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CONTENTS

EDITORIAL 165

TRIBUTES
JOHN R MARIER (1925 - 1992) Jeff Marier 166-167
A TRIBUTE TO JOHN R MARIER Dyson Rose 167-168

RESEARCH REPORTS
REPORT ON THE FIFTH FLUORINE SYMPOSIUM IN SZCZECIN
A Machoy-Mokrzynska, Poland 169-170
INTRODUCTION TO THE FIFTH FLUORINE SYMPOSIUM IN POLAND
G W Miller, USA 170
INTERACTION OF FLUORIDE IONS WITH MILK PROTEINS STUDIED BY GEL FILTRATION
P Wieczorek, D Sumujlo, D Chlubek and Z Machoy, Poland 171-174
EFFECTS OF FLUORIDE TOXICITY ON LEAF AREA, NET ASSIMILATION RATE AND RELATIVE GROWTH RATE OF *HORDEUM VULGARE* AND *ZEA MAYS*
S Rathore, India 175-182
THE FLUOROSIS PROBLEM IN TROPICAL SHEEP
U N Samal and B N Naik, India 183-190

LETTERS TO EDITOR
VITAMIN C DEFICIENCY AND FLUOROSIS A Mithal and M M Godbole 191-192
REPLY TO MITHAL AND GODBOLE U K Misra 193-194

ABSTRACTS
RED CELL MEMBRANE ALTERATIONS IN HUMAN CHRONIC FLUORIDE TOXICITY
D Sarala Kumari and P Ramakrishna Rao, India 195
DIACYLGLYCEROL GENERATION IN FLUORIDE-TREATED NEUTROPHILS: INVOLVEMENT OF PHOSPHOLIPASE D D English, G Taylor and J G N Garcia, USA ... 195
SODIUM FLUORIDE INFLUENCE UPON ENERGY AND PROTEIN LIVER METABOLISM AFTER ITS EXPERIMENTAL ISCHEMIA M Tyrtyshnikov and V P Pedik, Ukraine 196
MULTIPLE ACTIONS OF FLUORIDE IONS UPON THE PHOSPHOINOSITIDE CYCLE IN THE RAT BRAIN G Tiger, P-E Björklund, G Brännström and C J Fowler, Sweden 196
FLUORIDE, CALCIUM AND PHOPHORUS METABOLISM IN THE RAT: COMPARISON OF 'NATURAL INGREDIENT' WITH SEMIPURIFIED DIETS G M Whitford, USA 197

ABSTRACTS (continued)

A COMPARITIVE STUDY OF FLUORIDE PHARMACOKINETICS IN FIVE SPECIES
G M Whitford, E D Biles and N L Birdsong-Whitford, USA 197-198

THE EFFECT OF CALCIUM ON DISODIUM MONOFLUOROPHOSPHATE ABSORPTION FROM THE GASTROINTESTINAL TRACT OF RATS
A Villa, G Carrasco, A Valenzuela, A Garrido, Chile 198

INHIBITION OF PROTON-TRANSLOCATING ATPASES OF *STREPTOCOCCUS MUTANS* AND *LACTOBACILLUS CASEI* BY FLUORIDE AND ALUMINUM
Michael G Sturr and Robert E Marquis, USA 198-199

ALUMINUM STIMULATES THE PROLIFERATION AND DIFFERENTIATION OF OSTEOBLASTS *IN VITRO* BY A MECHANISM THAT IS DIFFERENT FROM FLUORIDE
K-H William Lau, A Yoo and S P Wang, USA 199-200

EFFECT OF FLUORIDE ON BONE AND BONE CELLS IN OVARIECTOMIZED RATS
D Modrowski, L Miravet, M Feuga, F Bannie, PJ Marie, France 200

INCORPORATION OF SODIUM FLUORIDE INTO CORTICAL BONE DOES NOT IMPAIR THE MECHANICAL PROPERTIES OF THE APPENDICULAR SKELETON IN RATS
TA Einhorn, GK Wakley, S Linkhart, EB Rush, S Maloney, E Faierman, DJ Baylink, USA 201

A HISTOMORPHOMETRIC ANALYSIS OF THE EFFECTS OF FLUORIDE ON EXPERIMENTAL ECTOPIC BONE FORMATION IN THE RAT
H Mohr and J Kragstrup, Denmark 201

THE EFFECTS OF FLUORIDE ON BONE Helen E Gruber and David J Baylink 202

FLUORIDE THERAPY FOR OSTEOPOROSIA REVIEW OF DOSE RESPONSE, DURATION OF TREATMENT, AND SKELETAL SITES IN ACTION
B A Dure-Smith, M E Kraenzlin, S M Farley,
C R Libanati, E E Schultz and D J Baylink, USA 202-203

IS FLUORIDE TREATMENT JUSTIFIED TODAY? Jukka A Inkovaara, Finland 203

DOSE EFFECTS ON EWE BONE REMODELING OF SHORT-TERM SODIUM FLUORIDE ADMINISTRATION: A HISTOMORPHOMETRIC AND BIOCHEMICAL STUDY P Chavassieux, P Pastoureau,
G Boivin, M C Chapuy, P D Delmas, P J Meunier, France 203

FLUORIDE-INDUCED BONE CHANGES IN LAMBS DURING AND AFTER EXPOSURE TO SODIUM FLUORIDE P Chavassieux, P Pastoureau,
G Boivin, M C Chapuy, P D Delmas, G Milhaud and P J Meunier, France 204

STUDIES OF FLUORIDE RETENTION BY ORAL SOFT TISSUES AFTER THE APPLICATION OF HOME-USE TOPICAL FLUORIDES
D T Zero, R F Raubertas, A M Pedersen, J Fu,
A L Hayes and J D B Featherstone, USA 204-205

EFFECTS OF FLUORIDE ON SECRETORY AND POSTSECRETORY PHASES OF ENAMEL FORMATION IN SHEEP MOLARS
Guy E Milhaud DVM, Eliane Charles, Marie-Laure Loubière DVM,
Martine Kolf-Clauw DVM and Christophe Joubertt, France 205

INFLUENCE OF FLUORIDE AND CARBONATE ON *IN VITRO* REMINERALIZATION OF BOVINE ENAMEL
P C Lammers, J M P M Borggreven,
F C M Driessens and J W E Van Dijk, Netherlands 206

BENEFITS AND RISKS OF FLUORIDE SUPPLEMENTATION: CARIES PREVENTION VERSUS DENTAL FLUOROSIS
W Dhoore and J P Vannieuwenhuysen, Belgium 206

AUTHOR INDEX for 1992 207-208

SUBJECT INDEX for 1992 209-210

EDITORIAL

By common agreement of the 150 researchers who attended, the 19th Conference of the International Society for Fluoride Research, held in Kyoto in September, was a resounding success. Our gracious Japanese hosts well deserved the praises for their excellent organization. Reports presented to the conference, many of great interest and significance, will be published in this journal during 1993. As the term of office of the Society's Officers is four years, no elections took place at this conference, except to unanimously confirm the appointment of the new Treasurer and Editor, who had been acting in those capacities since the retirement of Mrs Waldbott last year. New subscription rates for the journal were approved. Though an increase has become necessary, the journal remains lowly priced in comparison with similar journals. It was also unanimously decided to accept the invitation of Chinese members to hold the next conference in Beijing in 1994.

In this issue of the journal we report on the fifth Fluorine Symposium in Szczecin, Poland, and some of the excellent research carried out by members in that country. It was intended to publish also the significant study by Chlebna-Sokol and Czerwinski on bone changes detected in male children with dental fluorosis, but an unfortunate mailing loss has caused its postponement to the next issue.

We also publish tributes to the late John Marier, by his son Jeff Marier and by his close scientific colleague Dyson Rose. John Marier was mainly known to readers of this journal through his published work on fluoride. However, in addition to achieving distinction as a scientist, John had earned recognition as a music composer, playwright and poet. His epic poem "A North American Indian Looks at the Past 100 Years" was published in 1967 by the Indian Press and sponsored by the Canada Centennial Commission. He was a remarkable man.

At the Kyoto conference interesting discussions took place around some controversial aspects of fluoride research - including the use of fluoride therapy for osteoporosis and the dental benefit (or alleged benefit) of water fluoridation. Many such aspects, insofar as they involve differing scientific interpretations of data related to them, can be ventilated through letters to this journal. Two such letters in this issue are examples. The view held is that the stature of the Society and its journal will be maintained by "avoiding any activities that are not scientific in nature" and that *as ISFR members* we should not endorse or oppose any particular political stance on issues related to fluoride pollution or therapeutic use (Minutes, ISFR meeting 1992). However, scientific issues themselves lead to debate and discussion. Scientific journals can and should provide avenues for such scientific exchanges.

John Colquhoun

JOHN R MARIER (1925 - 1992)

Jeff Marier
Ottawa, Canada

On March 4 1992 John Marier died suddenly, at home in his sleep, of massive cardiac and pulmonary failure. This is rather ironic, as he had achieved some degree of notoriety for many publications dealing with the importance of dietary magnesium as a cardio-protectant. In fact, daily magnesium supplements, which he started after cardiac problems in 1974, probably prolonged and enhanced his life. It is difficult to condense his long and diversified career into a few short paragraphs, so I will try to concentrate on those highlights of which he was proudest.

In August 1943 he joined the staff of the Applied Biology Division of the National Research Council of Canada (NRCC) as a "Laboratory Helper". Over the next quarter century, he was promoted "Laboratory Technician" and worked on a variety of projects in food and dairy chemistry. The culmination of this work was in 1967, when the NRCC Food Chemistry Group was awarded the American Chemical Society's Borden Award "for the outstanding decade of research on the chemistry of milk". During this same period he developed several methods for the quantitative analysis of elements or compounds in biological tissues. In 1967 he was awarded the Canada Centennial Medal "for outstanding research on trace elements". The 1958 Marier and Boulet method for citric acid analysis (*Journal of Dairy Science 41* 1683-92 1958) was identified as one of the most cited articles in its field and was designated a "Citation Classic" in 1983. (*Current Contents 14* (46) 21-2 1983).

In 1970 he became a member of the Environmental Secretariat where he authored or co-authored five reports on various environmental topics, including *Environmental Fluoride* (1971, 1977). He also assisted in the development of a computerized database of pollution-relevant bibliographic references (PIP) in 1971. By 1979 he had been promoted to the "Professional" staff, as far as I know the only member of NRCC to achieve this feat without a university degree. Also in that year, he was appointed to the panel charged with supervision of the health studies relating to pollution of Cornwall Island, on behalf Canada's Minister of Health.

He retired from NRCC in March 1985, but continued to actively pursue his scientific interests. That same year he was awarded a Certificate of Merit from the American Society for Magnesium Research for "the furtherance of knowledge on the frontiers of magnesium research"; and elected a Fellow of the American College of Nutrition. He was a Consulting Editor for that College's Journal from 1986-1991; a member of the Editorial Board of *Magnesium and Trace Elements*, 1982-1991; and a member of the editorial Board of *Magnesium Research* from 1988 until his death. In February 1988, he was awarded the distinguished title of Researcher Emeritus by NRCC "in recognition of his outstanding contribution to the advancement of research". This was his most cherished award.In 1963 he co-authored both "Accumulation of skeletal fluoride and its implications" (*Archives of Environmental Health 6* 664-71) and "Hard waters and heart disease" (*British Medical Journal* Sept 14 686-687). His last publication was "Intakes of magnesium and fluoride and some systemic effects" (*Proceedings of the Finnish Dental Society 87* 581-94 1991).

In between, he investigated dietary intakes of both magnesium and fluoride, separately and in combination; fluoride in connection with osteoporosis and renal osteodystrophy; halogenated anesthetics; magnesium in connection with hard water and the human heart; and various magnesium interralations with fluoride, calcium, sodium, potassium, selenium, cobalt and aluminum. He was an invited keynote speaker on both fluoride and magnesium at many international symposia.

In addition to his scientific achievements, John Marier was an award-winning music composer and dedicated family man. Many people will miss him.

A TRIBUTE TO JOHN R MARIER

Dyson Rose
Alcove, Quebec, Canada

In the early 1950's resignation of a more senior staff member in the Division of Applied Biology (later The Division of Biosciences) of the National Research Council of Canada led the Director to ask me to assume the leadership of a small group known as the Food Chemistry Section. The staff of that section included a young Technician* named John R. Marier, and affectionally called Johnny. At the time Johnny made no particular impression on me, but I liked to include technical staff in the selection and planning of research projects, and as the years passed I became more and more aware of his ability to contribute meaningfully to our discussions. Over the 20 or so years of our Section Head/ Technician relationship, I learned that John had a most remarkable ability to read scientific papers, to retain details of what he had read, to mull them over in his mind seeking the inter-relations of items from different authors and to formulate therefrom a suggestion for further research or even a new scientific hypothesis.

Probably the earliest tangible evidence of this outstanding ability is a paper published by Marier, Rose and Boulet in the May 1963 issue of the *Archives of Environmental Health* (Vol. 6, pages 664-671). In the months leading up to this article, the Food Chemistry Section had become interested in the stability of the casein and calcium phosphate components of milk, and Johnny had been asked to search the literature for information on factors affecting the solubility of calcium phosphates. Fluoride is one of the known factors affecting calcium phosphate solubility, and that led Johnny into the literature on skeletal phosphate. To quote a later article by John Lear, Science Editor for *Saturday Review*, a New York magazine (Jan. 4, 1964 issue) Johnny "read with eager appetite, on his own time, before and after work, during lunch hours and over weekends". One result of this intense effort was the above mentioned paper, which was entitled "Accumulation of Skeletal Fluoride and Its Implications".

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* "Technician" was a ranking used to designate employees who lacked scientific training: "Professional" was the ranking for employees having at least one University degree, and preferably a doctorate in a scientific field.

As implied above, this paper caught the attention of John Lear of *Saturday Review*. Mr Lear came to Ottawa to interview Johnny and myself, and used our paper as a major information source in an article calling for caution in the drive to fluoridate city water supplies. In 1967, Canada's Centennial year, he approached us with a request that we write an article on Canadian scientific efforts. We obliged with "Is a Great Tradition Eroding", which appeared in the Sept. 2, 1967 edition of *Saturday Review*.

During 1967 - 68, the Food Chemistry Section was phased out and Johnny moved to a newly formed group mandated to review the potential environmental hazard of various chemicals, and was promoted to the Professional level. To the best of my knowledge, Johnny was the only member of NRC's staff to achieve Professional ranking without any University training.

That terminated our Technician/Section Head relationship, but not our friendship nor our co-operation on projects. In 1971 fluoride was one of the chemicals the environmental group chose to review, and, to my delight, Johnny approached me with a request that I co-author a paper entitled "Environmental Fluoride". This last joint effort appeared as NRC Publication No. 12,226 in December 1971.

I retired in 1977, but Johnny continued working, and it was during this period that he developed his interest in magnesium and its function during muscle contraction. He also earned a further honour:- appointment to an "Emeritus" position. He retained that position and continued an active interest in magnesium until his recent death at age 66.

He is sorely missed.

REPORT ON THE FIFTH FLUORINE SYMPOSIUM IN SZCZECIN

A Machoy-Mokrzynska,
Szczecin, Poland

On September 25, 1992, Szczecin, Poland, was the venue of the fifth Fluorine Symposium. The subject was: *Biological risks posed by fluorine compounds.* Participants were affiliated with 15 national and international Centers. The program comprised 8 reports and 20 posters, and opened with a message from Professor Gene Miller (USA), Secretary of the International Society for Fluoride Research.

Z Machoy (Szczecin) presented a report, "Biological risk to fallow deer by fluorine compounds in the area of the future Odra Riverside Park", on a plan by Poland and Germany to organize a national park in the Lower Odra river basin. The environmental hazard has prompted the Polish Hunters' Association to set up a deer protection Foundation for the Western Pomerania regions.

U Kierdorf (Göttingen) and H Kierdorf (Cologne) in the next report, "Pathological tooth changes in roe deer (*Capreolus capreolus*) caused by chronic fluoride intoxication", presented analyic data concerning increased fluoride accumulation in the bones of roe deer stemming from the highly industrialized Ruhr Basin in Germany. The animals displayed the characteristic pathogenic changes in teeth which result from protracted fluoride poisoning: increased wear, poorly mineralized enamel and discoloration.

M Borysewicz-Lewicka (Poznan) in "Influence of fluoride on mineralization in the oral cavity" reported an investigation of fluoride influences on mineralization of immature supragingival dental calculus.

D Chlebna-Sokół (Lódz) and E Czerwinski (Kraków) in their paper "Computerized X-ray image analysis in assessment of bone mineralization in children with dental fluorosis" reported on a part of their long-term and complex studies on the effects of overoptimal fluoride concentrations in potable water on the health and physical development of school-age children. They reported greater height and area of bone trabeculae in male children with dental fluorosis compared with the control group.

E Renner (Giessen) in "Effect of calcium intake on the bone mineral content and incidence of osteoporosis" provided data which support the hypothesis that appropriate consumption of calcium with milk and dairy products in childhood and during the maturation period are decisive both for achieving maximal bone mass and for protection against osteoporosis.

J Markiewicz (Kraków) in "Some analytical problems in determination of trace amounts of fluorine compounds" reported and discussed results of interlaboratory testing for fluorine compounds in soil and plant material samples.

J Krechniak (Gdansk) in "Hair as an index of exposure to fluoride" concluded that hair provides a useful global indicator of environmental and occupational exposure and is a valuable first screening method - much less inconvenient than monitoring of blood and urine.

Finally D Chlubek (Szczecin) in "Significance of placental transfer of fluoride" discussed viewpoints on placental fluoride transport and the difficulties with implementation of gained knowledge on this subject in clinical practice.

A commission of three evaluated the posters and discussed the contributions with each author. The subjects presented included: fluoride content of body fluids and its share in mineralization of bones and teeth; intoxication of experimental animals; and interaction of fluoride and enzymes.

In summary, the Symposium found that in Poland fluorine continues to be a major contaminant of the environment. The proceedings have been published in a book, (in Polish): *Metabolism of Fluorine '92*, with summaries in English.

INTRODUCTION TO THE FIFTH FLUORINE SYMPOSIUM IN POLAND

Gene W Miller, Secretary
International Society for Fluoride Research
Logan, Utah USA

Congratulations to all participants in the Fifth Fluorine Symposium to be held in Poland. On behalf of the ISFR I wish you successful meetings, interesting discussions and fruitful research. In the past I have followed with interest your Fluorine Symposia of 1979, 1986 and 1988.

Accounts of these meetings were published in the journal *Fluoride*. I encourage you again to submit a summary of your Symposium, so that it may be published in *Fluoride* and enable our members throughout the world to be informed about fluoride research in Poland. I am very appreciative of the efforts of Professor Dr Zygmunt Machoy from Szczecin who has kept us informed about the fluoride meetings and research in Poland. Professor Machoy has been a member of ISFR for many years and has actively participated in many of the international conferences. He is presently serving on the the Editorial Board of ISFR as does Dr Edward Czerwinski (Kraków Academy of Medicine) and Dr Jerzy Krechniak from Gdansk. The research on fluorine that has originated in Poland is extensive in the areas of plant, animal and human studies. Many excellent papers from Poland on fluoride toxicology have been cited frequently by researchers world-wide.

I would like to encourage all researchers in the area of fluoride to become members of the International Society for Fluoride Research and submit manuscripts and abstracts for publication in the *Fluoride* journal. Dr John Colquhoun (New Zealand) is new editor of the journal and welcomes your input to *Fluoride*. The fluorine research that is being conducted in Poland is of much value to all that are concerned with effects of fluoride. We acknowledge the excellence of this research and your colleagues throughout the world congratulate you on your dedication.

INTERACTION OF FLUORIDE IONS WITH MILK PROTEINS STUDIED BY GEL FILTRATION

P Wieczorek, D Sumujlo, D Chlubek and Z Machoy
Szczecin, Poland

SUMMARY: The interaction of fluoride ions with the bovine milk protein a-lactalbumin, type I α-casein, β-casein, and κ-casein was studied at pH 6.6, 5.5 and 3.9. At pH 6.6 and 5.5 fluoride ions do not combine with any of these protein. However, at pH 3.9 they combine with α-lactalbumin.

Key words: Fluoride; Gel filtration; Milk proteins.

Introduction

Earlier work has shown that fluoride ions may combine with serum albumin (1). In enzymatic proteins such an interaction frequently leads to changes in activity and may be monitored by kinetic methods (2,3). Numerous studies have also revealed that non-enzymatic proteins, such as hen egg albumin and bovine albumin bind fluoride ions (4,5). Methods widely used in these investigations are the gel filtration technique, fluoride electrode implementation, and the equilibrating dialysis method.

A separate problem in nutrition is posed by the presence of fluoride in milk products. Duff ascertained that the fluoride content of milk of cows drinking fluoridated water is greater than in the milk of cows drinking non-fluoridated water (6). This author also noted that part of the fluoride in milk was in a bound state - with organic milk components or with calcium - and that the percentage goes up with increased time of storage.

The aim of the present research was to find out whether fluoride ions combine with the milk proteins α-lactalbumin, type I α-casein, β-casein and κ-casein. Since the pH of fresh milk is 6.6-6.8 and decreases during storage, the investigations were carried out at pH 6.6, 5.5 and 3..9.

Material and Methods

Bovine α-lactalbumin, type I α-casein, β-casein and κ-casein (all from Sigma) were dissolved in 0.02 M phosphate buffer with pH 6.6 and 0.02 M acetate buffer with pH 5.5. Additionally, a solution of α-lactabumin was prepared in 0.02 M acetate buffer with pH 3.9 (at this pH caseins fail to dissolve). To avoid eventual contamination by fluoride ions, these solutions were purified by passing them through a column of "Sephadex G-25" (Pharmacia, Uppsala) equilibrated by protracted rinsing in the afore-mentioned buffers. Protein concentrations in the resulting solutions were determined by measuring the light absorption at 280 nm.

In the studies on the binding of fluoride by proteins, Hummels and Dreyer's technique (7) was employed with minor modification. For this purpose a column (0.8 cm -70 cm) was prepared and filled with Sephadex G-25 fine and equilibrated with the appropriate buffer with added NaF (terminal concentration of fluoride ion was 3.0×10^{-4} M). Subsequently, 1.0 ml of previously purified protein solution (1.0×10^{-4} M) suspended in the same buffer, but without fluoride, was transferred onto the column, which was then rinsed with equilibrating buffer. The flow rate was adjusted to 0.3 ml/min, and 1.0 ml fractions were collected. The protein content in respective fractions was defined by measuring the light absorption at 280 nm.

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The fluoride concentration was measured with the aid of an ion-selective fluoride electrode (Radelkis, Hungary). For this purpose 0.5 ml of each fraction was mixed with 0.5 ml of TISAB buffer (μ = 5.0 M, pH 5.5). Under these conditions it was found that fluoride disssociates from the protein, resulting in measurement of total fluoride concentration.

Results and Discussion

The results presented in Figure 1 indicate that at pH 6.6 and 5.5 none of the proteins bind the fluoride ions. If fluoride combined with protein under the conditions used, the fluoride concentration in fractions containing protein should rise over the concentration value of fluoride in buffer equibrilated with the column, and thereafter fall below this value.

However, we observe a drop in fluoride concentration only in later fractions already deprived of protein. This decrease was due to the fact that a protein sample free of fluoride ions was transformed onto the fluoride-equilibrated column. In the acetate buffer with pH 3.9 the profile for fluoride assumes a characteristic course showing that fluoride ions do combine with lactalbumin (Figure 2).

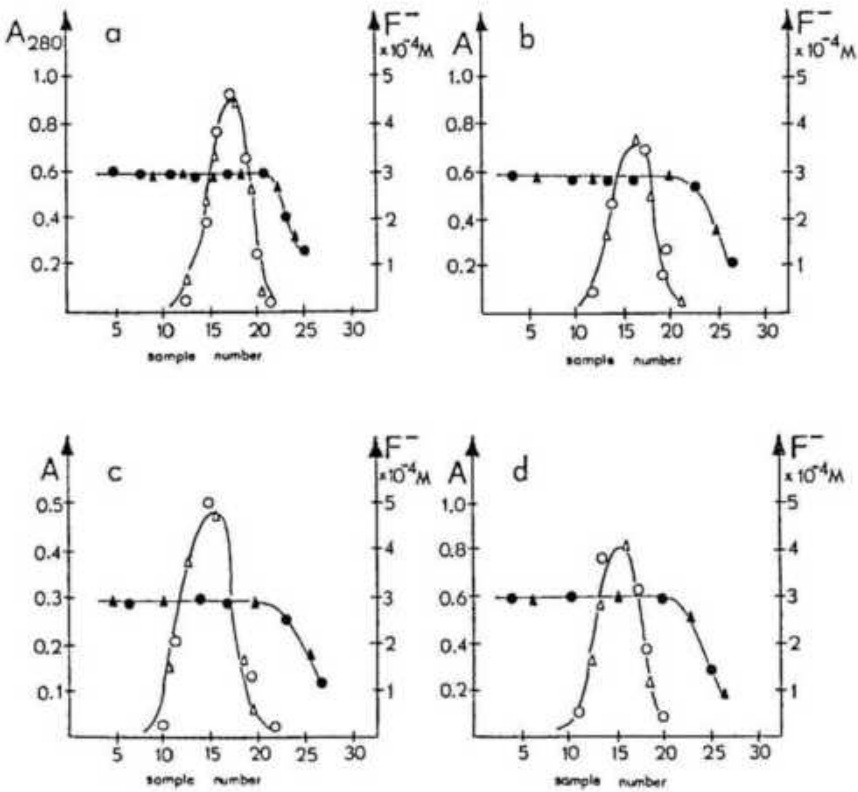

Figure 1. Elution profile of the 280 nm absorbancy (o,Δ) and F^- concentration (●,▲) accompanying the passage of: a) α-lactalbumin, b) type I α-casein, c) β-casein and d) κ-casein through a column of Sephadex G-25 gel which was equilabrated with 0.02 M phosphate buffer, pH 6.6 (o,●) and 0.02 M acetate buffer, pH 5.5 (Δ,▲). Both buffer contained 3.0 x 10^{-4} M NaF. All experiments were carried out at 20°C.

α- and β-Caseins are hydrophobic acid proteins with isoelectric points at pH 4.1 and 4.6, respectively (8). At both pH 6.0 and 5.5 they appear in anion form, which can account for their lack of interaction with fluoride anions. κ-Casein is also a slightly acidic protein (pK 6.0), and at pH 6.0 exclusively negative charges are present on its surface (9).

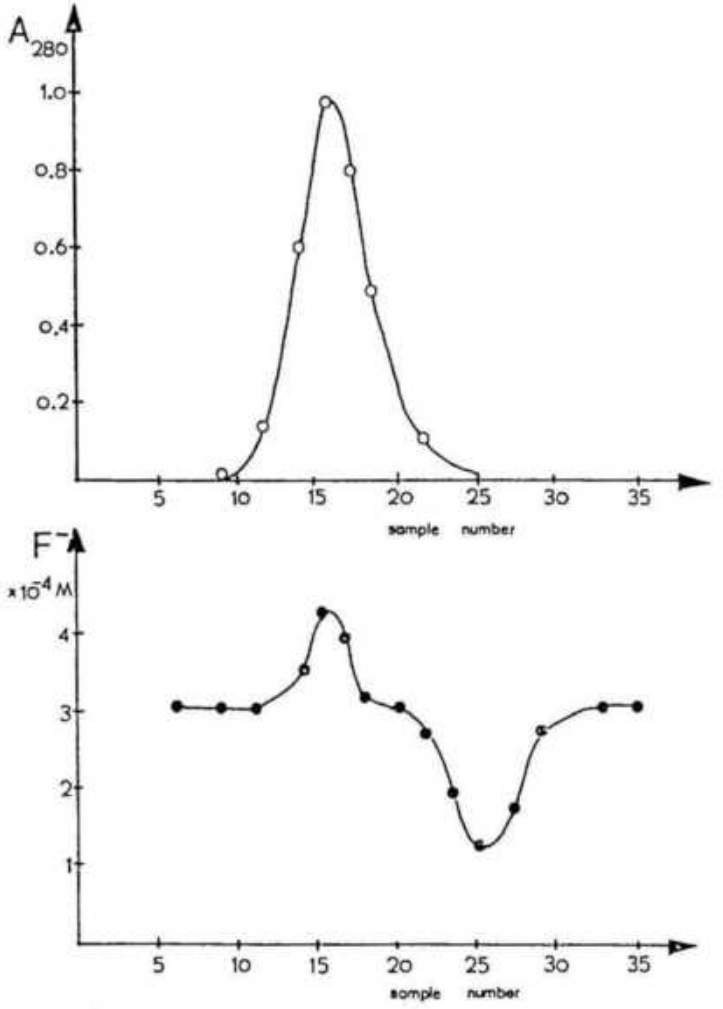

Figure 2. Elution profile of the 280 nm absorbency (o) and F^- concentration (•) accompanying the passage of α-lactalbumin through a column of Sephadex G-25 gel which was equilibrated with 0.02 M acetate buffer pH 3.9 containing 3.0 x 10^{-4} M NaF.

It is surprising why α-lactalbumin (pK 6.5) does not bind fluoride ions at pH 5.5 but only on reaching pH 3.9. Apparently this pH dependence stems from the residue of asparaginic acid (side chain pK 3.9). Only a pH below this value effects dissociation of the carboxylic groups in the side chain of this amino acid and makes the fluoride ion binding possible by positively charged side chains of other amino acid residues e.g. lysine. α-Lactalbumin also contains in its molecule one atom of calcium (10). It has been shown that the primary agent binding calcium in milk is the residue of asparaginate as well as lysine, and that calcium dissociates from the protein below pH 4 (11,12).

In the light of the above studies, the results established by Duff seem to bear out the idea that fluoride is bound in milk by calcium rather than by proteins. α- and β-Caseins are phosphoproteins with a considerable content of calcium, which passes from the proteins into the solution as pH decreases (13). This fact could also explain the drop in free fluoride ion content in milk during storage, which is accompanied by a lowering of pH.

Acknowledgement

The authors wish to thank Professor E Schlimme (Federal Dairy Centre, Kiel, Germany) for the gift of milk proteins.

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EFFECT OF FLUORIDE TOXICITY ON LEAF AREA, NET ASSIMILATION RATE AND RELATIVE GROWTH RATE OF *HORDEUM VULGARE* AND *ZEA MAYS*

Sunita Rathore
Jaipur, India

SUMMARY: Various concentrations of sodium fluoride (5-500 ppm) were sprayed every two weeks on *Hordeum vulgare* K-24 and *Zea mays* local dwarf. The observations, recorded at two week intervals, indicated that significant reductions in leaf area, net assimilation rate and relative growth rate were induced by higher concentrations of fluoride in both crop plants. Control plants remained healthy. *Hordeum vulgare* could not tolerate a fluoride concentration beyond 100 ppm while *Zea mays* tolerated up to 500 ppm.

Key words: Barley; Leaf area; Maize; NaF toxicity; Net Assimilation Rate; Relative Growth Rate.

Introduction

Hordeum vulgare and *Zea mays* belong to the Graminae family. In India barley is sown in *Rabi* season (November) and maize in *Kharif* season (June-July). Both these crop plants are used as fodder for animals and the seeds as concentrates for cattle. Barley seeds are also utilized in the malting industry (1). Both crop plants are sensitive to fluoride pollution.

Generally fumigation of plants with hydrogen fluoride (HF) and spraying with sodium fluoride (NaF) with a back sac sprayer are adopted for the application of fluoride (2). Sodium fluoride salt is readily soluble in water and easy to handle.

Air pollution is a serious problem to human life and agriculture. Gross symptoms of fluoride injury are necrotic lesions, burning of leaf tips and margins and chlorosis (3). Large necrotic markings appear on leaves (4). Growth and productivity of crops are adversely affected by fluoride toxicity (5-7). Accumulation of fluoride in the soil, surrounding plant roots and mesophyll cells disturbs the mineral metabolism, reduces the chlorophyll pigments and other morphological and physiological characters (4,8,9). Worst injury has been reported in tomato when fluoride entered through the roots (10). Chronic fluoride injury and a degradation of chlorophyll with an accompanying reduction in photosynthesis have been reported (11). The effects of fluoride on growth and yields, tissue destruction, photosynthesis rates, inhibition of enzymes, respiration and chlorophyll destruction have been discussed (12).

Phytotoxic fluoride emissions released from several industries reach the soil through precipitation, fog and dew. Fluoride ions are absorbed by plants through the mesophyll cells of the leaves and also by their root system. Sodium fluoride has been used in various concentrations as a source of fluoride. Watson (13) has analyzed the growth and variation of yield in wheat, barley and oats on the basis of leaf area, net assimilation rate and relative growth rate.

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Materials and Methods

Alluvial loam soil having 64.5% sand, 21.3% silt and 14.2% clay with water holding capacity 42.7% was selected for study. The pH of the soil was 7.33, *i.e* slightly alkaline in reaction. After presoaking for 24 hours in different concentrations of sodium fluoride, the seeds of barley were sown directly in the field at appropriate distances and the maize seeds in pots. Barley was sown in November and maize in June. The temperatures at sowing time were:

1. Barley - 30.6°C (maximum) and 13.5°C (minimum)
2. Maize - 41.0°C (maximum) and 28.5°C (minimum)

Manures and fertilizers were applied in the form of urea, single superphosphate and potassium chloride.

1. Barley - 60 kg/ha N, 50 kg/ha P_2O_5 and 40 kg/ha K_2O
2. Maize - 80 kg/ha N, 50 kg/ha P_2O_5 and 40 kg/ha K_2O

The crop plants selected for study were *Hordeum vulgare* K-24 and *Zea mays* local dwarf. Different concentrations of sodium fluoride were 0, 10, 25, 50, 100, 250, and 500 ppm and were applied at two week intervals. For each sample collection of four plants, the following were measured at time of maturity, *i e* 120 days after sowing of seeds.

1. Leaf area was calculated with the help of a planimeter. The leaves of the plants were arranged into three representative samples - large, medium and small.

2. Net Assimilation Rate (NAR) was calculated by the formula given by Watson (13):

$$NAR = \frac{(\log_e L_2 - \log_e L_1)}{(L_2 - L_1)} \times \frac{(W_2 - W_1)}{(t_2 - t_1)}$$

where L_1 and L_2 are total leaf area and W1 and W_2 are the dry weight at t_1 and t_2.

3. Relative Growth Rate (RGR) was calculated by the formula (13):

$$RGR = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}$$

where W_2 and W_1 are the dry weights; t_2 and t_1 are the times or intervals. The value of $\log_e = 2.306$.

The first fluoride spray was done one month after sowing and the subsequent treatments were at two week intervals. Randomized Block Design with 4 replications was followed. The data were analyzed statistically by F test and CD (Critical Difference) (14).

Results

Sodium fluoride treatments significantly reduce the leaf area per plant in comparison to control (Table 1). From CD values it was observed that all NaF concentration treatments from 5 to 100 ppm significantly reduced leaf area per plant in *Hordeum vulgare*. In both species the control was significantly superior to other treatments. Results at 25 and 50 ppm were similar with significantly reduced leaf area in *Zea mays*. Significant reduction in leaf area was observed from 50 to 500 ppm NaF treatments. Thus, normal plants showed highest production of leaf area while maize plants with 100 to 500 ppm showed lowest leaf area.

Regression analyses (15) of mean leaf area on sodium fluoride concentration were carried out (Figure 2). The regression equations were as follows:

Hordum vulgare $y = 215.3 - 1.048x$ $(r = -0.90, n = 6)$
$b = -1.048$ units of mean leaf area per NaF concentration

Zea mays $y = 380.3 - 0.41x$ $(r = -0.85, n = 7)$
$b = -0.41$ units of mean leaf area per NaF concentration

(x = Fluoride concentration, y = Leaf area per plant,
b = regression coefficient, r = correlation coefficient)

On plotting the data between mean leaf area on y-axis and log NaF concentration on x-axis, the resultant graph is a straight line (Figure 2). This graph indicates that the mean leaf area decreases with increasing NaF concentration logarithmatically.

Maximum NAR was produced by the normal plants and NAR was lower at concentrations of NaF from 100-500 ppm in both species (Table 2).

Sodium fluoride reduced RGR (Table 3). Control plants had the maximum RGR and treated ones had a decreased minimum RGR.

Discussion

The height of plants is also reduced at higher concentrations of fluoride. Dwarfism in Norway Spruce and Birch was noted due to the effect of fluoride emissions (16). Thus fluoride prevents a height gain and leads to a dense bushy growth. The reduction in height was due to the decrease in number as well as the size of the cells (20). Fluoride suppresses root growth of germinating corn seedlings. Fluoride induces changes of RNA structures. Adenine is depressed significantly only in the root tissue treated by the highest fluoride concentration (17).

The size of some citrus leaves may be reduced at NaF concentrations of 50-60 ppm (18,19). Growth, biomass, productivity, dry matter and leaf area were significantly reduced in pea, tomato, pinto bean and alfalfa with 10-500 ppm NaF concentrations (20-22). In comparison with normal plants, 49.6% and 53.9% reductions in leaf area were observed at 100 and 500 ppm concentrations in *Hordeum vulgare* and *Zea mays* respectively. The observed reduction in leaf area was due to the drying up of leaves at higher concentrations of sodium fluoride (burning effect of sodium fluoride was visible on margins, apex and lamina of the leaves). The variation

Table 1
Effect of NaF on Mean Leaf Area (sq.cm.) per Plant

NaF concentrations in ppm	*Hordeum vulgare* K-24 sown in field	Percent reductions for fluoride treatments	*Zea mays* local dwarf sown in pots	Percent reductions for fluoride treatments
Control	246	-	453	-
5	214	13.0	-	-
10	191	22.3	399	11.9
25	170	30.9	379	16.3
50	148	39.8	379	29.6
100	124	49.6	282	37.7
250	-	-	238	47.5
500	-	-	209	53.9
CD at 5%	2.0216		2.517	

Table 2
Effect of NaF on Net Assimilation Rate (mg/day)

NaF concentrations in ppm	*Hordeum vulgare* K-24 sown in field	*Zea mays* local dwarf sown in pots
Control	0.949	0.701
5	0.970	-
10	0.941	0.681
25	0.951	0.673
50	0.951	0.550
100	0.732	0.542
250	-	0.562
500	-	0.577
CD at 5%	0.0547	0.0068

Table 3
Effect of NaF on Mean Relative Growth Rate (mg/day)

NaF concentrations in ppm	*Hordeum vulgare* K-24 sown in field	*Zea mays* local dwarf sown in pots
Control	52.7	60.33
5	52.5	-
10	52.5	59.99
25	51.8	59.18
50	50.4	58.08
100	49.7	57.08
250	-	48.90
500	-	48.70
CD at 5%	0.6116	0.3004

Figure 1

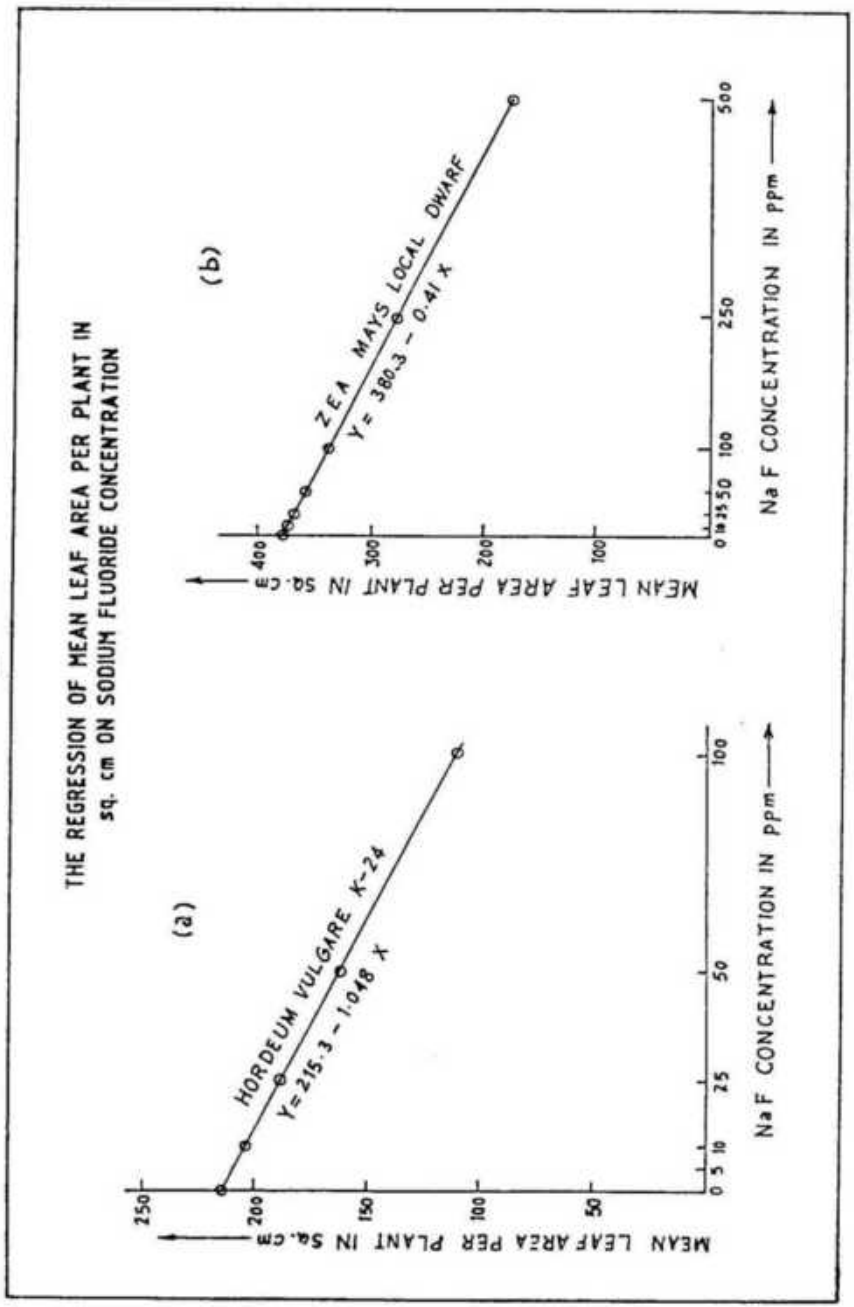
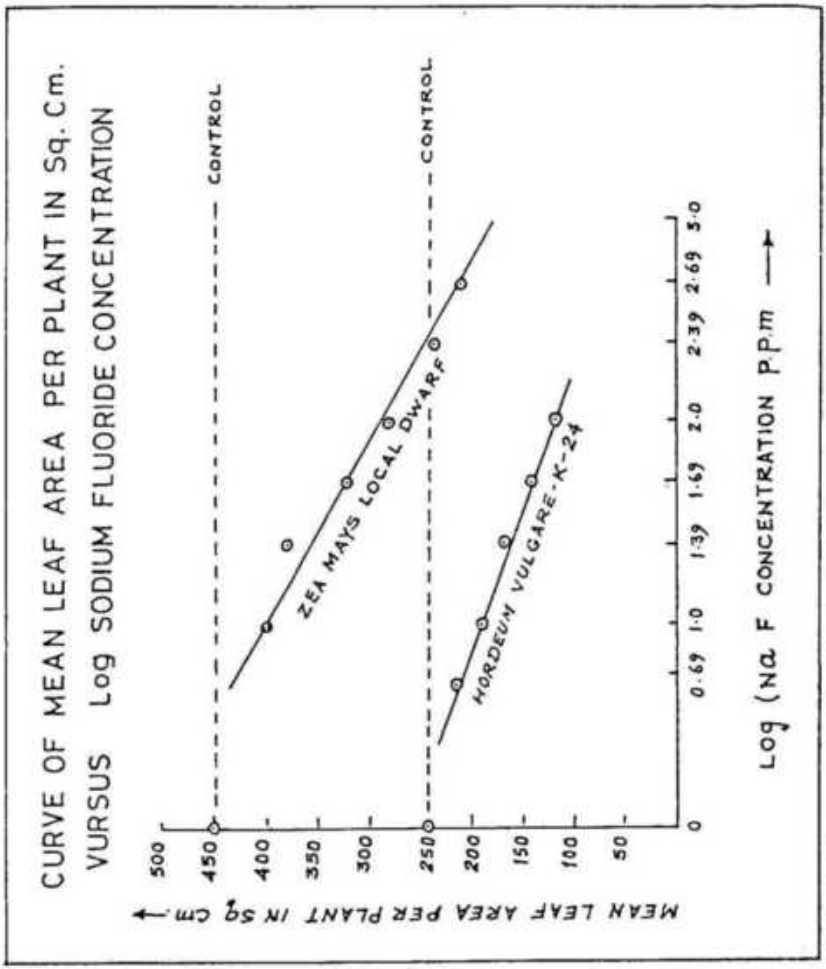

THE REGRESSION OF MEAN LEAF AREA PER PLANT IN
sq. cm ON SODIUM FLUORIDE CONCENTRATION
(a)
HORDEUM VULGARE K-24
Y= 215.3 - 1.048 X
MEAN LEAF AREA PER PLANT IN Sq. cm
NaF CONCENTRATION IN ppm
(b)
ZEA MAYS LOCAL DWARF
Y= 380.3 - 0.41 X
MEAN LEAF AREA PER PLANT IN Sq. cm
NaF CONCENTRATION IN ppm

Figure 2


CURVE OF MEAN LEAF AREA PER PLANT IN Sq. Cm.
VURSUS Log SODIUM FLUORIDE CONCENTRATION
CONTROL
CONTROL
ZEA MAYS LOCAL DWARF
HORDEUM VULGARE-K-24
MEAN LEAF AREA PER PLANT IN Sq cm
500
450
400
350
300
250
200
150
100
50
0
0.69 1.0 1.39 1.69 2.0 2.39 2.69 3.0
Log (Na F CONCENTRATION P.P.m

in the total leaf area of a plant is supposed to be due to the toxicity of sodium fluoride which is brought about through change in either leaf number or leaf size. Variation in leaf size may arise from effects on cell division, resulting in differences in cell number or cell extension (13,17).

Net assimilation rate was also affected at higher concentrations of sodium fluoride. NAR varies with mineral nutrition (23). The effect of mineral nutrition on the growth and the sensitivity to HF of Gladiolus has been studied (23). Tip burn was associated with K, P, and Mg deficiency in plants. Decreased tip burn was correlated with Ca and N deficiency. Symptoms of foliar fluorosis in nitrogen deficient bean plants fumigated for 20 days were observed (12). A possible combination of fluoride with Mg in chlorophyll was studied (18). Variation in NAR may also be due to the accumulation of F^- in the root zone (6). Fluoride affects the root system adversely. The roots become pulpy, pithy, succulant and also rotten when plants are treated with concentrations of sodium fluoride, which cuts off the supply of mineral nutrients and water. The photosynthetic activity of manufacturing food of leaves as well as the carbohydrate metabolism are severely affected by higher doses of sodium fluoride (20). From CD values it was observed that RGR was also affected at the highest concentrations, *i.e.* 100 ppm sodium fluoride in *Hordeum vulgare* and 500 ppm in *Zea mays*. From the above observation it is evident that barley is more susceptible to fluoride toxicity than maize (2).

Conclusion

It is concluded that the toxic effects of sodium fluoride on *Hordeum vulgare* K-24 and *Zea mays* local dwarf are similar: reduction of leaf area, NAR and RGR with higher doses of sodium fluoride. Significant effects were obtained at 5% CD values. Control plants showed the best performance and plants subjected to 100-500 ppm doses were the poorest.

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THE FLUOROSIS PROBLEM IN TROPICAL SHEEP

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SUMMARY: A study of sheep subsisting on fluoride-contaminated forage (up tp 390.0 ppm) in the vicinity of an aluminium factory was undertaken to determine fluorotic lesions in their incisors. Of 83 sheep examined, 67.5% (aged 1 to 3, 3 to 5, 5 to 7 and above 7 years) showed mild to severe degrees of dental lesions and ruptured/chipped-off edges. Their serum and milk fluoride levels were increased during the study period. In addition, urinary fluoride was 10.2 to 57.6 ppm, whereas a maximum of 2.40 ppm fluoride was recorded from controls.

Key words: Aluminium factory; Fluorotic lesions; Mottling; Tropical sheep.

Introduction

Sheep foraging in the vicinity of fluoride-contaminating sources such as ceramic works, phosphate fertilizer plants and aluminium factories often develop fluorosis (1,2), an ailment due to prolonged ingestion of fluoride-contaminated vegetation. The severity of symptoms depends on the fluoride content of forage and duration of ingestion. According to Pierce (3), daily ingestion of 60 mg or more of fluoride caused changes in the teeth of sheep roughly proportional to the amount of fluoride ingested.

Industrial fluorosis in sheep has been extensively studied in Europe (4,5) and Australia (6), and in India some work has been done by Naik and Samal (7). This study assesses the percentage of fluorotic animals (sheep) and fluoride content of their milk, blood serum and urine related to fluoride distribution in forage in the Hirakud area of Orissa.

Materials and Methods

Collection of samples: Sixteen sites around the aluminium factory were selected for collection of forage (legumes and graminoids) samples commonly grazed by local sheep during 1985 through 1986. Plants were air-dried and stored in paper bags. Blood, milk and urine samples of 60 lactating ewes of various age groups from polluted and control areas were also collected monthly in polythene bottles between 5 and 7 o'clock during the same years.

Dental examination: Sheep maintained in and around the Hirakud area were examined for lesions on their incisors. Eighty-three animals of various age groups (1 to 3, 3 to 5, 5 to 7 and above 7 years) were studied and the dental lesions were scored as follows (Figure 1): A, normal appearance; B, well-established brown stain on the surface; C, mottled with black streaks and ruptured edges; D, pitted anamel and chipped-off edges.

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Determination of fluoride: The air-dried plant samples were oven-dried and ground to pass an 85-mesh sieve. Five grams of the powdered sample were made alkaline with calcium oxide suspension, ashed in a muffle furnace at 600-650°C, and fused with sodium hydroxide pellets. The fused material was distilled with perchloric acid and silver perchlorate (8).

About 30 ml milk and 5 ml blood serum were placed in nickel crucibles. The milk was dried with calcium phosphate (9) and the serum with magnesium oxide (10) in a hot water bath. The samples were then ashed in a muffle furnace and fused with sodium hydroxide pellets. The cake of materials was distilled according to Willard and Winter (8).

Fluoride concentrations in distillates of plant, blood serum, milk and urine were determined with a spectrophotometer using Zirconium-eriochrome cyanine-R reagent as described by Megregian (11).

Results

Of the 83 sheep examined, 67% had characteristic markings on their incisors. Animals aged 1 to 3 years were suffering from mild to moderate fluorotic lesions while those older had teeth with dark brown streaks, pitting of the enamel and sometimes edges that ruptured or chipped-off (Figures 1 and 2).

Fluoride in forage has a marked monthly variation and ranged from 24.0 to 360.0 ppm and 27.0 to 390.0 ppm during the peak period (July to December) of metallic aluminium production for 1985 and 1986, respectively (Figure 3). Fluoride accumulation was higher in samples collected nearer to the production sites.

Fluoride levels in milk were lower than in blood serum (Figure 4). The maximum level of 1.60 ppm was in milk from the sheep older than 7 years, whereas samples from the control area ranged from 0.01 to 0.17 ppm. Fluoride in urine varied with ranges of 10.2 to 21.7, 14.6 to 26.5, 15.4 to 42.0, and 19.5 to 57.6 ppm in sheep 1 to 3, 3 to 5, 5 to 7 and above 7 years of age, respectively (Figure 4). In the control area urinary fluoride ranged from 1.10 to 2.40 ppm.

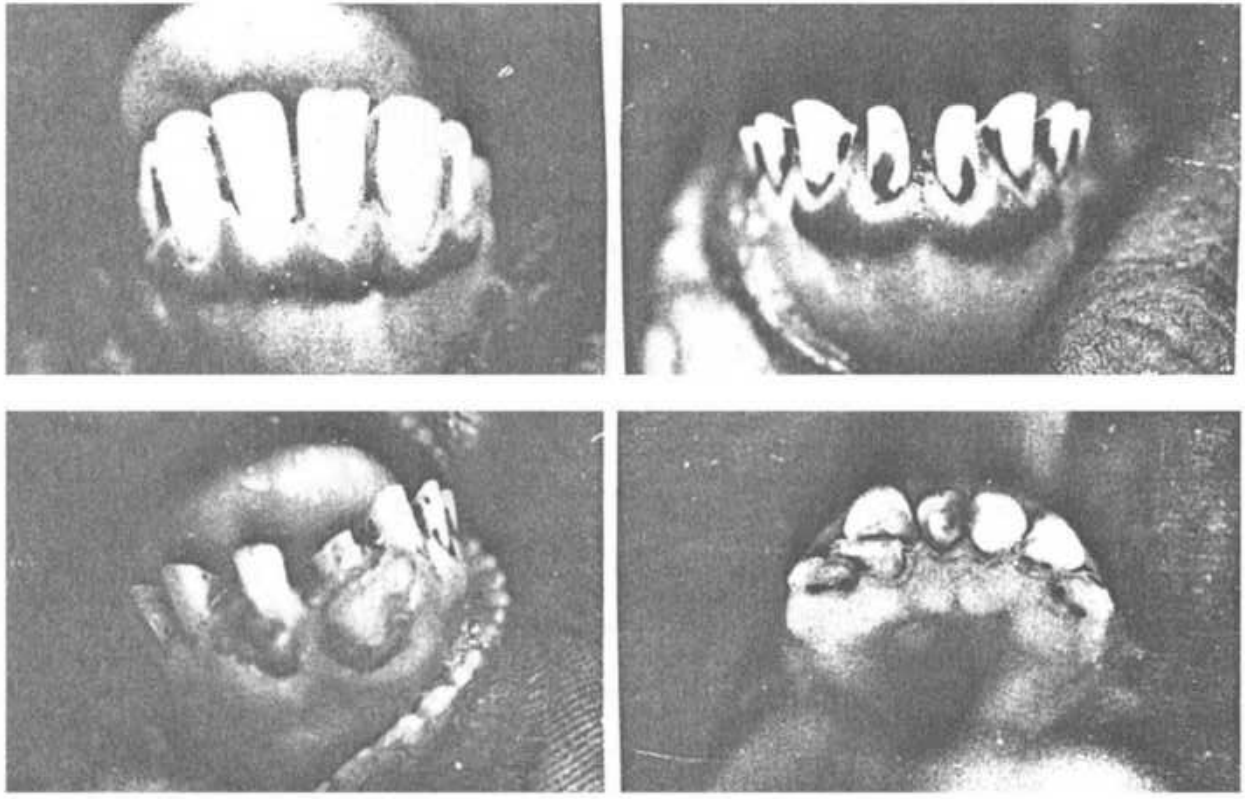
Discussion

The presence of fluoride in developing teeth interferes with calcium metabolism and oxidizes the organic material in the dental structures. When the tooth erupts it bears the characteristic markings of mottling, staining, hypoplasia and hypocalcification (12). The presence of these lesions in a large number of sheep subsisting on the fluoride-contaminated forage grown in the vicinity of an aluminium factory at Hirakud indicates the sheep had dental fluorosis. No dental lesions could be detected in the sheep that came into the endemic area after eruption of their permanent teeth.

Dietary fluoride influences the fluoride content of milk (13,14). According to Sally *et al* (15) milk from treated ewes contained more total fluoride than control ewes. In the present study milk from fluorotic ewes contained 10 times as much fluoride as that from controls.

Figure 1

Teeth of sheep showing various degrees of dental fluorosis



Upper left: Teeth with normal appearance. Upper right: Teeth with well-established brown stain on the surface. Lower left: Teeth mottled with black streaks and ruptured edges. Lower right: Teeth with pitted enamel and chipped-off edges.

Figure 2

Dental fluorosis in sheep foraging within 3 km radius of the aluminium factory at Hirakud

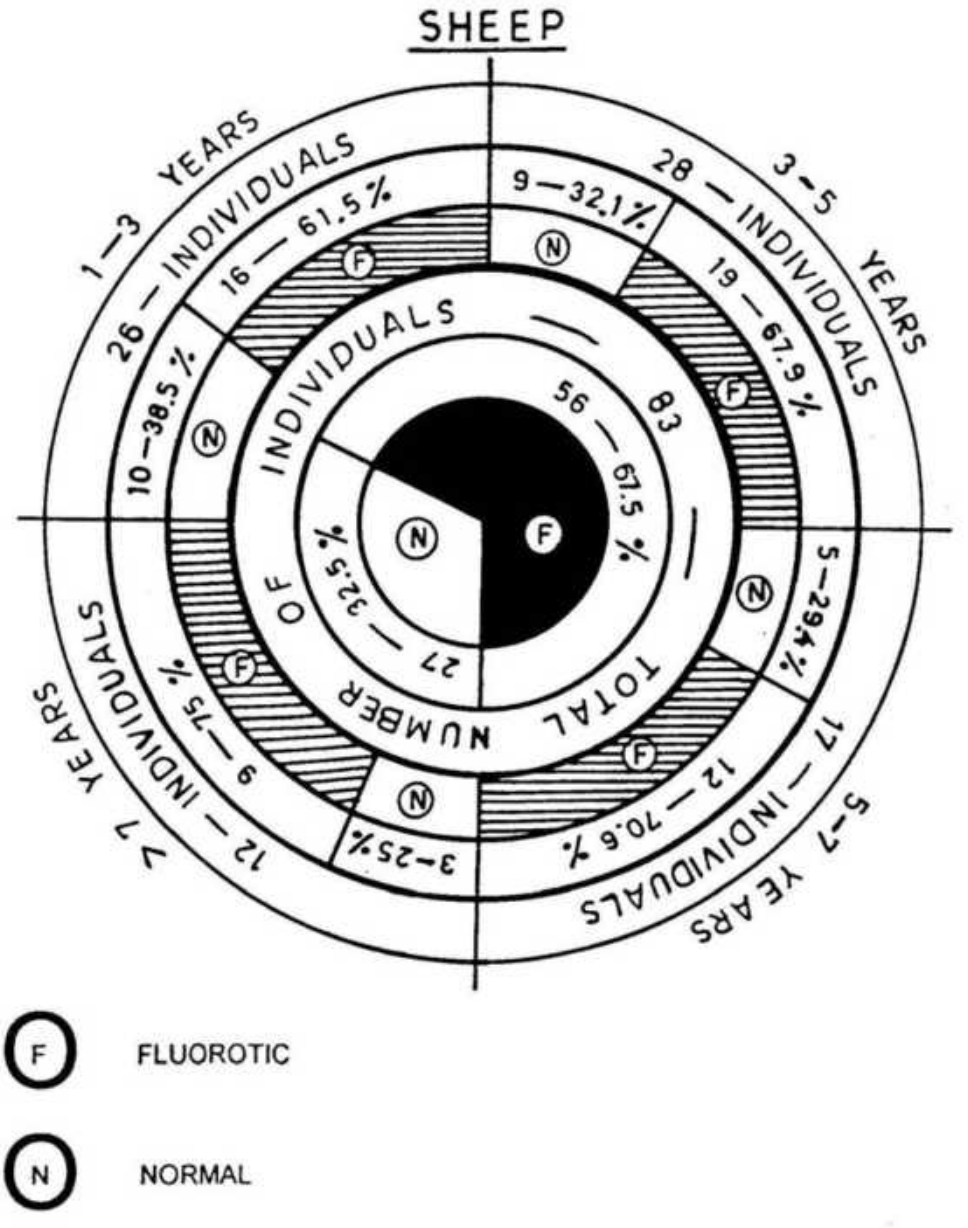


Figure 3

Monthly variation in fluoride content (ppm) of forage collected from different sites around the factory at Hirakud

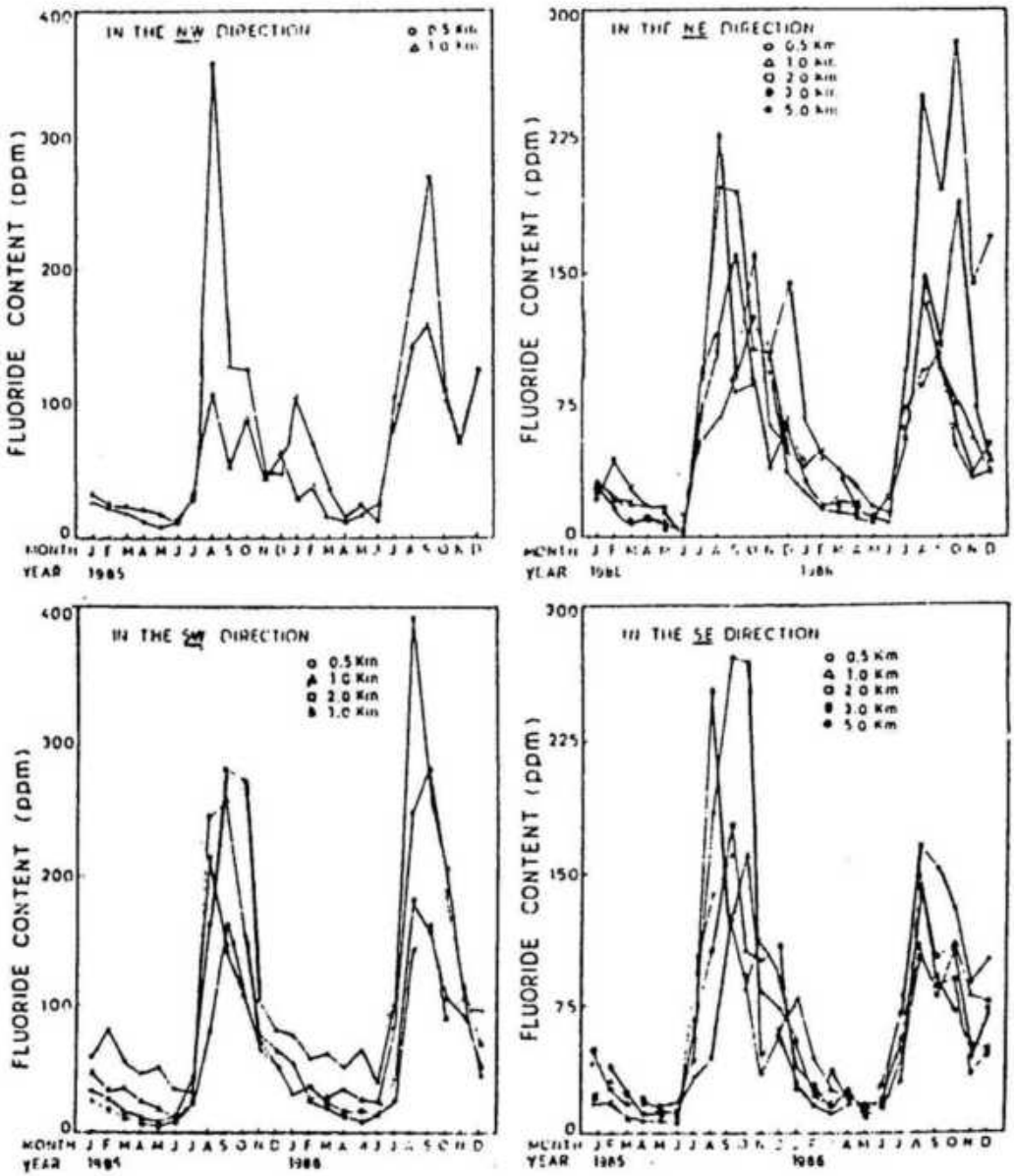
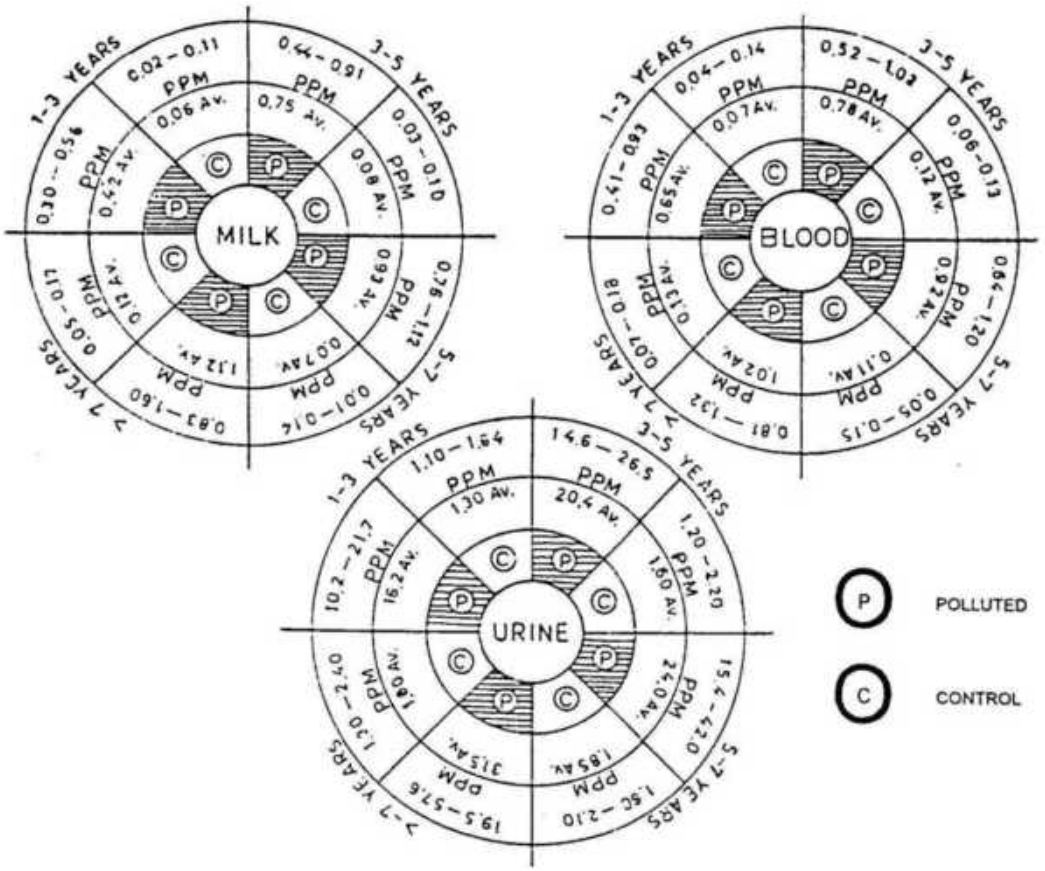


Figure 4

Fluoride concentration in certain biological materials collected from sheep within 3 km radius of the aluminium factory at Hirakud



Ingestion of fluoride-contaminated vegetation results in increased concentrations of blood fluoride (16) and relates to the development of anaemia (17). Hoogstratten *et al* (18) found a correlation between serum fluoride and dietary fluoride. The present investigation reveals that sheep maintained in and around the Hirakud area had increases in the fluoride concentration of their blood that correlated with the fluoride in their forage diet and with their age.

Urinary fluoride can be a good indicator of fluorosis in sheep. According to Boddie (2) fluoride tests of urine are useful to detect fluoride intoxication. He found 19 to 33 ppm F in the urine of cattle with continuous intake of vegetation containing 776 ppm F. Shupe *et al* (16) showed a direct linear relationship between the fluoride concentration in the total ration and that in the urine. They also recorded urinary fluoride concentrations from 8.04 to 14.78 ppm in mild fluorosis, from 10.54 to 20.96 ppm in moderate fluorosis, and from 14.71 to 30.09 ppm in severe fluorosis. In our study urinary fluoride ranged from 10.2 to 57.6 ppm (Figure 4), which indicates that most of the ewes suffered from moderate to severe degrees of fluorosis. Fluoride concentrations in urine also increased with age. Shupe *et al* (16) in their experiments and Rao and Pal (19) in their field investigations made similar observations. A major portion of the fluoride ingested by young and growing sheep is retained by the mineralizing bone structure, whereas the amount of fluoride eliminated through urine is lowered. As the animal grows older, its assimilation capacity is lowered and most of the ingested fluoride is excreted in urine.

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VITAMIN D AND ENDEMIC FLUOROSIS

We read with interest the article "Association of vitamin D deficiency with endemic fluorosis in India" by Mishra *et al* published in the Journal (1). The authors' conclusion that vitamin D deficiency in endemic fluorosis occurs due to lack of dietary vitamin is not supported adequately by their own data or that of others. The major source of vitamin D is sun exposure (2). This is even more true of inhabitants of rural India, the majority of whom are manual labourers exposed to the tropical sun throughout the year. Their diet is cereal based, a poor source of vitamin D. There is poor intake of milk and dairy products. Food products fortified with vitamin D are not available in rural areas. Vitamin D nutritional status of these subjects can not be accurately assessed from calculations based on diet. The accepted method of assessing vitamin D nutrition in any individual is by estimation of serum 25(OH)vitamin D level (2). Serum 25(OH)vitamin D has been reported to be normal in Indian subjects suffering from fluorosis (3,4), including some with radiological evidence of osteoporosis and growth arrest lines.

The data about diet (protein, calories) is inadequately presented. Does 89% deficiency of vitamin D mean that all the habitants of the village were consuming diets 89% deficient in vitamin D, thereby implying that there was no variation in the diet within the village? A similar statement has been made about calories and protein. Was there any correlation of these dietary factors with clinical, biochemical and radiological spectrum, *i e* did subjects with clinical or radiological evidence of vitamin D deficiency have different vitamin D intakes from the others? In the absence of this information it seems difficult to accept the conclusion that dietary vitamin D could have played a major role in determining disease manifestations in the subjects studied.

There is no mention of dietary calcium intake in the subjects studied. Poor calcium intake is rampant in such populations because of low consumption of dairy products. Several reports of rachitogenic effects of low calcium intake are available in literature (5). Calcium deficiency (daily intake less than 150 mg) has been found the causative factor in the pathogenesis of clinically and radiologically proven rickets in Nigerian children. The serum 25(OH) vitamin D and 1,25 (OH)2 vitamin D levels in rachitic children were not significantly different from those in controls but the ratio of 1,25(OH)2 vitamin D to 25(OH) vitamin D was significantly higher than that in controls. Evidence of osteomalacia was present on bone biopsy. Treatment of these children with calcium gluconate (1 gm/d) led to clinical, radiological and biochemical healing of rickets (6). It has been shown in rats that the rate of inactivation of vitamin D in liver is increased by calcium deprivation. The effect is mediated by 1,25 (OH)2 vitamin D produced in response to secondary hyperparathyroidism, which promotes hepatic conversion of vitamin D to polar inactivation products (7).

Furthermore, poor calcium intake has been shown to have a modifying effect on the clinical presentation and radiological pattern of skeletal fluorosis (8,9).

High phytate content in unleavened bread (chupatty) may contribute to development on late rickets and osteomalacia in Asian population (10). Chupatty is the staple diet in North India. Substitution of chupatty with leavened bread of lower extraction in these subjects led to healing of rickets. Sodium phytate enhances the hepatic conversion of 25 (OH) vitamin D, an effect which is similar to calcium deprivation (7). Thus the implication of dietary calcium in study of rickets and osteomalacia is particularly important in North India where the the diet is primarily cereal based with low intake of dairy products.

It has not been mentioned in what percentage of subjects, children and adults, serum alkaline phosphatase levels were high. Fluoride intoxication or calcium deprivation alone in absence of subnormal 25 (OH) vitamin D levels can lead to elevation of serum alkaline phosphotase (4,6). In two of the water samples analysed, the fluoride content has been reported to be normal. It would be of interest to know whether subjects consuming water with normal fluoride had manifestations of skeletal fluorosis, as has been reported from other countries (11).

We feel that the conclusions the authors have derived from their data are not justified. A typical example of how such information may be misleading to the public is evident from the enclosed newspaper write up (12).

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REPLY TO MITHAL AND GODBOLE

Drs Mithal and Godbole suggest the possible role of dietary calcium deficiency in producing rickets and osteomalacia in endemic fluorosis instead of vitamin D deficiency as reported by us. Low dietary calcium leading to rickets is a rare condition. Only a few reports are available and those only in children. It is associated with severe calcium deprivation, dietary intake ranging from 180-150 mg/day (1,2) but such changes have not yet been reported in adults (1-3). In the study from Unnao, rickets and osteomalacia were found in 3 out of 70 patients. Calcium intake of this group was 284±46 mg/day (4). In our study 4 out of 21 adults also had radiological changes suggestive of osteomalacia. Are they implicating the role of dietary calcium deficiency in adults leading to osteomalacia? Daily calcium requirement has been difficult to define (5). Selective deficiency of calcium in human diet is virtually unknown to produce osteomalacia (6). No clear cut disease has been documented even under the condition of low intake as low as 300 mg/day (7). If vitamin D intake is satisfactory osteomalacia and rickets are unlikely with moderate calcium deficiency because of compensatory mechanisms.

In our study we did look at daily calcium and phosphorus intake of 10 families. The average intake was 450 mg and 1000 mg respectively. The radiological changes of rickets and osteomalacia were attributed to vitamin D deficiency which is common in rural areas of the state of Uttar Pradesh. In our subjects serum calcium level was low in 20%, phosphorus was low in 10.5%, alkaline phosphatase was high in 80% of adults and all the children. No doubt serum vitamin D level estimation would have been the ideal method for confirming the deficiency but in its absence dietary analysis with all its limitations was the only way to investigate this problem. The clinical picture, dietary history, radiological features and the commonness of the condition all favoured the possibility of vitamin D deficiency rickets and osteomalacia in our patients.

It seems that the method of dietary analysis has not been understood. The questionary method recommended by the expert group of ICMR was used. Different members of the family were converted into average man coefficient. The nutritive value of all the food items consumed by the family was calculated on the basis of average man coefficient. The results have been compiled into average family requirements of nutrients, actual consumption of nutrients and the difference thereof. Individual correlation of nutritional status with the radiological changes thus was not possible.

Normal serum 25 OH D levels in two Indian studies on fluorosis have been quoted (8,9) and their results have been used to exclude vitamin D deficiency in endemic fluorosis. Both these studies were aimed at studying the endocrinal changes in fluorosis and the subjects in these studies were not reported to be having significant nutritonal deficiency. However, in the study of Srivasta *et al*, one out of five patients did have severe protein and caloric deficiency, low serum calcium and subnormal levels of 25 OH D and 1, 25 (OH) D concentration highlighting the presence of vitamin D deficiency in endemic fluorosis patients (9). Lack of low serum 25 OH D and 1, 25 (OH) D levels in the patients with growth arrest lines in radiographs does not exclude the possible role of vitamin D deficiency. Growth arrest lines indicate nutritional or metabolic abnormality interfering with bone growth at the specific time. They persist even after the deficiency has been made up and hence they may not correlate with present vitamin D level or other parameters of nutritional status. It is surprising that the authors consider dietary calcium deficiency as a cause of osteomalacia and rickets though the dietary calcium intake in their patients was 284±46 mg/day which does not seem to be low enough to produce rickets or osteo-

malacia based on the information currently available (1-3). They presume that vitamin D level in their subjects was normal though neither its serum level nor the dietary intake was estimated (3). Vitamin D deficiency is the commonest cause of nutritional rickets and osteomalacia as mentioned in standard medical texts (10).

Fluorosis produces complex changes in the bone which are because of secondary calcium deficiency produced by fluorosis itself, endocrinal changes which have been extensively reported and the nutritional aspects which have been highlighted by us. The latter may be especially important in India and other developing countries where fluorosis is endemic. Excluding the role of dietary vitamin D because of tropical sunshine, and extrapolating the rare reports of calcium deficiency rickets to the problem of nutritional rickets, and even osteomalacia which is presently regarded to be due to vitamin D deficiency, may be misleading. The suggestion of primary role of dietary calcium deficiency in rickets and osteomalacia should await more scientific evidence.

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RED CELL MEMBRANE ALTERATIONS IN HUMAN CHRONIC FLUORIDE TOXICITY

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Abstract from *Biochemistry International 23* (4) 639-48 1991

Red cells from humans exposed chronically to toxic levels of fluoride through drinking water showed significant increase in lipid peroxidation and membranous cholesterol and phospholipids. Additionally, electrophoretic patterns of ghost membrane proteins revealed the presence of a new brand in the range of ≃ 66 Kd and increase in the high molecular weight protein and predominance of bands with a molecular weight of ≃ 93 Kd and ≃ 20 Kd. The activities of total, Na^+ -K^+ -, Mg^{2+} - and Ca^{2+} - ATPases were significantly decreased in the red cell ghosts of fluorotic patients.

Key words: Chronic fluoride toxicity; Erythrocytes; Red cells.
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DIACYLGLYCEROL GENERATION IN FLUORIDE-TREATED NEUTROPHILS: INVOLVEMENT OF PHOSPHOLIPASE D

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Abstract from *Blood 77* (12) 2746-56 1991

Neutrophils exposed to fluoride ion (F^-) respond with a delayed and sustained burst of superoxide anion release that is both preceded by and dependant on the influx of Ca^{2+} from the extracellulair medium. The results of this study demonstrate a similarly delayed and sustained generation of 1,2-diglyceride in F^--treated neutrophils, over 90% of which was 1,2-diacylglycerol. Diacylglycerol generation was not dependant on the presence of extracellular Ca^{2+}. Conversely, in contrast to results obtained with other agonists, removal of extracellular Ca^{2+} markedly potentiated synthesis of diacylglycerol in F^--treated neutrophils. This effect was accompanied by a corresponding decrease in the recovery of phosphatidic acid. In either the presence or absence of extracellular Ca^{2+}, phosphatidic acid accumulated before diacylglycerol in F^--treated cells, suggesting the latter was derived from the former. Consistent with this hypothesis, the phosphatidic acid phosphohydrolase inhibitor, propranolol, suppressed generation of diacylglycerol as it potentiated the accumulation of phosphatidic acid in F^--treated neutrophils. This effect was observed both in the presence and absence of extracellular Ca^{2+}. Moreover, high levels of propranolol (160μmol/L) effected complete inhibition of diacylglycerol generation in F^--treated neutrophils with a corresponding increase in phosphatidic acid generation. Phosphatidylethanol accumulated in neutrophils stimulated with F^- in the presence of ethanol. The extent of phosphatidylethanol accumulation at all time points after addition of F^- corresponded to decreased levels of both phosphatidic acid and diacylglycerol, indicating that phosphatidylethanol was derived from the phospholipase D-catalysed transphosphatidylation reaction. The results indicate that F^- activates a Ca^{2+}-independant phospholipase D, which appears to be the major, if not sole, catalyst for both phosphatidic acid and diacylglycerol generation in F^--treated neutrophils. Ca^{2+}, mobilized as a result of F^- stimulation and possibly as a consequence of phospholipase D activation, exerts a profound effect on cellular second messenger levels by modulating the conversion of phosphatidic acid to diacylglycerol.

Key words: Diacylglycerol; Fluoride-treated; Neutrophils; Phospholipase D.
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SODIUM FLUORIDE INFLUENCE UPON ENERGY AND PROTEIN LIVER METABOLISM AFTER ITS EXPERIMENTAL ISCHEMIA

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Abstract from *Fiziologicheskii Zhurnal 38* (1) 42-6 1992

The experimental study of 88 white rats has stated that peroral introduction of sodium fluoride at a rate of 1.2 mg per 100 g of mass in animals during 3 month period is followed by the development of fluoride intoxication, that causes a considerable decrease of liver resistance to ishemia and more vivid disturbances of its energy and protein metabolism. The activity of the restoration plastic processes after ishemia decreases. A conclusion is drawn that fluoride can influence the seriousness of illness, ishemia undelying it.

Key words: Sodium fluoride; Liver metabolism; Ischemia
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MULTIPLE ACTIONS OF FLUORIDE IONS UPON THE PHOSPHOINOSITIDE CYCLE IN THE RAT BRAIN

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Abstract from: *Brain Research, 573* 93-101 1990

The effects of sodium fluoride upon basal and agonist-stimulated inositol phospholipid breakdown have been investigated in rat brain miniprisms. NaF concentration independently increased basal inositol phospholipid breakdown, with a maximum effect being seen at 20 mM. NaF reduced the inositol phospholipid breakdown responses to stimulation by carbachol, noradrenaline, serotonin and quisqualate, but not to the stimulation produced by raising the assay (K^+) from 6 to 18 mM. More detailed study demonstrated NaF to have a 'leveling' effect, reducing all InsP/(Lipid+InsP) values > 0.15 (*i.e.* produced by carbachol at raised [K^+], noradrenaline and by 50 mM K^+) to about this value. Time-course experiments indicated that NaF treatment reduced the rate of carbachol-stimulated inositolphospholipid breakdown up to this InsP/(Lipid + InsP) level and thereafter blocked further breakdown. Inhibitory effects upon carbachol-stimulated inositol phosopholipid breakdown were not seen with forskolin, sodium nitroprusside or 8BrcGMP. Under conditions where there is no de nova synthesis of phosphoinositides from [^{3}H]myo-inositol, NaF reduced the total Lipid+InsP labelling by about 20%. NaF in addition inhibits the activity of Ins(1,4)P_2-phosphatase in cerebral cortical homogenates. It is concluded that fluoride ions inhibit agonist-stimulated inositol phospholipid breakdown via actions not only on G-proteins but also on phosphoinositide-specific phopholipase C substrate availability.

Key words: Fluoride ion; G-protein; Muscarinic receptor; Polyphosphoinositide metabolism; Rat brain.
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FLUORIDE, CALCIUM AND PHOPHORUS METABOLISM IN THE RAT: COMPARISON OF 'NATURAL INGREDIENT' WITH SEMIPURIFIED DIETS

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Abstract from: *Archives of Oral Biology 36* 291-7 1991

Three groups of weanling female rats were fed different, commercial available, 'natural ingredient' diets containing 12, 28 or 45 parts/10^6 F, mainly as bone meal, for six weeks. Two other groups were fed a low-fluoride (0.76 parts/10^6) semipurified diet. They received fluoride doses, either in the drinking water or by daily intraperitoneal injection, which were approximately equal to the average dose of the other three groups. Rats on the 'natural ingredient' diets ingested more food and water and excreted more faeces and urine, effects which were attributed to the higher amounts of dietary fibre, Na, K and Cl. Thus, at any given concentration of fluoride in the food or water, the level of fluoride ingestion and the ensuing effects would be influenced by the type of diet used. The values for fractional fluoride absorption (45-49%) and retention (38-47%) were similar among the groups given 'natural ingredient' diets. In the groups given semipurified diet, the corresponding values were about twice as high with the exception that fractional absorption was negative (-41%) in the injected group, which indicated net intestinal secretion of fluoride. Fluoride balances and tissue concentrations were highest in the groups fed the semipurified diet, even though the level of intake was not always higher. The fractional values for calcium and phosphorus absorption (41-51%) and retention (33-43%) were also similar among the groups given 'natural ingredient' diets. The corresponding values were about twice as high in the groups fed the semipurified diet. In terms of supporting maximum bone calcification, phosphorus absorption was marginal in two of the groups on the 'natural ingredient' diets. Because of their variable fluoride concentrations and ill-defined compositions, the use of 'natural ingredient' diets in research should be avoided.

Key words: Absorption; Balance; Bone fluoride; Bone mineralization; cAMP; Dental fluorosis; Enamel fluoride; Enamel mineralization; Faecal excretion; Intake; Osteoporosis; Plasma fluoride; Retention; Urinary excretion.

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A COMPARITIVE STUDY OF FLUORIDE PHARMACOKINETICS IN FIVE SPECIES

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Abstract from: *Journal of Dental Research 70* (6) 948-51 1991

This study was designed to quantitate and compare the major features of the short-term pharmacokinetics of fluoride - *i.e.*, the plasma (Cp), renal (Cr), and extra-renal (Cer) clearances - in young adult dogs, cats, rabbits, rats, and hamsters. Plasma and urine samples were collected for seven h after the iv administration of fluoride (0.5 mg F/kg). Cp ranged from 3.5 to 8.6 mL/min/kg in the dog and hamster, respectively. Cr ranged from less than 1.5 mL/min/kg in the dog and rabbit to about 3.5 mL/min/kg in the rat and hamster. Cer ranged from 2.1 mL/min/kg in the dog to over 4.5 mL/min/kg in the cat,

rabbit, and hamster. It was concluded that 1) there are major quantitative differences in the metabolic handling of fluoride among the five species, and that 2) Cp, Cr, and Cer values of the young adult dog, when factored for body weight, resemble those of the young adult human most closely.

Key words: Cats; Dogs; Fluoride pharmacokinetics; Hamsters; Rabbits; Rats.
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THE EFFECT OF CALCIUM ON DISODIUM MONOFLUOROPHOSPHATE ABSORPTION FROM THE GASTROINTESTINAL TRACT OF RATS

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Abstract from *Research Communications in Chemical Pathology and Pharmacology 77:* (3) 367-74 1992

The absorption of fluoride from disodium monofluorophosphate with or without added calcium has been studied in ligated stomachs and duodena of rats, *in vivo*. Measurements of fluoride absorption from sodium fluoride were also carried out for comparative purposes. The formation constant of the soluble, neutral calcium monofluorophosphate 0 complex has been determined at 20-degrees, 25-degrees and 37-degrees-C. Its value at 37-degrees-C being 315 +/- 10(molar units). The influence of increasing concentrations of calcium on alkaline phosphatase (E.C.3.1.3.1) activity whith disodium 0 monofluorophosphate as substrate has been also studied. Gastric absorption of 2mM disodium monofluorophosphate in the presence of 50mM calcium was much slower than that of 2mM disodium monofluorophosphate alone. The latter was slower than that of 2mM sodium fluoride. The opposite situation has been found for the duodenal fluoride absorption. Results obtained are interpreted in terms of the occurrence of an intestinal monofluorophosphate hydrolysis prior to its absorption as fluoride. In addition, data presented suggest an independent and parallel pathway of fluoride absorption in the form of the liposoluble calcium monofluorophosphate complex.

Key words: Calcium; Disodium monofluorophosphate; Gastrointestinal tract; Rats.
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INHIBITION OF PROTON-TRANSLOCATING ATPASES OF *STREPTOCOCCUS MUTANS* AND *LACTOBACILLUS CASEI* BY FLUORIDE AND ALUMINUM

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Abstract from *Archives of Microbiology 155* 22-7 1990

One of the major effects of fluoride on oral bacteria is a reduction in acid tolerance, and presumably also in cariogenicity. The reduction appears to involve transport of protons across the cell membrane by the weak acid HF to dissipate the pH gradient, and also direct inhibition of the F_1F_o proton-translocating ATPases of the organisms, especially for *Streptococcus mutans*. This direct inhibition by fluoride was found to be dependent on aluminum. The dependence on aluminum was indicated by the protection against fluoride inhibition afforded by the Al-chelator deferoxamine and by loss of protection after addition of umolar levels of Al^{3+}, which were not inhibitory for the enzyme in the absence of fluoride. The F_1 form of the enzyme dissociated from the cell membrane previously had

been found to be resistant to fluoride in comparison with the $F_1 F_0$ membrane-associated form. However, this difference appeared to depend on less aluminum in the F_1 preparation in that the sensitivity of the F_1 enzyme to fluoride could be increased by addition of umolar levels of Al^{3+}. The effects of Al on fluoride inhibition were apparent when enzyme activity was assayed in terms of phosphate release from ATP or with an ATP-regenerating system containing phosphoenolpyruvate, pyruvate kinase, NADH and lactic dehydrogenase. Also, Be^{2+} but not other metal cations, *e.g.* Co^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2}, Sn^{2+}, and Zn^{2+}, served to sensitize the enzyme to fluoride inhibition. The differences in sensitivities of enzymes isolated from various oral bacteria found previously appeared also to be related to differences in levels of Al. Even the fluoride-resistant enzyme of isolated membranes of *Lactobacillus casei* ATCC 4646 could be rendered fluoride-sensitive through addition of Al^{3+}. Thus, the $F_1 F_0$ ATPases of oral bacteria were similar to $E_1 E_2$ ATPases of eukaryotes in being inhibited by Al-F complexes, and the inhibition presumably involved formation of ADP-Al-F^{-}_3 complexes during catalysis at the active side of the enzymes.

Key words: Aluminum; $F_1 F_0$ ATPase; Fluoride; *Lactobacillus casei*; Oral bacteria; *Streptococcus mutans.*

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ALUMINUM STIMULATES THE PROLIFERATION AND DIFFERENTIATION OF OSTEOBLASTS *IN VITRO* BY A MECHANISM THAT IS DIFFERENT FROM FLUORIDE

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Abstract from *Molecular and Cellular Biochemistry 105* 93-105 1991

Micromolar cocentrations of aluminum sulfate consistently stimulated [^{3}H]thymidine incorporation into DNA and increased cellular alkaline phosphatase activity (an osteoblastic differentiation marker) in osteoblast-line cells of chicken and human. The stimulations were highly reproducible, and were byphasic and dose-dependent with the maximal stimulatory dose varied from experiment to experiment. The mitogenic doses of aluminum ion also stimulated collagen synthesis in cultured human osteosarcoma TE-85 cells, suggesting that aluminum ion might stimulate bone formation *in vitro* The effects of mitogenic doses of aluminum ion on basal osteocalcin by normal human osteoblasts could not be determined since there was little, if any, basal secretion of osteocalcin by these cells. 1,25 Dihydroxyvitamin D_3 significantly stimulated the secretion of osteocalcin and the specific activity of cellular alkaline phosphatase in the human osteoblasts. Although mitogenic concentrations of aluminum ion potentiated the 1,25 dihydroxy-vitamin D_3-dependent stimulation of osteocalcin secretion, they significantly inhibited the hormone-mediated activation of cellular alkaline phosphatase activity. Mitogenic concentrations of aluminum ion did not stimulate cAMP production in human osteosarcoma TE85 cells, indicating that the mechanism of aluminum ion does not involve cAMP. The mitogenic activity of aluminum ion is different from that of fluoride because (a) unlike fluoride, its mitogenic activity was unaffected by culture medium changes; (b) unlike fluoride, its mitogenic activity was nonspecific for bone cells; and (c) aluminum ion interacted with

fluoride on the stimulation of the proliferation of osteoblastic-line of cells, and did not share the same rate-limiting step(s) as that of fluoride. PTH interacted with and potentiated the bone cell mitogenic activity of aluminum ion, and thereby is consistent with the possibility that the *in vivo* osteogenic actions of aluminum ion, might depend on PTH. In summary, low concentrations of aluminum ion could act directly on osteoblasts to stimulate their proliferation and differentiation by a mechanism that is different from fluoride.

Key words: Aluminum; Bone formation; Differentiation; Fluoride; Osteoblasts; Proliferation.
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EFFECT OF FLUORIDE ON BONE AND BONE CELLS IN OVARIECTOMIZED RATS

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Abstract from *Journal of Bone and Mineral Research 7* (8) 961-9 1992)

To evaluate whether treatment with a mitogenic agent may increase bone formation and bone mass in osteopenia induced by estrogen deficiency, we determined the effect of oral fluoride treatment on bone and bone cells in ovariectomized rats. Sodium fluoride (NaF) was administered to 3-month-old ovariectomized rats 1 day after ovariectomy (OVX) for 1, 3, and 6 months. NaF was given in drinking water at the dose of 1 mg/kg body weight per day. Fluoride administration led to a partial prevention of the bone loss induced by OVX as shown by histologic analysis of tibial metaphysis and by evaluation of femoral calcium content. These beneficial effects of fluoride were more striking at early time points (1 and 3 months postovariectomy) than after 6 months of treatment. The increase in trabecular bone volume in OVX rats treated with fluoride was associated with a rise in the osteoblast surface, which was increased by 60, 72, and 235% at 1, 3, and 6 months postovariectomy compared to untreated OVX rats. In OVX rats and in sham-operated rats plasma osteocalcin was increased in correlation with the osteoblast surface. However, these two parameters were not correlated in OVX rats treated with fluoride. The heat-labile bone-specific alkaline phosphatase in plasma was decreased in OVX rats treated with fluoride compared to OVX rats, suggesting that both the number and the activity of osteoblasts were affected by NaF treatment. To examine the effect of fluoride on the osteocalcin production and the proliferative capacity of bone cells, osteoblastic cells were isolated by collagenase digestion from the bone surface of tibia in treated and untreated OVX rats. In OVX rats DNA synthesis by cultured bone cells was markedly increased compared to sham rats. In OVX rats treated with fluoride DNA synthesis tended to be further increased compared to untreated OVX rats as evaluated by thymidine incorporation into DNA. Osteocalcin production by osteoblastic cells in vitro was comparable in the different groups. The results of this study show that oral treatment with fluoride partially prevents the bone loss induced by estrogen deficiency in OVX rats. This beneficial effect of fluoride results from a further stimulation of bone formation as shown in vivo by an increased extent of bone-forming cells and in vitro by an enhancement of the proliferative capacity of osteoblastic cells isolated from the bone surface.

Key words: Bone; Bone cells; Estrogen deficiency; Fluoride; Ovariectomy; Rats.
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INCORPORATION OF SODIUM FLUORIDE INTO CORTICAL BONE DOES NOT IMPAIR THE MECHANICAL PROPERTIES OF THE APPENDICULAR SKELETON IN RATS

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Abstract from *Calcified Tissue International 51* (2) 127-31 1992

Clinical studies on the use of sodium fluoride (NaF) in osteoporotic patients have demonstrated increased spinal bone mass without a reduction in vertebral fracture incidence, and a trend towards reduced appendicular bone mass with an increase in peripheral fracture incidence. As previous reports have suggested that NaF becomes incorporated into bone's crystal structure, possibly affecting bone strength, we sought to examine the relationship among bone fluoride content, bone mass, and skeletal fragility. Twenty-one-day-old female Sprague-Dawley rats were treated with four different doses of NaF. The tibiae were subjected to histomorphometric and biochemical analyses, and the femora were tested in torsion for the properties of strength, stiffness, energy storage capacity, and angular deformation. The results showed that over 50% of the skeleton in these rats was turned over in the presence of NaF. The four different doses resulted in a linear increase in bone F concentration and suggested excellent absorption and incorporation of this drug. No changes in histomorphometric indices of bone formation or turnover were found. Despite the large fraction of bone formed during NaF treatment, and the linear increase in bone fluoride content in relation to dose, there were no changes observed in any of the mechanical properties. These results suggest that, even extensive incorporation of fluoride into bone, in the absence of an effect on bone mass or remodeling, does not significantly alter its capacity to withstand mechanical loads.

Key words: Sodium fluoride; Cortical bone; Mechanical properties; Histomorphometry; Bone mass.

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A HISTOMORPHOMETRIC ANALYSIS OF THE EFFECTS OF FLUORIDE ON EXPERIMENTAL ECTOPIC BONE FORMATION IN THE RAT

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Abstract from *Journal of Dental Research 70* (6) 957-60 1991

Ectopic bone formation was induced in 14 rats receiving 100 ppm fluoride in drinking water and in 14 control animals. Sections from ossicles removed after 14 and 20 days were sampled for stereological analysis. Bone volume density and bone volume were reduced in experimental animals on day 14 ($p < 0.05$). This difference was no longer present after 20 days. On day 20, surface density and areas of formative surfaces were increased in the fluoride group ($p < 0.05$). Osteoid seam thickness was higher in the fluoride group on both days ($p < 0.01$). In conclusion, fluoride induced quantitative alterations in ectopic bone formation, and the presented model may prove a useful addendum to previous methods for investigation of fluoride effects on mineralization processes *in vivo*.

Key words: Ectopic bone formation; Fluoride; Histomorphometric analysis; Rats.

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THE EFFECTS OF FLUORIDE ON BONE

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Abstract from: *Clinical Orthopaedics and Related Research 267* 264-7 1991

Fluoride has often been used as a treatment for osteoporosis, a metabolic bone disease of considerable importance in the elderly population. The techniques currently used to monitor a patient's response to fluoride are outlined. New findings concerning 1) a mechanism for interaction of fluoride with osteoblasts (via mitogenic signals or growth factors); 2) toxicity and carcinogenesis;(3) recent clinical trial data; and 4) the importance of dosage, administration regimens, and side effects in an effective fluoride treatment protocol are reviewed. Some recent clinical data challenge the efficacy of fluoride in the treatment of postmenopausal osteoporosis. Because of the implications of these recent studies with respect to fracture incidence during fluoride therapy, fluoride cannot be recommended at this time for general use in the treatment of osteoporosis.

Key words: Bone; Fluoride; Osteoporosis.
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FLUORIDE THERAPY FOR OSTEOPOROSIS:A REVIEW OF DOSE RESPONSE, DURATION OF TREATMENT, AND SKELETAL SITES IN ACTION

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Abstract from *Calcified Tissue International 49* (Suppl) S64-7 1991

Osteoporosis is a disease characterized by a reduction in bone density which predisposes to fracture after even minimal trauma. Fluoride, because it has consistently been shown to stimulate bone formation and increase trabecular bone density, has been widely studied for the treatment of osteoporosis. The article focuses on the dose response, duration of treatment, and skeletal sites of action of fluoride; we also include comments on the effect of fluoride on vertebral and appendicular fracture rates. The skeletal response to fluoride doses, ranging from 15 to 43 mg elemental fluoride per day, included a linear increase in spinal bone density at an average rate of 1.25 ± 0.91 mg/cm^3 per month. The rate of increase in spinal bone density was related to the dose of fluoride ($r = 0.34$, $P < 0.03$). Spinal bone density had increased above the fracture threshold in 44% of patients treated with fluoride for 32 ± 10 months. The time required to achieve this goal was, however, influenced by the pre-treatment spinal bone density and interpatient variation in response to fluoride treatment. Patients whose spinal bone density remained below the fracture threshold had lower pretreatment bone densities and/or slower rates of increase in spinal bone density ($P < 0.001$). The osteogenic effect of fluoride was not limited to the spine. After 2 years of fluoride therapy, we found bone density in the femoral condyle (measured by QCT) to have increased by 13 ± 2.5 mg/cm^3 ($n = 38$, $P < 0.001$); bone density in the hip (measured by DPA) was increased by 0.0261 ± 0.015 g/cm^2 ($n = 55$, $P < 0.025$). The efficacy of fluoride therapy to reduce fractures is not well established. Recently, investgators from the Mayo Clinic and Henry Ford Hospital reported fluoride had no effect on the vertebral fracture rate despite a significant increase in spinal bone density, but this finding has not been supported by findings in other studies. Moreover, our preliminary analysis of over 500 fluoride-treated patients found a time-dependant decrease in vertebral fracture rate related to a corresponding increase in spinal bone density. We conclude that these data, together with the many other positive

international findings related to fluoride, justify continued investigation of this potent agent for the treatment of osteoporosis.

Key words: Fluorides; Osteoporosis.
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IS FLUORIDE TREATMENT JUSTIFIED TODAY?

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Abstract from *Calcified Tissue International 49* (suppl) 568-9 1991

Fluoride has been used for the treatment of osteoporosis since 1961, because it increases trabecular bone mass in the spine and may be effective in the treatment of spinal osteoporosis. Fluoride treatment is still controversial because of its side effects, the high rate of non-responders, possible osteomalacic effect on bone, deleterious effects on cortical bone, and especially because of its uncertain effect on fracture rate. At present, fluoride therapy is highly questionable in the prophylaxis and treatment of osteoporosis.

Key words: Fluoride; Osteoporosis.
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DOSE EFFECTS ON EWE BONE REMODELING OF SHORT-TERM SODIUM FLUORIDE ADMINISTRATION: A HISTOMORPHOMETRIC AND BIOCHEMICAL STUDY

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Abstract from *Bone 12*: (6) 421-7 1991

The early effects of two doses of sodium fluoride (NaF) on bone remodeling were studied in 14 ewes divided into two groups. Group I received orally 1 mg NaF/kg/day and group II received a five-fold greater dose. No calcium supplement was given. Transiliac bone biopsies and blood samples were taken before treatment (T0) and after 45 (T45) days of treatment. Bone fluoride content significantly increased in group II. In both groups, a significant decrease of serum calcium and phosphorus, and a slight but nonsignificant augmentation in serum parathyroid hormone were noted. Osteoid perimeter and area were significantly increased. The osteoid width significantly increased in both groups, but was twice higher in group II than I. At T45, the osteoblast perimeter increased in both groups. Osteoid perimeter was significantly correlated with serum osteocalcin values ($r = 0.74$, $p < 0.001$) and bone fluoride content ($r = 0.64$; $p < 0.01$). The bone formation rate at tissue level tended to increase in both groups. Concerning the apposition rate, a decrease was noted which was 1.5-fold higher in group II than in I. The increased formation period resulted from a prolonged inactive period in group II. These results point out a stimulatory effect of fluoride on the birth rate of osteoblasts. However, fluoride prolonged the lifespan of osteoblasts that had reduced activity.

Key words: Biochemical investigations; Bone remodeling; Dose; Ewe; Histomorphometry; Sodium fluoride
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FLUORIDE-INDUCED BONE CHANGES IN LAMBS DURING AND AFTER EXPOSURE TO SODIUM FLUORIDE

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Abstract from *Osteoporosis International 2* 26-33 1991

The evolution of bone changes induced by fluoride after the end of exposure was investigated in lambs. Sodium fluoride (NaF) was given orally at a dose of 3.5mg/kg per day to 14 animals for 120 days. A group of 7 controls and 7 treated lambs was slaughtered at the end of NaF administration (T120) and another group 120 days after the end of NaF exposure (T240). At T120, the bone fluoride content (BFC) was very significantly increased in treated animals. The histomorphometric analysis confirmed that fluoride induces an increase in bone formation (the osteoid perimeter and area were 3-fold and 4.5-fold higher respectively in treated than in control animals). The number of osteoblasts was significantly augmented. Serum osteocalcin level was twice as high in treated animals compared with controls. The bone formation rate at the tissue level (BFR) doubled after treatment, but the apposition rate (Aj.AR) was half that in the control group. The mineralization lag time (Mlt) was 120 days in treated animals compared with 42 days in controls. At T240, BFC had decreased by 50% compared with the level at T120, but it was still significantly higher than in controls. The osteoid and osteoblastic parameters were 2 and 1.3 times higher than in control animals. BFR remained significantly increased in treated animals, but Aj.AR and Mlt were similar in control and treated animals. In conclusion, after 4 months of NaF exposure fluoride induced an increase in osteoblast natality and bone formation at the tissue level, associated with a toxic effect at the individual cell level. Four months after the end of NaF exposure, positive effects on bone formation were still present but the evidence of cellular toxicity had disappeared.

Key words: Bone fluoride content; Bone remodeling; Fluoride; Histomorphometry; Lambs.
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STUDIES OF FLUORIDE RETENTION BY ORAL SOFT TISSUES AFTER THE APPLICATION OF HOME-USE TOPICAL FLUORIDES

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Abstract from *Journal of Dental Research 71* (9) 1546-52 1992

Previous studies have focused on enamel and plaque as the primary sites of fluoride (F) retention in the mouth. The present study was undertaken to evaluate the role of oral soft tissue as a site of F retention by comparing an edentulous subject panel ($n = 9$) with a fully dentate panel ($n = 10$). Unstimulated whole saliva samples were collected by having subjects pool saliva for two min. Samples were collected over a 24-hour period after application of a placebo dentifrice (PD; 0.4 ppm F), fluoride dentifrice (FD; 1 100 ppm F), fluoride rinse (FR; 226 ppm F), or fluoride gel (FG; 5000 ppm F) delivered in custom trays. There was no statistically significant difference in salivary flow rate between the two panels for any of the treatments. The edentulous panel had higher salivary F levels than the dentate panel, which reached statistical significance ($p < 0.05$) for the FD and FG treatments. In a separate study involving the same treatments, F levels at specific soft-tissue sites were measured over a one-hour period by use of absorbent discs placed in

different soft-tissue areas of the mouth. The tongue and lower posterior vestibule retained the highest F levels, followed by the upper posterior buccal vestibule and upper anterior the highest F levels, followed by the upper posterior buccal vestibule and upper anterior labial vestibule, with the lowest F levels retained in the lower anterior vestibule and the floor of the mouth. There was a strong-to-moderate correlation between whole saliva F concentration and F levels at specific soft-tissue sites. This study establishes the importance of oral soft tissue as the major site of F retention in the mouth.

Key words: Fluoride retention; Oral soft tissues; Topical fluorides.
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EFFECTS OF FLUORIDE ON SECRETORY AND POSTSECRETORY PHASES OF ENAMEL FORMATION IN SHEEP MOLARS

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Abstract from *American Journal of Veterinary Research 53* (7) 1241-7 1992

Effect of fluoride was assessed on molars during and after mineralization. Two groups of 7 sheep each were dosed orally with 3.5mg of fluoride/kg of body weight daily for 4 months (from 5 to 9 months after birth). Sheep of the first group were slaughtered immedately after fluoride administration; those of the second group were slaughtered 4 months later at the age of 13 months. Three control groups of 7 sheep each were slaughtered at 5 months (to determine the state of the teeth at the beginning of fluoride administration), and at 9 and 13 months.

During fluoride administration, plasma fluoride concentration rapidly increased to about 0.50 µg/ml; after fluoride administration, it stabilized at 0.20 µg/ml in treated sheep, whereas controls had concentration of 0.10 µg/ml ($P<0.01$).

Parts of the molars that were in the process of mineralization during fluoride administration (mainly second molars) had thinning enamel, with pits, mainly close to the apex, marked decrease in hardness throughout the layer (< 100 Vickers U, compared with 240 Vickers U), and fluoride accumulation twice as high as that in controls. (1,000 to 2,500 mg/kg [dry weight]). Fluoride accumulation was higher in dentine (2,700 to 4,200 mg/kg), but hardness was less affected.

On parts of the molars that were already mineralized (mostly, the first molar), changes in the appearance of the enamel and cementum, decreased hardness (less important than in teeth during mineralization) affecting outer enamel more than inner enamel, high fluoride concentration (4,000 to 5,500 mg/kg [dry weight]) in outer enamel extending over 200 µm were observed. Thus, in sheep, fluoride has a substantial post-secretory effect that may be explained by a slower maturation phase of enamel in this species.

Because molar wear is correlated to enamel hardness (dentine at the occlusal surface has low resistance - 30 Vickers U), abnormal abrasion of molar teeth that have mineralized before and during fluoride intakes can be observed.

Key words: Fluoride; Enamel formation; Sheep molars.
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INFLUENCE OF FLUORIDE AND CARBONATE ON *IN VITRO* REMINERALIZATION OF BOVINE ENAMEL

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Abstract from: *Journal of Dental Research 70* (6) 970-4 1991

The influence of fluoride, carbonate, and fluoride in combination with carbonate on the *in vitro* remineralization of bovine enamel was investigated with the use of a sandwich technique. After demineralization, enamel slices were subjected for 610 h to remineralizing solutions with 0.03 or 1.0 ppm fluoride. At each fluoride level, either 0, 1, 10, 20, or 25 mmol/L carbonate was tested. After 0, 22, 62, 126, 192, 329, and 610 h of remineralization, contact microradiographs were made by Cu Kα-radiation. At 0.03 ppm fluoride, carbonate had an inhibiting influence on remineralization. At 1.0 ppm fluoride, the inhibiting influence of carbonate changed into a stimulation of remineralization at 20 and 25 mmol/L carbonate. At 0, 1, and 10 mmol/L carbonate, fluoride had an inhibiting influence on remineralization. The differences in remineralization between the groups were explained by events concerning crystal growth, *i.e.*, different types of minerals might have precipitated with differences in precipitation rates, and retardation of a precipitation step might have occured under the various remineralization conditions. There was a mutual influence of fluoride and carbonate on the remineralization process. We conclude that the composition of the remineralizing solution with respect to fluoride and carbonate concentrations is important for the remineralization process.

Key words: Carbonate; Fluoride; Remineralization.
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BENEFITS AND RISKS OF FLUORIDE SUPPLEMENTATION: CARIES PREVENTION VERSUS DENTAL FLUOROSIS

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Abstract from *European Journal of Pediatrics 151* (8) 613-6 1992

To assess the risks (dental fluorosis) and the benefits (caries prevention) of fluoride (F) tablets and F toothpaste, we surveyed 2003 schoolchildren aged 5-20 years old (mean = 10.82, SD = 3.40). Children were scored for dental caries by means of the decayed, missing, filled teeth index (DMFT index). Frequent use of F toothpaste (toothbrushing frequency) is poorly linked to caries (Spearman r = 0.05, P = 0.02) and dental fluorosis (r = 0.05, P = 0.03). Children who use F tablets regularly and appropriately exhibit mild fluorosis more often than non- or occasional users (odds ratio = 9.58), and have a mean DMFT index 50% lower than other children. We conclude that using F tablets is an effective means of preventing caries. When used appropriately in non fluoridated areas, using F tablets results in minor damage.

Key Words: Caries; Caries prevention; Dental fluorosis, Fluoride.
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Asta J 96
Bannie F 200
Barker D J P 48-49, 49-50
Barot J M 101-110
Baylink D J 201, 202, 202-203
Belandria G 96
Berry H 92
Bhargava S K 65-70
Biles E D 197-198
Birdsong-Whitford N L 197-198
Björklund P-E 196
Boivin G 203, 204
Borggrreven J M P M 206
Brännström G 196
Brody J A 47-48
Brown R H 95
Butler J E 97
Cai J 123-128
Carrasco G 198
Cedel S L 45
Chao E Y S 45
Chapuy M C 203, 204
Charles E 205
Chavassieux P 203, 204
Chellappah N K 94
Chen Y 123-128
Chinoy N J 71-76, 101-110
Chlubek D 171-174
Chowdhury N G 95
Clark M K 50
Colquhoun J 1-4
Cooper C 48-49, 49-50
Czarnowski W 129-134
Dahiya I S 135-142
Danielson C 161
Das T K 5-22
Deka R C 5-22
Delmas P D 203, 204
Dhoore W 206
Diagbouga P S 85-91
Dote T 55-64, 97
Driessens F C M 206
Dure-Smith B A 202-203
Egger M 161
Einhorn T A 201
Ekstrand J 97
English D 196
Eymontt M J 92
Faierman E 201
Farley S M 202-203
Featherstone J D B 204-205
Feuga M 200
Fowler C J 196
Fu J 204-205
Garcia J G N 196
Garrec J-P 96
Garrido A 198
Gear K A 93
Ghodasara N B 101-110
Ghosh P 5-22
Gikunju J K 37-43, 111-114
Githui K 111-114
Godbole M M 191-192
Goldberg J 47-48
Goodenough G K 161
Grewal M S 135-142
Gritsan N P 115-122
Gruber H E 202
Gujral R B 65-70
Gupta I P 5-22
Gupta S 143-148
Hayes A L 204-205
Hodgson S F 45
Hong J 123-128
Ibrahim Th A 23-36
Inkovaara J A 203
Inoue S 55-64, 97
Ishii T 160-161
Jacobsen S J 47-48, 49-50
Jannausch M L 50
Joseph-Enriquez B 85-91
Joshi S M 101-110
Joubertt C 205
Kacker S K 5-22
Karam M H 23-36
Keltjens H M A M 94-95
Koch W 93
Kolf-Clauw M 205
Kono K 55-64, 97
Kraenzlin M E 202-203
Kragstrup J 201
Krechniak J 129-134
Kumari D S 195
Lacey R F 48-49
Lammers P C 206
Lau K-H W 199-200
Lee J R 162-164
Li J 123-128
Libanati C R 202-203
Lindsay R 46
Linkhart S 201
Lo G L 94
Loubière M-L 205
Lyon J L 161
Machoy Z 99-100, 171-174
Machoy-Mokrzynska A 96, 99-100, 169-170
Maitho T E 111-114
Maloney S 201
Marie P J 200
Marier J 166-167
Marquis R E 198-199
Martin B 51-52
Martin J H 92

Mehta U 143-148
Melton III L J 45, 47
Meunier P J 203, 204
Miles T P 47-48
Milhaud G 85-91, 204, 205
Miller G W 53-54, 170
Miravet L 200
Misra U K 65-70, 193-194
Mithal A 191-192
Muhs J M 45
Modrowski D 200
Moller F 93
Münzenberg K J 93
Nagaie H 55-64, 97
Naik B N 183-194
Narayana M V 101-110
O'Fallon W M 45
Orita Y 55-64, 97
Parikh D J 101-110
Pastoureau P 203
Pedersen A M 204-205
Pedik V P 195
Pitt P 92
Purohit R M 101-110
Rao P R 195
Rathore S 175-182
Raubertas R F 204-205
Riggs B L 45
Rimm A A 47-48
Robson H J 93
Rose D 167-168
Rush E B 201
Samal U N 183-190
Satam M 97
Schaeken M J M 94-95
Schnitzler C M 93
Schultz E E 202-203
Sequeira E 71-76, 101-110
Sharma V P 65-70
Shashi A 77-84, 149-158,
Shepherd M G 95
Singh A 143-148
Singh J P 155-158
Small J S 164
Sowers MF R 50
Steltz M D 9
Stiers W 47-48
Sturr M G 198-199
Suckling G 160-161
Sumujlo D 171-174
Susheela A K 5-22
Sutton P R N 159-160
Tandon R K 5-22
Tanioka Y 55-64, 97
Taylor G 196
Thapar S P 155-158
Tiger G 196
Tyrtyshnikov M 195
Umebayashi K 55-64, 97
Valenzuela A 198
Van Der Hoeven J S 94-95
Van Dijk J W E 206
Vannieuwenhuysen J P 206
Vignehsa H 94
Villa A 198
Wahner H.W 45
Wakley G K 201
Wallace R B 50
Wang J 123-128
Wang S P 199-200
Wang W 123-128
Watanabe H 55-64, 97
Watanabe M 55-64, 97
Weingrad T R 92
Whitford G M 197, 197-198
Wickham C 48-49
Wickham C A C 49-50
Wieczorek P 171-174
Wig J R 93
Wing J R 93
Wrzesniowska K 129-134
Yoo A 199-200
Yoshida Y 55-64, 97
Zero D T 204-205
Zhan C 123-128

Absorption 197
Acrosomal integrity 71-76
Adjuvant 97
Aging 55-64
Agro-climatic zones 135-142
Albino rabbits 149-158
Aluminium, Aluminum 199-200
  factory 183-190, 198-199
Alluvial planes 135-142
Alpine valley 96
Amino acids 149-158
Anaemia 23-36
Antibody 97
Balance 197
Barley 115-122, 175-182
Bioaccumulator 96
Biochemical investigations 203
Biological index 97
Bone(s) 50, 123-128, 129-134, 200, 202
  cells 200
  cortical 201
  fluoride 197
  formation 199-200
  fluoride content 204
  fractures 93
  fragility 1-4, 93
  marrow 159-160
  mass 201
  mineralization 197
  remodeling 203, 204
  scanning 92
Boreholes 111-114
Brain 77-84
Calcium 50, 198
Camels 23-36
Carbonate 206
Caries 206, 207
  prevention 206
Cats 197-198
Cattle 85-91
Chlorhexidene varnish 94-95
Cholesterol 77-84, 149-158
chronic fluoride toxicity 195
Cortical bone 201
Dental caries 206
Dental fluorosis 160-161, 169-170, 183-190, 206
Dialcylglycerol 195
Differentiation 199-200
Diet 37-43
Disodium monofluorophosphate 198
Dose 203
Drinking water 129-134
Ecologic studies 49-50
Ecological analysis 47-48
Ectopic bone formation 201
Enamel
  defects 94
  formation 205
Environment 96
Erythrocytes 195
Estrogen deficiency 200
Ewe 203
Experimental animals 149-158
Experimental fluorosis 149-158
Faecal excretion 197
Fertility impairment 71-76
F_1F_0 ATPase 198-199
Fish 37-43
Fluoridated water 159-160
Fluoridation 47-48, 48-49, 49-50, 50, 51-52, 95, 159-160, 161, 162-164, 171-174
Fluoride 77-84, 94, 97, 101-110, 198-199, 199-200, 200, 201, 202, 203, 204, 205, 206, 206
  adsorbtion 96
  accumulation 37-43, 96
  administration 97
  binding 85-91
  bone 197
  ingestion 5-22
  in nails 96
  intake 143-148
  in urine 129-134
  ion 196
  pharmacokinetics 197-198
  pollution 96
  potable water 143-148
  retention 204-205
  therapy 45, 46, 47, 92
  -treated 195
  92, 93, 93
  varnish 94-95
  water 111-114, 143-148
Fluorides 50, 202-203
  topical 204-205
Fluorine 99-100
  Symposium, Poland 169-170, 170
Fluorine-containing industrial
  emissions 115-122
  water soluble soil 135-142
Fluorosis 37-43, 65-70
  experimental 149-158
Fluorotic lesions 183-190
Fractures 50
Free fatty acid 77-84, 149-158
Gastrointestinal discomfort 5-22
Gastrointestinal tract 198
Gel filtration 171-174
Goats 123-128
G-protein 196

Grain crops 115-122
Haemogram 23-36
Hair color 55-64
Hamsters 197-198
Haryana, India 135-142
Hip fracture 45, 46, 47, 47-48, 48-49, 49-50, 92, 161
Hisar 143-148
Histomorphometry 201, 203, 204
Histomorphometric analysis 201
Human population 101-110
Immunosuppressive 159-160
India 65-70
Industrial fluorosis 23-36, 123-128
Infant fluoride intake 95
Intake 197
International Society for Fluoride Research 53
Ischemia 196
Japan 160-161
Kenya 37-43
Lactobacillus casei 198-199
Lake Naivasha 37-43
Lambs 204
Latitude 47-48
Leaf area 175-182
Lichens 96
Liver metabolism 196
Marier, John R 166-168
Maurienne 96
Mechanical properties 201
Mehsana 101-110
Metal shift 97
Mice 71-76
Milk proteins 171-174
Mottling 160-161, 183-190
Muscarinic receptor 196
NaF 71-76 (see Sodium fluoride) toxicity 175-182
Nairobi 111-114
Net Assimilation Rate 175-182
Neutrophils 195
New Zealand 95
Nonulcer dyspepsia 5-22
Nutrition 65-70
Oral bacteria 198-199
Oral soft tissues 204-205
Oreochromis leucostictus 134
Osteoblasts 199-200
Osteofluorosis 5-22
Osteomalacia 65-70
Osteoporosis 45, 46, 47, 48-49, 92, 92, 93, 93, 162-164, 197 202, 202-203, 203
Osteosclerosis 5-22
Ovariectomy 200
Peyers' patches 97
Phospholipase D 195
Phospholipids 77-84, 149-158
Phosphorus 99-100
Phytotoxic effects 115-122
Plasma constituents 85-91
Plasma fluoride 197
Poland 169-170, 170
Pollution 115-122
Polyphosphoinositide metabolism 196
Potable water fluoride 143-148
Poverty 47-48
Proliferation 97, 199-200
Proteins 149-158
milk 171-174
Rabbit(s) 77-84, 149-158, 197-198
Rat brain 196
Rats 129-134, 197-198, 198, 200, 201
Red cells 195
Relative Growth Rate 175-182
Remineralization 206
Retention 197
Root-surface caries 94-95
Root surface microflora 94-95
Rural location 47-48
Scientific knowledge 51-52
Sex 55-64
Sheep molars 205
Singapore 94
Sodium fluoride 71-76129-134, 149, 158, 196, 201, 203
hydrogen carbonate 129-134
toxicity 175-182
Social dynamics 51-52
Spatial variability 135-142
Sperm count 71-76
Sperm motility 71-76
Streptococcus mutans 198-199
Stress fractures 93
Sunlight 47-48
Survay study 101-110
Teeth 123-128, 129
Tilapia Fish 134
Testes 149-158
Topical fluorides 204-205
Total lipids 77-84, 149-158
Triglycerides 77-84, 149-158
Tropical sheep 183-190
Ukraine 115-122
Underground water 143-148
Urinary excretion 197
Utah 161
Vertebral fractures 92
Vitamin D 65-70
Water fluoride level 143-148
Water hardness 47-48
Water soluble soil fluorine 135-142
Wheat 115-122
X-ray fluorescence analysis 55-64

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Papers should present original investigations. Review papers are also accepted.

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4. **Key words.** List the major themes or subjects.

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