October, 1989 Vol. Twenty-two, No. Four

FLUORIDE

OFFICIAL QUARTERLY JOURNAL

OF

INTERNATIONAL

SOCIETY for

FLUORIDE

RESEARCH

Vol. Twenty-two, No. Four October, 1989 Pages 155-220

FLUORIDE

Quarterly Reports

Issued by

THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH

TABLE OF CONTENTS

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

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

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

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

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

FLUORIDE, WATER AND VEGETATION

There is considerable information on the uptake of fluoride from soil and air, but little has been presented on uptake of fluoride from water (1). Plants can accumulate fluoride when their aerial parts are exposed, either by flooding or sprinkling, to fluoride-containing water.

Experiments in our laboratory showed the following accumulation in barley leaves with daily foliar sprays of different fluoride concentrations after 15 days.

Accumulation of Fluoride in Barley Leaves from Foliar Sprays

ppm Fluoride in Spray	0	1	5	10	20
ppm Fluoride in tissue (dry wt.)	10	13	104	130	240

The fluoride accumulation in plants was very high, at concentrations in the water over 5 ppm, exceeding the tolerance levels proposed for domestic animals (heifers, 30 ppm; dairy cattle, 40 ppm; beef cattle, 50 ppm; breeding ewes, 60 ppm; horses, 60 ppm) (2). These tolerance levels are a suggested guide only when the feed is essentially the sole source of fluoride. Scientists at Cornell University have strongly questioned the above tolerance levels and showed injury to cattle at levels below those established by the National Research Council (3,4).

Many naturally occurring and developed geothermal waters in the world contain significant quantities of the pollutant fluoride and other salts. There is considerable motivation to locate, develop and utilize geothermal water for agricultural purposes. In assessing the problems of fluoride toxicosis in Western United States, data from over 300 geothermal wells were compiled (5). At least 68 percent of the waters contained fluoride ranging from 2-30 ppm. Fluoride content of ranch waters in Idaho were as follows:

Water Source	Fluoride ppm
Ranch – Grandview	
Regular Well	0.8
Warm Well	14.2
Ranch – Bruneau	
Culinary	8.4
Pump	9.7
Ditch from Flowing Well	11.6
Flowing Well Outlet	11.5
Ranch – Raft River	
Geothermal Wells	5.4-10.4

Fluoride content in vegetation ranged from 5 to 430 ppm.

Another potential source of fluoride pollution in water may come from cooling towers of coal-fired electrical plants. Although the water may originally contain 0.2 ppm fluoride, after release from the cooling process it may reach

5-10 ppm fluoride. Such water directly used for sprinkle irrigation would lead to high concentrations in the folliage.

Greater demands will be made on agriculture to supply food needs of a rapidly expanding world population. In arid and semi-arid regions (over 30% of the world) sprinkle irrigation is very common. The use of wells containing high fluoride is prevalent and will increase with added pressures for food production on agriculture. Animals, both domestic and wild, will drink this water (warm and attractively palatable) and consume forage containing high fluoride. There is a need for additional information on the effects of high fluoride waters on vegetation and effects on animals consuming water and vegetation irrigated with such water. Fluoride is still a serious pollutant and much research remains to be done. Support by Federal, State and Private agencies is important to alleviate and avoid current and future problems associated with fluoride.

— G.W. Miller

References

1. Peterson, H.B.: Some Suggested Research Needs. In: Shupe, J.L., Peterson, H.B. and Leone N.C. (Eds.): **Fluorides, Effects on Vegetation, Animals and Humans.** Paragon Press, Inc., Salt Lake City, Utah, USA, 1983.
2. Peterson, H.B. and Shupe, J.L.: A Study of Fluoride from Geothermal Water. Fourth Biannual Veterinary Toxicology Workshop, Utah State University, Logan, Utah, 1978.
3. Krook, L. and Maylin, G.A.: Industrial Fluoride Pollution. Chronic Fluoride Poisoning in Cornwall Island Cattle. **Cornell Vet. 69**, Suppl. 8:1-70, 1979.
4. Eckerlin, R.H., Maylin, G.A. and Krook, L.: Milk Production in Cows Fed Fluoride-Contaminated Commercial Feed. **Cornell Vet.**, 76:403-414, 1986.
5. Peterson, H.B., Shupe, J.L. and Miller, G.W.: A Study of Fluorides From Geothermal Water. 11th Annual ISFR Conference, Dresden, GDR, 1981.

****

RADIOLOGICAL MODIFICATIONS OF THE SKELETAL SYSTEM AMONG ALUMINUM SMELTER WORKERS

A 15-Year Retrospective Study

by

H. Runge* and J. Franke
Halle and Erfurt, GDR

SUMMARY: Previously by the time skeletal fluorosis among aluminum smelter workers due to high fluoride exposure was diagnosed numerous cases of bone fluorosis had already reached stages II and III according to Roholm. Today, as a result of improved working conditions and continuous health care, the picture has changed. This paper reports the frequency of occurrence of bone changes caused by fluoride in a population of 358 aluminum smelter workers who had been fluoride exposed for more than 5 years and whose diagnosis had not been made prior to 1971. In the examination, particular attention was paid to degenerative changes of the skeleton and the frequency of spondylosis, arthrosis of the hip and elbow joints as well as changes in the form of diffuse idiopathic skeletal hyperostosis (spondylosis hyperostotica Forestier). A population of 81 foundry workers in aluminum smelters under similar working conditions, but not fluoride exposed, served as controls.

KEY WORDS: Aluminum smelter; Arthritis; Bone changes, radiologically visible; Fluorosis, skeletal; Foundry workers; Spondylosis.

Introduction

More than 50 years have passed since Møller and Gudjonsson (1) described fluorosis, a new occupational disease, for the first time. Roholm (2) subsequently described this disease in detail in cryolite workers. He divided roentgenologically visible changes into three stages; Fritz (3) added two prestages (vague symptoms and stage 0-1). In the fourth to seventh decades of our century description of typical skeletal fluorosis with distinct sclerosis of the spine and pelvis and marked formation of appositions at long bones (radius and ulna, as well as tibia and fibula) in the range of the lower legs and forearms played a central role. In recent years, reports on less distinct fluorosis cases is incresing (4-12), in which hyperostosis at the spine and bone appositions at muscular and ligamental attachments dominate. Also Carnow and Conibear (13) concluded from a study of 1242 aluminum smelter workers that fluoride exposures were related to a history of musculo-skeletal diseases and other abnormalities in the absence of radiologically apparent skeletal fluorosis. They suggested that non-specific changes could comprise an early stage of fluorosis.

In view of these facts we examined carefully about 500 aluminum smelter workers of an aluminum factory near Halle with respect to roentgenological

* Department of Othropedic Surgery, Faculty of Medicine, Martin-Luther-University, Halle, GDR.

symptoms. Previously Franke et al. (14) and Specht (15) had classified all cases of fluorosis of the same factory, which had occurred up to the early 1970s. Thus in this paper only those workers are included whose disease had begun during the last 15 years (1971-1986) who have been fluoride-exposed for more than 5 years.

This group of 358 aluminum smelter workers was compared with the fluoride-exposed subjects examined by Specht (15) taking into consideration exposure time, age when starting the occupation and age when the disease began.

Materials and Methods

The roentgenograms of 358 aluminum smelters, having been fluoride-exposed for more than 5 years were analysed. X-ray photographs were available of the thoracic and lumbar spine at two levels, of the pelvic region ap. and lateral, of the forearms with elbow and of the lower legs ap. and laterally with the knee joint. Moreover, in the course of the last two years lateral x-ray photographs of the heel bone of all aluminum smelter workers were registered by screening. According to the health-care program for fluoride-exposed persons in the G.D.R., the above-mentioned x-ray photographs are taken every four years. In addition, workers are thoroughly examined clinically. A population of 81 foundry workers from different factories of the G.D.R. (in Halle and Karl-Marx-Stadt) served as controls. They worked under similar conditions as well as under the influence of extreme heat, without being exposed to fluoride. Their ages were approximately the same as the aluminum smelter workers (49.2 yrs ±6.87 for the workers in the foundry and 47.5 years ±10.14 for workers in aluminum smelters) particularly important in connection with the assessment of the frequency of various degenerative skeletal symptoms.

Results

Table 1 shows the frequency in percent (%) of the various stages of fluorosis in three different groups of fluoride-exposed subjects. Whereas Fritz (3) carried out his examinations in cryolite workers, Specht (15) and we investigated aluminum smelter workers of the same factories near Halle.

Fritz found that over 40% of the cases were suffering from fluorosis stage 0-I up to stage III; Specht found that 45.7% fell into these groups, whereas in our present examination only 18.7% of fluoride-exposed persons were in these stages. Among Specht's patients only 21.6% showed no fluoride-caused changes of the skeleton, in other words he found 73.5% without skeletal changes.

Table 2 shows medium fluoride-exposure time leading to fluorosis, in comparison with examinations by Fritz and Specht. According to this table the time leading to fluorosis stage I, II or III is longer, on the average, in Fritz's than in Specht's workers. In our investigation it is somewhat longer. Medium exposure time of workers who developed the disease between 1971 and 1986 does not differ markedly with reference to the individual stages of fluorosis; it is always around 20 years without statistical significance, with a range of 10 to 43 years (for example in stage I). In Table 3 we did not demonstrate the dependence on the worker's age when entering the produc-

Table 1

Frequency (%) of Various Stages of Fluorosis in Three Different Fluoride-Exposed Groups.

Fluorosis Stage	Fritz [1958] (n = 156)	Specht [1975] (n = 300)	Runge [1986] (n = 358)
No Changes	53.2	21.6	73.5
Vague Symptoms	4.7	32.7	7.8
0-I	17.6	17.3	7.8
I	13.6	16.0	5.3
I-II	—	7.0	3.9
II	5.7	2.3	0.6
II-III	—	2.3	1.1
III	5.2	0.8	—

Table 2

Mean Fluoride Exposure (Years) for Developing Fluorosis.

Fluorosis Stage	Fritz [1958]	Specht [1975] (mean/range)	Runge [1986] (mean/range)
No Changes	—	9.7 (0-20)	19.9 (5-35)
Vague Symptoms	11	12.8 (2-23)	19.2 (10-37)
0-I	12-13	14.1 (5-33)	22.6 (10-30)
I	12-26	15.0 (6-36)	21.1 (10-43)
I-II	—	17.2 (12-27)	21.1 (10-33)
II	21-31	17.6 (11-21)	17.5 (16-19)
II-III	—	18.9 (14-25)	21.3 (15-26)
III	25-35	19.5 (19-20)	—

tion process or on the worker's age at the time of diagnosis, nor could exposure time be related to the development of a certain stage of fluorosis. Therefore occurrence of bone fluorosis does not depend upon whether the worker starts working in such a factory at age 20 or at age 40. Nor is duration of exposure significant, since stages II-III can be demonstrated after 15 years. On the other hand, in some cases, more than 30 years may be necessary to develop vague symptoms or stage 0-I.

Individual differences in sensitivity to noxious fluoride seems to be more important. The three tables demonstrate that it is quite possible to be an aluminum smelter worker for 30 years or longer without showing fluoride-caused bone changes, whereas others develop symptoms of fluorosis after only 10 years; the varying effect of fluoride has been demonstrated by therapy tests for osteoporosis.

Table 3

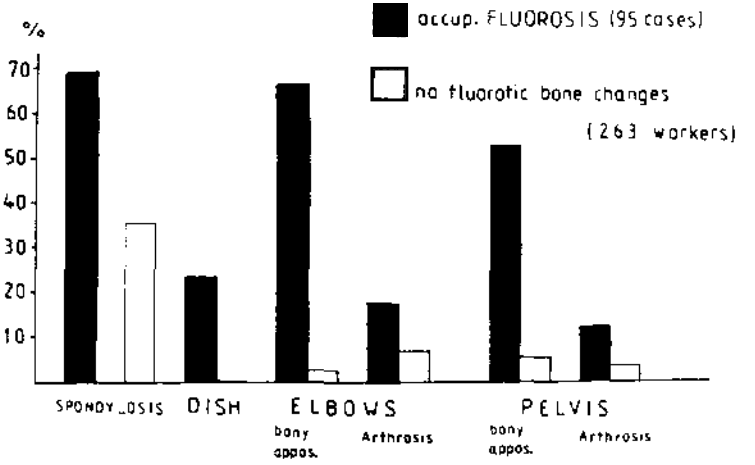
Worker's Age When Entering Production Process and Age of Diagnosis (n = 358 Aluminum Smelter Workers with over 5 Years Exposure Time).

Fluorosis Stage	n	Age (in Years) At Start of Employment (mean/S.D.)	Age (in Years) At Confirmation of X-ray Diagnosis (mean/S.D.)	Duration of Exposure (mean/S.D.)
No Change	263	22.9 ±15.30	—	19.9 ±9.26
Vague Symptoms	28	29.4 ±7.42	51.0 ±9.52	19.2 ±6.21
0-I	28	27.9 ±7.07	51.5 ±7.40	22.6 ±4.96
I	19	32.0 ±8.04	53.8 ±9.10	21.1 ±7.67
I-II	14	31.6 ±8.27	54.6 ±5.68	21.1 ±6.40
II	2	32.5 ±12.02	50.0 ±9.90	17.5 ±2.12
II-III	4	28.3 ±2.36	50.5 ±7.33	21.3 ±5.19

For this reason we examined the causes of this difference in sensitivity to fluoride by studying the degenerative changes in fluoride-exposed persons, the frequency of which is illustrated in Figure 1. Spondylosis, elbow and hip joint arthrosis occur more often in the 95 cases diagnosed as fluorosis. Also bony appositions at the epicondyles of the humerus and in the pelvic region occur more frequently in the fluorosis-group than in fluoride-exposed subjects with symptoms of fluorosis. Like Boillat et al. (7) we observed hyperostosis

Figure 1

Frequency in Percent (%) of Spondylosis, Diffuse Idiopathic Skeletal Hyperostosis (DISH), Bony Appositions and Arthrosis of Elbow and Pelvic Regions (n = 358 Aluminum Smelter Workers, Exposure-time over 5 Years; 95 Workers with Signs of Fluorosis, 263 without Fluorotic Bone Changes).



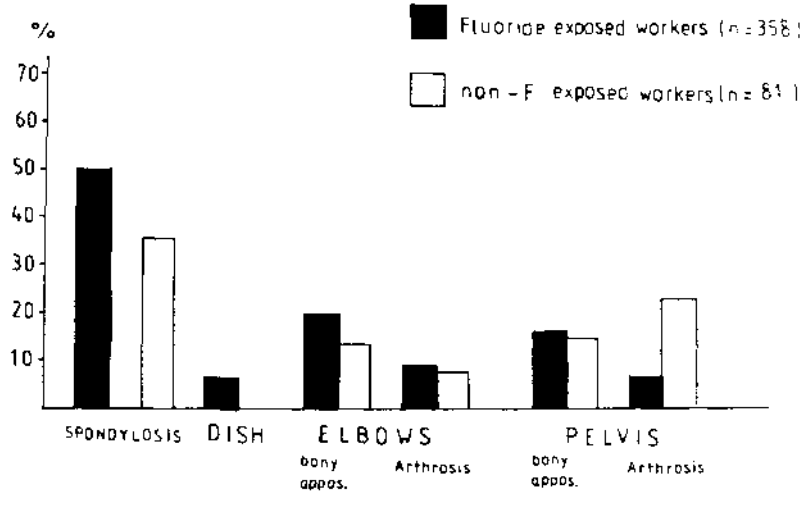
of the spine or of peripheral skeletal parts in 22 cases, a particular form of fluorosis. The final evidence that the changes are really fluoride-caused can only be produced by a biopsy of these areas, a measure not desired by the workers. In the Anglo-American literature these generalized hyperostoses are referred to as Diffuse Idiopathic Skeletal Hyperostoses (DISH). In Germany and France they are described as spondylosis hyperostotica or Forestier desease (16-24). Neither Boillat et al. (5) nor we found differences between the fluoride-induced hyperostosis and the form which (at a much lower frequency) can occur spontaneously without external cause (idiopathic). In our 22 cases, bone density was normal in 12, i.e. no sclerosis of the spine nor of the pelvis could be identified.

Figure 2 compares the frequency of spondylosis, elbow and hip joint arthrosis as well as bony appositions in the elbow and pelvic region of fluoride-exposed aluminum smelter workers to that of iron foundry workers. The average age of the two groups examined was approximately the same (47.48 yrs in the fluoride-exposed group; 49.21 yrs in the controls).

It can be noted that spondylosis, DISH (no case occurred among workers not exposed to fluoride) as well as bony appositions occurred more often in aluminum smelter workers. Arthrotic changes of the elbow joint likewise occurred often, but were more distinctly developed among aluminum smelter workers; coxarthrosis was found more frequently in non-fluoride exposed foundry workers than in aluminum smelter workers. In the majority of these DISH-like cases the hyperostotic changes in the region of the spine, pelvis, elbow joints and heel bones, were observed simultaneously with a normal density of the skeleton.

Figure 2

Frequency in Percent of Spondylosis, DISH, Bony Appositions and Arthrosis of Elbow and Pelvic Region in Population of Fluoride-Exposed Workers (n = 358) compared with Non-Fluoride Exposed Population of Foundry Workers (n = 81).



Discussion

Our study shows that hyperostosis of the spine and peripheral skeletal parts occurs more frequently among fluoride-exposed aluminum smelter workers. It is similar to diffuse idiopathic skeletal hyperostosis (or spondylosis hyperostotica Forestier) (16-24). Separation of the fluoride-caused form from that etiologically unexplained idiopathic is only possible through bone biopsy. DISH is accompanied by a disturbance of the vitamin A metabolism (16). In the literature there are no similar examinations of fluoride exposed subjects with hyperostosis. As in the 43 aluminum smelter workers suffering from fluorosis examined by Boillat et al. (6) we found no pathology in our aluminum smelter workers other than movement restrictions and cases of asthmoid bronchitis.

Regarding the question raised by Boillat et al. (6) on the role of physical stress in the development of fluorosis and its osteoarticular manifestation, we have no final answer. In our patients vertebral changes even exceeded the 70% of Boillat et al. (6), when DISH-like hyperostoses of the spine were added. Hyperostotic changes at the knee joint, which occurred in the reports of the above-mentioned authors in 43% of cases were found in less than 5% of ours.

In numerous cases, we noted considerable arthrosis of the elbow joints, recognized as an occupational disease in our country; aluminum smelter workers are obliged for years to use a steel rod to break the smelt crust. Boillat et al. (6) reported 0.5-2.3 mg F/m^3 in the air in the electrolysis area. In comparison in the factory where our patients work the values were partly over 5 mg F/m^3 around 1970. In recent years, air F^- was 2.5 mg F/m^3. Nevertheless, the formerly typical sclerosis does not occur as frequently. On the other hand, skeletal changes similar to degenerative diseases are observed.

Czerwinski and Lankosz (8) who question whether mechanical factors play a role in developing fluoride-caused bone changes consider the possibility of synergistic action of fluorine and mechanical factors. The frequency of vertebral changes could be facilitated largely by prolonged exposure apart from mechanical factors. Since the crust breaking equipment was formerly poorly suspended, the whole body was exposed to considerable shaking, resulting in spondylosis.

Conclusion

In the last 15 years due to improvement in working conditions (new crust breaking equipment, with a cabin, improvements of suction devices) and intensive health care, the frequency of fluorosis has markedly diminished in the factory supervised by us and only incipient stages of fluorosis are encountered. However, the number of workers showing degenerative spinal changes beyond the normal level is still high. As fluoride is largely used in the nickel, copper, coal, gold and silver industries, in the production of fertilizers, narcotic gas as well as in the production of steel, iron, glass, ceramics, enamel and numerous other production processes, the possibility of developing occupational fluorosis must always be considered. The total number of American workers potentially exposed to fluorides, according to the National Institute for Occupational Safety and Health (25), is 350,000. Although the severity of industrial

fluorosis is decreasing in some aluminum factories, at present, due to improved control and working conditions, the danger caused by fluoride is increasing as its use in industry becomes more extensive.

References

1. Møller, I.J. and Gudjonsson, S.V.: Massive Fluorosis of Bones and Ligaments. Acta Radiol., 13:269-294, 1932.
2. Roholm, K.: Fluorine Intoxication. A Clinical-Hygienic Study. H.K. Lewis and Co., London, 1937.
3. Fritz, H.: Röntgenpathologische und pathologische Beobachtungen zum Fluoroseproblem. Med. Habil. Schrift., Dresden, 1958.
4. Boillat, M.A., Rouget, A., Curati, W., Dettwiler, W., Maillard, J.M., May, P. and Demeurisse, C.: Quelques aspects de la fluorose industrielle en Suisse. II: Radiologie et fluor osseux. Arch. mal. prof., 36:412-420, 1975.
5. Boillat, M.A., Garcia, J., Detwiler, W., Burckhardt, P. and Courvoisier, B.: Clinical Aspects of Industrial Fluorosis. In: Fluoride and Bone. B. Courvoisier et al., eds., Berne, Hans-Huber-Verlag, 1978, pp. 155-162.
6. Boillat, M.A., Baud, C.A., Lagier, R., Garcia, J., Rey, P., Bang, S., Boivin, G., Demeurisse, C., Goessi, M., Tochon-Danguy, H.H., Very, J.M., Burckhardt, P., Voinier, B., Donath, A. and Courvoisier, B.: Fluorose industrielle. Etude multidisciplinaire de 43 ouvriers de l'industrie de l'aluminium. Schweiz. Med. Wschr., 109:suppl.8:1-28, 1979.
7. Boillat, M.A., Garcia, J. and Velebit, L.: Radiological Criteria of Industrial Fluorosis. Skeletal Radiol., 5:161-165, 1980.
8. Czerwinski, E. and Lankosz, W.: Industrial and Endemic Skeletal Fluorosis. In: Fluoride and Bone. Courvoisier et al, eds., Berne, Hans-Huber-Verlag, 1978, pp. 144-154.
9. Grandjean, P.: Classical Syndromes in Occupational Medicine. Occupational Fluorosis through 50 Years. Clinical and Epidemiological Experience. Am. J. Industr. Med., 3:227-236, 1982.
10. Grandjean, P.: Long-term Significance of Industrial Fluoride Exposure. A Study of Danish Cryolite Workers. In: Fluoride Toxicity. Proceedings of the 13th Conference of the ISFR, New Delhi, November 13-17, 1983. Susheela, A.K., ed., New Delhi. All India Institute of Medical Sciences, 1985, pp. 5-16.
11. Hodge, H.C. and Smith, F.A.: Occupational Fluoride Exposure. J. Occup. Med., 19:12-29, 1977.
12. Lagier, R.: Pathology of Skeletal Industrial Fluorosis. In: Fluoride and Bone. Courvoisier et al., eds., Bern, Hans Huber Verlag, 1978, pp. 163-167.
13. Carnow, B.W. and Conibear, S.A.: Musculoskeletal and Respiratory Diseases in Aluminum Smelter Workers. Arch. Hig. Rad. Toksiko., 30:967-981, 1979.
14. Franke, J., Rath, F., Runge, H., Fengler, F., Auermann, E. and Lenart, G.: Industrial Fluorosis. Fluoride, 8:61-85, 1975.
15. Specht, A.: Über die Bedeutung des Eintrittsalters und der Fluorexpositionsdauer für das Aftreten der Knockenfluorose bei Aluminiumschmelzern des CKB–Bitterfeld. Diplomarbeit, Halle. Martin-Luther-Universität, 1975.
16. Abiteboul, M., Lussier, A., Billon, B., Drapeau, G. and Petitcler, C.: Hyperstose vertebrale et troubles du metabolisme du retinol. Rev. Rhum., 52:141-143, 1985.
17. Arbiteboul, M., Mazieres, B. and Menard, H.: A propos de deux nouveaux cas familiaux d'hyperostose vertebral ankylosante. Rev. Rhum., 52:645-647, 1985.

18. Abiteboul, M., Arlet, J., Sarrabay, M.A., Mazieres, B., and Thouvenot, J.P.: Etude du metabolisme de la vitamine A au cours de la maladie hyperostosique de Forestier et Rotes-Querol. Rev. Rhum., 53:143-145, 1986.
19. Houk, R.W., Hendrix, R.W., Lee, C., Lal, S. and Schmid, F.R.: Cervical Fracture and Paraplegia Complicating Diffuse Idiopathic Skeletal Hyperostosis. Arthritis Rheum., 27:472-475, 1984.
20. Lagier, R. and Baud, C.A.: Diffuse Enthesopathic Hyperostosis – Anatomical and Radiological Study on a Macerated Skeleton. Roe. Fo., 129:588-597, 1978.
21. Ott, V.R.: Spondylosis hyperostotica. Stuttgart, Enke, 1982.
22. Resnick, D., Shaul, S.R. and Robins, J.M.: Diffuse Idiopathic Skeletal Hyperostosis (DISH). Forestiers Disease with Extraspinal Manifestation. Radiology, 115:513-524, 1975.
23. Tsukamoto, Y., Onitsuka, H. and Lee, K.: Radiological Aspects of Diffuse Idiopathic Skeletal Hyperostosis of the Spine. Am. J. Roentgenol., 129:913-918, 1977.
24. Yagan, R. and Khan, M.A.: Confusion of Roentgenographic Differential Diagnosis between Ankylosing Hyperostosis (Forestier's Disease) and Ankylosing Spondylitis. Clin. Rheumatol., 2:285-292, 1983.
25. NIOSH (National Institute for Occupational Safety and Health) Recommended Standard for Occupational Exposure to Inorganic Fluoride. Washington, D.C., U.S. Department of Health, Education and Welfare, 1979.

****

EFFECT OF FLUORIDE ON REPRODUCTION IN MICE

by

K.S. Pillai*, A.T. Mathai and P.B. Deshmukh
Valvada, India

SUMMARY: Mice were administered daily 17.3 and 5.2 mg F/kg b.w. ($^1/_3$rd and $^1/_{10}$th LD_{50}, respectively) orally from day 6 of mating until day 15 (10 days). Body weight and amount of food consumed were measured daily, until day 21, when the experiment was terminated. Fluoride-treated mice showed no sign of pregnancy; body weight and RBC counts declined. Hemoglobin declined significantly in mice given 5.2 mg F/kg b.w.

KEY WORDS: Blood parameters; Body weight; Mice; Reproduction.

Introduction

Available reports on administration of fluoride in pregnant laboratory animals are few. Sodium fluoride and calcium fluoride, administered in Webster mice during gestation, affected the matrix of calcified tissues of neonatal mice (1). Similar results have been demonstrated in Wistar albino rats (2). Administration orally of 25 mg F/kg b.w. to pregnant rats caused fetal abnormalities in liver, kidney, skull, jawbones and teeth (3). The foregoing studies clearly indicate that fluoride is a teratogenic agent. Shepard (4) likewise included it in his book **Catalog of Teratogenic Agents.**

This study aims to determine the effect of sublethal concentrations of fluoride on pregnant mice.

Materials and Methods

Laboratory inbred Swiss albino mice (30-35 g in weight) of Haffkine strain of our colony (Jai Research Foundation, Valvada, Dist. Valsad, Gujarat) were used. Female mice were placed overnight with males (in the ratio of 1:2) The following morning female mice were examined for the sign of mating (presence of vaginal plug). The day on which the vaginal plug was observed in mice was considered the first day of pregnancy (day 1 post coitum).

Sodium fluoride, 17.3 mg F/kg b.w. dissolved in distilled water, was administered orally (single dose administration) to each mouse daily to 4 mice of Group 1, and 5.2 mg F/kg b.w. to 4 mice of Group 2 from day 6 post coitum until day 15 post coitum. The two doses are considered $^1/_3$rd and $^1/_{10}$th of LD_{50} (5), respectively. Groups 3 and 4, two control groups, consisting of 4 mated and 4 non-mated mice, respectively, received distilled water. Throughout the experiment, animals of all groups were given pelletted mice feed and water ad libitum.

* From the Jai Research Foundation, Off N.H. No. 8, Valvada, Taluka Umbergam, Dist. Valsad, Gujarat 396108, India.

Body weight of individual animals and amount of food consumed were noted from day 6 post coitum until day 21 post coitum, when the experiment was terminated. On this day blood cell count (RBC and WBC) and hemoglobin estimation (6) were carried out on mice following which the animals belonging to Groups 1 and 2 were sacrificed to observe the uterus.

Relationship of day, body weight and food consumed was studied by multiple regression equation and partial correlation coefficients (7). Blood parameters were compared using Student-Newman-Keul's test (8).

Results and Discussion

Group 3, control animals, delivered on the 21st day. Groups 1 and 2 showed no sign of pregnancy. Examination of uterii of these animals showed no evidence of implantation of embryos. Administration of fluoride in conceived mice on day 6 post coitum might have affected implantation of blastocytes. However, this view is not proven.

Pregnant mice (Group 3) showed a significant increase in body weight during 6-20 days (from 32.5 ±1.4 g to 51.3 ±0.4 g). Group 4 mice showed a body weight gain from 23.9 to 27.4 g. Data on mice belonging to the control group when compared to changes in body weight and food consumption in fluoride-treated mice (Figure 1) showed that food consumption, and body weight of mice given 17.3 mg F/kg b.w. were significantly related (Table 1). However, in mice given 5.2 mg/kg b.w. the relationship was not significant. Control animals showed a highly significant multiple correlation coefficient. The significant $r_{x_1 y.x_2}$ indicates that the body weight of controls increased day by day.

Table 1

Multiple Regression Analysis

Animals	Multiple Regression Equation*	Multiple Correlation Coefficient (R)	Partial Correlation Coefficients
17.3 mg F/kg b.w.	$Y = 27.18 - 0.06\ X_1 - 0.07\ X_2$	0.66**	$r_{x_1 x_2 .y} = 0.48$
			$r_{x_2 y.x_1} = -0.29$
			$r_{x_1 y.x_2} = 0.38$
5.2 mg F/kg b.w.	$Y = 21.18 - 0.11\ X_1 + 0.18\ X_2$	0.54 (N.S.)	(N.S.)
Control	$Y = 24.28 + 0.27\ X_1 - 0.07\ X_2$	0.97**	$r_{x_1 x_2 .y} = 0.19$
			$r_{x_2 y.x_1} = -0.34$
			$r_{x_1 y.x_2} = 0.96$**

*$Y = a + b_1X_1 + b_2X_2$, where X_1 and X_2 are days and food consumption (g/100 g b.w.), respectively and Y is the body weight of mice (g); "a" is the intercept and "b_1" and "b_2" are the slopes.

**Correlation coefficients are significant at 5% probability level. N.S. — Not significant.

Compared to controls (Group 4) RBC (in fluoride-treated mice) decreased significantly (Table 2), but changes in WBC were insignificant ($p > 0.05$). In mice treated with 5.2 mg F/kg b.w. hemoglobin decreased significantly. Decrease in both RBC (9) and hemoglobin (10) in rats due to fluoride toxicity has been reported previously and many researchers (11,12) have shown that fluoride exerts an adverse effect on WBC.

Table 2

RBC, WBC and Hemoglobin in Mice

Mice	RBC	WBC	Hemoglobin (g/100 mL)
17.3 mg F/kg b.w.	12.47×10^{6}* $\pm 57.80 \times 10^{4}$	90.67×10^{2} 4.63×10^{2}	11.42 ±1.91
5.2 mg F/kg b.w.	11.83×10^{6}* $\pm 10.60 \times 10^{5}$	95.43×10^{2} $\pm 8.81 \times 10^{2}$	8.59* ±1.42
Control	13.29×10^{6} $\pm 27.87 \times 10^{6}$	95.87×10^{2} 3.20×10^{2}	11.76 ±0.98

Values expressed as mean ±S.D. ($n = 4$). Asterisks indicate significant difference from control ($p < 0.05$).

In this study, during the initial phase of the experiment, body weight of the animals declined but following discontinuance of fluoride administration they started to gain body weight (Figure 1). By the time cell counts were done (6 days after the last fluoride dose) the mice might have already started to recover from fluoride's toxic effect.

Conclusion

Administration of fluoride to pregnant mice adversely affects the mothers. To understand the mechanism of fluoride toxicity on implantation of embryos, requires further study.

Acknowledgement

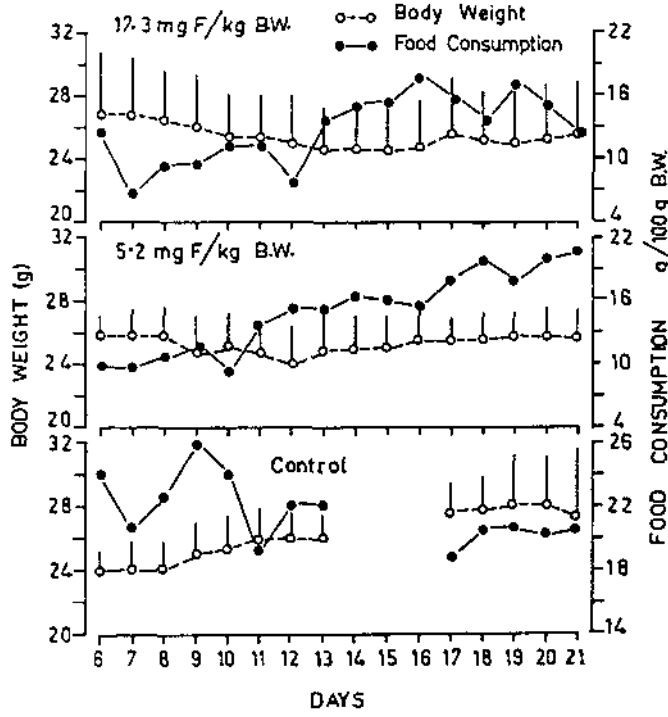
Gratitude is expressed to Mrs. Sandra R. Shroff and Mr. Rajju D. Shroff, Managing Trustees of Jai Research Foundation for their interest in fluoride research and for the necessary laboratory facilities.

References

1. Fleming, H.S. and Greenfield, V.S.: Changes in the Teeth and Jaws of Neonatal Webster Mice After Administration of NaF and CaF_2 to the Female Parent During Gestation. **J. Dent. Res.**, 33:780-788, 1954.
2. Goto, K.: Effect of Sodium Fluoride on the Fetuses and Sucklings in Wistar Strain Albino Rats (Abstract). In: **Proceedings of the Congenital Anomalies Research Association of Japan**, Eighth Annual Meeting, Tokyo, Japan, April 9-10, 1986, pp. 46.
3. D'Angelo, M. and Esposito, U.: Osservazioni Istoloiche su Neonati da Ratte Intossicate con Dosi Elevate di NaF. **Ann. Stamatol.** (Rome), 14:835-842, 1965.

Figure 1

Changes in Body Weight (b.w.) and Food Consumption of Mice.
b.w. Expressed as Mean Weight of Mice. Bars show Standard Deviation (n = 4).



4. Shepard, T.H.: **Catalog of Teratogenic Agents.** The Johns Hopkins University Press, Baltimore, 1973, pp. 211.
5. Pillai, K.S., Mathai, A.T. and Deshmukh, P.B.: Acute Toxicity of Fluoride to Mice. **Fluoride,** 20:68-70, 1987.
6. Wong, S.Y.: Colorimetric Determination of Iron and Hemoglobin in Blood. II. J. **Biol. Chem.,** 77:409-412, 1928.
7. Snedecor, G.W. and Cochran, W.G.: **Statistical Methods.** Oxford and IBH Publishing Co., New Delhi, 1968, pp. 381-416.
8. Woolf, C.M.: **Principles of Biometry.** D. van Nostrand Co., Inc., Princeton, 1968, pp. 101-109.
9. Kahl, S., Wojcik, K. and Ewy, Z.: Effect of Fluoride on Some Hematological Indices and Iron — 59 *Distribution in the Blood and Iron* — Storing Tissues of Rats. **Bull. Acad. Polonaise des Sci. (Ser. Sci. Biol.),** 21:389-393, 1973.
10. Danilov, V.B. and Kas'yanova, V.V.: Experimental Data on the Effect of Hydrofluoric Acid on Embryogenesis of White Rats. [in Russian] **Gig. Tr. Prof. Zabol.,** 1:57-58, 1975.
11. Greenberg, S.R.: Leucocyte Response in Young Mice Chronically Exposed to Fluoride. **Fluoride,** 15:119-123, 1982.
12. Greenberg, S.R.: The Response of Murine Leucocytes to Extended Fluoride Exposure. **Anat. Rec.,** 196:232, 1980.

****

EFFECTS OF ENVIRONMENT UPON FLUORIDE CONTENT IN NAILS OF CHILDREN

by

Z. Machoy*
Szczecin, Poland

SUMMARY: This report aims to evaluate the content of fluoride in nails of children, aged 10-12 years, who attend schools in several localities in Poland. Preliminary studies have shown that the method used, namely gas chromatography, may be applicable.

KEY WORDS: Environment; Fluoride determination; Fluoride in nails.

Introduction

To assess the health risk to people and animals, the extent of environmental pollution must be determined. For this purpose fluorine bioindicators were utilized. To date, body fluids, and/or hard and soft tissues have been analyzed, to study fluorine metabolism. Since all fluids and tissues are not easily accessible, analysis is not always approved by physician or patient. Our earlier report focused on the fact that material for fluoride examination could be provided by human nails and animal claws (1), which are within easy reach and, as supporting substances, exhibit a certain similarity to hard tissues. The main organic components of hard tissues (bones) is collagen, whereas the principal organic component of nails is keratin. The objective has been to find out whether or not the variable amounts of fluorine in the environment are reflected in the level of fluoride in children's nails.

Materials and Methods

The nails were supplied by children, aged 10-12 years, attending elementary schools in Poland. The children were divided into 5 groups, each with about 50 subjects. Group I came from a locality where a big industrial plant is engaged in processing phosphorites and apatites; the drinking water is not fluoridated. Ocassionally, in this locality, the norm in Poland calculated for 24 hours for fluoride content in air namely 0.01 mg F/m^3 was exceeded. Group II came from a locality where the drinking water was artifically fluoridated at 0.64 ppm. Groups III, IV and V resided in localities with 400,000, 80,000 and 10,000 inhabitants respectively where water was not fluoridated nor were there any large industrial plants. Altogether the nails of 284 children, 138 boys and 146 girls, were studied.

The fluoride in nails was determined by gas chromatography (2) with minor modification. Nails (10-50 mg) were dissolved in 200 µL NaOH containing 670 g $NaOH/dm^3$, in polypropylene test-tubes and heated in boiling water bath. After cooling, 650 µL of concentrated HCl were added slowly to the solution. It was cooled again and 500 µL of reagent were added. The basic composition of the reagent was 1 cm^3 benzene + 0.6 mg trimethylchlorosilane,

* Direct correspondence to Z. Machoy, Pomeranian Medical Academy, Department of Biochemistry, Szczecin, Poland.

2-5 µg isopentane (amount of latter employed depended on the content of fluoride in the nails). The mixture was shaken on a microshaker for about 20 minutes, and subsequently centrifuged at 40 g/5 min. to obtain separation of the benzene layer from the aqueous layer. Two and a half µL of fluid were collected from the benzene layer by means of a Hamilton syringe and injected to determine fluoride by gas chromatography against the standard solution (3).

Results

The investigations were intended to evaluate: 1] the gas chromatography method for determining fluoride in the nails; 2] the level of fluoride in children living in different, variable conditions of the natural environment.

Table 1 shows the results of fluoride determination in nails taken from 1 individual, aged 60 years, to establish repeatability of the method. Table 2 reports the fluoride analyses for nails of fingers and toes obtained from a single subject. Table 3 presents mean value for determinations of fluoride in nails of children in the 5 groups described in the previous section.

Discussion

The results presented in Tables 1 and 2 were meant to evaluate the usefulness of the gas chromatography method in determining fluoride in nails. The exactness of weighing the nails was up to 0.01 mg with 10 to 50 mg samples; therefore, we consider any weight error to be relatively small. To determine the error due to measuring the volume of the solution sample with

Table 1

Repeatability in Determinations of Fluoride in Nails from a Single Subject with the Gas Chromatography Method

Sample No.	Level of Fluoride (ppm) Analysis No. 1	2	3	Mean value of three analyses
1	1.53	1.45	1.49	1.49
2	1.43	1.37	1.37	1.39
3	1.64	1.84	1.72	1.73
4	1.21	1.17	1.22	1.20
5	1.14	1.14	1.17	1.15
6	1.44	1.49	1.37	1.43
7	1.59	1.71	1.62	1.64
8	1.53	1.35	1.63	1.50
9	1.88	1.72	1.57	1.72
10	1.20	1.17	1.19	1.18

Mean value: 1.45; Standard Deviation: 0.216

Table 2

Level of Fluoride in Nails of One Subject Determined Separately for Fingers and Toes

Sample No.	Date	Level of Fluoride (ppm) Fingers	Toes
1	07/13/1985	2.44	
2	07/18/1985		3.38
3	08/25/1985	3.65	3.91
4	09/15/1985	3.76	3.65
5	10/06/1985	4.37	2.31
6	10/27/1985	3.81	4.15
7	11/23/1985	4.15	3.55
8	12/15/1985	3.95	4.26
9	01/05/1986	3.67	3.74
10	01/26/1986	6.53	3.12
11	02/20/1986	2.80	3.31
12	03/14/1986	3.35	2.87
13	04/03/1986	3.02	3.63
14	04/22/1986	3.15	4.24
15	05/10/1986	2.50	3.96

Fingers: Mean ±S.D. 3.65 ±1.02; total mass 1.24853
Toes: Mean ±S.D. 3.58 ±0.55; total mass 2.17672

the Hamilton syringe, employed in the gas chromatography method, the nails from fingers and toes of a subject were dissolved in NaOH solution. Next, a maximum number of analyses were carried out as permitted by the available material (Table 1). The magnitude of the error made is indicated by the standard deviation. We regard the results satisfactory. Table 2 contains the values established by determination of fluoride in nails of fingers and toes of the same individual with analyses performed over some months. The subjects lived in an area where water was fluoridated to 0.64 ppm F^-. After the nails had been collected they were analyzed for their fluoride content. The determination of fluoride in these samples over a number of days is inclined to provide greater dispersion of the results than that depicted in Table 2. The standard deviation for fluoride in toe nails is smaller than that in finger nails.

The second part of the paper deals with the effect of environmental changes on the level of fluoride in nails of children (Table 3). The highest level of fluoride in nails was found in Group I residing in the environs of a large industrial plant processing phosphorites and apatites. Likewise, fluoride was elevated in nails of children from the locality where water is fluoridated. Standard deviations in both groups were highest. Only in Group II, compared

Table 3

Level of Fluoride in Nails of School Children, Aged 10-12 Years

Locality	No. of Children	Mean ±S.D.	"t"	Degrees of Freedom	Significance
I	53	12.2 ±8.7			
II	66	8.6 ±5.1	2.66	83	$p < 0.01$
III	55	2.5 ±1.5	8.00	57	$p < 0.001$
IV	60	6.4 ±2.6	4.67	63	$p < 0.001$
V	50	7.6 ±2.8	3.65	66	$p < 0.001$

Group I differed statistically compared with remaining groups.
The differences are highly significant by Student's "t" test.

with Group I, was the significance $p < 0.01$. Upon comparing groups III, IV and V the level of fluoride in nails was highest in localities with a low number of inhabitants (10,000) and lowest in the residential district of a large town (400,000). One of the causes may be attributed to the difference in living standard of people inhabiting small and large towns in Poland. The flats in large towns are heated centrally through installations connected with the municipal boiler-houses, while natural gas is used mainly for cooking purposes. On the other hand, in small towns great amounts of coal are burned in every household. Coal in Poland contains, besides other impurities, considerable quantities of fluorine compounds which escape into the atmosphere. More exact interpretation of results is also hindered by the fact that all school children are required once a year, for the purpose of fluoride prophylaxis, to brush their teeth 5 times with Elmex-gel preparation at intervals of 10 days.

It should also be emphasized that fluorine metabolism in nails of humans, in age groups other than those studied, may take a different course (4,5). In our opinion, the numerical strength of groups consisting of about 50 children, is rather low for drawing more precise conclusions on changes involving the level of fluoride in nails.

Conclusion

Nonetheless, on the basis of performed studies it may be concluded that environmental changes do influence the level of fluoride in children's nails.

Acknowledgements

Gratitude is expressed to Technical Assistants A. Durda, S. Wolski and A. Machoy for help in determining fluoride by the gas chromatography method and for preparing samples.

References

1. Elsair, J., Merad, R., Denine, R., et al.: Fluoride Content of Urine, Blood, Nails and Hair in Endemic Skeletal Fluorosis. **Fluoride**, 15:43-47, 1982.
2. Fresen, J.A., Cox, F.H. and Witter, M.J.: The Determination of Fluoride in Biological Materials by Means of Gas Chromatography. **Pharm. Weekblad**, 103:909-914, 1968.
3. Durda, A., Machoy, Z., Siwka, W. and Samujło, D.: Evaluation of Methods of Preparing Exploratory Materials for Fluoride Determination. [Polish] **Bromat. Chem. Toksykol.**, 19:209-213, 1986.
4. Machoy, A. and Szczešniak, W.: Accumulation of Fluorine in the Nails. [Polish] **Czas. Stomat.**, 39:705-709, 1986.
5. Niewiarowska-Pawlus, A., Durda, A., Noceń, I., et al.: Comparative Estimation of Fluorine Content in the Nails of Police Inhabitants. **Folia Medica Cracoviensia**, XXVIII:83-87, 1987.

****

DETERMINATION OF TISSUE FLUORIDE IN RATS FOLLOWING ADMINISTRATION OF AN ORGANIC COMPOUND (DIFLUNISAL)

by

Misako Tomita, Takako Sugimura, Mie Takokoro and Yoshihiro Kaneko*
Tokoyo, Japan

SUMMARY: The purpose of this study: to find a suitable method for determination of total and ionic fluoride (F) in rats exposed to an organic F compound.

Male rats were given an organofluorine compound Diflunisal by gastric intubation. Fluoride was determined by the fluoride ion electrode method and gas chromatography following pretreatment with low temperature ashing, pyrohydrolysis and microdiffusion for plasma and soft tissues, and direct extraction and microdiffusion for hard tissues. Pyrohydrolysis was found useful for determination of total F in biological samples containing organofluorine compounds, whereas microdiffusion using a strong acid was unsatisfactory. Doses of the water-insoluble Diflunisal had little effect on the ionic F level in either soft or hard tissues of the rat.

KEY WORDS: Animal experiment; Fluoride analysis; Hard and soft tissues; Organofluorine compound.

Introduction

For determination of fluoride in a biological sample, it has become increasingly important in recent years to include not only the ionic fluoride but also the total fluoride level. However, determination of total F content in a biological sample is more difficult and less reliable than that of ionic F (1). Reports on the fate of fluoride in biological systems exposed to organofluorine compounds are limited. This study was initiated in an attempt to find a suitable method for determination of total and ionic F in animal tissue in an animal exposed to an organofluorine compound (Diflunisal).

Materials and Methods

Animal Experiment: Nineteen 8-week-old male Wistar rats randomly divided into four groups: the control (n = 5), F1 (n = 5), F2-a (n = 4), and F2-b (n = 5) received fluoride (as Diflunisal) at a dose of 0, 0.5, and 1.52 mg per kg body weight per day, respectively (Table 1). Diflunisal is one of the organofluorine compounds sold as an anti-inflammatory and analgesic drug under the trade name of Dolobid (2). Treatment solutions were made by suspending pulverized Difunisal in a 0.5% carboxymethyl cellulose (sodium salt) solution. The suspension was administered by gastric intubation and the experiments were carried out for 14 days. All rats except those in F2-b group were sacrificed 24 hours following the last Difunisal administration; those in F2-b group

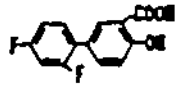
* Department of Hygiene and Oral Health, School of Dentistry, Showa University, 1-5-8, Hatanodai, Shinagawa-Ku, Tokyo 142, Japan.

were sacrificed three hours after the final dosage (Table 1). The organs and tissues were removed for analysis.

Table 1

Animal Weight and Dose Level

Group	N	Animal weight (g)				Dose (DF gastric intubation)		
		Pretest		Gain		D F	F	
		$\bar{x}$	±SD	$\bar{x}$	±SD	Daily (mg/kg/day)	Daily (mg/kg/day)	Total (mg/times)
Cont	5	340	19	71	11	–	–	–
F1	5	336	20	70	17	3.3	0.5	15.7/13
F2-ⓐ	4	343	9	63	12	10	1.52	47.5/13
F2-ⓑ	5	336	15	41	7	10	1.52	49.1/14

DF : Diflunisal  m.p. 200-214℃

Fluoride Analysis: The procedures employed for fluoride analysis by low temperature plasma ashing (LTA), the fluoride ion selective electrode (IE) method, the microdiffusion (Dif) method, and gas chromatography (GC), have been reported previously (3,4). In this study, three methods for analysis of F in plasma and soft tissues were employed. The IE method was used for ionic F; a pyrohydrolytic pretreatment (PyH) (4) and LTA method were used for total F analysis. For analysis of ionic F in soft tissues alone, the Dif method was also used for comparison. The PyH method consisted of prehydrolytic F separation in a quartz tube at a high temperature (94° C) with a supply of oxygen and steam. Fluoride in the sample was separated as HF condensed with water vapor. The sample was placed on a sample boat and preheated for 1 min, followed by heating in a mainheater for 7 min. A 12 mL condensate, collected in a polypropylene tube, was then analyzed for F by GC.

For hard tissues, only ionic F was determined. For this purpose, the tissues were first dried and ground to a fine powder. The powdered sample was then subjected to direct extraction (DE) and the Dif method. In the DE method, a portion of the sample was dissolved in 25% HCl. After standing for 2 hr, the acidic extract was diluted ten times with distilled water. The resultant solution and the sample prepared by the Dif method were analyzed for F by GC.

Results and Discussion

Figure 1 shows the mean values of F in rat plasma samples determined

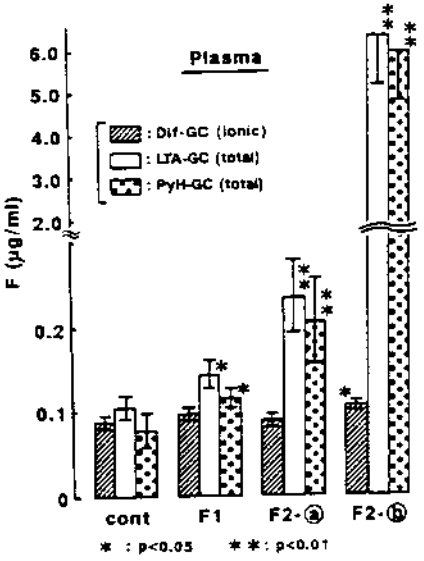
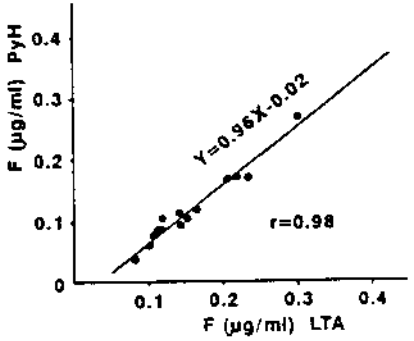


Figure 1

F Level (Ionic and Total) in Plasma

Figure 2

Correlation of F in Plasma Determined by LTA and PyH



by the three methods. Total F levels of the high dose groups were significantly higher than those of the controls; only the F2-b group showed a significant difference in ionic F from the control group. Fluoride concentrations in the plasma determined by both LTA and PyH methods were compared with each other the results of which are shown in Figure 2. The values obtained from both methods were closely correlated with each other, although the LTA method generally gave higher values than the PyH method.

Figure 3

F Level (Ionic and Total) in Soft Tissues

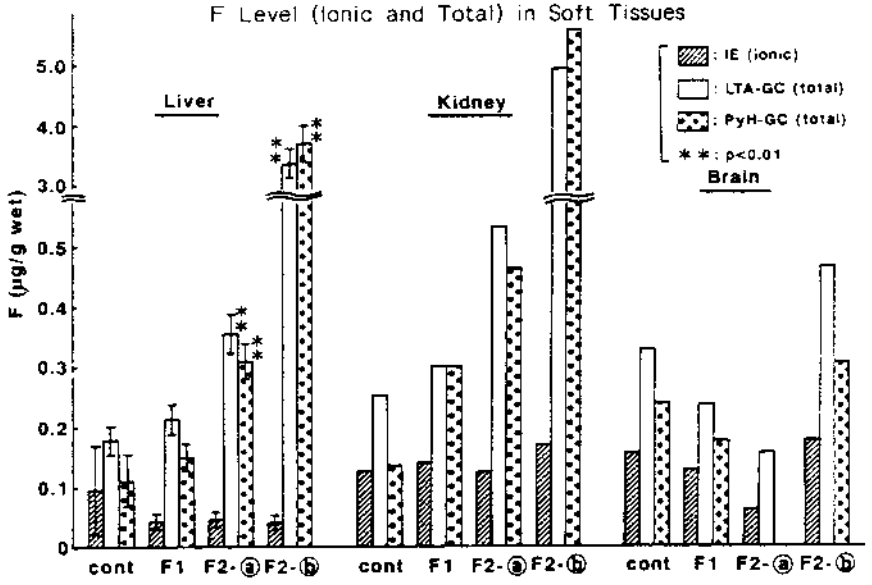
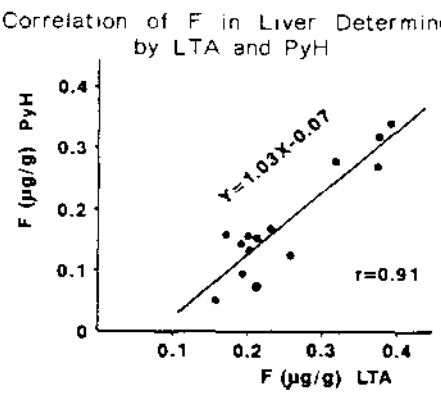


Figure 4



Fluoride concentrations in soft tissues including the liver, kidney, and brain determined by the three methods are shown in Figure 3. Tissue ionic F concentrations between the control and F-treated groups did not differ significantly. Average total F in the tissues, however, from high F dose groups were markedly higher than those of the control. The relationship between the total F levels in the liver determined by LTA method and those by the PyH method is shown in Figure 4. Correlation between values obtained from both methods was good.

The total F level in the brain was about one-tenth of that in other tissues of the high F dose groups (Figure 3). Concerning F distribution in tissue, Armstrong and Singer (6), and Whitford et al. (7) reported in their radiofluoride studies that most of the soft tissues were kinetically homogeneous with plasma fluoride; this however was not true for brain. Furthermore, they supported the concept of the existence of blood-brain barrier, restricting F movement. In this study, we used an organofluorine compound which differs from ionic F compounds with regard to F distribution and metabolism. Nevertheless, our results support the concept of the blood-brain barrier.

The average ionic F levels in the incisor and femur, determined by the two methods, are shown in Figure 5. The ionic F levels in hard tissue of F-treated groups were about the same as those of controls which suggests that administration of Diflunisal, which is extremely insoluble in water, had little effect on the ionic F level of soft or hard tissues of the rat. However,

Figure 5

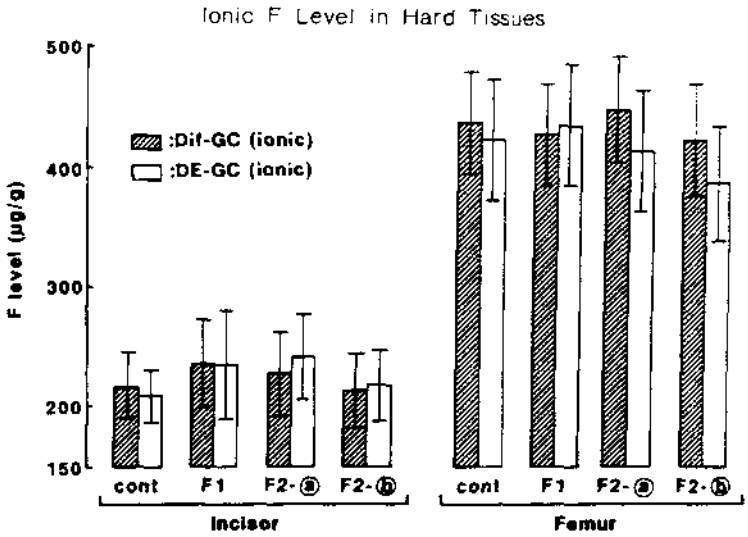
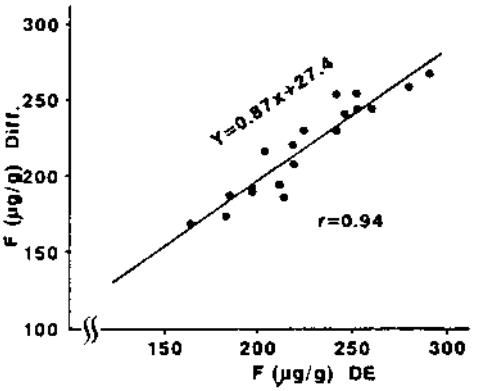


Figure 6
Correlation of F in Incisor Determined by DE and Diffusion



in our previous studies with a water-soluble organofluorine compound, we observed an increase in ionic F level in the plasma (8). The F concentration in the tooth determined by the two methods was similar (Figure 6) which suggests that, because it is simple and less time-comsuming, the DE method may be preferable to other methods for determination of fluoride in hard tissues.

Conclusion

1. The PyH method was useful for determination of total F in biological samples containing organofluorine compounds. The Dif method however was unsatisfactory, even though it involves use of a strong acid.

2. The DE method was rapid and useful for determination of ionic F in hard tissue.

3. Average total F levels in plasma and soft tissue of the high F dose groups were markedly higher than those of the control group.

4. Administration of Difunisal had little effect on the ionic F level of either soft or hard tissues of the rat.

Acknowledgement

This work is supported by a Grant-in-Aid for Scientific Research (No. 63570968) for 1988-1989 from the Ministry of Education, Science and Culture of the Government of Japan.

References

1. Singer, L. and Ophaug, R.: Ionic and Nonionic Fluoride in Plasma (or Serum). Clin. Reviews in **Clin. Lab. Sci.**, 18:111-140, 1982.
2. Seki, T. and Miyazaki, M.: Effects of a New Anti-Inflammatory, Analgesic and Antipyretic Drug MK-647 (Difunisal) on Healthy Japanese Volunteers. **Japan. J. Clin. Pharmacol. Ther.**, 9:169-177, 1978.
3. Bessho, Y., Tomita, M. and Kaneko, Y.: Determination of total Fluorine Levels in Blood Sera. In: Tsunoda, H. and Yu, M.-H., Eds.: **Fluoride Research 1985.** Elsevier Science Publishers, Amsterdam, 1986, pp. 73-80.
4. Bessho, Y., Tomita, M. and Kaneko, Y.: Fluoride Analysis of Milk in Japan. **Fluoride**, 21:30-35, 1987.
5. Itai, K. and Tsunoda, H.: Determination of Submicrogram Quantities of Fluoride by a Rapid and Highly Sensitive Method. In: Tsunoda, H. and Y, M.-H., Eds.: **Fluoride Research 1985.** Elsevier Science Publishers, Amsterdam, 1986, pp. 25-29.
6. Armstrong, W.D. and Singer, L.: Fluoride Tissue Distribution: Intracellular Fluoride Concentrations. **Proc. Soc. Exp. Biol. Med.**, 164:500-506, 1980.
7. Whitford, G.M., Pashley, D.H. and Reynolds, K.E.: Fluoride Tissue Distribution: Short-term Kinetics. **Am. J. Physiol.**, 236:141-148, 1979.
8. Tadokoro, M., Tomita, M. and Kaneko, Y.: Fluorine Analysis in Serum in Pyrohydrolysis-Gas Chromatography. **Japan. J. Hyg.**, 43:419, 1988.

****

THE INFLUENCE OF BIOMASS INCREASE, RAIN AND WIND ON THE CONCENTRATION OF AIRBORNE FLUORIDES IN PERENNIAL RYE GRASS

by

Ludwig O. deTemmerman* and H. Baeten
Tervuren, Belgium

SUMMARY: Perennial rye grass (Lolium perenne) grown in containers equipped with a semi-automatic watering system, was exposed to ambient fluorides for four weeks. The fluorides emitted by the pollution source, a phosphate fertilizer plant, were mainly in gaseous form.

After exposure the containers were transported to a low ambient-fluoride level area. In some containers the evolution of the fluoride concentration in the grass was controlled at several time intervals. An exponential decrease in the fluoride concentration as a function of the time was found.

The effect of rain was studied by using a rain simulator and the impact of wind by using a fan. The results clearly show the major importance of the studied climatic parameters in the fluoride release process.

Washing the samples prior to analysis, indicated that this procedure always removes a part of the accumulated airborne fluorides, even after exposure to large amounts of artificial rain.

KEY WORDS: Airborne fluorides; Lolium perenne; Fluoride losses; Rain effect; Wind effect.

Introduction

Grass is a well-known accumulator of airborne fluorides (1). Especially gaseous fluorides, such as HF, are absorbed and accumulated by the leaves to a large extent. After exposure, the fluoride concentration in grass, however, decreases rapidly as shown by Hitchcock et al. (2) and McCune (3).

In this study, grass was grown in containers and exposed during four weeks in a polluted area. A part of the grass was harvested immediately after exposure. The other containers were transported to a low-level fluoride area, and harvested at three time intervals in order to determine the evolution of the fluoride content. Each time, the biomass per unit surface as well as the dry weight was determined for the detection of an eventual growth dilution effect.

In a second series of experiments, the grass was treated with artificial rain and wind after exposure in the polluted area in order to determine the effect of climatic parameters on the fluoride losses of grass.

* Institute for Chemical Reseach, Ministry of Agriculture, Museumlaan 5, B-1980 Tervuren, Belgium.

The polluted area is situated at approximately 500 m from a phosphate fertilizer plant. The yearly average ambient fluoride concentrations ranged from 0.85 up to 1.44 $\mu g/m^3$ with daily averages up to more than 10 $\mu g/m^3$. In the low level area, the average fluoride concentrations in ambient air are lower than 0.05 $\mu g/m^3$. More than 90 percent of the fluorides present in ambient air are in gaseous form as measured with a denuder system (1).

Materials and Methods

Perennial rye grass, Lolium perenne cv Melino RVP was grown in containers under standardized conditions; the containers used were 48 x 30 cm and 20 cm deep. A water reservoir was available underground and the water supply was provided by two filter candles located in the soil substrate (peat soil) (4).

Four rows of four containers were placed in a 4 x 4 latin square configuration to be exposed to ambient fluorides. A series of four containers were harvested before transporting the others to the low level area. Once in this area, the remaining three groups of four containers were harvested at increasing time intervals.

The experiments with artificial rain were carried out with a rain simulator (5). Several grass containers were exposed to rain (demineralized water) for different durations immediately after exposure to fluoride pollution. The diameter of the rain droplets was approximately 6 mm. Mist was produced with a pesticide spraying device and a fan with an oscillating head was used to simulate wind effect.

Fluoride Analysis: The grass samples were weighed and dried to determine biomass and dry weight per container. One gram of dried and ground grass was extracted with 0.1 N nitric acid and the fluoride content was measured by using an ion specific electrode after addition of a buffer solution (6). All grass samples were divided into two subsamples, one being subjected to a washing experiment with a mixture of 0.05% NaEDTA and 0.05% detergent (7).

Results and Discussion

Fluoride Losses Under Ambient Conditions: Five experiments were carried out at different periods of the growing season. As shown in Figure 1, there is a rapid decrease in the fluoride concentration in grass after exposure. As the decrease is exponential as a function of time, it can be described by the equation:

$$y = ae^{bx}.$$

The calculated functions as well as the correlation coefficients are given in Table 1 for unwashed as well as washed samples.

The higher the value of "b" in Table 1, the quicker the fluoride concentration decreases in the grass. The coefficients obtained in the conducted experiments ranged from 0.027 to 0.045. The average is 0.035, which is closely related to the coefficient obtained by McCune (3) and by Hitchcock et al. (2) after fumigation experiments: $e^{0.032x}$.

Figure 1

Decrease in the Fluoride Concentration in Grass After Exposure

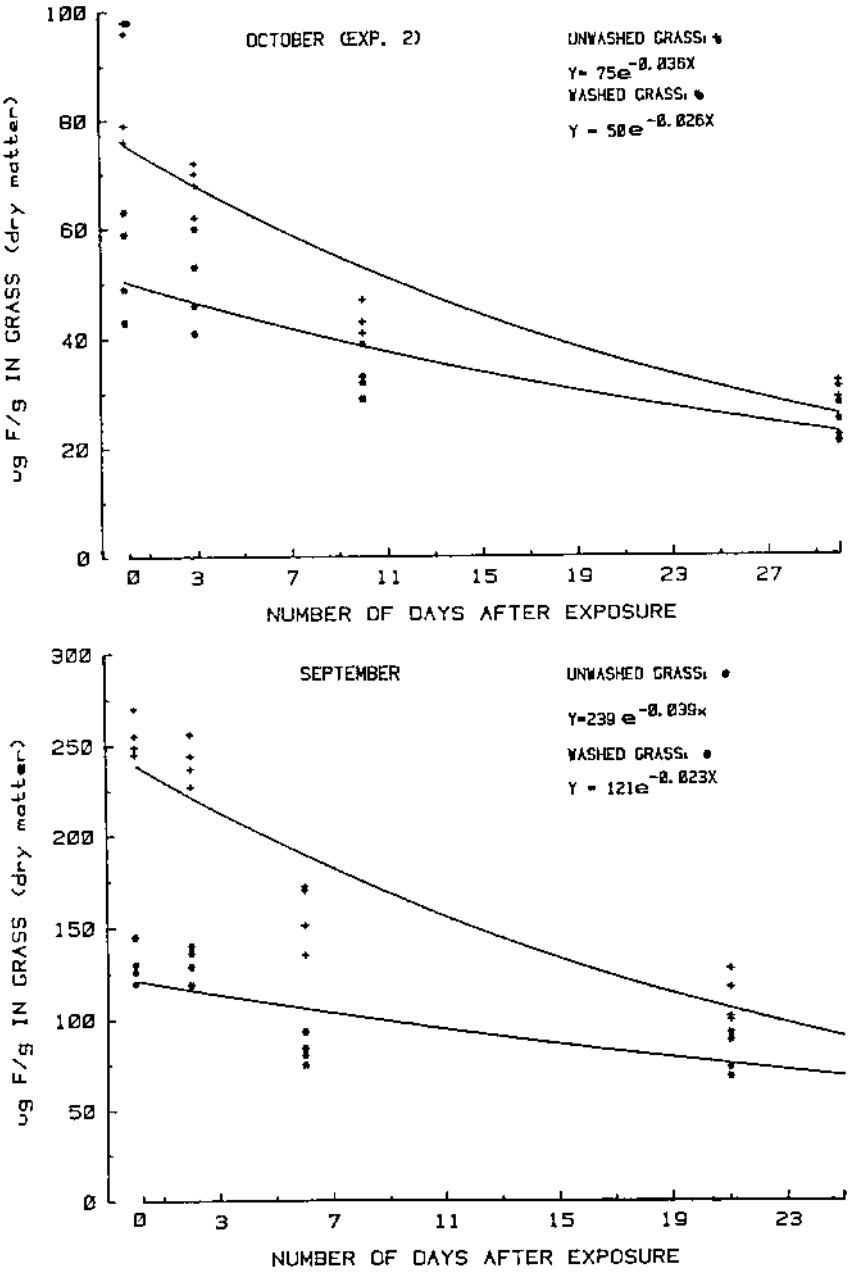


Table 1

Decrease of the Fluoride Concentration in Grass After Exposure to Ambient Fluorides

Experiment	Unwashed Samples $y = ae^{-bx}$	r^2	Washed Samples $y = ae^{-bx}$	r^2
July	$y = 56e^{-0.027x}$	0.56	$y = 32e^{-0.027x}$	0.50
August	$y = 174e^{-0.045x}$	0.80		
September	$y = 239e^{-0.039x}$	0.85	$y = 121e^{-0.032x}$	0.56
October 1	$y = 87e^{-0.036x}$	0.70	$y = 57e^{-0.021x}$	0.45
October 2	$y = 75e^{-0.036x}$	0.86	$y = 50e^{-0.026x}$	0.79

x: number of days after exposure
y: concentration in grass at time x.
r: correlation coefficient

From Figure 1 it is clear that a part of the fluoride present is removable by washing the grass. The curve of washed grass is, however, less steep.

Different facts can explain the observed exponential decrease, such as re-emission of fluorides by grass, removal of fluorides by rain, and also growth dilution.

Growth dilution is caused by an increase of biomass and, consequently, a decrease of the fluoride concentration because there is not further accumulation of fluorides in the low level area. Growth dilution can be determined by calculating the fluoride mass per unit of soil surface.

The evolution of the concentration and the fluoride mass as a function of time is presented in Figure 2. The percentage of growth dilution as a part of the total decrease of the fluoride concentration in grass is calculated in Table 2 and ranges from 0 to 79%, depending on the growth rate of the grass and on climatic parameters. The average is 23% during the growing season. The growth rate in the summer months was rather high during the exposure in the polluted area, but slowed down drastically in the low-level area. This caused a relatively low increase in biomass (as percent of total biomass) and consequently a low-growth dilution.

The Effect of Rain and Wind on Fluoride Losses by Grass Cultures: Four experiments were carried out to determine the effect of rain on the fluoride losses of grass. The results are summarized in Table 3 and presented in Figure 3. Heavy rain can cause more than 50% fluoride loss in grass. This must be attributed to a washing effect because growth dilution does not play any role in such a short-time experiment.

The second experiment shows an increasing fluoride loss as a function of increasing amounts of rain. The remaining part of the fluoride in grass

Figure 2

Fluoride Losses of Grass After Exposure

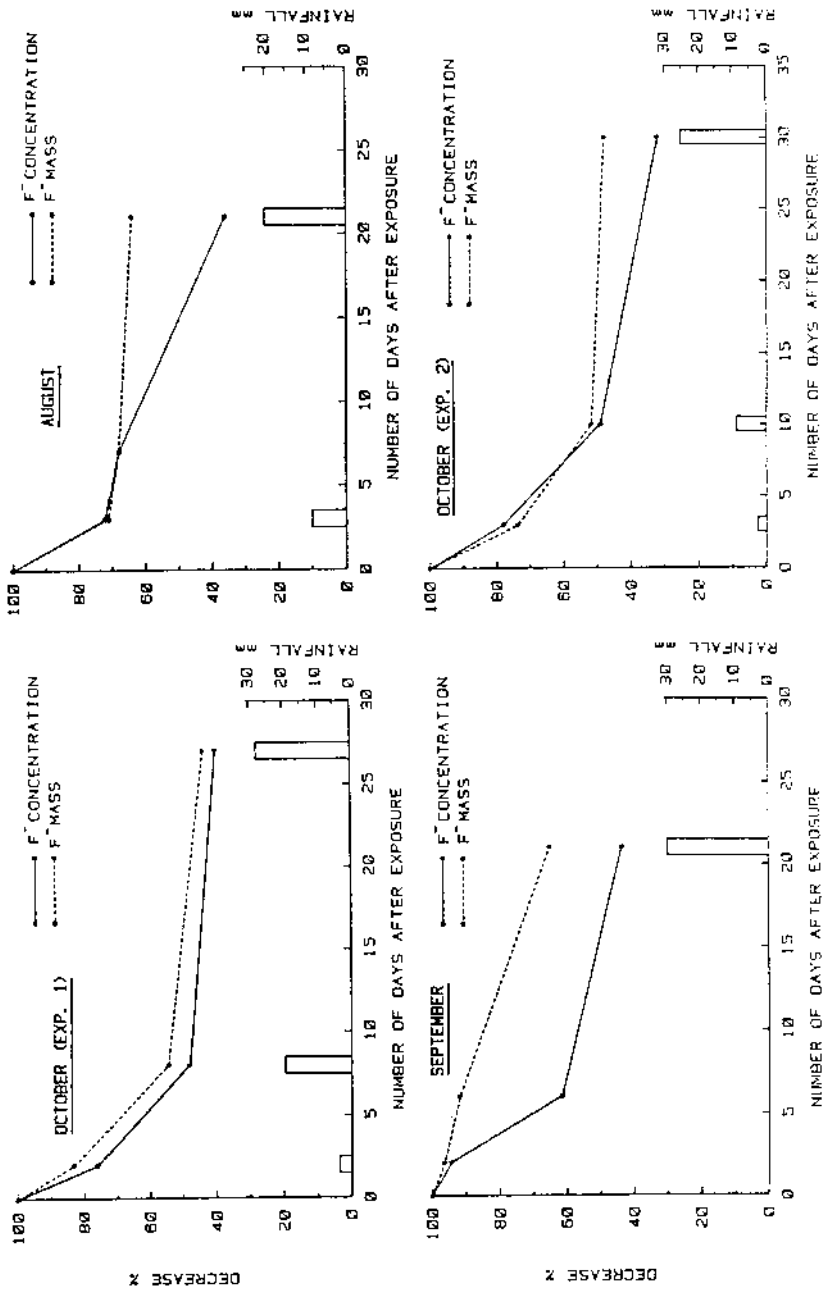


Table 2

Estimation of Growth Dilution

Experiment	T.I.	%				
		D.W.*	ΔC*	ΔM*	D.E.	rel. D.E.
July	0	100 a	0 a	0 a	–	–
	2	107 a	-21 b	-17 a	4	19
	6	106 a	-26 b	-21 a	5	20
	14	118 a	-36 a	-25 a	11	31
August	0	100 a	0 a	0 a	–	–
	3	101 a	-28 b	-29 a	–	–
	7	96 a	-32 b	-32 a	–	–
	21	122 b	-64 c	-36 a	28	44
September	0	100 a	0 a	0 a	–	–
	2	93 a	-6 a	-4 a	2	36
	6	150 b	-39 b	-8 a	31	79
	21	150 b	-57 b	-35 b	22	38
October 1	0	100 a	0 a	0 a	–	–
	2	111 a	-24 a	-17 ab	7	29
	8	113 a	-52 b	-46 bc	6	12
	27	107 a	-60 b	-56 c	4	6
October 2	0	100 a	0 a	0 a	–	–
	3	97 a	-22 b	-27 ab	–	–
	10	106 a	-51 c	-48 b	3	5
	30	151 b	-68 d	-52 b	16	23

* Data followed by the same character are now significantly different at the 5% level according to Duncan's Multiple Range Test.

T.I.: time interval, number of days after exposure;
D.W.: Dry weight of grass per container;
ΔC: decrease of the fluoride concentration;
ΔM: decrease of the fluoride mass;
D.E.: dilution effect ΔM-ΔC;
rel. D.E.: dilution effect relative to the total decrease of the fluoride concentration in grass.

is rather constant and less dependent on the rain treatment. Abundant rain apparently removes the part of the accumulated fluoride in grass which can be eliminated by washing. This indicates that part of the fluoride accumulated is readily exchangeable and removable on a short term. Misting the plants also removes a part of the fluoride present in and on the grass more or less equivalent to the percentage of fluoride removed by the same amount of rain.

There must also be an emission of fluorides by the grass as shown in the third experiment. By keeping some containers in a greenhouse, the grass already lost half of the fluoride content potentially removable by rain. Consequently, a lesser percentage could be removed by artificial rain.

Figure 3

The Influence of Rain and Mist on the Fluoride Content of Grass

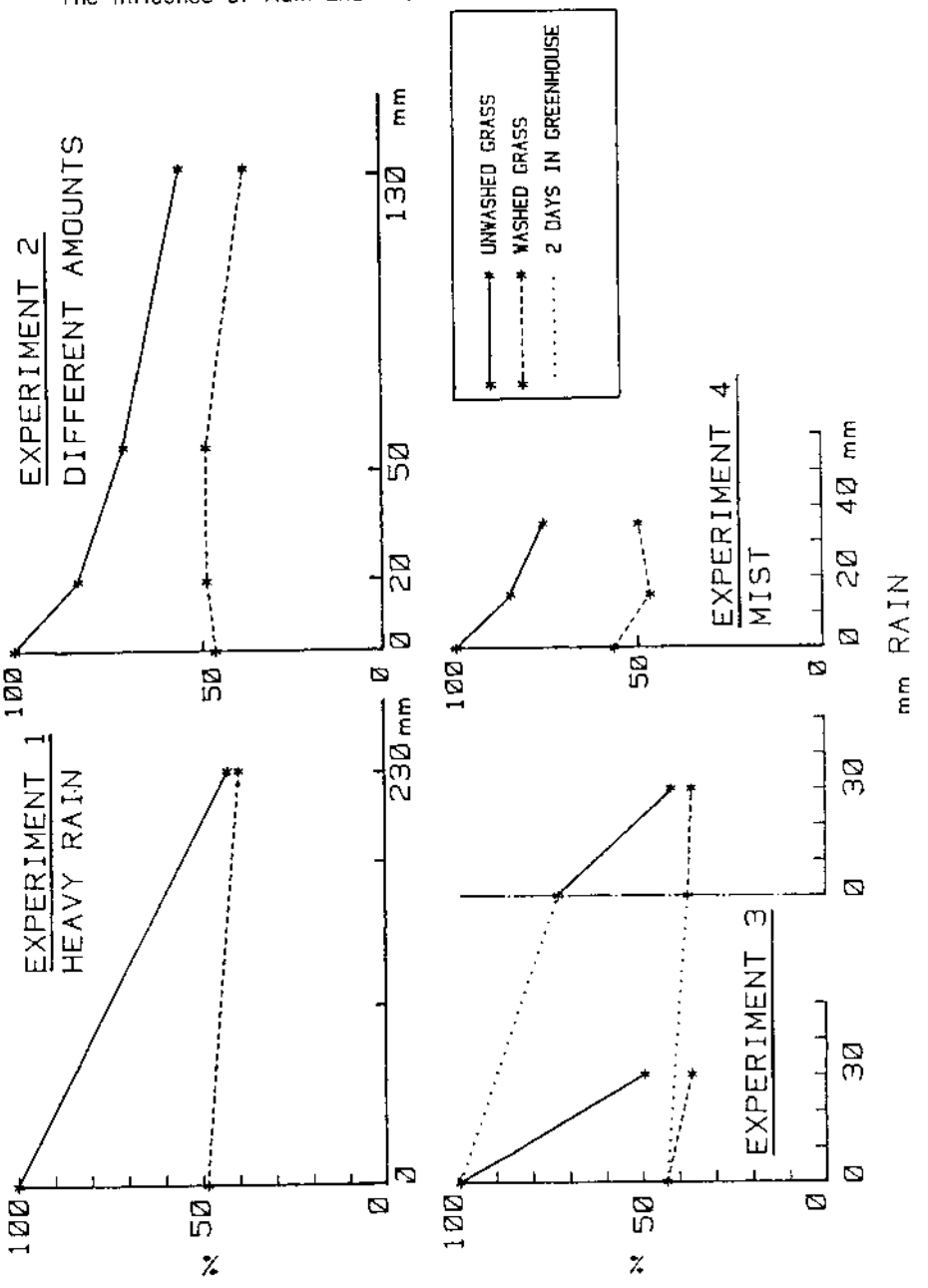


Table 3

The Influence of Rain and Mist on the Release of Fluoride by Grass ($\mu g\ F^-/g$ dry matter)

	Treatment	Unwashed*	Washed*
Experiment 1 (Heavy Rain)	No Rain	238 a	116 a
	230 mm rain/3 h, 30 min.	104 b	95 b
Experiment 2 (Intervals)	No Rain	46 a	21 a
	20 mm Rain/20 min.	38 b	22 a
	60 mm Rain/1 hr.	32 b	22 a
	140 mm Rain/3 hr.	25 c	17 b
Experiment 3 (Repeated)	No Rain	85 a	37 a
	30 mm Rain/45 min.	42 b	31 b
	After 2 Days (No Rain)	62 c	32 b
	27 mm Rain/45 min.	35 d	31 b
Experiment 4 (Mist)	No Mist	108 a	62 a
	12 mm/20 min.	92 b	51 a
	35 mm/60 min.	82 b	54 a

* Data followed by the same character are not significantly different at the 5% level according to Duncan's Multiple Range Test.

Release of fluorides to the gas phase could be demonstrated by blowing over the grass with a fan. A significant amount of fluorides could be removed by blowing during 19 hours with a wind speed oscillating between 0 and 4 m/s (Table 4).

Conclusion

The fluoride content of grass decreases as a function of time after exposure to ambient fluorides. In periods with rapid grass growth, the effect

Table 4

The Influence of Wind on the Release of Fluorides by Grass ($\mu g\ F^-/g$ dry matter)

Treatment	Unwashed*	Washed*
No Wind	108 a	62 a
0-4 m/s/2 Os cycle during 19 hours	76 b	51 a

• Data followed by the same character are not significantly different at the 5% level according to Duncan's Multiple Range Test.

of growth dilution can be very important, amounting to about 79% of the total decrease of the fluoride concentration. The decrease is highest immediately after exposure and slows down after some days. The process can be described as an exponential function. Rain and wind are two major factors influencing fluoride losses by grass.

It can be concluded from the experiments that up to more than 50% of the airborne fluorides accumulated by grass can be removed by wind and rain. This is equivalent to the amount of fluorides removed by washing the grass thoroughly. The results of the washing experiments give an idea of the readily removable part of the accumulated fluoride. The remaining part is more or less fixed in the grass and is relatively constant over a period of some hours or even days. Each treatment, however, decreases the remaining part of the fluorides in the grass.

References

1. DeTemmerman, L.O. and Baeten, H.: The Accumulation of Airborne Fluorides by Perennial Rye-Grass Cultures. **Fluoride**, 21:185-192, 1987.
2. Hitchcock, A.E., McCune, D.C., Weinstein, L.H. MacLean, D.C., Jacobson, J.S. and Mandl, R.H.: Effects of Hydrogen Fluoride Fumigation on Alfalfa and Orchard Grass: A Summary of Experiments from 1952 through 1965. **Contrib. Boyce Thompson Inst.**, 24:363-386, 1971.
3. McCune, D.C.: Problems Involved in Devising Air Quality Criteria for the Effects of Fluorides on Vegetation. **Am. Ind. Hygiene Assoc. J.**, 32:697-701, 1971.
4. DeTemmerman, L.O., Baeten, H. and Raekelboom, E.L.: Étude biologique de la pollution atmosphérique en fluorures dans une zone industrielle. **Revue de l'Agriculture**, 39:85-97, 1986.
5. Gabriels, D., DeBoodt, M. and Minjauw, W.: Description of a Rainfall Simulator for Soil Erosion Studies. **Med. Fac. Landbouwwet. Gent**, 38:294-303, 1973.
6. Roost, F. and Sigg, A.: Erfahrungen mit einer potentiometrischen Fluorbestimmungsmethode für biologische Materialen. **Staub-Reinhalt. Luft**, 38:363-366, 1978.
7. National Academy of Sciences: **Fluorides**. Washington, D.C., 1971, p. 295.

****

BIOAVAILABILITY IN SOIL FLUORIDE IN SHEEP

by

Guy Milhaud, Martine Clauw* and Brigitte Joseph-Enriquez
Maisons-Alfort Cédex, France

SUMMARY: Digestive absorption of soil fluoride was determined by balance technique, during 4 days on groups of 4 ewes which for 4 weeks had been given concentrated feed containing 30 percent earth.

Seven soils of various origins were used: 4 were collected in the vicinity of different aluminum plants, 3 in an area far distant from any source of fluoride. Total fluoride levels ranged between 490 and 1000 ppm/dry in the soils collected near the plants and between 235 and 1030 ppm/dry in the non-polluted soils. The levels of extractable fluoride (water, $CaCl_2$, amberlite) were significantly higher in the polluted soils.

Digestive absorption of soil fluoride ranges between 5 and 25 percent, with 4 values close to 20 percent. It is positively correlated (0.59) to soil total fluoride but independent of soil extractable fluoride. In some cases, to assess the hazards of fluorosis in ruminants, it is necessary to differentiate between soil fluoride and vegetable fluoride.

KEY WORDS: Bioavailability; Fluoride; Sheep; Soil.

Introduction

Fluoride is a major air pollutant in industrial areas although control systems have been installed in many plants. Soils always contain considerable amounts of natural fluoride (200 to 3,500 ppm) to which air-borne fluoride fallout is added. Even if the amount of soil fluoride absorbed by plants is small, the ration for ruminants always contains a certain amount of earth.

Therefore, it is necessary to determine the bioavailability of soil fluoride to assess accurately the hazards linked to the fluoride in the ration and to withdraw over-polluted feed from consumption, if necessary. The data obtained through the determination of the digestive absorption of fluoride from various soils are presented in this study: soil fluoride bioavailability proves to be low but varies with the nature of soils.

Materials and Methods

Digestive absorption of soil fluoride was determined by balance studies over a 4-day period in animals which had been fed a concentrated feed containing 30 percent earth for 4 weeks.

Soil Selection: The experiment was carried out with 7 soils, different in origin

* Laboratoire de Pharmacie et Toxicologie, École Nationale Vétérinaire d'Alfort, 94704 Maisons-Alfort Cédex, France.

and nature, collected either in the vicinity of aluminum plants (Table 1, soils 1, 2, 3, 4) or far from any source of fluoride pollution (Table 1, soils 5, 6, 7). A single 100 kg sample of each type of soil was collected, 30 cm deep, which was incorporated into the feed.

Animals: Every balance study was carried out with four "Prealpes" 2- to 5-year-old ewes, which came from an INRA Experimental Station. The animals selected for the experiment presented no detectable clinical disability. The experiment extended over a 3-year period, from 1985 to 1988.

Animal Husbandry Before the Balance Study: The animals were given concentrated feed, forage and water ad libitum. Two types of concentrated feeds were prepared: regular feed and "with-earth" feed. The two types of feed were prepared with barley, oats and dehydrated alfalfa from the same lot. The regular feed contained 37.5, 25 and 37.5 percent of the different ingredients, respectively. The "with-earth" feed was prepared by incorporating 30 percent p/p of dried and pulverized earth in the previous feed. From the onset, each ewe received 1 kg concentrated feed per day; the "with-earth" feed was started four weeks before the balance study.

Carrying Out the Balance Study: The balance study was carried out from Monday morning (9 o'clock) to Friday morning (9 o'clock). At 9 o'clock the ewes were given 500 g of "with-earth" feed, 200 or 300 g of forage, 1.8 g of water, then 500 g of "with-earth" feed in the evening. Feed rejections were collected and measured the next morning, previous to new feed intake. From Tuesday to Friday, every morning, feces were collected and weighed; urine was also collected and its volume was measured.

The "with-earth" feed was given until euthanasia was performed a few days or a few weeks after the end of the balance studies (T61R: Distrivet).

Sample Collecting: An earth sample was collected before its incorporation into the concentrated feed. The regular feed samples were collected on opening the bags for feeding. "With-earth" feed, forage and drinking water were sampled every day during the four-day experiment. After weighing the total amount of feces collected, a sample corresponding to 1/10th of the initial weight was collected every day so as to obtain an average sample representative of the four days of the study.

Fluoride Determination: All fluoride determinations were carried out with a fluoride specific ion electrode (Cambridge, USA), after dilution in an ionic buffer containing CDTA (TISAB, Orion, Cambridge, Mass.).

Total fluoride from the earth, the feed and the feces was determined in the liquid obtained by a technique in which the simple distillation described by Willard and Winter was preceded by a distillation in phosphoric acid at 290 °C (2). Each sample was analyzed three times to obtain an average value.

In the earth, besides total fluoride, the fluoride extractable by the methods described by Supharungsun and Wainwright (3) was determined (distilled water, 0.01M $CaCl_2$ amberlite IRA-40).

Calculation of Bioavailability of Soil Fluoride: The following equation was

used to calculate the bioavailability P (percentage of digestive absorption of soil fluoride)

$$P = \frac{S - C}{S} \times 100$$

where:

S = amount of soil fluoride ingested (fluoride from the "with-earth" concentrated feed minus fluoride in the regular concentrated feed).

C = amount of soil fluoride in feces (total amount of feces fluoride minus fluoride from feed ingredients other than soil, whose bioavailability was evaluated at 75 percent).

Statistical Tests: All results were submitted to various analysis, then compared by the Newman-Keuls technique. Correlations between certain parameters were investigated.

Results

Soil Fluoride: Total soil fluoride ranged between 490 and 1000 mg/kg in the soils collected in polluted areas and between 235 and 1030 mg/kg in the non-polluted soils (Table 1). The fluoride extractable by H_2O or 0.01M $CaCl_2$ is usually below 1%, with particularly low concentrations in non-polluted soils (less than 0.25% of total fluoride). The fluoride extracted by amberlite is significantly higher.

Table 1

Nature of the Soils and Fluoride Levels

Number of Balance Studies	Nature of the Soils*	Total Fluoride mg/kg/dry	Extractable Fluoride mg/kg/dry		
			H_2O	$CaCl_2$	Amberlite
1	Clayey, Fine Alluvium	490	2.45 (0.50%)	1.20 (0.24%)	12.80 (2.60%)
2	Alluvium	535	1.45 (0.27%)	0.80 (0.15%)	21.50 (4.01%)
3	Fine Alluvium	1000	15.90 (1.59%)	10.65 1.00%)	50.10 5.01%)
4	Fine Alluvium	730	6.00 (0.82%)	4.05 (0.55%)	21.05 (2.88%)
5	Fine Alluvium	710	0.40 (0.06%)	0.35 (0.05%)	3.50 (0.50%)
6	Fine Alluvium	1030	0.70 (0.07%)	0.25 (0.03%)	1.95 (0.20%)
7	Sandy Alluvium	235	0.60 (0.25%)	0.15 (0.06%)	6.75 (2.87%)

• According to the textural triangle suggested by D. Hillel (1).
( ) Percentage with respect to total fluoride.

Table 2

Fluoride Concentrations in Feed and Drinking Water

Balance Study	Regular Feed mg/kg/dry	Feed "With Earth" mg/kg/dry	Forage mg/kg/dry	Water mg/L
1	5.5	137.5	2.5	0.20
2	2.1	216.5	8.0	0.25
3	9.5	312.5	1.5	0.20
4	5.0	244.5	1.0	0.30
5	8.5	236.5	13.0	0.15
6	28.2	321.5	1.0	0.25
7	ND	70.0	2.0	0.25

ND: No Determination

Ingested Fluoride: Table 2 shows the mean values of all analyses carried out on each type of feed used in the course of each experiment. The amounts of fluoride ingested by each animal during the 4-day experiment were calculated by multiplying these values by the amounts of the different feeds ingested by each ewe.

Excreted Fluoride and Soil Fluoride Bioavailability: The fluoride excreted in the feces (data in Table 3) indicate the amounts of feces collected for each ewe during the four days of the experiment and their fluoride concentration. Soil fluoride bioavailability (P) range from 3.4% (Earth 7) to 22.9% (Earth 3) (Table 3, Figure 1).

Figure 1

Soil Fluoride Bioavailability

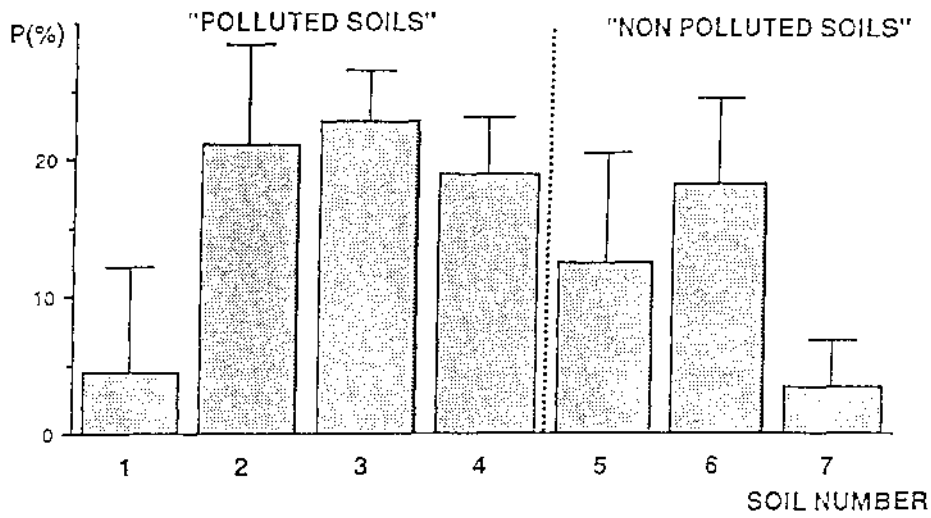


Table 3

Fluoride Excretion in Feces and Soil Bioavailability (P)

Balance Study	Ewes	Age (years)	Feces		P	Mean = x S.D. = σ
			weight (kg)	mg F/kg/dry		
1	2211	3	2.145	202.0	6.7%	x = 4.5% σ = 7.7%
	605	3	1.550	233.5	-6.9%	
	1471	2	2.240	194.0	8.8%	
	84781	5	1.720	200.0	9.3%	
2	2460	3.5	2.690	258.5	21.0%	x = 21.2% σ = 7.3%
	3809	3	1.945	283.5	23.2%	
	3815	3	2.355	270.0	29.1%	
	3351	3	1.390	299.0	11.5%	
3	2303	2	1.800	439.5	19.6%	x = 22.9% σ = 3.7%
	2315	2	2.175	435.5	25.6%	
	2467	2	2.055	425.5	26.5%	
	2848	2	2.230	489.0	19.8%	
4	5307	2	2.145	353.5	21.7%	x = 18.9% σ = 4.2%
	5743	2	2.020	368.0	23.3%	
	5804	2	2.300	356.5	15.3%	
	5810	2	2.180	376.5	15.3%	
5	4023	2	2.250	345.5	2.0%	x = 12.5% σ = 8.0%
	4086	2	2.415	307.0	20.7%	
	4327	2	2.280	364.0	11.1%	
	4347	2	2.420	323.0	16.3%	
6	4096	3	2.380	371.5	26.3%	x = 18.2% σ = 6.4%
	4480	3	2.365	427.0	18.0%	
	5319	3.5	2.370	426.5	17.9%	
	5481	2	2.200	499.0	10.7%	
7	6317	2	2.535	101.5	1.1%*	x = 3.4%* σ = 3.5%*
	6343	2	2.030	106.0	6.1%*	
	6557	1.5	2.365	110.0	-0.2%*	
	6485	1.5	2.310	105.0	6.7%*	

* Values calculated on the assumption that regular feed contained 10 ppm F/dry.

Discussion

The percentages which are presented (Table 3) must be strictly considered as apparent coefficients of absorption. Yet, as fluoride is not subject to any important digestive re-elimination (Wöhlbier et al. (4)), the value obtained is very close to the fraction really absorbed, referred to as digestive bioavailability. The assessment of mineral elements retention, through balance studies is, according to Duncan (5), always superior to the real retentions measured by corporal analysis. In our experiment, the error in the determination of ingested and excreted fluoride can be estimated at ±5 percent, which can result in an error of ±50 percent for a digestive absorption close to 20 percent.

Statistical analysis shows that two groups of soils can be distinguished ($p < 0.05$): four soils with a percentage of absorption near 20 percent (soils 2, 3, 4, 6); two soils near 5 percent (soils 1, 7).

Cross-checking of a certain number of elements reveals the consistencey of the results. In Experiment 3 (St-Jean-de-Maurienne) and 6 (Le Collet), the amount of ingested fluoride (95 to 97 percent as earth) ranges from 5 to 6 mg/kg/day. Blood fluoride levels are low — about 0.25 ppm — whereas they reach 0.5 ppm (Milhaud et al. (6)) when 3.5 mg F/kg/day, as sodium fluoride, is administered. A 20 percent bioavailability is likely.

There is a very significant link ($p < 0.01$) between the total fluoride concentration of intake and bioavailability of soil fluoride. The analysis of the other parameters does not permit correlations between the values of P and the soil pH, its clay content, its water, calcium chloride or amberlite-extractable fluoride.

The nature of the soil is thus proved to influence digestive bioavailability as shown in the very close results obtained with two soils of similar geological nature (schist and red limestone), which are macroscopically alike and have very close total fluoride content and in which the amounts of extractable fluoride are different (soils 3 and 6).

Our results are lower than those obtained by balance studies in other species: Wöhlbier et al. (4) investigated the fluoride bioavailability of a sandy silt containing 718 ppm total fluoride — 2.3% of which was fluoride soluble in water at 20° — collected in the vicinity of an aluminum plant, in three cattle over a period of 316 days: the percentage of absorbed fluoride ranged from 30 to 41 percent, with an average value of 38 percent.

Clay and Suttie (7) studied the apparent fluoride bioavailability of various phosphates and of NaF in the goat: the figures are also markedly higher than our own, with 75% for NaF, 65% for natural phosphate, 33% for defluorided phosphate, 38% for dicalcic phosphate.

Measurement of bone retention is another experimental approach which can be exploited to assess the bioavailability of ingested fluoride; it was carried out in cattle (Oelschlager et al. (8)), in sheep (Clay and Suttie (7)), and its results agree with balance studies.

The bioavailability of feed fluoride can also be assessed by measuring the area under the curve of variations of blood fluoride levels in relation to time after a single administration. By this technique, conducted on 7 ewes Joseph-Enriquez (9) obtained a fluoride bioavailability of 60.8% with sodium fluoride and of 5.9% with Soil 1.

It is clear that, to assess the hazards of fluorosis, one must distinguish fluoride from the soil, where bioavailability is low (25 percent maximum in sheep) and fluoride from forage, whose bioavailability is comparable to sodium fluoride, i.e. 75% (Shupe et al. (10)).

According to Zach and Mayoh (11), grazing cattle and sheep can consume up to 14 percent of dry matter ingested as earth, particularly in poor quality pasture.

It should also be noted that leaf and beetroot neck ensilage usually contains from 10 to 25 percent earth. Yet, dairy cows which are given concentrated feed, consume less than 1 percent/MS earth.

Acknowledgments

This experiment was made possible thanks to financing by INRA.

References

1. Hillel, D.: **L'eau et le sol. Principes et processus physiques.** (Translated from English). Vander Editeur, Louvain, Belgium, 1974, 288 pages, p. 19.
2. Willard, H.H. and Winter, O.B.: Volumetric Methods for Determination of Fluorine. **Ind. Eng. Chem. Anal.**, 5:7, 1933.
3. Supharungsun, S. and Wainwright, M.: Determination, Distribution and Absorption of *Fluoride in Atmospheric Polluted Soils.* **Bull. Environ. Contam. Toxicol.**, 28:632-636, 1982.
4. Wöhlbier, W., Oelschlager, W., Gronbach, G. and Giessler, H.: **Die Resorption von Fluor durch Ochsen aus Erde und Flugstaub einer Aluminiumhütte.** Forschungsber. 14 der DFG „Fluor-Wirkung", Fr. Steiner, Wiesbaden, 1968, p. 114.
5. Duncan, Dorothy L.: The Interpretation of Studies of Calcium and Phosphorus Balance in Ruminants. **Nutr. Abstr. Rev.**, 28:695-715, 1958.
6. Milhaud, G., Borba, M.A. and Krishnaswamy, S.: Effect of Fluoride Ingestion on Dental Fluorosis in Sheep. **Am. J. Vet. Res.**, 48:873-879, 1987.
7. Clay, A. and Suttie, J.W.: The Availability of Fluoride from NaF and Phosphorus Supplements. **Vet Hum. Toxicol.**, 27:3-6, 1985.
8. Oelschlager, W., Löffler, K. and Opletalova, L: Retention von Fluor in Knochen und Knochenabschnitten von Ochsen bei gleich hohen Fluorzulagen in Form von Erde, Flugstaub einer Aluminiumhütte und Natriumfluorid. **Landwirtsch. Forsch.**, 23:214-226, 1970.
9. Joseph-Enriquez, Brigitte: **Pharmacocinétique du fluor chez la Brebis. Application à la détermination de la biodisponibilité du fluor d'échantillons de terre.** Thèse de doctorat (Université Paris VII), 1987, 191 pages.
10. Shupe, J.L., Miner, M.L., Harris, L.E. and Greenwood, D.A.: Relative Effects of Feeding Hay Atmospherically Contaminated by Fluoride Residue, Normal Hay plus Calcium Fluoride and Normal Hay plus Sodium Fluoride to Dairy Heifers. **Am. J. Vet. Res.**, 23:777-787, 1962.
11. Zach, R. and Mayoh, K.R.: Soil Ingestion by Cattle: A Neglected Pathway. **Health Phys.**, 46:426-431, 1984.

****

FLUORIDE AND ASH CONTENT OF BONE IN VARIOUS STAGES OF HUMAN FLUOROSIS

by

J. Franke*
Erfurt, GDR

SUMMARY: A special method was reported earlier to confirm the diagnosis "fluorosis" in doubtful cases in early stages, or in sporadically occuring fluorosis: During iliac crest biopsy a second bone cylinder is removed, the ash content as well as the fluorine content in this cylinder is determined. From 1969 to 1986 44 cases in various stages of industrial or neighborhood-fluorosis were studied. In 8 normal cases the ash content was 42.6 ±8.5 percent, the F-content in dry bone was 0.42 ±0.09 percent fluoride in iliac crest ash. With increasing F-content ash content increased significantly. In radiological stages II and III, according to Roholm, the ash content was more than 57 percent.

In a case of stage II fluorosis, the fluoride content in the bone ash was 0.78 percent. According to a second iliac crest biopsy, 13 years after removal from the fluoride-exposed areas of employment, fluoride content had dimminished to 0.45 percent.

KEY WORDS: Bone ash content; Bone biopsy; Bone fluoride content; Human fluoride.

Introduction

In 1972 (1) we developed a special method of confirming the diagnosis of "fluorosis" in doubtful cases, in early stages or in sporadically occurring fluoroses of varying origin. Two bone cylinders were removed by iliac crest biopsy for histological investigation and fluoride analyses. At that time only 10 patients had been studied by this method. Now the series includes 44 human fluorosis cases.

Material and Methods

Between 1969 and 1986 40 cases of industrial fluorosis and 4 cases of neighborhood fluorosis of various stages were studied. Eight individuals of the same age without fluoride exposure served as controls (Table 1). The industrial fluorosis cases were due to F-exposure from air fluoride concentrations in the plant of more than 3 mg F/m^3 in the fifties and early sixties, subsequently 2-3 mg F/m^3, and most recently were slightly above 1 mg F/m^3.

The average exposure time ranged between 15.7 and 18.7 years, with a minimum of 6 and maximum of 30 years.

Moreover, 2 patients without roentgenologic findings and 4 cases of

* Department of Orthopedic Surgery, Medical Academy Erfurt, DDR-5010 Erfurt, GDR.

Table 1

X-ray Findings, Average Age, and Duration of F^--Exposure of 44 Cases of Fluorosis and 8 Normal Individuals

X-ray Stages	n	Age (years)	Duration of F-Exposure (years)
Normal	8	53.5 ±9.8	–
Subtle Signs	7	43.3 ±11.8	15.7 ±5.9
0-I	8	49.0 ±12.9	16.3 ±5.9
I	10	50.3 ±9.5	16.9 ±2.5
II	9	53.9 ±10.8	18.7 ±6.5
III	4	51.3 ±8.1	18.3 ±4.1
	[38]		
Fluoride Contact	2	46.5 ±2.5	15
Old Fluoroses	4	59.8 ±5.4	9.0 ±4.3 (without F^-)
	[44]		

fluorosis of long standing (Stage II, II-III, two times subtle signs) were examined.

The conditions of 4 cases of neighborhood fluorosis from a small Saxon town near an HF-factory were described by Schmidt and Franke (2) and Schmidt (3).

Radiological findings were graded according to Roholm (4) in Stages I to III; to Fritz (5) who added two prestages: subtle signs and stage 0-I. Two bone cylinders were removed by iliac crest biopsy the first served for histological examinations, the findings of this were reported earlier (1,6-9).

The second bone cylinder was dried at a temperature of 105° C with the addition of magnesium acetate to bind volatile F ions up to weight constancy (dry weight) and ashed for 2 hours at 450° C until the ash was white and porous (ash weight). Fluoride was determined according to a modified Megregian (10) method (determination of fluorine by vapor distillation with perchloric acid and formation of a complex of circoneriochromecyanine, with subsequent spectrophotometry), 2-3 analyses were performed per sample. The fluorine content could be related to the individual stages of fluorosis.

Results

Normal F-values were 0.042 percent in dry bone or 0.09 percent in bone ash. With increasing radiological changes bone F content increased. The first distinct histological and radiological changes (subtle signs) were observed in bone ash which contained approximately 0.42 percent (4,200 ppm) fluoride (Table 2).

In the most severe cases (Stage III) F-values were around 1.0 percent in bone ash. Figure 1 shows the ash content of dried bone in relation to

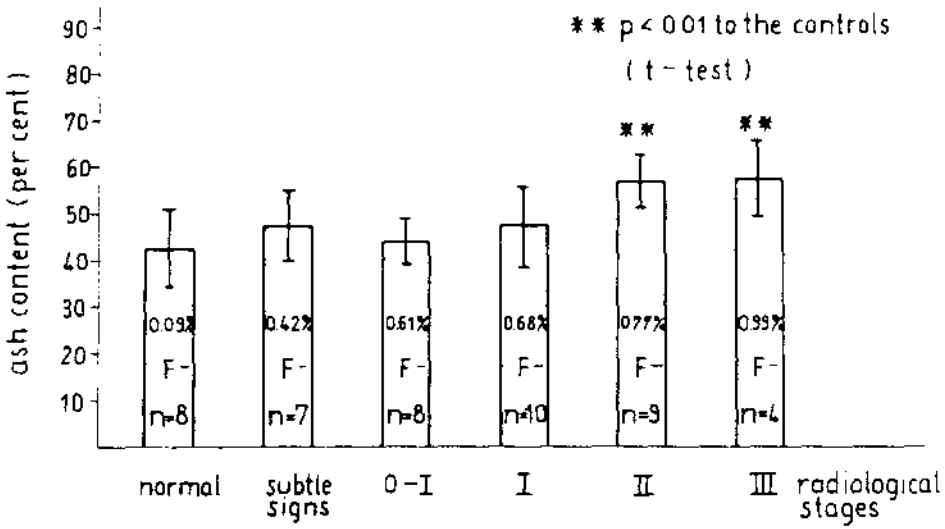
Table 2

Correlation of Radiological Findings of Fluorosis to the Fluoride Content of Dry and Ashed Iliac Crest Bone.

X-ray Stages	n	F-Content in dry bone (%)	F-Content in bone ash (%)
Normal	8	0.042 ±0.015	0.09 ±0.02
Subtle Signs	7	0.20 ±0.03	0.42 ±0.09
0-I	8	0.27 ±0.08	0.61 ±0.13
I	10	0.33 ±0.09	0.68 ±0.08
II	9	0.44 ±0.05	0.77 ±0.05
III	4	0.57 ±0.11	0.99 ±0.11

Figure 1

Relations Between the Ash Content of the Dry Bone and the X-ray Stages, Respectively the Fluoride Content of Bone Ash.

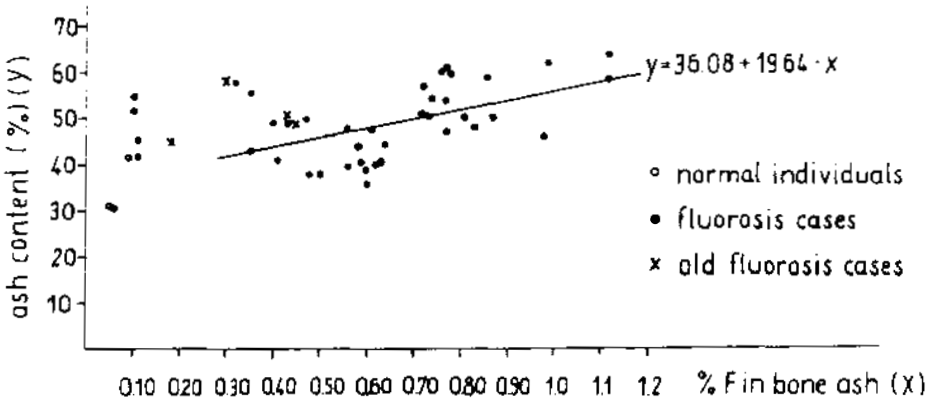


the F-content and roentgenological stage. As F-content and roentgenological changes increases ash content increased up to 35 percent, significantly in stages II and III.

The ash content of all 42 fluorosis cases was 50.18 percent, about 18 percent above that of control bones (42.8 percent) ($p < 0.05$). As seen in Figure 2 the two measured values were directly correlated. The dependence was secured statistically by the coefficient of correlation ($R = 0.4597$, therefore: $R > 0$ with $p < 0.01$) (11).

Figure 2

Relations Between The Ash Content and Fluoride Content in the Ash of Iliac Crest Bone.



Figures 3 and 4 show that F-exposure time and F-content in bone ash are only slightly correlated. The same applies to ash content of iliac crest bones.

In as case of State II fluorosis (Figures 5 and 6), the fluoride content in bone ash was 0.78 percent. 13 years after removal from the fluoride-

Figure 3

Correlation Between F-Content in the Bone Ash and Duration of F-Exposure.

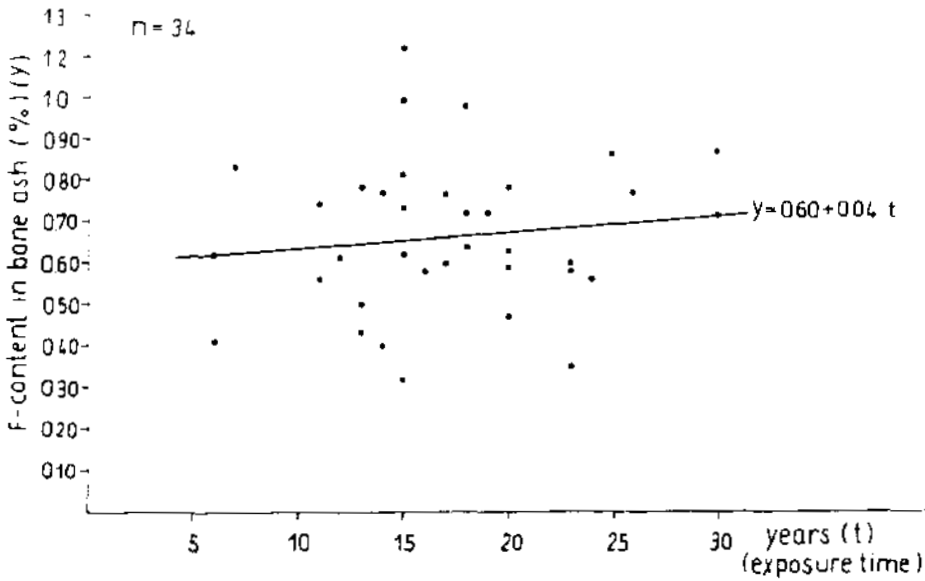
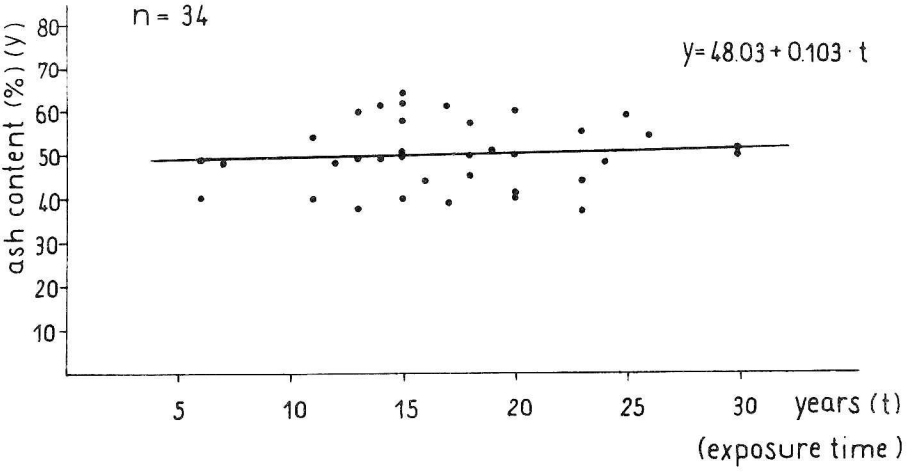


Figure 4

Relations Between Ash Content of Bone and Duration of F-Exposure

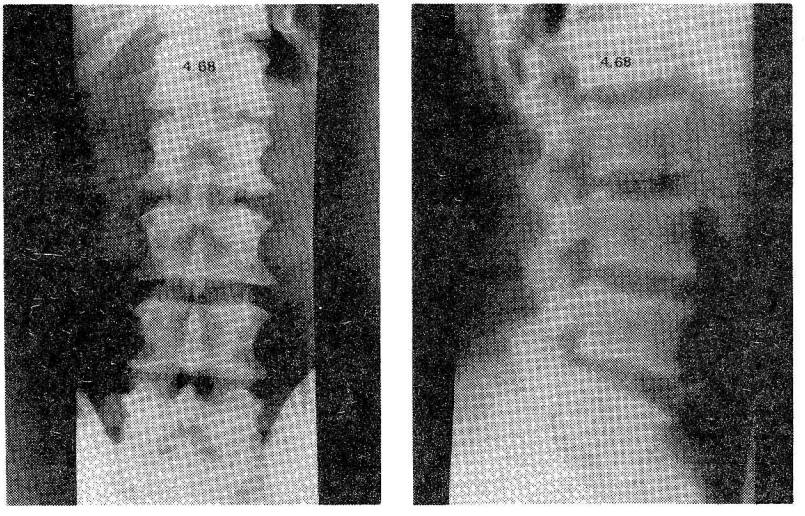


contaminated place of employment; the X-ray indicates a diminution of osteosclerosis (Figures 7 and 8).

In the second iliac crest bone cylinder, ash fluorine content was diminished to 0.45 percent.

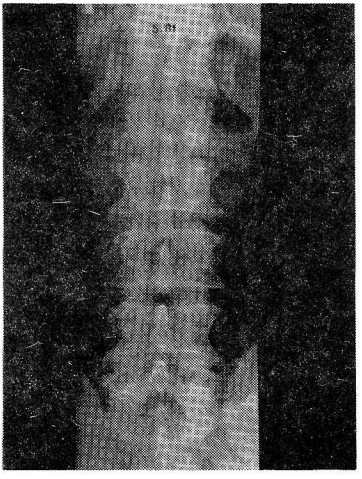
Figures 5 and 6

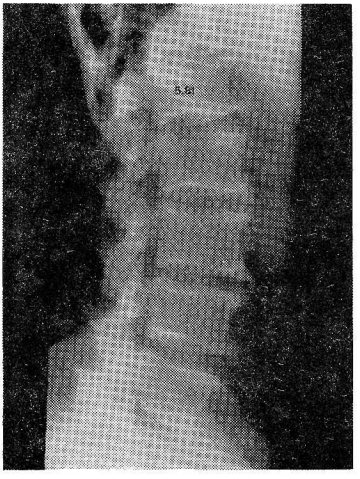
Lumbar Spine (1986). Radiological Stage III Fluorosis



Figures 7 and 8

Same Patient 13 years after retirement. Osteosclerosis is deminishing but spondolosis is increasing.





Discussion

As previously has been stated (1,6,8) the first distinct radiological and histological changes in cases of human fluorosis were found in bone ash F-content of 0.42 ±0.09 percent. Much the same limits were recorded by Sankaram and Gadekar (12) in human endemic fluorosis, and by Shupe (13) in cattle. Jackson and Weidmann (14) found higher values (0.6 percent) whereas Freitag et al. (15) reported lower ones (0.23 percent) in humans.

Boillat et al. (16) noted initial changes in the microradiogram in 43 cases of industrial fluorosis of which F-content in iliac crest bone was 2,000 ppm, which corresponds approximately to our 4,200 ppm in bone ash. For diagnosis of fluorosis, however, values must be 4,000 ppm F^- with histological investigation or 2,000-4,000 ppm F^- in bone, with changes of the osteocyte lacunae (mottled periosteocystic lacunae) in the microradiogram.

High fluoride values up to 0.9 percent in iliac crest, rib, or lumbar vertebral bodies ash of persons living in areas where drinking water contains 4-8 ppm, without histological and radiological changes, have been reported by McClure et al. (17), Zipkin et al. (18), McCann (19) and Posner et al. (20) from the U.S.A.

As early as 1937 Roholm (4) found, in autopsies of fluorosis cases, F-values in bone ash of pelvic bones of 0.92 percent (Stage II) or, 1.31 percent (Stage III) in the lumbar vertebra. In the meantime many other reports have appeared on the F-content of single or several cases of endemic or industrial fluorosis.

Our analyses are comparable with those by Schlegel (21), who studied

Table 3

Relations Between the Degree of Radiographic Changes, Years of Exposure and Bone Fluoride Concentration (Comparison: Schlegel [1974] and Franke [1986])

X-ray Stages	Number of Cases		Average Years of Exposure		Percent F in Bone Ash	
	Franke	Schlegel	Franke	Schlegel	Franke	Schlegel
Normal	8	–	–	–	0.09	–
Fritz Pre-stages: Subtle Signs	7	–	15.7	–	0.42	–
0-I	8	7	16.3	22	0.61	0.69
Roholm: I	10	23	16.9	29	0.68	0.52
II	9	27	18.7	29	0.77	0.75
III	4	5	18.3	40.5	0.99	0.84

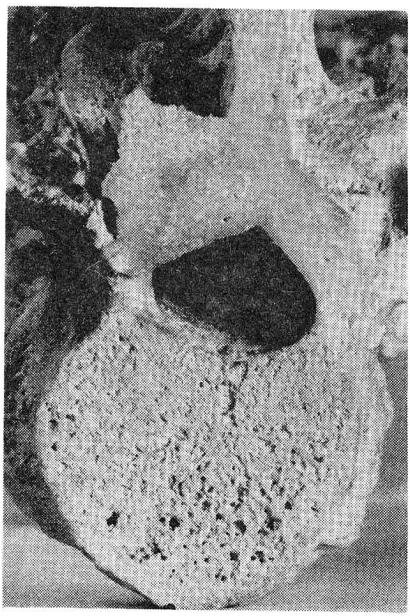


Figure 9

Cross Section of Lumbar Vertebrae of Stage III Fluorosis Case.

61 cases of industrial fluorosis orginating in Switzerland. Table 5 indicates similar observations. Due to the low F-concentrations at the place of employment (2-4 mg F^-/m^3) exposure time in Schlegel's (21) cases was 70 percent longer for the same roentgenological stages to develop.

Boillat et al. (22) found a good correlation between F-content in iliac crest and ossification of ligaments, tendons and muscles (hyperostosis), but no correlation to bone tissue density.

The increase in ash content is not surprising considering the spongiosa density of the vertebra in severe fluorosis (Figure 9).

In 43 cases of industrial fluorosis Band et al. (23) found a significant increase in trabecular volume in the iliac crest (19 ±5.5 percent compared to 15.9 ±3.7 percent of control bones), associated with hypermineralization.

Our bone mineral analyses of distal radius by means of ^{125}J-photon-absorptiometry according to Cameron and Sorensen (24) also showed increased mineral content and bone width depending on the fluoride concentration at the work place (8).

F-content and ash content was not related to exposure time. Average exposure time between beginning roentgenological changes and stage III was only 3 years. Since the fluoride concentration at the place of employment and working conditions were similar for most of the workers, strong individual differences pointed out again and again in our other studies, are of pivotal important (6-9,25).

Obviously a group of highly flouride sensitive individuals, are found in the Roholm's stages II and III.

Also Bang et al. (23) found no correlation between F-content and exposure time in the Swiss fluorosis cases.

The fluoride content in iliac crest ash at a stage II fluorosis decreased by only one half during the 13 years following termination of F-influence. According to Bang et al. (23) the half-life period is 20 years. Since the entire skeleton is rebuilt in a period of 25 years, this phenomenon can be explained by the fact that the fluoride released by bone resorption is partly incorporated into new osteones again and again.

References

1. Franke, J. and Auerman, E.: Die Bedeutung der Beckenkammpunktion mit histologischer und mikroanalytischer Untersuchung des gewommenen Knochenmaterials bei der Diagnostik der Fluorose. **Int. Arch. Arbeitsmed.**, 29:85-94, 1972.
2. Schmidt, Chr. W. and Franke, J.: Kasuistische Darstellung eines Falles von sog. Nachbarschaftsfluorose. **Z. ges. Hyg.**, 22:611-615, 1976.
3. Schmidt, Chr. W.: Auftreten von Nachbarschaftsfluorose unter der Bevölkerung einer sächsischen Kleinstadt. **Dt. Gesundh.-Wesen**, 31:1271-1274, 1976.
4. Roholm, K.: **Fluorine Intoxication.** Lewis, London, 1937.
5. Fritz, H.: **Röntgenpathologische und pathologische-anatomische Betrachtungen zum Fluoroseproblem.** Med. Hab.-Schr., Dresden, 1958.
6. Franke, J., et al.: Industrial Fluorosis. **Fluoride**, 8:61-83, 1975.
7. Franke, J.: Wirkungen von Fluoriden auf das Skelettsystem. Med. Diss. (B), Halle, 1976.
8. Franke, J., Runge, H. and Fengler, F.: Endemic and Industrial Fluorosis. In: Courvoisier, B., Donath, A. and Baud, C.A., (Eds): **Fluoride and Bone.** Edit. Médecine et Hygiène, Geneva, 1978, pp. 129-143.
9. Franke, J.: Ossifications and Calcifications of Muscle and Tendon Insertion in Human Industrial Fluorosis. In: Havelka, S. and Trnavsky, K. (Eds.): **Bone-Cartilage-Joint.** Avicenum, Prague, 1985, pp. 201-120.
10. Megregian, S.: Critical Factors in Fluoride Distillation Techniques. **J. Amer. Water Works Ass.**, 45:1110-1116, 1953.
11. Afifi, A. and Azen, S.P.: **Statistical Analysis – A Computer Oriented Approach.** Academic Press, New York and London, 1972.
12. Sankaram, B. and Gadekar, N.G.: Skeletal Fluorosis. In: Blackwood, H.S.S. (Ed.): **Bone and Tooth. Proceedings of the First European Symposium,**

Oxford, 1963. Pergamon Press, Oxford, London, New York, Paris, 1964, 1964, pp. 357-362.
13. Shupe, J.L.: Fluorine Toxicosis and Industry. **Amer. Ind. Hyg. Assoc. J.**, 31:240-247, 1970.
14. Jackson, D. and Weidmann, S.M.: Fluorine in Human Bone Related to Age and the Water Supply of Different Regions. **J. Path. Bact.**, 76:451-459, 1958.
15. Freitag, V., Oelschläger, W. and Loeffler, K.: Fluoride Content and Microradiographic Findings in Skeletal Fluorosis. **Fluoride**, 3:167-174, 1970.
16. Boillat, M.-A., et al.: Fluorose industrielle. Suppl. No. 8 to **Schweiz. med. Wschr.**, 109, 1979.
17. McClure, F.G., McCann, H.C. and Leone, N.C.: Excessive Fluoride in Water and Bone Chemistry (Comparison of Two Cases). **Publ. Health Rep.**, 73:741-746, 1958.
18. Zipkin, I. et al.: Fluoride Deposition in Human bones After Prolonged Ingestion of Fluoride in Drinking Water. **Publ. Health Rep.**, 73:732-740, 1958.
19. McCann, H.G.: Comparison of the Physiologic and Pathologic Characteristics and of the Fluoride Content of the Skeletal Tissues of Two Persons of Similar Experience Except for Exposure to Fluoride, Part 2: The Fluoride Content of the Skeletal Tissues. **Arch. Ind. Health**, 21:336-337, 1960.
20. Posner, A.S., Eanes, E.D. and Zipkin, I.: X-ray Diffraction Analysis of the Effect of Fluoride on Bone. In: Richelle, L.J. and Dallemagne, M.J. (Eds.): **Calcified Tissues 1964.**, Collection des Colloques de l'Univ. Liege, 1965, pp. 79-88.
21. Schlegel, H.H.: Industrielle Skelettfluorose. Vorläufiger Bericht über 61 Fälle aus Aluminiumhütten. **Sozial- und Präventivmed.**, 19:269-274, 1974.
22. Boillat, M.A., Garcia, J. and Velebit, L.: Radiological Criteria of Industrial Fluorosis. **Skeletal Radiology**, 5:161-165, 1980.
23. Bang, S. et al.: Morphometry and Biophysical Study of Bone Tissue in Industrial Fluorosis. In: Courvoisier, B., Donath, A. and Baud, C.A. (Eds.): **Fluoride and Bone.**, Edit. Médecine et Hygiène, Geneva, 1978, pp. 168-175.
24. Cameron, J.R. and Sorenson, J.: Measurement of Bone Mineral in vivo. **Science**, 142:230-232, 1963.
25. Franke, J.: A New Concept of the Effect of Fluoride on Bone. **Fluoride**, 12:195-208, 1979.

****

THREE-YEAR CARIES INCREMENTS AFTER FLUORIDE RINSES OR TROPICAL APPLICATIONS WITH A FLUORIDE VARNISH

by

C. Bruun, J. Billie, K.T. Hansen, J. Kann, V. Qvist, A. Thylstrup
Copenhagen, Denmark

(Abstracted from **Community Dent. Oral Epidemiol.**, 13:299-303, 1985)

A 3-yr, double-blind, clinical trial of two caries preventive fluoride programs was completed by 251 9-12-yr-old children. Caries increments and progression patterns were compared in two groups of children who rinsed every fortnight with a 0.2% NaF solution or received biannual topical application with a fluoride varnish (Fluor-Protector). Clinically recorded mean DFS increments were 3.3 ±0.2 (SE) in the rinse group and 3.5 ±0.2 in the varnish group. In both groups nearly half of these increments were recorded in the occlusal surfaces of second molars.

The mean incremental DFS recorded radiographically on approximal surfaces of posterior teeth were 1.1 ±0.2 and 1.5 ±0.2 in the rinse and varnish group, respectively. None of the inter-group differences were statistically significant ($p < 0.05$). Detailed analyses of the radiographic scores revealed a similar and extremely slow caries progression in the two study groups. They strengthened the conclusion of equal clinical efficacy of the two treatments. None of the fluoride programs had changed the preestablished patterns of caries development among the children.

KEY WORDS: Dental caries, increments; Fluoride rinses; Fluoride varnish.

REPRINTS: Carsten Bruun, Department of Cariology and Endodontics, Royal Dental College, 160 Jagtvej, DK-2100 Copenhagen Ö, Denmark.

****

FLUORIDE EFFECTS ON THE ACTIVITY OF RHUS LACCASE AND THE CATALYTIC MECHANISM UNDER STEADY-STATE CONDITIONS

by

Gerald B. Koudelka and Murray J. Ettinger*
Buffalo, New York, USA

(Abstract from **J. of Biological Chemistry**, 263:3698-3705, 1988)

The copper-bearing enzyme Rhus Laccase uses three types of Cu(II) sites to catalyze the reduction of O_2 to H_2O. Fluoride binds to the type 2 site. The effects of F^- on the kinetics of O_2 reduction were examined to determine the catalytic roles of the copper sites. Under steady-state conditions, F^- rapidly inhibits the oxidation of dimethylphenylenediamine. Both reductant-dependent and -independent steps are inhibited. Rapid-freeze ESR spectra

under steady-state conditions showed that F^- decreased the steady-state concentrations of oxidized type 1 copper and oxidized type 2 copper while increasing the concentration of an oxygen radical intermediate. Stopped-flow kinetic experiments were used to determine the catalytic step(s) affected by F^-. The most significant effect of F^- was on the reductant-dependent rate of reduction of the type 3 site. While a stricly first-order dependence was observed in the absence of F^-, a hyperbolic dependence was detected in the presence of F^- indicating a limiting reductant-independent step. The steady-state kinetic rapid-freeze ESR and stopped-flow kinetic data are consistent with the implicated step being the reduction of the oxygen radical in an intermediate containing reduced type 1 and reduced type 2 copper. The results suggest a role for the type 2 Cu(II) site in binding the oxygen radical and catalyzing its reduction to H_2O. — Authors' Abstract

KEY WORDS: Copper; Fluoride; Rhus Laccase.

REPRINTS: Department of Biochemistry, State University of New York at Buffalo, Buffalo, New York 14214, USA.

****

FLUORIDE AND FLUORIDATION

by

Geoffrey E. Smith
Melbourne, Victoria, Australia

(Abstracted from **Soc. Sci. Med.**, 26:451-462, 1988)

The prevalence of dental caries is declining both in communities where water is fluoridated and where it is not. The author believes that this decrease may be related to an increase in the use of fluoridated water and tablets, topical application and fluoride-containing toothpaste as well as mouth rinses now being used in many places to control dental caries; likewise to an increase of fluoride in the food chain, the unintentional ingestion of fluoride-containing dental health products, and increasing contamination of the total environment with fluoride emissions and solid wastes from many industries.

In man, excessive intakes of fluoride over many years may lead to a well-defined disorder — skeletal fluorosis. In addition, a number of recent studies have suggested that fluoride may be genotoxic.

KEY WORDS: Environmental fluoride; Fluoridation; Fluoride ingestion; Fluoride products.

REPRINTS: 56 Surrey Rd., South Yarra, Melbourne 3141, Victoria, Australia.

****

SKELETAL FLUOROSIS IN HUMANS: A REVIEW OF RECENT PROGRESS IN THE UNDERSTANDING OF THE DISEASE

by

K.A.V.R. Krishnamachari
Hyderabad, India

(Abstracted from **Prog. Food Nutr. Sci.** 10:279-314, 1986)

Endemic skeletal fluorosis is a chronic metabolic bone and joint disease caused by ingesting a cumulative toxin which can alter accretion and resorption of bone tissue, and affect the homeostasis of bone and mineral metabolism. The total quantity of ingested fluoride determines the clinical course of the disease which is characterized by immobilization of joints of the axial skeleton and of the major joints of the extremities. A combination of osteosclerosis, osteomalacia and osteoporosis of varying degrees, as well as exostosis, characterizes the bone lesions. Secondary hyperparathyroidism is observed with associated characteristic bone changes.

Increased metabolic turnover of bone, impaired bone collagen synthesis and increased avidity for calcium are features in fluoride toxicity. Osteosclerosis is evident when small doses of fluoride are ingested over a long period of time during which calcium intake is apparently normal; osteoporotic forms are common in the pediatric age group associated with a higher body load of NaF.

In fluorosis, hormones concerned with bone mineral metabolism are altered. The kidney is the primary organ for excretion of fluoride. Age, sex, calcium intake in the diet, dose, duration of fluoride intake and renal efficiency in handling fluoride influence the outcome. Serum parameters rarely help in the diagnosis. Elevated urinary and bone fluoride indicate fluoride toxicity but no effective therapeutic agent is known. Bone density measurement assist in early diagnosis.

Industrial fluorosis is increasing on a global basis.

KEY WORDS: Fluorosis review; Fluorosis, skeletal.

REPRINTS: National Institute of Nutrition, Indian Council of Medical Research, Jamai Osmania P.O., Hyderabad 500 007, A.P., India.

****

THE EFFECT OF FLUORIDATED WATER ON DMF SCORES OF FIRST PERMANENT MOLARS IN MIXED DENTITIONS

by

Eeva Linkosalo
Kuopio, Finland

(Abstracted from **J. of Dentistry for Children**, 53:354-358, 1986)

Registration of caries status of all teeth in young people during eruption periods as well as differences in diagnosis may result in differences in findings between studies. Likewise, distribution of caries and the effectiveness of fluoride on cariogenicity varies depending upon the type of tooth. Although occlusal surfaces receive some protection from fluoridation its effect on them is weakest.

In two groups of Finnish children, born and residing in towns with or without fluoride added to drinking water, the health status of first permanent molars was studied. At ages eleven and fifteen, in the fluoridated area, the groups with only one DMF surface per tooth were still the largest, 54.1 percent and 53.3 percent, respectively. In the low-fluoride area, at age fifteen, three or more DMF surfaces were found in 55.4 percent of first permanent molars. Likewise at age fifteen, the number of intact first permanent molars, even where water contained fluoride was hardly that reported without fluoride by King et al. Especially in the low fluoride area, in this study, intact first permanent molars were few. Since topical prophylaxis with fluoride was negligible, the most important protective effect in children was that due to F in drinking water. The results of this study show that fluoride obtained from drinking water is not sufficient for an effective preventive regimen.

KEY WORDS: DMF scores; Fluoridation in Finland; Molar DMF data.

REPRINTS: Institute of Dentistry, University of Kuopio, Kuopio, Finland.

****

THE MUTAGENICITY OF SODIUM FLUORIDE TO L5178Y [WILD-TYPE AND TK+/-(3.7.2c)] MOUSE LYMPHOMA CELLS

by

Jane Cole, Wendy J. Muriel and Bryn A. Bridges
Brighton, UK

(Abstracted from **Mutagenesis**, 1:157-167, 1986.)

L5178Y wild-type and TK+/-(3.7.2c) cells were treated with sodium fluoride over a range of concentrations (10-500 μg/mL) and treatment times (4, 16 and 48 h) covering <10-100% survival. The mutant frequency at five genetic loci (resistance to ouabain, 6-thioguanine, excess thymidine, methotrexate and

1-ß-D-arabinofuranosyl cytosine) was assayed in wild-type cells and trifluorothymidine in TK+/- cells. No significant induced mutation at any locus was observed after 4 h of treatment. Sixteen hours of treatment with high concentrations of sodium fluoride did not induce resistance to ouabain, but resulted in some significant induction of 6-thioguanine, 1-ß-Darabinofuranosyl cytosine and methotrexate resistance, although the results were variable between experiments and no dose-response was observed. At the thymidine kinase locus, a dose-related increase in mutant frequency to excess thymidine and trifluorothymidine resistance was observed. The maximum induction was approximately eight times the control frequency after TK+/- cells were treated with the highly toxic concentration of 500 µg/mL of sodium fluoride for 16 h. These observations, and an analysis of the colony size of trifluorothymidine-resistant mutants in TK+/- cells, suggest that sodium fluoride is clastogenic to dividing cultured mammalian cells at high, toxic concentrations. Further work is desirable to investigate the mechanism by which chromosomes were damaged at high concentrations of fluoride, since without such a mechanistic understanding, extrapolation of our data to the human situation must be insecure.

— modified Authors' Abstract

KEY WORDS: Chromosomes; Mutation; Sodium fluoride; United Kingdom.

REPRINTS: MRC Cell Mutation Unit, University of Sussex, Falmer, Brighton, Sussex BN1 9NR, UK.

****

MARKED SKELETAL FLUOROSIS FROM DIMINISHED KIDNEY FUNCTION

by

Chr.W. Schmidt, P. Würgatsch* and E. Auermann
Heidenau and Karl-Marx-Stadt, GDR

(Abstracted from **Z. Urol. u. Nephrol.**, 78:173-176, 1985)
[in German]

Massive skeletal fluorosis was manifested in bones and teeth of an 86 year old man who was chronically exposed to fluoride in air and in drinking water which was contaminated by industry. Renal insufficiency developed, caused by arteriosclerosis in both kidneys.

This case accentuates the importance of knowing the renal functional parameters in cases of therapeutic use of fluoride.

KEY WORDS: GDR; Kidney function; Skeletal fluorosis.

REPRINTS: CA Dr. sc. med. Chr. W. Schmidt, Innere Abteilung am Kreiskrankenhaus, German Democratic Republic 8312, Heidenau, Dohnaer Strasse.

****

AUTHORS INDEX

CORRECTION

January, 1989 (Vol. 22, No. 1) paragraph 3, line 12. In the "Report of a Meeting," International Fluoridation Symposium in Brazil the word "agreed" should have been "argued."

INSTRUCTIONS TO AUTHORS

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

PREPARATION OF PAPERS

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

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

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

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

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

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

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

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

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

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

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

THE INTERNATIONAL SOCIETY for FLUORIDE RESEARCH

P.O. BOX 692 WARREN, MICHIGAN 48090