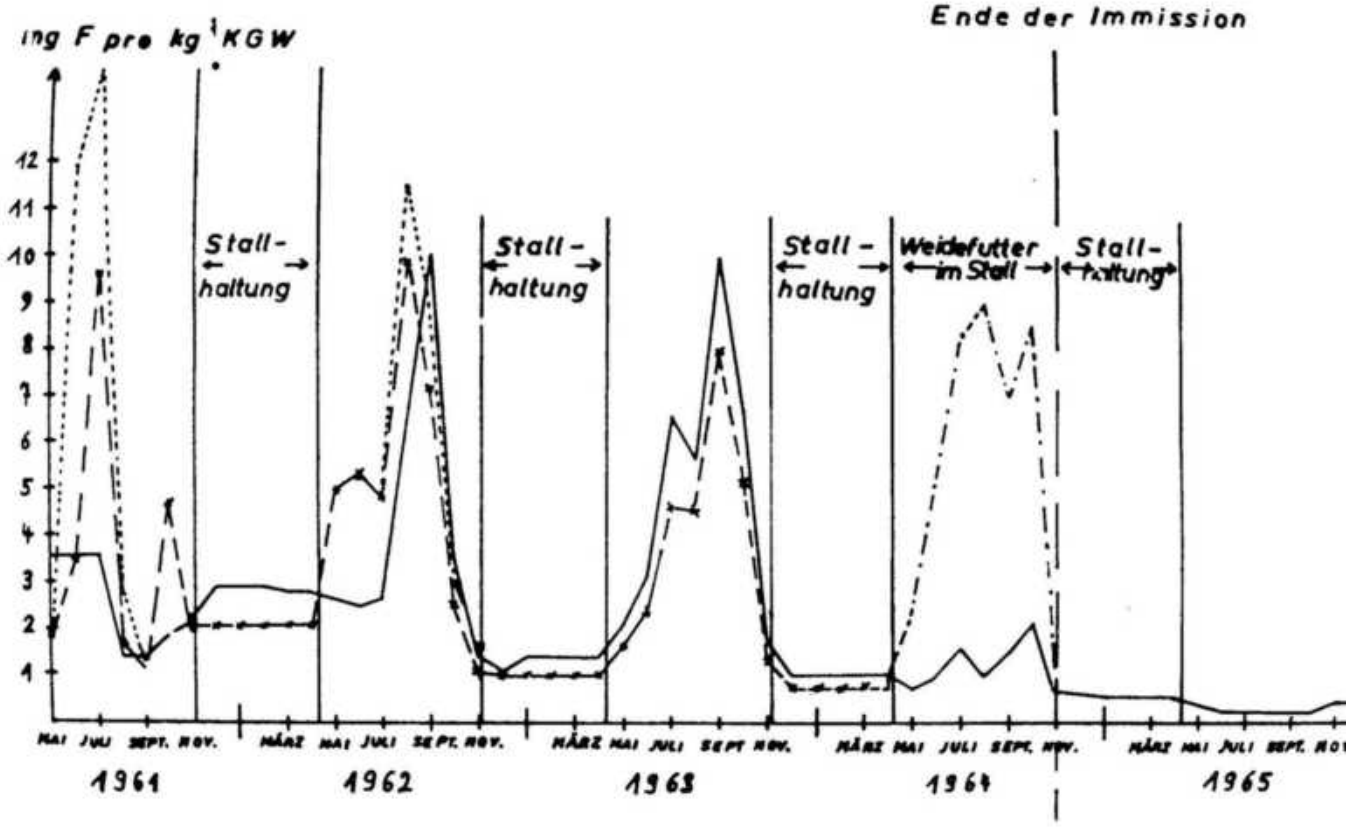


From Water: F from drinking water accounted for an average of only 5 mg per animal per day (0.01 mg/kg body-weight) in stabled animals, for 25 mg per animal per day or 0.05 mg/kg in animals on pasture.

From Forage: F intake from forage is subject to errors, because of many unknowns pertaining to F absorption from various food items. Water solubility of a F compound in forage is most significant. Whereas F in soil is usually not readily soluble, the F compounds in plants exposed to F emissions are very soluble and therefore readily absorbable. Therefore, if forage contained more than 1% polluted soil, its F uptake had to be corrected. We followed the directions of Mortensen et al. (3). The nutrient elements of various forage items were determined on the basis of our own methods and those of Wohlbier (1). Figure 4 presents the mean monthly F uptake in mg/kg per day.

Fig. 4

Average F Uptake by Cattle
(mg F/kg Weight - Day (Monthly Mean)



Cows Lactating or in advanced pregnancy
Beef cattle
5 Cows with high F intake during summer 1964

F uptake varies with different F content of forage. The seed in the barn and forage on pasture differ in their content because the effect of F emissions varies from one field to another. The greatest consumption of F polluted forage occurred from July to Sept. on "factory-near" pastures, the lowest at the time when the cows were in the barns and were feeding on forage grown on "factory-distant" fields. During summer 1964 the F uptake of the experimental cows from hay cut from "factory-distant" fields was similar to that fed to animals in the barn during the winter months. The group ingesting "factory-near" hay was exposed to high F intake as in the previous year.

Additional differences in F uptake are related to age differences. Up to August 1962 F uptake of cows could not be compared to that of heifers because the animals were not separated. After September 1962 the curves ran parallel in cows and heifers, but the non-lactating and non-pregnant cows took up distinctly less F. Heifers in advanced pregnancy took up especially high amounts of F during summer 1961 and 1962 while on "factory-near" pastures, namely as much as 10 mg/kg. This constitutes a 50 to 100 fold higher than "normal" uptake. Wohlbier et al. (1) calculated the average F uptake of a normal cow (550 kg) in a non-F area as 0.17 mg/kg on pasture and as 0.2 mg/kg in the barn. Non-lactating cows (500 kg) averaged 0.14 or respectively 0.13 mg/kg F per body-weight. The minimal F uptake of the experimental animals in the barn in 1962-63 and 1963-64 averaged 1.0 and 0.7 mg/kg. This represents 5 to 10 times the "normal" values. In the emission area the average F uptake of all experimental animals during the 3½ year period amounted to 3.4 mg/kg, and during the two subsequent years outside of the emission area 0.4 mg/kg. A slight increase in F intake above the calculated "normal" values is due to the proximity of the field station to the city of Hannover.

Effects of Fluoride Emissions

The following parameters were studied in relation to the effect of F upon the organism:

1. The animals' ability to absorb F compounds; 2. The daily F uptake per kg body-weight; 3. The duration of F uptake; 4. Age of the animals, feeding and management.

On the basis of the literature, Grieser and Brunch (5) established 2.5 mg/kg F (as NaF or F containing water) taken up during a period of two years as toxic. They consider 1.5 mg/kg tolerable, less than 1.0 mg/kg innocuous. Judging by the F uptake, therefore, we must consider the average F emission as toxic to our cows.

We studied particularly the manifestations of fluorosis, their effect upon productivity of the animals and the F levels of body tissue, blood and excretions.

Clinical Manifestations: During the experimental period intercurrent diseases in the cattle, such as birth defects, pneumonia and mastitis, were in no way connected with F emission. Two cows died, one from a liver disease, the other from an abscess.

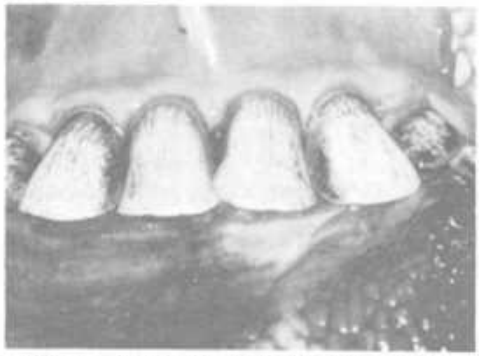
The characteristics of fluorosis were the morphological dental and skeletal changes and disturbed gait.

a. Dental Changes: Animals, in which the teeth were already developed at the beginning of the experiment, showed only lines of roughness of enamel, pin-point sized defects on labial surfaces of the enamel and transitory somewhat yellow discoloration (Fig. 5a). More pronounced changes were noted on the incisors which were in the growing stage during F emission. Those which broke through during the first experimental year showed only surface changes with foci of extensive brownish discoloration and roughness

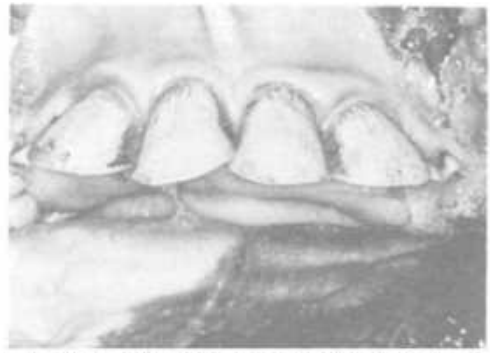
Fig. 5

Dental Fluorosis in a 2½ year old Steer Exposed to F Emission

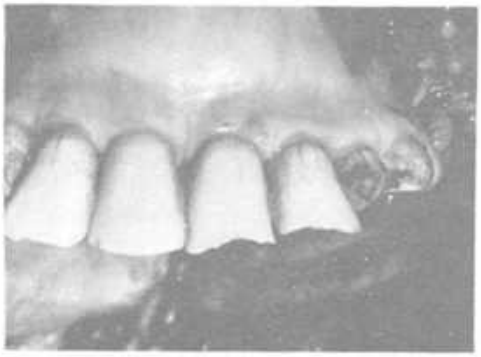
(a) ½ year: slight stain, yellow color of incisors contracted prior to initiation of the experiment.



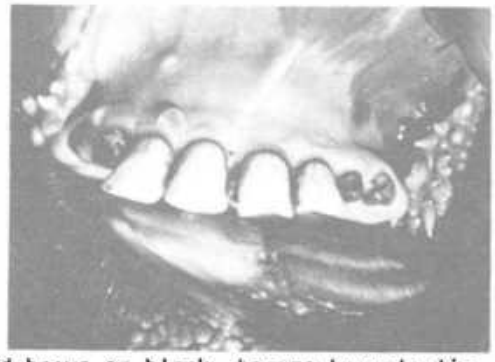
(b) 1 year: black-brown color, poorly developed permanent external incisors.



(c) After 3 years: black-brown discoloration; external incisors hypoplastic and worn.



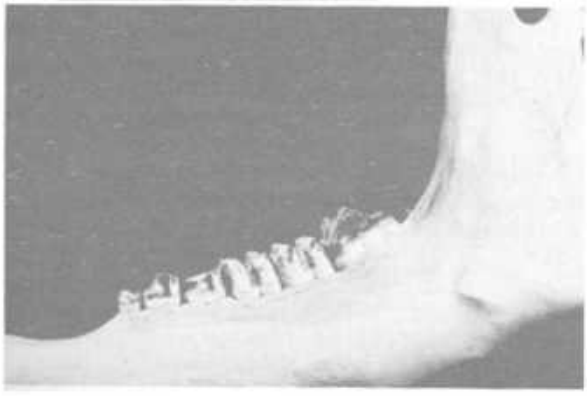
(d) 1 year after leaving emission area: external incisors and canines stunted.



of the enamel. Teeth which developed later turned brown or black, became hypoplastic and exhibited serious defects due to usage (Fig. 5). In spite of marked morphological changes there was no impairment of mastication, because the molars were only slightly affected (Fig. 6).

Fig. 6

Mandible of 5 year old cow. Hyperostosis due to Fluoride.



b. Skeletal Manifestations: The typical skeletal changes are periosteal hyperostosis which lead to exostoses. The bones exhibit round, extended, smooth or rough protrusions (Fig. 6). In the living animal these changes can be demonstrated by palpation or by x-rays only after they reach a certain size. Such exostoses were barely palpable in four cows at their hind metatarsal bones after the second pasture period (Oct. 1962). More extended lesions developed especially in old cows during the last experimental year. Among the youngest heifers no such changes occurred. The exostoses localized mainly on the metatarsal bones, were of the size of a bean or of pigeon eggs. These lesions were not associated with lameness. Five cows also exhibited hard, plum-sized nodes on the last

4 ribs. At first we considered them to be exostoses. After the animals were slaughtered they proved to represent incompletely healed spontaneous rib fractures (Fig. 7).

Fig. 8

Incompletely healed Rib Fracture in Skeletal Fluorosis in a 13 year old Cow.

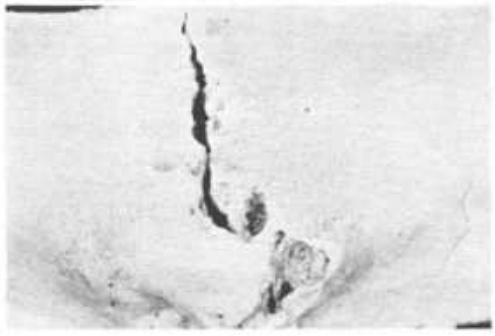
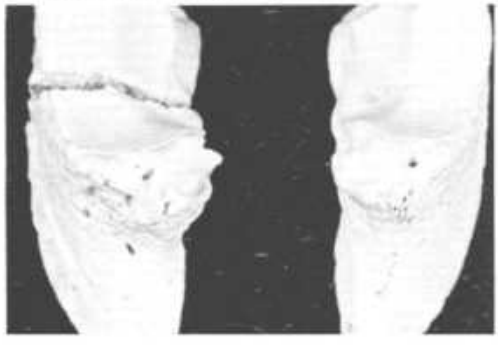


Fig. 8

Recent Transverse Fracture of Metatarsal Bone of Cow with Skeletal Fluorosis.



c. Lameness: Seventeen animals showed disturbances in movement. In 5 they were due to an infectious purulent disease of the hoofs and therefore unrelated to the F effect. In the other 12 animals they were the result of F emission; they occurred on pasture during or after periods of high F intake. During summer 1964 no paralysis occurred in group II. F intake was equally as high as in the previous years in the barn-fed animals.

Three different kinds of paralysis were noted:

1. Four cases exhibited stiffness in walking which lasted 1 to 3 weeks. The animal avoided movement without evidence of any localized defect.

2. In 3 cases minor impairment of gait occurred on all extremities which lasted 4 to 6 weeks. It was due to painful hoofs which, however, showed no x-ray changes. This condition, like the previous one, disappeared during the period of decreased F uptake.

3. In 5 animals moderate or severe palsy of the anterior extremities occurred, due to roentgenologically demonstrable fractures or fissures of metatarsal bones (Fig. 8).

One cow, age 4, exhibited metatarsal fractures in all four extremities. Because of extremely severe pain this animal was constantly standing on the carpal joints and was later unable to rise at all. In all 5 animals an orthopedic appliance designed to take the weight off the diseased bone improved the condition. After 2 to 3 months all cases were completely cured. When the animals were slaughtered 1 to 3 years later the metatarsals showed only slight evidence of dealed fractures. In a cow slaughtered during the acute paralysis, the fracture was distinctly recognizable.

Fluoride Levels of Body Tissues and Excretions

Whereas increased F levels are promptly eliminated from blood and soft tissue organs, fluoride accumulates in hard tissues. F determinations of the vertebrae of the tail and of urine are therefore important in establishing the diagnosis of fluorosis. Otherwise useless small vertebrae of the tail can easily be removed surgically. They are small enough to be analyzed in toto. Based on the examination of 28 calves and 12 cows, the following relationwhip of F in hard tissue was established when the F content of 100 mg% in tail vertebrae was used as a base:

Pelvic (pubic) bones	106
Rib	91
Mandible	85
Metatarsal bones	80
Teeth	50

In spite of wide variations in F uptake during summer and winter, the F levels of tail vertebrae differed only slightly. After removal of cattle from the emission area the average F content of bones decreased by about 25% after one year, 30% after 2 years. Most F,however, remained in the bones for a long time.

Effect Upon Productivity

The weight of the 3/4 to 2½ year old animals, their nutritional state and milk production were recorded. In 10 animals the fertility appeared to be normal, based on an insemination index of 1.8. Two cows remained sterile because of congenital malformations of their genital organs; 2 others were eliminated because of permanent sterility due to puerperal infection and Cesarean section. The periods between calving averaged 460 days.

The nutritional state of the cows as indicated by their weight showed consistent improvement during the 3 year period. No weight loss occurred even during heavy F emissions. Lactating animals or cows in advanced pregnancy, however, lost 50 to 100 kg in weight regularly both on pastures and in the barns, which was also clinically discernible. Within 2 to 3 months after their removal from the emission area their nutritional state became normal.

Milk production was not directly affected even during the highest F uptake unless the F uptake by the mothers during the last 3 to 4 months of pregnancy. However, the total F concumption of the mothers was not related to the F levels of bones in calves. At no time did F uptake in calves through placenta or lactation appear to be toxic to the offspring.

The most important effects of industrial F contamination, of an average order of magnitude of 3.4 mg/kg body weight, consisted of disturbances in movement due to progressive skeletal fluorosis and of marked loss of weight in the lactating cows during periods of excessive F uptake. Dental fluorosis did not appear to affect the productivity of the cows.

Summary

During a 3½ year experiment, 12 cows aged 3/4 to 9 years, were placed on a farm near an HF producing factory. F uptake from food, drinking water and air, as well as F levels in urine and in bones were studied. The animals' state of health and their productivity were observed. F levels in forage showed wide variations, depending upon the season and the distance of the pastures from the factory. F intake through consumption of F containing air and water was less significant.

Monthly F uptake by the cows varied between 1.0 and 9.5 mg F/kg body-weight, with an average of 3.4 mg/kg during the 3½ year experiment. Lactating cows and those in advanced pregnancy showed greater F retention.

All animals exhibited typical evidence of fluorosis in teeth and bones and disturbances in movements due to fissures and fractures of the metatarsal bones. Lactating cows showed a marked decline in nutrition during periods when their F uptake averaged 3.0 - 9.5 mg/kg.

There was no indication that F emission affected fertility or milk production, which reached as much as 20 liters a day. Calves from cows with chronic fluorosis showed no evidence of damage although the F content of their bones was high.

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We wish to acknowledge the cooperation of Dr. Oelschlager, Institut für Tierernährungslehre, Hohenheim and Chemierat Dr. Kopp, Tierhygien Inst., Univ. of Freiburg/Brg who carried out the fluoride analyses.

EXPERIMENTS WITH FLUORINE ALLEVIATORS

by

J. L. Flatla, Fr. Ender and M. Aas Hansen

Author's Abstract

In a 6 year experiment seven groups of sheep were given daily doses of sodium fluoride mixed in the feed at a level of 4.5 mg per kilo body weight. A supplement of either Al-lactate or Al-sulphate were fed to each group, using four different levels of Al-lactate and three different levels of Al-sulphate. Two control-groups were included in the experiment. None of the control groups were supplemented with an aluminum compound, but one of them had sodium fluoride included in the ration at the same level as the experimental groups.

The addition to the ration of Al-lactate or Al-sulphate at the lower levels provided a very slight protection against fluorosis. When given in adequate amounts Al-lactate produced approximately 50% reduction in F storage in the bones and to some extent prevented development of dental fluorosis. The effects of this compound was much superior to that of Al-sulphate at the same level of supplementation.

The optimal dose of Al-lactate in this experiment appeared to be 24 mg per kg body weight. Extremely high doses (64 mg/kg) clearly alleviated to a large extent the harmful effects of fluorine, but resulted in an impairment of growth in the animals.

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FLUOROSIS IN CATTLE IN ENGLAND AND WALES: INCIDENCE AND SOURCES

by

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Weybridge, England

The first recorded account of fluorosis in farm livestock in Britain, described the investigation of "a mysterious lameness of cattle" in the vicinity of a large concentration of brick kilns in Bedfordshire (1). During the next 10 years several other outbreaks were confirmed, the sources of fluoride being emissions from the open-air calcining of ironstone, colour and enamel works, and steel and aluminum producing plants (2-6). Subsequent sporadic outbreaks continued to occur, chiefly in the steel-producing areas of Yorkshire (7) and also in the vicinity of the ceramic industries in Staffordshire. During 1954 to 1957 the Central Veterinary Laboratory of the Ministry of Agriculture carried out a survey of herds in industrial areas in England and Wales (8) to obtain more complete information on sources, location, extent and degree of fluoride contamination of herbage by particulate and gaseous industrial emissions.

The effects of high fluoride intakes in cattle have been studied in many long-term experiments and are well documented by workers in Europe and the U.S.A. I shall not refer to these reports but shall try to give a summary of the diagnostic procedures used and the results obtained in this survey.

Diagnosis and Assessment of Clinical Signs

The diagnosis was based on:

(1) recognition of the clinical effects of fluorosis. These consist of characteristic dental lesions, lameness, bone lesions and loss of production.

(2) Chemical analyses showing increased fluorine concentrations in samples of urine, bone and feedingstuffs. These analyses are essential to confirm diagnosis, especially as sometimes only one set of symptoms may be found. For example, dental lesions do not occur in animals exposed to a high intake of fluoride after completion of the development of the enamel of all the permanent teeth, but bone changes and lameness may develop.

(3) Recognition of the source of fluoride. This is usually easy but may be difficult if clinical effects are due to past exposure which may have been from a non-industrial source such as a mineral mixture with a high fluorine content (9).

Dental Lesions are probably the most widely known and characteristic features of fluorosis and there is no need to describe them here. In our survey, the dental lesions were given the numerical ratings described by Phillips et al. (10) in the Report of the Committee on Animal Production of the National Research Council, National Academy of Sciences, U.S.A., Publication No. 824, 1960.

Lameness or stiffness is usually the first abnormality noticed by owners, and it is this which focuses attention on the possibility of fluorosis.

There are two main types of lameness symptomatic of fluorosis: (1) A generalized stiffness and "moving" lameness which may be exhibited first in one leg and then in another (7). It has been suggested that this type is probably related to periosteal tenderness or mild ossification of the tendons and periarticular structures (11).

(2) The other type is an acute, severe lameness usually in a foreleg, occurring particularly in early summer and associated with fracture of the pedal bone or 3rd phalanx. This is a characteristic type of lameness observed in some areas in England, the incidence usually being higher in districts and during seasons in which the ground is hard (8). Recovery usually results after housing the animal for several weeks in spite of a continued excess fluorine intake via contaminated hay and cut grass or other herbage.

It is important to differentiate the lameness of fluorosis from other types of bovine lameness caused by infective and non-infective conditions and also by traumatic fracture of the os pedis.

Loss of Production: Among the effects most consistently associated with fluorosis is loss of production, which includes reduced milk yield, loss of bodily condition in adult cows and retarded growth in young stock. These are non-specific effects and it is important to determine whether they are attributable only to a high fluoride intake.

There are three types of loss of production which should be considered in making a diagnosis: (1) that secondary to lameness or severe dental damage; (2) that which is primary or systemic resulting from a high continuous fluorine intake and (3) that which occurs in many industrial environments where the fluorine intake is relatively low and production loss is due to the difficulties of farming in such an area.

Results

Information obtained from the survey may be summarized as follows:

A total of 832 farms in 21 industrial areas of England and Wales were investigated. Fluorosis, severe enough to cause economic loss, occurred on 170 farms in 17 districts. Of these, 9 were so badly affected that cattle were no longer kept; 61 were classed as severely and 100 as slightly affected. All of them fell into the "damaging fluorosis" category, i.e. fluorosis of sufficient severity to damage animal health with consequent economic loss. A very much larger number of farms had cattle showing only dental lesions without disability, lameness or loss of production. Table 1 indicates affected areas and farms. Some, but not all, of the farms with dental lesions only have been included.

TABLE 1

Affected Areas and Farms

Area (alphabetical)	No. of Farms	No. of Farms with Damaging Fluorosis			
		Abandoned Grazing	Severely Affected	Slightly Affected	Total Affected
Bedford	43	3	8	8	19
Bletchley	13	-	-	1	1
Edgcott	6	-	-	1	1
Grantham	1	-	1	-	1
Middlesbrough	30	-	4	6	10
Peterborough and Whittlesey	38	1	6	13	20
Rotherham 5 mile radius	155	5	15	26	46
Rotherham N.E. area	14	-	-	9	9
Sheffield South	11	-	1	1	4
St. Helens	1	-	1	-	1
Stocksbridge	79	-	8	9	17
Stoke-on-Trent	334	-	12	10	22
Stoke-on-Trent, Cresswell	39	-	4	10	14
Tamworth	1	-	1	-	1
Wales, N.	12	-	-	2	2
Wales, S.	18	-	-	1	1
Wolverhampton	1	-	-	1	1
Total	796	9	61	100	170

Affected farms were in the vicinity of industries likely to emit fluorine compounds into the atmosphere. These industries and the sources of fluoride are listed as follows:

(1) Steel and metal works, when the method of production involves the use of fluorspar as a flux.

(2) Brickworks, where the source is usually the local clay, but coal used for the kilns can be a contributory factor.

(3) Glass, enamel and certain colour works where fluorine compounds are often added to the raw material to facilitate melting and to give the finished products certain properties.

(4) Potteries, heavy ceramic industries and tile works where the sources are the clay, some other materials used in manufacture, and coal if used for heating the ovens.

(5) Collieries and power stations which use a high proportion of low-grade coal containing fluorine-rich shale. Such sources usually cause dental lesions but do not cause damaging fluorosis.

Our investigations showed that the most common cause of loss of production was attributable to pain and reduction of movement and grazing resulting from lameness. Dental damage was not an important contributory factor and in only a few instances was it severe enough to interfere with grazing and mastication.

During the 4 year period of the survey, it became increasingly evident that there were factors other than fluorosis which influenced the occurrence and severity of clinical effects under investigation. This was particularly noted when it was attempted to assess how much loss of milk production, loss of bodily condition in adult cows, and retarded growth in young stock could be attributed specifically to high F intake and how much could be attributed to other factors such as the general industrial environment, level of nutrition and inefficient management.

For example, in districts like Bedford, where dental and skeletal lesions and lameness occurred in association with a high fluorine contamination of the herbage but with a minimum of the difficulties of industrial environments, it was notable that there was very little loss of production in non-lame animals and that it occurred only in herds severely affected by lameness. In areas where the general industrial environment caused difficulties, as in Yorkshire and Stoke-on-Trent, the percentage of herds affected with loss of production was higher in all lameness classifications and was greatest in Stoke-on-Trent, where the industrial difficulties were most marked. The occurrence of loss of production in herds not affected by lameness was particularly notable in Stoke-on Trent. It therefore seems reasonable to conclude that, in animals in which fluorosis lameness occurred, the loss of production was a consequence of the lameness, whereas in non-lame animals in industrial environments providing a moderate fluorine contamination of herbage, loss of production was the result of the general effects of that environment.

The difficulties of farming in an industrial district have been described in a report by Leeds University (12) on farming in the industrial Pennines. Among the factors enumerated are poor natural conditions, smoke pollution, inadequate grassland management and a low level of livestock management and feeding. Milk yields were low and cows were reported to deteriorate after 2 years in the district.

As well as these factors, others were observed, such as the small size of the farms, rapid corrosion of fencing wire, trespassing nuisance and progressive deterioration of grazing swards caused by the dying out of productive grasses in the industrial atmosphere. Farmers were often reluctant to improve grassland because of the probability of the land being taken for building in the near future.

Evidence that copper deficiency was also a contributory cause of loss of production emerged from the survey. Low blood copper values indicative of copper deficiency in cattle were found in many of the industrial districts. There are now reports from many different countries and areas that copper deficiency in cattle can cause loss of production involving reduced milk yields (13,2) and severe retardation of growth in young stock (14, 15).

Infertility was not a feature of herds in fluorosis areas. Some individual cows, however, which had been severely lame and emaciated, failed to conceive after recovery.

It was noticed during the survey that in some animals with skeletal fluorosis, joint infections of a type which would have been comparatively mild in normal animals, were severe, progressive and resistant to treatment with a tendency to form massive exostoses, resulting in crippling lameness.

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