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TABLE OF CONTENTS

GUEST EDITORIAL

Is Fluoridation Effective 51-53

ORIGINAL ARTICLES

Elimination of Excess Fluoride in Potable Water with Coacervation by Electrolysis Using an Aluminum Anode — by Liu Ming, Sun Rui Yi, Zhang Jun Hua, Bina Yuan; Wei Lei, Liu Ping, and Kei Chiro Fuwa; Chang Zhou, Hebei, China and Tokyo, Japan 54-63

A Native Index of Defluoridation by Serpentine — by M. Maruthamuthu and J. Venkatanarayana Reddy; Madras, India . . . 64-67

Acute Toxicity of Fluoride to Mice — by K.S. Pillai, A.T. Mathai and P.B. Deshmukh; Valvada, India 68-70

Alteration in Gastric Enzymes of Rats Following *in situ* Administration of NaF — by Ravi Rastogi, R.K. Upreti and A.M. Kidwai; Lucknow, India 71-74

Analysis of Fluoride Content in 1376 Water Samples and Endemic Fluorosis in Jiang Xi Province — by Yanyu-fu, Wanfeng-gen, Zhaurong-shun; NanChang, Jiang Xi, China 75-78

Urinary Fluoride in Workers and Rats Exposed to Phosphorites — by W. Czarnowski, J. Krechniak and K. Szeleżyński; Gdańsk, Poland 79-83

Changes in Metabolites and Physiological Activities in a Freshwater Mussel, *Indonaia caeruleus* (Preshad) due to Short-term Exposure to Fluoride — by U.H. Mane, K.S. Pillai, S.R. Akarte, D.A. Kulkarni and K.R. Rao; Aurangabad, India 84-91

ABSTRACTS

Fluoride Supplementation — by Committee on Nutrition 1985-1986; Elk Grove, Illinois, USA 92

Occurrence and Distribution of Fluoride in Groundwaters of Kenya — by K.R. Nair, F. Manji and J.N. Gitonga; Nairobi, Kenya 92-93

Respiratory Survey of North American Indian Children Living in Proximity to an Aluminum Smelter — by P. Ernst, D. Thomas and M.R. Becklake; Montréal, Québec, Canada 93-94

Hydrogen Fluoride Permeability of Astomatous and Stomatous Plant Cuticles — by Jean-Pierre Garrec and Roger Plebin; Champenoux and Grenoble, France 94

Influence of Dithiocarb, (+)-Catechin and Sylbine on Halothane Hepatotoxicity in the Hypoxic Rat Model — by C.P. Siegers, A. Frühling and M. Younes; Lübeck, W. Germany 95

Fluoride in Mixed Human Saliva after Different Topical Fluoride Treatments and Possible Relations to Caries Inhibition — by C. Bruun, D. Lambrou, M.J. Larsen, O. Fejerskov and A. Thylstrup; Copenhagen, Denmark 95-96

Effects of Fluoride on Membrane Permeability and Brush Border Enzymes of Rat Intestine *in situ* — by R.M. Shayiq, H. Raza and A.M. Kidwai; Srinagar, Kashmir, India 96

Effect of Fluoride on the Phosphodiesterase on Bovine Photoreceptors — by Enrico Bignetti, Robert T. Sorbi and Roberto Tirindelli; Parma, Italy 97

Ultrastructure of Fluoride-induced Cysts in the Rat Molar Enamel Organ — by Anita Lange Norlund, James W. Simmelink, Fredrik Henell and Lars Hammarstrom; Stockholm, Sweden 97-98

Fluoride Absorption: Independence from Plasma Fluoride Levels — by G.M. Whitford and J.L. Williams; Augusta, Georgia, USA . . . 98

BOOK REVIEW

Studies in Environmental Science 27: *Fluoride Research* 99-100

The International Society for Fluoride Research (ISFR) extends a special invitation to you to participate in the 16th Conference. This will be held in the Conference Hall of Zyma at Nyon (30 km from Geneva) Monday, August 31st through Wednesday, September 2nd, 1987. Professor C.A. Baud will host this Conference and he has nominated Christiane Demeurisse as secretary of the Conference. The Fluoride journal will carry information about the Conference in future issues.

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

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IS FLUORIDATION EFFECTIVE?

Over the past six years or so, a large and rapidly growing body of scientific evidence has been published which suggests that the alleged benefits of fluoridation in reducing dental caries may have been over-estimated by its proponents. This evidence falls into five distinct, mutually-supporting categories.

Reduction in Unfluoridated Areas

There are now over 20 studies from eight different developed countries showing large reductions in caries over the past one to three decades in unfluoridated communities. The magnitude of these reductions is often comparable with that reported in fluoridated communities (1-3). Some of the reductions (e.g. in prefluoridation Sydney, in Glouchestershire and in 5-year-olds in New Zealand) occurred before the use of any form of fluoride, topical or systemic, became widespread.

Reductions in "Optimally Exposed" Children in Fluoridated Areas

In several communities which have been fluoridated for a long period of time, caries is continuing to decline in children who are considered "optimally exposed," namely children of a given age who have received fluoridated water since birth. Such reductions have occurred after the maximum possible benefit (if any) would have been achieved from fluoridation. Areas where this has been observed include Canbera and Tamworth in Australia, and part of Glouchestershire in the U.K. (3) where fluoride occurs naturally in water. Other examples of this phenomenon can be seen in the caries reductions in 5- and 15-year-old Anglesey children between 1974 and 1983 (4) and in 5-year-olds in north-east England between 1976 and 1981 (5). In a number of these communities the caries reductions, occurred too late to have been caused by fluoridation. However they have been incorrectly attributed to fluoridation, either by the authors or by proponents of fluoridation other than the authors. Thus there are clear cases where the alleged benefits of fluoridation have been over-estimated.

Absolute Values of Caries Sometimes Contradict the "Inverse Relationship"

There are several observations which contradict the notion that the absolute value of caries prevalence in fluoridated areas is always lower than in unfluoridated areas of the same country. In particular, primary school children in the Australian state of Queensland, which is only 5% fluoridated, have the same average value of caries prevalence as in the states of South Australia (70% fluoridated since 1971) and Western Australia (80% fluoridated since 1968) (3). Children in unfluoridated Christchurch, New Zealand, have equal or less caries than children in all the other major cities of New Zealand, which are fluoridated (6). Time-independent surveys in natural fluoride areas in India, Sweden, Japan, the USA, and New Zealand are also inconsistent with the alleged "inverse relationship" between caries prevalence and fluoride concentration of drinking water (3).

The Poor Still Have the Most Caries

An ethical argument, commonly used by proponents of fluoridation to

justify what opponents consider compulsory medication with an uncontrolled dose, is that fluoridation serves the poor. Yet there is evidence that, in fluoridated areas, the poor are still the socio-economic class with the highest prevalence of dental caries (7,8). This observation is consistent with the hypothesis (see below) that the overall dietary pattern is the main determinant of dental caries.

Dubious Scientific Quality of Existing Fluoridation Trials

Scientific re-examination of existing fluoridation trials raise serious doubts about the magnitude of the benefits which are traditionally claimed for fluoridation:

- For instance, in the "classic" North American studies at Newburgh, N.Y., Grand Rapids, Michigan, Brantford, Ontario, and Evanston, Illinois, large secular variations in caries prevalence in the control cities (for those trials which actually had controls for the duration of the experiment) which may invalidate the methods used and hence the conclusions of these studies (9).

  Not one of the many Austrialian fluoridation studies is a properly-controlled longitudinal trial (10).

- The fluoridation experiment in Hastings, New Zealand, has recently been re-examined, by drawing upon archives obtained under the New Zealand Official Information Act. It has been found that a substantial part of the large reduction in caries in Hastings could actually have been the result of changes in diagnostic procedures which were not mentioned in official reports of the study (11).

  For a scientific test of the dental effects of fluoridation, it is necessary to perform several properly controlled longitudinal studies, with annual, blind examinations of children's teeth. These studies should include several years of baseline examinations before the test communities were fluoridated, and follow-up measurements conducted for several years after all age groups have been "optimally exposed." To reduce the possibilities of examiner bias, the choice of which community becomes the test group and which the control should be made randomly at the end of the baseline period. Moreover, each test and control group to be compared should be similar in environment, lifestyle and socio-economic parameters. These simple measures, I suggest, are the minimum basic requirements for a scientific fluoridation trial (10). Yet, as far as I can determine, not one of the 95 or so studies listed by dental proponents of fluoridation as allegedly proving or demonstrating enormous benefits from fluoridation (12), meets these elementary requirements of sound experimental design.

If, as the considerations above suggest, there are factors other than fluoridation which may be playing an equal or greater role in the reduction of caries, then it is likely that much funding for dental health education and research programs is being wasted. Moreover, until such factors are properly understood, it is possible that a reversion to the "bad old days" of high caries prevalence could reoccur, despite the widespread fluoridation of water supplies in several English-speaking countries.

My own hypothesis, which seems to be consistent with the available scientific data, is that fluoridation has played, at best, a minor role in the observed reductions in caries in developed countries. The main causes of these reductions, I suggest (3,10), are:

i) changes in broad dietary patterns, such as the increased consumption of wholemeal bread, unpolished rice, cheese and, in the case of infants, breast milk;

ii) possible changes in the immune status of populations;

iii) use of topical fluorides.

This hypothesis should, of course, be subjected to rigorous scientific experiments in order to verify or refute it.

References

1. Leverett, D.H.: Fluorides and the Changing Prevalence of Dental Caries. Science, 217:26-30, 1982.
2. Glass R.L. (Ed.): First International Conference on the Declining Prevalence of Dental Caries. J. Dent. Res., 61:(special issue) 1304-1360, 1982.
3. Diesendorf, M.: The Mystery of Declining Tooth Decay. Nature, 322:125-129, 1986.
4. Jackson, D., James, P.M.C., Thomas, F.D.: Fluoridation in Anglesey. Br. Dent. J., 158:45-49, 1985.
5. French, A.D., Carmichael, C.L., Rugg-Gunn, A.J., Furness, J.A.: Fluoridation and Dental Caries Experience in 5-year-old Children in Newcastle and Northumberland in 1981. Br. Dent. Jr., 156:54-57, 1984.
6. Colquhoun, J.: Dental Objections to Fluoridation. NH&MRC Symp. on Fluoridation, Adelaide, 28 Aug., 1986, Univ. of Auckland preprint.
7. Colquhoun, J.: Influence of Social Class and Fluoridation on Child Dental Health. Commun. Dent. Oral Epidemiol., 13:37-41, 1985.
8. Bradnock, G., Marchment, M.D., Anderson, R.J.: Social Background, Fluoridation and Caries Experience in a 5-year-old population in the West Midlands. Br. Dent. J., 156:127-131, 1984.
9. Sutton, P.R.N.: Fluoridation: Errors and Omissions in Experimental Trials. Melbourne University Press, 2nd ed. Melbourne, Australia, 1960.
10. Diesendorf, M.: A Re-examination of Australian Fluoridation Trials. Search, 17:256-261, 1986.
11. Colquhoun, J., Mann, R.: The Hastings Fluoridation Experiment: Science or Swindle? The Ecologist, 16:243-248, 1986.
12. Murray, J.J., Rugg-Gunn, A.J.: Fluorides in Caries Prevention (2nd ed.) Wright, Bristol, UK, 1982.

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ELIMINATION OF EXCESS FLUORIDE IN POTABLE WATER WITH COACERVATION BY ELECTROLYSIS USING AN ALUMINUM ANODE

by

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SUMMARY: Potable water in Chang Zhou, China, contains rather high levels of fluoride (4 to 5 mg/L), and elimination of excess fluoride from it has been a serious problem. In this study, a new method to remove excess amounts of fluoride from potable water has been developed based on the coacervation induced by electrolysis using an aluminum anode. By this method, the concentration of fluoride decreases from 4-5 mg/L to 0.5-1.0 mg/L, without significant changes in levels of other ions. Furthermore, 90-95% of E. coli and other bacteria were removed; the turbidity of the water was also markedly decreased. Experimental parameters in this method such as the ratio of fluoride to aluminum, pH, effect of agitation, etc., are discussed in detail. The method is easier, safer, and less expensive than use of activated alumina.

KEY WORDS: Aluminum anode; Coacervation induced by electrolysis; Elimination of excess fluoride; High concentration of fluoride; Potable water.

Introduction

Excessive intake of fluoride is known to be poisonous, although fluorine is an important trace element in man (1-4). Fluoride in potable ground water at Chang Zhou city, China, may be as high as 5 ppm. Previously, we have treated ground water for potable purposes by different techniques (5), including calcium superphosphate, bone black, activated alumina and membrane electrolysis processes for elimination of excessive amounts of fluoride, introduction of other anions to the treated water, and simultaneous reduction of other essential elements, with some success; but these methods mentioned are time-consuming and costly.

In this study, an electrocondensation technique which has been applied in the Soviet Union, Japan, and other countries for sterilizing potable water and decreasing its turbidity has been used to eliminate excessive fluoride with an aluminum anode. This method not only successfully reduces fluoride content from 5 ppm to 1 ppm or below, but it also has the advantages of killing germs and bacteria and lessening turbidity.

Electrocondensation is based on solvation of a metallic anode under the action of direct current. When pure aluminum anodes are placed in water containing fluoride, the major electrolysis reactions are shown in equations 1-5

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Experimental Apparatus

Home-made Electro-Condensation Equipment

Program	I	II
Area of plates	15 x 15 cm^2	40 x 40 cm^2 x 20
Power	DC	DC (parallel connection)
Current density	14 A/m^2	14 A/m^2
Water flow mode	static	continuous
Distance between plates	3 mm	3 mm
Plate material	aluminum	aluminum
pH (raw water)	6.5	6.5-7.0
pH (treated water)	7.0	7-7.5
C_F (raw water)	4.7	4.7
C_F (treated water)	0.8	0.5-0.8
ratio (Al/F)	8	6-8

Materials: Raw ground water from Chang Zhou city was used in this study.

Figure 1

Fluoride content of some natural waters in China and Japan (not containing spring water).

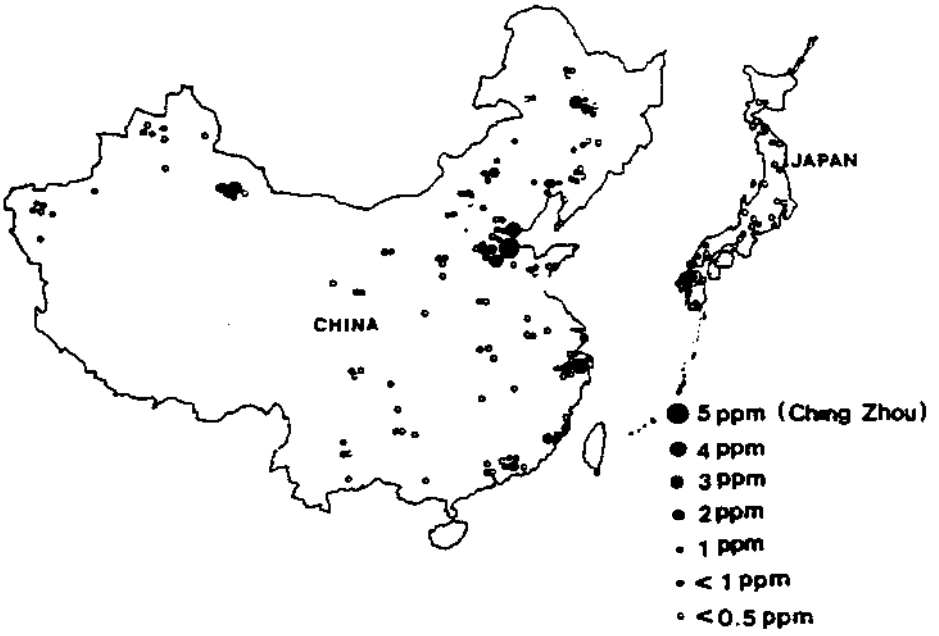


Figure 2

The basic reaction of ions on the electrocondensation process.

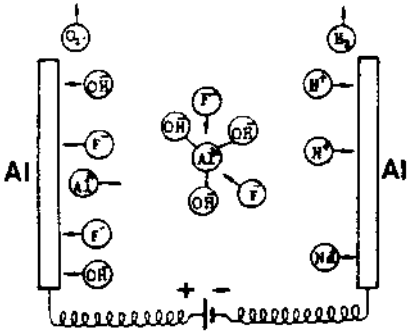
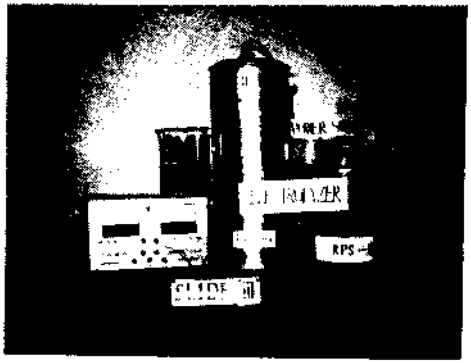


Figure 3

Photograph of Apparatus to reduce fluoride for home use.



and in Figure 2.

$$Al - 3e = Al^{3+} \quad \ldots 1$$

$$Al + 6F^- - 3e = AlF_6^{3-} \quad \ldots 2$$

$$AlF_6^{3-} + 3Na^+ = Na_3AlF_6 \quad \ldots 3$$

$$Al + 3(OH)^- - 3e = Al(OH)_3 \quad \ldots 4$$

$$nAl(OH)_3 = Al_n(OH)_{3n} \quad \ldots 5$$

Fluoride may be segregated in water as Na_3AlF_6 (cryolite) or other complex compounds with complex ion AlF_6^{3-}. Removal of F^- in these compounds is very economical in terms of aluminum consumption: Al/F (mass ratio) = 1/4.2, colloidal aggregates positively charged will be produced at appropriate pH.

The apparatuses for test and home family use are shown in the photograph (Figure 3).

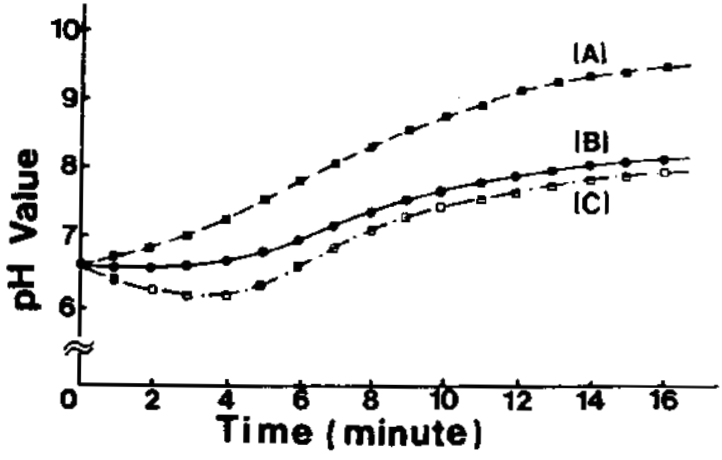
Result and Discussion

pH value: Since aluminum hydroxide is an amphoteric hydroxide, regulating water pH is important for reducing the fluoride concentration by electrolysis. More rapid liberation of H_2 at the cathode than liberation of O_2 at the anode causes a rise in pH. Figure 4 shows the changes in pH in water and around the electrodes.

When pH < 4, aluminum remains the form of Al^{3+}, at lower pH no precipitation occurs, the fluoride concentration cannot be reduced. When 4 < pH < 5.5, the hydrogen ion concentration remains relatively high. Consequently, a high ζ(zeta) potential and a strong repulsive force between aluminum hydroxide colloidal clots is maintained, which is not conducive to flocculation and precipitation. When 5.5 < pH < 7.0, the concentration of H^+ and OH^-, which are free states, is lower, a part of F^- and Al^{3+} will produce the complex ion AlF_6^{3-} at the electrode. The AlF_6^{3-} and F^- would be segregated from water

Figure 4

Changes in pH with the time in the bulk solution and around the electrodes.



(A): around cathode.
(B): in solution.
(C): around anode.

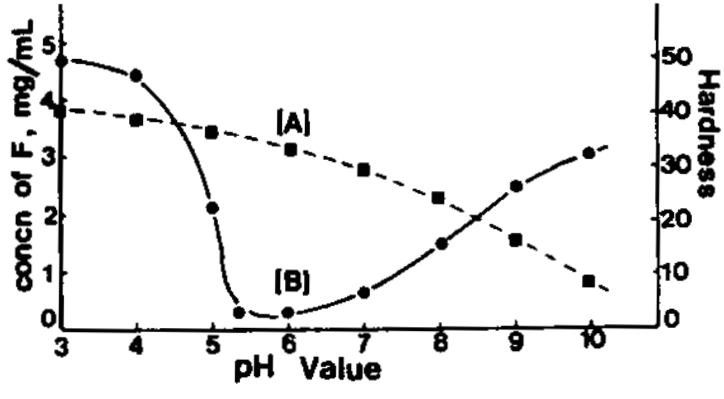
reaction conditions:
current 0.9 A/m²/L water.
distance of plates 24 mm.

with the colloid of aluminum hydroxide carrying positive potential. At this condition removing effect of fluoride is the best.

When pH > 7.0, OH^- ions increase in water. OH^- and F^- ions are alike in mass, charge and ion radius, and due to the principle of nomogenesis and nomophase, they tend to substitute for each other. In water, OH^- can make

Figure 5

Dependence on pH values of the solution for reducing fluoride and hardness



(A): hardness (CaO, mg/L).
(B): concentrations of fluoride (F^-, mg/L).

transition by like leaps adopting, its mobility is greater than that of F^-. In the anodic adsorption layer, the concentration of OH^- ions is higher than the concentration of F^-, so the production of the complex ion AlF_6^{3-} becomes difficult; therefore, the effect of reducing fluoride begins to decrease. Figure 5 shows the relation between pH and efficiency of fluoride reduction. When we tested waters with the method of similar fluoride contents, e.g. about 5 ppm, under the same electrolysis conditions of and varied initial pH values, the fluoride concentrations varied, as shown by a line with some points in Figure 5. We noticed that when $5.5 < pH < 7.0$, best results are obtained, which can reduce the fluoride concentration to less than 1 ppm. In this study we used vinegar concentrate produced in China or edible hydrochloric acid for regulation of the original water to about 5.5 to 7.0. After electrolysis the pH is about 6.5 to 7.5.

Hardness: Upon electrolysis, water hardness often undergoes changes which vary at different initial pH conditions, as shown in Figure 5. Reports from India, America, and China have confirmed that appropriate concentrations of calcium and magnesium in potable water may reduce the incidence of cardiovascular disease, and that they are antagonistic to fluoride intoxication.

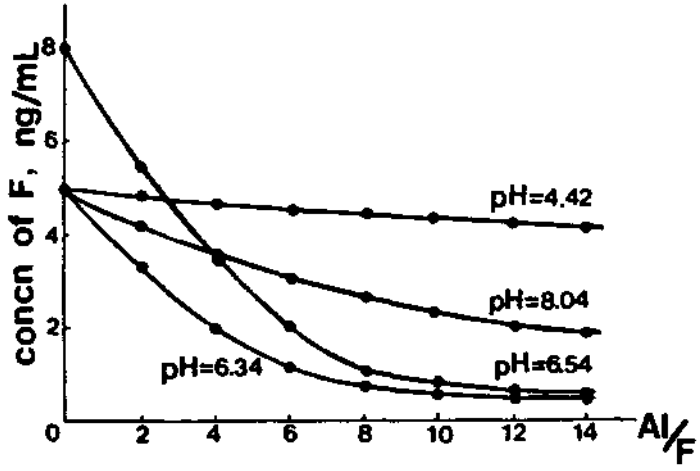
In this study, calcium and magnesium ions are partially segregated from water by coprecipitation with the aluminum hydroxide leading to a somewhat lowered water hardness. Total ionic concentration of HCO_3^- and CO_3^{2-} is about 300 ppm. In different conditions of pH value, HCO_3^- ion can mutually change, the pH value of water rises on electrolysis as HCO_3^- gradually converts to CO_3^{2-}. The solubility of $CaCO_3$ or $MgCO_3$ is less than of $Ca(HCO_3)_2$ or $Mg(HCO_3)_2$. The formation and precipitation of $CaCO_3$ and $MgCO_3$ can be expected when the pH rises, causing a decrease in hardness. The pH during the electrocondensation method must therefore be controlled.

Al/F: Here Al/F represents the ratio of aluminum consumed in electrolysis to the quantity of fluoride ions removed, important in both technical and economic criteria. If hydrolysates of aluminate are used to reduce fluoride, Al/F ratio should be within the range 10-15 to ensure a satisfactory result. The relationship between aluminum consumed and residual fluoride concentration for different pH values (shown in Figure 6) illustrates that, for each water sample with fixed pH, a lower limit to fluoride reduction by electrocondensation exists. The reason is that, the pH of water will increase with electrolysis and lead to different processes of hydrolysis and polymerization at high pH, where Al^{3+} will be converted into AlO_2^-, which cannot reduce the level of fluoride. Figure 6 shows that electrocoagulation is more advantageous in treating water of higher concentration of fluoride. It is estimated that, in the range of 5-10 ppm, similar results could be obtained with equal aluminum consumption. In short, satisfactory results of fluoride reduction are possible in the range of 4-6 of Al/F, provided the electrocoagulation conditions are properly controlled.

Stirring Effect: In the electrolysis, hydrogen is released at the cathode and oxygen at the anode, floating up as micro-bubbles, which are adsorbed into flocs produced by electrolysis, lifting up the flocs to the water surface. This layer of micro-bubbles partly cuts off the adsorption of fluoride to colloids, causing a reduction in the effectiveness of fluoride removal. Therefore, it is advisable to break up the bubbles by moderate stirring.

Figure 6

Effect of Al (Al/F) on Fluoride Concentrations at Various pH Values.



Al/F: The ratio between the amount of aluminum consumed on electrodes to the quantity of fluoride ions removed.

As the speed of stirring is increased, a shearing stress will be produced on colloidal clots due to uneven distribution of motion in water, which can destroy flocs and, more significantly, cause the fluoride adsorbed on aluminum hydroxide colloidal clots to be released into the water by the force of static electricity, as indicated by the following equation:

$$N = \frac{1}{6} G\, n' n''\, (d' + d'')^3 \qquad \ldots 6$$

and the shearing stress (L) will be

$$L = uG \qquad \ldots 7$$

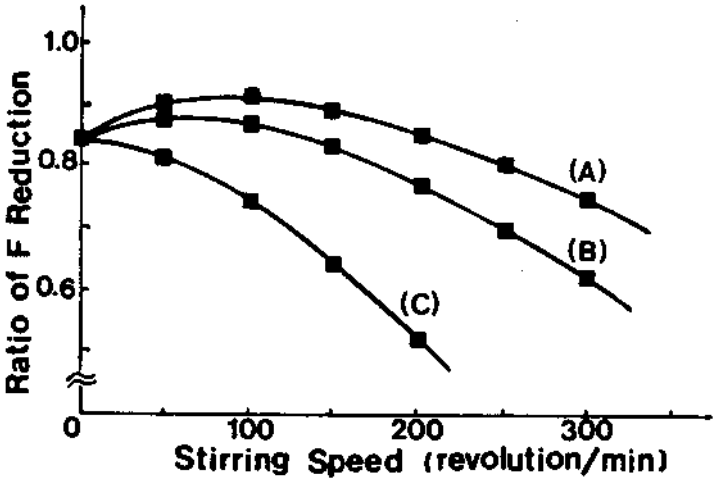
where G: velocity gradient; n', n'': number of particles with diameters d' and d'' in unit of water respectively; d', d'': the diameter of particles of different sizes; u: coefficient of velocity.

Since the formation of flocs (alumen ustum) takes time, the interval between the end of electrolysis and the beginning of stirring will affect the effectiveness of fluoride reduction. For details, refer to Figures 7 and 8.

Efficiency of Current Density: Variations in current density can be seen in Figure 9, where the sharp peak is a result of the capacity charging between plate electrodes, whereas the subsequent elevation of current is due to the solvation of oxidized layer on aluminum surface accelerating the process of electrolysis. Under the influence of the electric field, ionic concentrations between two plates and the conduction of current increase, contributing to

Figure 7

Effect of Stirring on Reducing Fluoride.



reaction conditions: 1 L water, stirring 1 minute, pH 6.4

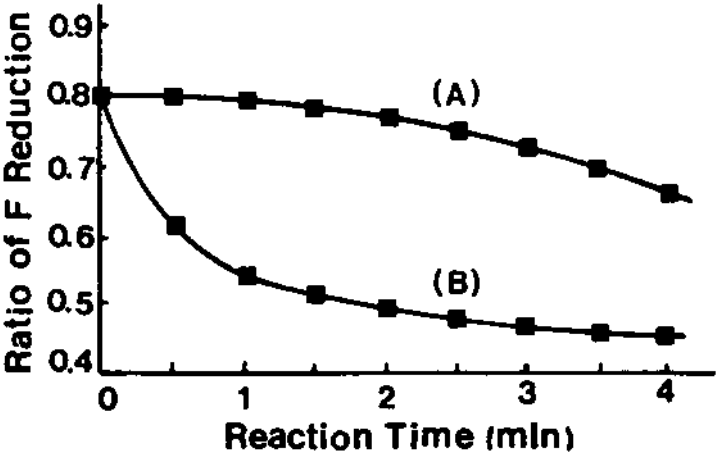
(A): begins stirring after 0 minutes.

(B): begins stirring after 2 minutes.

(C): begins stirring after 4 minutes.

Figure 8

Effect of Stirring Speed on the Reduction of Fluoride.



(A): by rapid stirring.

(B): by slow stirring.

a further rise in current which finally leads to an ultimate current under a given potential. The ultimate current is determined by ion concentration, charge numbers and mobility.

Figure 9

Changes in Current Density with Reaction Time.

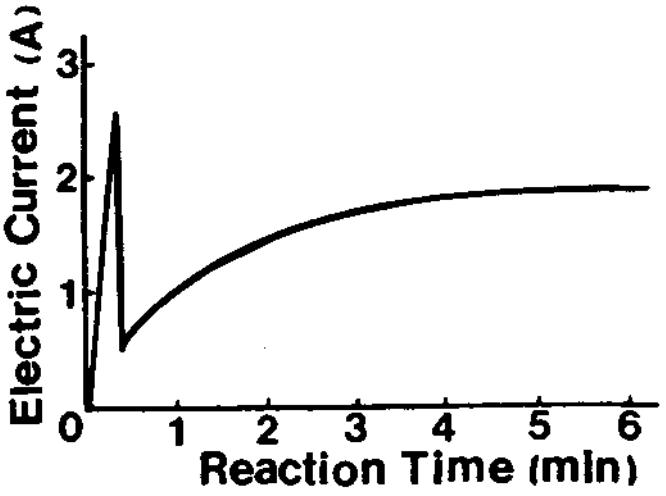
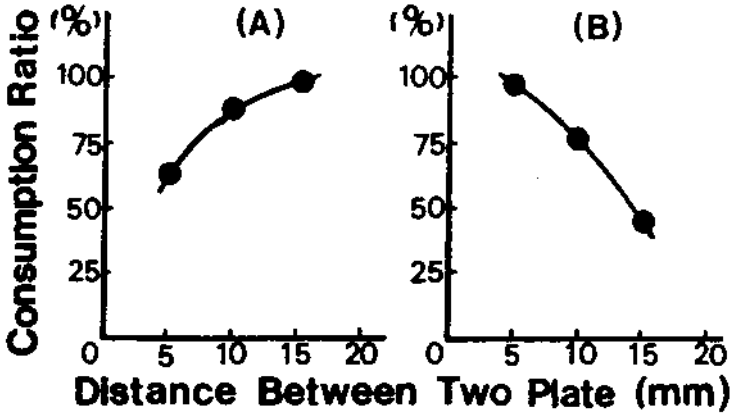


Figure 10

Consumption of the Cathode and Anode Plates with the Changes in Distance Between Electrode Plates.



(A): consumption ratio of anode plate to theoretical consumption.

(B): consumption ratio of cathode plate to anode consumption.

Consumption of cathodic and anodic plates is shown in Figure 10. Experiments have shown that, when the distance between two plates is decreased,

anodic current efficiency approaches unity, while solvation of cathode becomes more intense with about 1/6 - 1/3 of anode consumption; when the distance is increased, anodic current efficiency rises to 120%-130%, while cathode consumption decreases to 1/10 - 1/20 of anode consumption.

Plate current density depends on factors of field strength, ion mobility, stirring intensity and fluoride ion concentrations. Under conditions of field strength 4 V/cm, pH 7, without stirring, and plate current density below 14 A/m^2, is estimated to be optimal for the purpose of effectively reducing fluoride content from 5 ppm to 1 ppm. As the water flow rate between two plates increases, current density can be moderately increased.

Table 1

Comparison of Hygienic Indexes with and without Treatment

Water	Bacteria	Original	Treated
River	coliform (count/L)	2380	230
	bacteria (count/mL)	2000	150
Drinking	coliform (count/L)	<3	<3
	bacteria (count/mL)	<100	<100

Table 2

Comparison of the Elemental Concentrations With and Without Treatments

Ion	Raw Water (mg/L)	Treated Water (mg/L)
F	4.45	0.85
I	0.15	0.14
Cl	99	198
Se	0.005	0.001
SO_4	139	135
K	1.6	1.6
Na	279	275
Ca	11.5	6.5
Mg	11.8	10.3
Al	0.028	0.041
Fe	0.36	0.08
Mn	0.03	0.05
Cu	0.003	0.005
Cr	0.002	0.001
Cd	0.008	0.008
Pb	0.009	0.009
pH	8.0	7.2

Application: Tables 1 and 2 show the comparisons of characteristics of raw water to treated water in Chang Zhou. The concentration of fluoride is decreased to 1 ppm from 5 ppm, and the hardness remained almost the same as before treatment with some other qualified indices. On the other hand, upon electrolysis, chlorine ions in water produce sodium hypochlorite at the anode, which dissociates and releases oxygen atoms; aluminum hydroxide colloidal clots, by adsorption and trapping, have the functions of killing and clearing away germs and colon bacilli.

Conclusion

To sum up, our electrocondensation method is a new efficient treatment of potable water. At present, we have made a small set for use in a family. Sets are now used in public. Sets of medium size, suitable for use in plants and schools, have become commercially available.

Acknowledgement

The authors thank Gao Chunhui, Wei Baoquan and Zheng Li for assistance in this work.

References

1. Fuwa, K.: Biogeochemistry of Fluoride and Its Minute Analysis. Dental Review, Japan, 427:111-116, 1978.
2. Matuura, S. and Kokubu, N.: Fluorine – Its Distribution and Role in Nature. University of Tokyo Press, Tokyo, Japan, 1972, pp. 94-106.
3. Hu-Ying: Physical Chemistry. Ren Ming Press, Peking, China, 1978, pp. 2-302-311.
4. Underwood, E.J.: Trace Elements in Human and Animal Nutrition. Maruzen Co., Ltd., Academic Press, New York, 1975, pp. 369-400.
5. Sun-Licheng: Removing Fluorine from Drinking Water Using Electric Coagulation Method. Technology of Water Treatment, 10:13-18, 1984.

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CORRECTION:

Fluoride, January, 1987. Page 2: In Table 1, Column 8, first line: 14.0 not 4.0. Page 3: first paragraph, third line: applies not applied. Paragraph 2, line 5: supplies not supplied. Reference 2, line 6, Arnala not Armala. See enclosed corrected reprint.

A NATIVE INDEX OF DEFLUORIDATION BY SERPENTINE

by

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SUMMARY: A native index of defluoridation has been identified as the ratio of fluoride content in two varieties of serpentine. The index has a value of 1.3 indicating that the green variety of serpentine is 1.3 times more efficient than the yellow variety for defluoridation. This has been proved experimentally by showing the agreement between the native index and the ratio of experimental defluoridating efficiencies. Adsorption of fluoride on serpentine obeys the Freundlich adsorption isotherm and the ratio of the slopes of the adsorption isotherms for the two varieties of serpentine and agrees nicely with the native index.

KEY WORDS: Defluoridation; India; Serpentine, 2 varieties.

Introduction

Serpentine is a naturally occurring metasilicate of magnesium and it is represented as $3\ MgO \cdot 2\ SiO_2 \cdot 2\ H_2O$ or $Mg_3Si_2O_5(OH)$. Rather than conducting the study with the pure mineral, it is worth-while to employ the naturally occurring serpentine as such for defluoridation. Defluoridation with serpentine has been the subject of research interest (1,2) in recent years. The proxy of F^- for OH^- has been suggested to be the principal contributing factor in the mechanism of fluoride removal. Serpentine has also been studied as an antidote for fluorosis (3,4) and the shortcomings have and been discussed (5).

Two varieties of serpentine have been used in the present study. On the basis of color, the symbols SPG and SPY are used to indicate the samples, SPG referring to the green colored serpentine and SPY, the yellow colored serpentine. The samples, collected from the Pulivendala asbestos belt in the Cuddapah district of Andhra Pradesh, India, are associated with dolomites (6) and the chemical compositions are the same as observed by Rao et al (2). The significance of differing amounts of fluoride, 0.17 wt % in SPG and 0.13 wt % in SPY has led us to realize the native index of defluoridation in the two varieties of serpentine. Even though the fluoride is present in very small amounts, the fluoride impurity present in the serpentine mineral serves as a guide indicating the fluoride uptake ability of serpentine. The origin of fluoride in serpentine may be solid-state reactions or the circulating waters. The relatively higher fluoride level in SPG may be taken to indicate its higher defluoridating capacity compared to SPY. In fact, the ratio of fluoride content of th· two serpentines may be identified as the native index of defluoridation in serpentines.

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$$\frac{\text{Fluoride content of SPG}}{\text{Fluoride content of SPY}} = \text{Native Index of Defluoridation} = \text{NID} \quad \ldots 1$$

Substituting the fluoride contents for SPG and SPY, we get

$$\text{NID} = \frac{0.17}{0.13} = 1.3 \quad \ldots 2$$

If we define

$$\frac{\text{Defluoridating efficiency of SPG}}{\text{Defluoridating efficiency of SPY}} = r \quad \ldots 3$$

then, r should be equal to NID. Even without performing experiments, we can infer from the magnitude of NID that SPG should be 1.3 times more efficient than SPY. Our chief objective is therefore to prove the validity of the following relationship,

$$r = \text{NID} \quad \ldots 4$$

The other objective of the study is to test the Freundlich adsorption isotherm for SPG - F^- and SPY - F^- systems.

Materials and Methods

Serpentine samples: Powdered serpentine samples passing 200 mesh have been used for the study. The quantity of serpentine employed in the present work ranges from 0.2 to 2 g.

Defluoridation experiment: The equilibrium contact time is first determined by studying the adsorption of fluoride as a function of time. Then the actual experiment is performed at various serpentine dosages maintaining a contact time, well above the equilibrium contact time. A known weight of the adsorbent, i.e., serpentine is added to 20 mL of 9.75 ppm fluoride solution and stirred for 24 hours at constant temperature (30°C). After centrifuging and filtering, the clear filtrate is analyzed to determine the free equilibrium concentration of fluoride $[F^-]_{eq}$. The initial concentration of fluoride $[F^-]_o$, is maintained in the range of 10 ppm usually so that it closely parallels the average fluoride concentration in the endemic fluorosis area.

Fluoride estimation: The concentration of fluoride is determined using the fluoride-ion selective electrode (JAS Chemical Corporation, India).

Results and Discussion

The native index of defluoridation which has been identified in serpentine minerals should be equal to the ratio of the experimental defluoridating efficiencies as pointed out in the introduction. A very simple experimental quantity which can be regarded as a measure of the defluoridating efficiency of serpentine is the % of defluoridation. The % of defluoridation for SPG and that for SPY are compared at the same serpentine dosage (Table 1).

Table 1

Defluoridating Efficiencies of SPG and SPY

Serpentine dosage (g/mL)		0.01	0.02	0.025	0.03	0.04	0.05	0.10
Percent of defluoridation	SPG	32.1	47.5	50.0	60.0	66.2	72.3	83.6
	SPY	20.5	34.6	40.5	48.7	58.5	64.1	81.0
r		1.56	1.37	1.23	1.23	1.13	1.13	1.03
Mean value of r at low serpentine dosage = 1.35 (≤ 0.03 g/mL)						Mean value of r at high serpentine dosage = 1.09		

There is good agreement between r at low serpentine dosage and NID. Literature data (2) also yield a value of r = 1.2 at the serpentine dosage of 0.05 g/mL. The results also suggest that the dosage of serpentine in nature is also low. The inference is quite reasonable since one has to consider the enormous volume of circulating water coming in contact with a specified amount of serpentine. Experiments with $[F^-]_o$ = 5.75 ppm yield a value of r = 1.1 at the dosage of 0.025 g/mL. The low value of r at high serpentine dosage (Table 1) as well as at low $[F^-]_o$ is due to the fact that the fluoride release from serpentine cannot be neglected under these conditions.

The performance of serpentine in defluoridation is also described by adsorption data. Freundlich adsorption isotherm (7)

$$\frac{x}{m} = kc^{1/n}$$

is applicable to the system, where x is the amount of adsorbate (fluoride) adsorbed, m is the weight of the adsorbent (serpentine), c is the equilibrium concentration of the adsorbate in solution after adsorption, $[F^-]_{eq}$, and k and n are empirical constants.

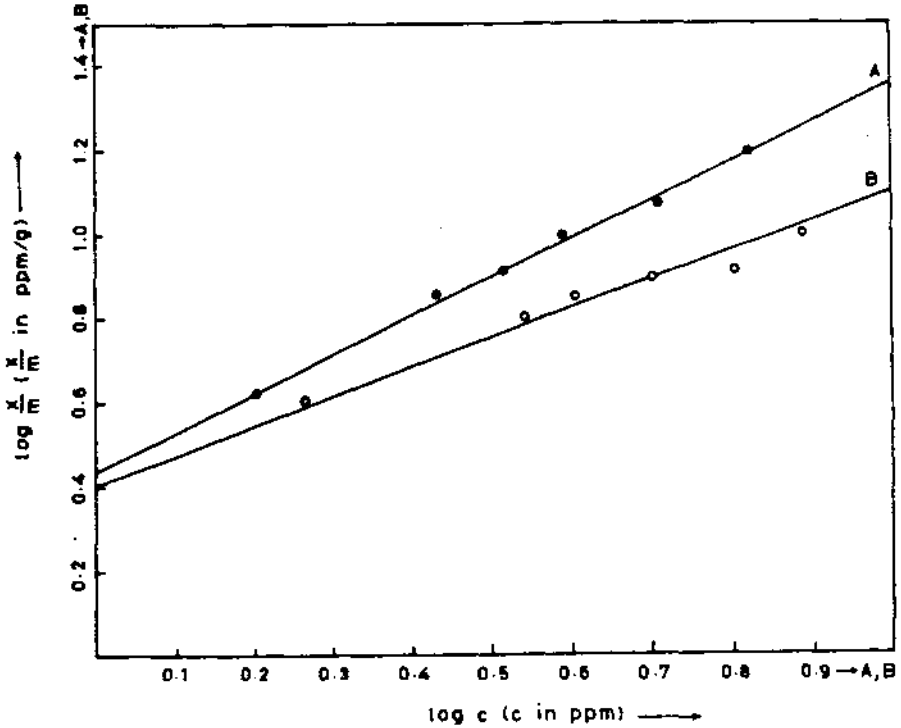
The linearity of the plot of log (x/m) versus log c indicates the applicability of the Freundlich adsorption isotherm (Figure 1). Both SPG and SPY give rise to flat adsorption isotherms indicating that serpentine can be employed over a wide range of fluoride concentration for defluoridation. The slope of the isotherms, 1/n (Figure 1), can also be taken to indicate the defluoridating efficiencies of SPG and SPY. In that case, the ratio of slopes of the adsorption isotherms should also agree with the native index. The ratio of slopes turns out to be 1.28, showing good accord with NID once again confirming the validity of the postulate.

Acknowledgement

One of the authors (J.V.) acknowledges gratefully CSIR (India) for the award of a research fellowship.

Figure 1

Freundlich adsorption isotherms for the adsorption of fluoride on Green Serpentine (plot A) and Yellow Serpentine (plot B). $[F^-]_o$ = 9.75 ppm.



Bibliography

1. Rao, K.V., Purushottam, D., Khandedar, A.K., Vaidyanadhan, D. and Francis, P.: Defluoridation of Natural Water by Serpentine. Curr. Curr. Sci., 41:841, 1972.
2. Rao, K.V., Purushottam, D. and Vaidyanadhan, D.: Uptake of Fluoride by Serpentine. Geochim. Cosmochim. Acta., 39:1403-1411, 1975.
3. Rao, S.R., Murthy, K.J.R., Murthy, T.V.S.D. and Reddy, S.S.: Treatment of Fluorosis in Humans. Fluoride, 8:12-24, 1975.
4. Teotia, S.P.S., Teotia, M. and Singh, R.: Treatment of Endemic Fluorosis in Human Beings – An Experimental Study. Fluoride, 9:98-104, 1976.
5. Waldbott, G.L.: Antidotes for Fluoride Intoxication. Fluoride, 13:96, 1980.
6. Krishnan, M.S. and Venkatram, M.S.: Asbestos and Barytes in Pulivendala Talug, Cuddapah District. Bull. Geol. Surv. Ind. Serv. A. Econ. Geol., 5:1-53, 1953.
7. Benefield, L.D., Judkins, J.F. and Weand, B.L.: Process Chemistry for Water and Wastewater Treatment. Prentice Hall, Inc., Englewood Cliffs, New Jersey, 1982.

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ACUTE TOXICITY OF FLUORIDE TO MICE

by

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SUMMARY: Mice were orally administered (single dose) 46, 49, 51, 54, 57 and 60 mg F^-/kg body weight of the animal. LD_{50} values for male and female mice were calculated as 54.4 and 51.6 mg F^-/kg body weight, respectively. The slopes (b) or regression equations (probit of mortality vs log F^- dose) of both sexes did not differ statistically; a common slope was established for both sexes to calculate the LD_{50} of F^- for mice (51.5 mg F^-/kg body weight).

KEY WORDS: Acute toxicity; Fluoride; LD_{50}; Mice.

Introduction

Acute toxic effects of a single large dose of a chemical are often strikingly similar to chronic effects (1). To study the chronic effects in laboratory animals, LD_{50} values are helpful in assessing the doses to be given them. Considerable data on fluoride toxicity to laboratory animals are now available. Unfortunately, in much research F^- doses administered to animals (2-4) have been at the scientist's discretion. LD_{50} values can be used as a base for selection of proper doses for chronic toxicity studies.

The present work is aimed at establishing the LD_{50} value of fluoride for mice.

Materials and Methods

Laboratory inbred Swiss albino mice (30-35 g in weight) of Haffkine strain of our colony (Jai Research Foundation, Valvada, Dist. Valsad, Gujarat) were used in the study. Food (obtained from Hindustan Lever Ltd., Bombay) was withdrawn for 20-24 hrs prior to administration of fluoride. Water (fluoride < 1 ppm/ hardness, 136-140 ppm as $CaCO_3$) was given ad libitum. Desired concentrations of fluoride were obtained by dissolving the appropriate amount of NaF in distilled water. Fluoride doses, which never exceeded 1 mL (1 mL/35 g b.w. of mice), were given by a single oral administration using a syringe of 1 mL capacity and a cannula as described in SOP/GTX/118 (5). Control mice were given distilled water.

Initially, 179 and 357 mg F^-/kg b.w. was given to two mice each. 100% mortality occurred within 5 hrs in both; when the experiment was repeated with 60, 66, 69, 71, 77 and 83 mg F^-/kg b.w. the result was again 100% mortality within 24 hrs; subsequently the doses were further lowered. Male mice (2-3 each) were given 46, 49, 51, 57, 60 and the female mice (2-3 each) were given 46, 51, 54, 57, and 60 mg F^-/kg b.w., respectively. These doses were again given to 5 mice each of either sex. Mortality in mice recorded due to each fluoride dose (excluding the doses which caused 100% mortality) were used to calculate the LD_{50} and fiducial limits of LD_{50} (6).

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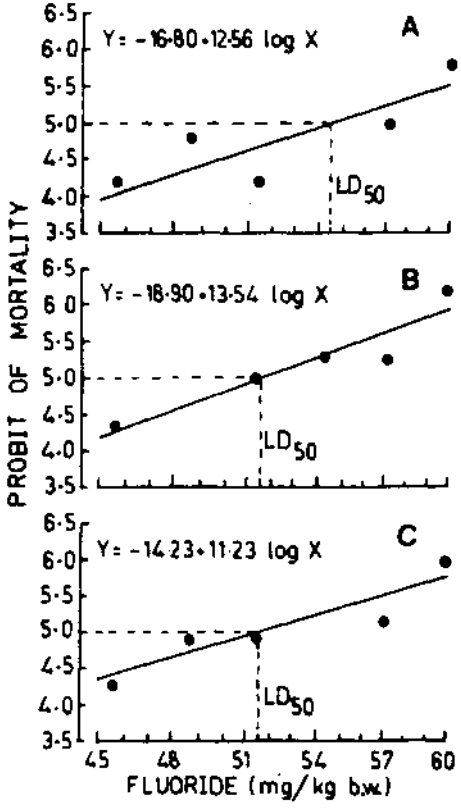
Figure 1

Regression Lines Established Between the Probit Values of Mortality of Mice and Fluoride Doses. A, B and C Denote the Regression Line for Male, Female, and Male + Female, Respectively. Abscissa is in Log Scale.



Results and Discussion

No mortality was observed in the control group. Mortalities in male mice, when they were given 46, 49, 51, 57 and 60 mg F^-/kg b.w. were 20, 40, 20, 50 and 80% respectively. The female mice showed 25, 50, 63, 63 and 88% mortality respectively, when they were administered with 46, 51, 54, 57 and 60 mg F^-/kg b.w.

The single oral dose of fluoride for laboratory animals is thus in the range of 20-100 mg/kg b.w. (7). The present study revealed an LD_{50} of 54.4 mg F^-/kg b.w. for male mice, slightly less for female mice (51.6 mg F^-/kg b.w.). The regression lines established (probit mortality vs log concentrations of fluoride dose) for male and female mice are parallel in nature (Figure 1A and 1B). The slopes (b) did not differ significantly from each other (Table 1). The common slope established for both sexes is in good agreement (Table 2). Hence, it can be stated that LD_{50} of fluoride for laboratory mice weighing 30-35 g is 51 mg/kg b.w. (single oral dose), with a fiducial limit of 47 and 56 mg/kg b.w. De Lopez et al. (8) determined the LD_{50} of fluoride for female rats weighing 80 and 150 g as 54 and 52 mg/kg b.w. The LD_{50} was low (31 mg F^-/kg b.w.) for female rats weighing 200 g. since the Ca^{++} and Mg^{++} ions in drinking water are capable of reducing the toxicity of fluoride (9), hardness of water given to the laboratory animals after the fluoride administration should be considered an important factor in fluoride toxicity studies.

Acknowledgement

We are grateful to Mrs. Sandra R. Shroff and Mr. Rajju D. Shroff, Managing Trustees of Jai Research Foundation for their encouragement and laboratory facilities.

Table 1

Heterogeneity and Parallelism of Regressions*

Nature of Variation	d.f.	ss	ms
Parallelism of Regressions	1	0.0074	0.0074
Heterogeneity	6	2.6141	0.4356
TOTAL	7	2.6215	

* (Y=a+b log X; Y = Calculated Probit; X = Concentration of Fluoride in Dose; a = Intercept; b = Slope)

d.f. = degrees of freedom, ss = sum of squares; ms = mean square

Table 2

LD_{50}, 95% Fiducial Limits of LD_{50} and χ^2 Value

Mice	LD_{50}*	95% Fiducial Limits of LD_{50}*	χ^2
Male	54.41	50.00-59.14	1.99
Female	51.60	47.71-55.71	0.62
Male & Female	51.53	47.71-55.71	2.09

* Expressed as mg F^-/kg b.w. of mice.

References

1. Waldbott, G.L., Burgstahler, A.W. and McKinney, H.L.: Fluoridation: The Great Dilemma. Coronado Press, Inc., Lawrence, Kansas, 1978, pp. 86-97.
2. Mohamed, A.H. and Chandler, M.E.: Cytological Effects of Sodium Fluoride on Mice. Fluoride, 15:110-118, 1982.
3. Greenberg, S.R.: Leukocyte Response in Young Mice Chronically Exposed to Fluoride. Fluoride, 15:119-131, 1982.
4. Soni, M.G., Kachole, M.S. and Pawar, S.S.: Changes in Hepatic and Renal Microsomal Mixed Function Oxidase System in Adult Male Rats During Administration of NaF. Fluoride, 15:132-136, 1982.
5. Paget, G.E. and Thomson, R. (Eds.): Standard Operating Procedures in Toxicology. University Park Press, Baltimore, 1979, pp. 260-263.
6. Finney, D.J.: Probit Analysis. Cambridge University Press, London, 1971, pp. 50-80.
7. Davis, P.K.: Fluorides — A Critical Review, V. Fluoride Intoxication in Laboratory Animals. J. Occup. Med., 3:593-601, 1961.
8. DeLopez, O.H., Smith, F.A. and Hodge, H.C.: Plasma Fluoride Concentrations in Rats Acutely Poisoned with Sodium Fluoride. Toxicol. Appl. Pharmacol., 37:75-83, 1976.
9. Abukurah, A.R., Moser, A.M., Jr., Baird, C.L., Randall, R.E., Jr., Setter, J.G. and Blanke, R.V.: Acute Sodium Fluoride Poisoning. J. Can. Med. Assoc., 222:816-817, 1972.

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ALTERATION IN GASTRIC ENZYMES OF RATS FOLLOWING IN SITU ADMINISTRATION OF NaF

by

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SUMMARY: Fluoride intoxication studies were carried out following in situ administration of 24, 48 and 96 mM NaF into the rat stomach. Gastric luminal fluid showed significantly enhanced levels of various cell membrane enzymes following NaF treatment. SDS-gel electrophoresis also revealed a concentration-dependent release of several polypeptide bands. The results indicate that the higher concentrations of fluoride could partially damage the gastric cell lining.

KEY WORDS: Rat; Stomach; Enzymes; NaF; In situ F^- effects.

Introduction

Mass intoxication from accidental over-fluoridation (126 mM) of drinking water has been reported. Symptoms most commonly observed were nausea, vomiting and diarrhea (1). Alterations caused by fluoride in the permeability of membranes and membrane-bound enzymes have been observed in rat liver and rabbit muscle (2,3). Fluoride consumed by man and animal is chiefly absorbed in the intestine (4). However, the effect of fluoride on gastric secretion, ion transport and other disorders have also been studied (5-8). Our earlier studies have shown significant alterations in the formation of lipid peroxides and on the uptake of D-glucose in rat intestine (9,10). Since the effect of fluoride on human health stems largely from fluoride taken through the oral route, the stomach automatically becomes the first obvious organ to be involved. The present study was undertaken to investigate the in situ effect of fluoride on rat gastric cell lining, the first and most exposed site of contact.

Materials and Methods

Male albino rats (150-170 g) were procured from animal breeding facility of Industrial Toxicology Research Center, Lucknow and fasted overnight to obtain a food-free stomach. Animals had free access to drinking water. Laparotomy on each rat was performed under light ether anesthesia. The stomach was thoroughly washed with distilled water through two cuts, one slightly distal to the gastro-esophageal junction and the other at the distal end of the gastro-duodenal junction. The stomach sac was prepared by ligating the upper end of the stomach using sterile threads after which the sac was filled with 7.0 to 9.0 mL of sodium fluoride solution through the proximal opening with the help of a syringe and blunt needle; the proximal opening was immediately ligated. Control animals received distilled water in place of sodium fluoride. The stomach was left in situ and the abdomen kept closed. The head of the

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animal was kept slightly uplifted; proper breathing and anesthesia was maintained throughout the experiment. After 30 min. of *in situ* incubation, the stomach sac was removed, gently blotted and luminal content drained into a graduated tube. The luminal fluid was made up to desired volume and used for various estimations.

Alkaline phosphatase was determined according to Weiser (11), Ca^{2+}-ATPase according to Hidalgo et al (12), sucrase according to Dahlquist (13) ν-glutamyl transpeptidase according to Boelsterle and Zbinden (14), and 5'-nucleotidase by the method of Aronson and Touster (15). Enzyme activities are expessed in terms of specific activity (μmoles of reaction product liberated/min/mg protein). Protein was determined according to the method of Lowry et al (16) using bovine serum albumin as standard. Mean values were compared by the student's 't'-test. A probability value of less than 0.05 was chosen as indicating significance. SDS-PAGE was carried out according to Laemmli (17) using 4% acrylamide concentration in the collecting gel and 8% in the separating gel. The gels were stained with coomasie-brilliant blue.

Results and Discussion

Experiments were designed to observe the release, if any, of various enzymes from stomach cell lining following NaF administration. Table 1 summarizes the results for the *in situ* effect of NaF on rat stomach enzymes. Following 24 mM NaF administration, ν-glutamyl transpeptidase, Ca^{2+}-ATPase and 5'-nucleotidase activities of stomach luminal fluid showed an elevated level of 31, 33 and 53%, respectively whereas, sucrase and alkaline phosphatase levels were enhanced to about 300% compared to their respective controls. However, following 48 mM NaF administration, alkaline phosphatase, sucrase and 5'-nucleotidase activities of luminal fluid still remained elevated but to a lesser extent compared to the 24 mM NaF concentration. Conversely, 96 mM NaF concentration lowered the elevated levels of enzyme activities. In addition, Ca^{2+}-ATPase and alkaline phosphatase were further inhibited to 26 and 45%, respectively, compared to their respective controls.

Table 1

Effect of Sodium Fluoride on Rat Stomach Luminal Fluid Enzymes following *in situ* Administration

Treatment	Ca^{2+}-ATPase	Alkaline Phosphatase	Sucrase	5'-nucleotidase	ν-glutamyl traspeptidase
Control	0.042 ±0.002	0.020 ±0.001	0.042 ±0.003	0.017 ±0.001	0.013 ±0.001
24mM NaF	0.056 ±0.004	0.080 ±0.005	0.160 ±0.011	0.026 ±0.002	0.017 ±0.001
48mM NaF	0.034 ±0.003	0.055 ±0.004	0.153 ±0.013	0.025 ±0.002	0.015 ±0.002
96mM NaF	0.031 ±0.002	0.011 ±0.001	0.116 ±0.009	0.014 ±0.002	0.010 ±0.001

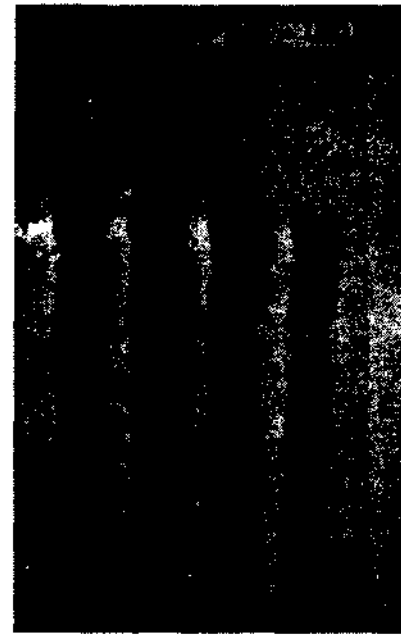
Values are mean ±S.D. from 3-5 animals. Control animals received distilled water. $P \cdot 0.05$.

The release of enzymes in luminal fluid with 24 mM NaF could be due either to partial damage of cell lining or to stimulation of the gastric surface. Fluoride is known to alter the permeability of the gastric cell lining to H^+ ion and also in mobilization of other ions such as Na^+, K^+ and Ca^{2+} (18).

Significant release of enzymes in the lumen, therefore, indicates a possible partial damage of gastric cells. The difference in the extent of increase between different enzymes can be related to their location in the membrane, – sucrase and some phosphatases are localized at the external surface of the membrane – whereas other enzymes are intimately associated with the hydrophobic core of membrane; they are situated deeper in the membrane. The lowering of enzyme activities including inhibition with higher NaF concentrations could be due to the presence of higher remaining amounts of fluoride in luminal fluid. Our in vitro studies with rat intestinal brush border enzymes have also shown significant inhibition of various enzymes due to higher fluoride concentrations (unpublished data).

Figure 1

SDS-gel electrophoresis pattern of stomach luminal fluid following NaF treatment.



To investigate the release of protein/polypeptides into the luminal fluid due to the possible damage of the gastric lining cells caused by NaF treatment, polyacrylamid gel electrophoresis of luminal fluid samples was carried out. SDS-PAGE pattern revealed a number of protein/polypeptide bands ranging in apparent molecular weights of 12,000 to over 300,000 daltons (Figure 1). NaF treatment indicated a concentration-dependent release of protein/polypeptide content. Compared to the control, significant alterations in the SDS-PAGE pattern of stomach luminal fluid following NaF administration clearly indicates damage of cell lining. Furthermore when equal amounts of proteins were used for electrophoresis, differences in some high molecular weight bands were also evident with administration of higher fluoride concentrations.

Conclusion

The present findings demonstrate the release of either membrane-bound or intracellular proteins into the lumen following fluoride administration suggesting at least partial damage of the stomach cell lining. In addition, the present technique (in situ) can also be used to evaluate possible damage to gastric or intestinal cell lining following administration of various environmental pollutants and other toxicants in an animal model.

Acknowledgement

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References

1. Waldbott, G.L.: Mass Intoxication from Accidental Over-Fluoridation of Drinking Water. Clin. Toxicol., 18:531-541, 1981.
2. Kaul, R.D. and Sushella, A.K.: Evidence of Muscle Fibre Degeneration in Rabbits Treated with Sodium Fluoride. Fluoride, 7:177-181, 1974.
3. Martin, B.R., Stein, J.M., Kemnaly, E.L. and Doberska, C.A.: The Effect of Fluoride on the State of Aggregation of Adenylate Cyclase in Rat Liver Plasma Membrane. Biochem. J., 188:137-140, 1980.
4. Stookey, G.K., Crane, D.B. and Muhler, J.C.: Further Studies on Fluoride Absorption. Prosc. Soc. Exp. Biol. Med., 115:295-298, 1964.
5. Waldbott, G.L.: Gastric Ulcer and Fluoride. Fluoride, 10:149-151, 1977.
6. Reed, J.D. and Smy, R.J.: The Effect of NaF on Gastric Acid and Electrolyte Output in Anaesthetized Rats. J. Physiol., 301:39-42, 1980.
7. Assem, E.S.D. and Wan, B.Y.C.: Stimulation of H^+ Ion Secretion from the Isolated Mouse Stomach by NaF. Eperimentia, 38:369, 1982.
8. Shayiq, R.M., Raza, H. and Kidwai, A.M.: Alteration in Gastric Secretion of Rats Administered NaF. Fluoride, 17:178-182, 1984.
9. Shayiq, R.M., Raza, H. and Kidwai, A.M.: Fluoride and Lipid Peroxidation: A Comparative Study in Different Rat Tissues. Bull. Environ. Contam. Toxicol., 37:70-76, 1986.
10. Shayiq, R.M. and Kidwai, A.M.: Effect of Fluoride on Uptake of D-glucose by Isolated Epithelial Cells of Rat Intestine. Environ. Res., 44 (in press), 1986.
11. Weiser, M.M.: Intestinal Epithelial Cell Surface Membrane Glyco-protein Synthesis. J. Biol. Chem., 248:2536-2541, 1973.
12. Hidalgo, C., Gonzalez, M.E. and Lagos, R.: Characterization of the Ca^{2+}- or Mg^{2+}-ATPase of Transverse Tubule Membranes Isolated from Rabbit Skeletal Muscle. J. Biol. Chem., 258:13937-13945, 1983.
13. Dahlquist, A. Methods for the Assay of Intestinal Di-saccharidases. Analyt. Biochem., 7:18-25, 1964.
14. Boelsterli, U. and Zbinden, G.: Application of Fine Needle Aspiration Biopsy for the Diagnosis of Dysplastic and Neoplastic Liver Cell Changes Induced by N-nitrosomorpholine in Rats. Arch. Toxicol., 42:225-231, 1979.
15. Aronson, N.N. and Touster, O.: Isolation of Rat Liver Plasma Membrane Fragments of Isotonic Sucrose. Methods Enz. 31:90, (Eds) Fleischer, S. and Packer, L., Academic Press, N.Y., 1974.
16. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein Measurement with the Folin Phenol Reagent. J. Biol. Chem., 193:265- 275, 1951.
17. Laemmli, U.K.: Cleavage of Structual Proteins During the Assembly of the Head of Bacteriophage T_4. Nature, 227:680-685, 1970.
18. Suketa, Y.: Effect of Fluoride on Physiological Ion Mobilization in Rats. J. Pharmac-Bio. Dyn. 3:85, 1980.

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ANALYSIS OF FLUORIDE CONTENT IN 1376 WATER SAMPLES AND ENDEMIC FLUOROSIS IN JIANG XI PROVINCE

by

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SUMMARY: This survey was to determine the fluoride content in drinking water and the extent of endemic fluorosis in the entire province, in order to prevent it. The fluoride content of major water samples is less than 1 ppm and distribution of fluorosis is spotty. The fluoride content in water and fluorosis were not significantly correlated.

KEY WORDS: China; Fluoride in water; Fluorosis; Jiang Xi.

Introduction

The occurrence and development of endemic fluorosis is directly related to the fluoride content in the environment, especially in water. To prevent fluorosis, in 1982 we determined the fluoride content in drinking water in the whole province, mainly in potable water and, in 1983, in parts of counties and cities with the following results:

Jiang Xi province is located in the Poyang Lake basin on the southern side of the Yangtze river. Terrain inclines gently from south to north and from border to interior. Mountain areas, flatlands and hilly lands are found within the boundaries of this province. Mainly the soil is red and loess; rice is the major grain. Most residents drink well water, a few drink from rivers and hot springs. The province is rich in mineral resources. High-fluoride rock such as fluorspar, mica, quartz and coal are plentiful; about one hundred hot springs are found within the boundaries. The annual climate is warm, and change of seasons is clearly marked. Mean annual temperature is 16-19 °C; mean annual precipitation is 1400-2000 mm.

Material and Method

To measure the fluoride content in water, at least 5 samples of 500 mL were taken from five different points in the east, north, south, west, and center of each county. Fluoride content in water was determined by the fluoride selective electrode and colorimetric analysis of alizarin zirconium; the two methods were tested prior to analysis of samples with no significant difference in the result. The epidemiologic survey of fluorosis was made in parts of hot spring and high-fluoride rock areas, on the basis of the diagnostic standard of endemic fluorosis issued by the central leading group of endemic disease.

Results

1376 water samples were analyzed: 866 were well water, 278 river water,

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Table 1

Fluoride Content in Water

Type of Water	No.	Range (ppm)	$\bar{X}$ ± SD	Proportion of Samples							
				≤ 1 ppm		> 1 ppm		> 2 ppm		> 4 ppm	
				No.	%	No.	%	No.	%	No.	%
well	866	0.012-6.0	0.18 ±0.32	855	98.73	7	0.81	1	0.11	3	0.35
river	278	0.014-1.8	0.21 ±0.26	276	99.28	2	0.72				
hot spring	25	0.060-14.0	4.48 ±3.38	1	4.00	4	16.00	9	36.00	11	44.00
other*	207	0.012-2.6	0.18 ±0.17	206	99.52	1	0.48				

* dyke, small stream, lake, tap water.

Table 2

Dental Fluorosis in Parts of Area

Place	F^- in Water			Crowd of Surrey		DF*		Type**
	No.	Range (ppm)	X (ppm)	No.	Composition	No.	%	
Xin Mei	1	0.08	0.08	31	nature	21	67.74	A
Lao Hu Shi	1	0.20	0.20	20	nature	15	75.00	A
Qian men	1	0.16	0.16	17	nature	7	41.18	A
He Tang Wei	1	0.21	0.21	12	pupils aged 10-15	12	100.00	A
Wen Tang	25	0.15-1.98	0.63	1114	nature	269	24.15	B
Long Wan Yuan	1	1.20	1.20	48	nature	13	27.08	B
Tang Hu	38	0.08-14.50	1.90	1508	nature	39	78.00	B
Bai Shi	2	1.20-2.60	1.90	50	nature	39	78.00	B
Ruo Tang	1	2.50	2.50	347	nature	240	69.19	C
Zhong Shao	2	0.10-6.00	3.05	70	nature	26	37.00	B
De An fluorspar area	2	2.40-4.00	3.20	13	pupils aged 11-14	12	92.31	C

Correlation analysis between fluoride content in water and incidence of dental fluorosis: $n = 11$, $r = 0.02$, $p > 0.05$

* DF indicates dental fluorosis.

** A indicates fluorosis caused by smoke pollution; B: by high-fluoride hot spring water; C: by fluorspar polluted water.

25 hot spring water, and 207 were other water samples. For the fluoride content of different types of water see Table 1.

The mean fluoride content in hot spring water is the highest of all water samples. The mean fluoride content in well, river and other water is less than 1 ppm. Differences are statistically significant between the fluoride content of hot spring and the other three types of water ($p < 0.01$). According to the national standard of fluoride content of drinking water (0.5-1.0 ppm), the fluoride content in hot spring water exceeds the standard markedly; of the 25 hot springs, all except one contain more than 1 ppm fluoride. The rate of exceeding the standard is 96 per cent. In those above standard, the fluoride content mainly exceeds 4 ppm; they comprise 44 percent of the total hot spring water samples. The rate of above standard well water is 1.27 percent and of river water is 0.72 per cent.

Table 2 shows the relationship between fluoride content in drinking water and dental fluorosis. The incidence of dental fluorosis is highest (100%) among pupils aged 10-15 in He Tang Wei village; second, among pupils aged 11-14 in De-An fluorspar areas (92.31%). The correlation between dental fluorosis and fluoride content in water is not significant ($p > 0.05$).

Table 3, which presents the fluoride content of water according to topography, shows that fluoride content of water in a mountainous area is higher than that in the flatlands, and the fluoride content of water in flatlands is higher than that in hilly land. The differences in fluoride content of water according to the three topographies are not statistically significant.

Table 3

Fluoride Content of Water According to Topography

Topography	Fluoride Content in Water			U	p
	No.	Range (ppm)	X ±S.D. (ppm)		
Flatland	47	0.045-0.74	0.152 ±0.14	0.579	> 0.05
Hilly	98	0.027-1.05	0.131 ±0.125	0.875	> 0.05
Mountain	54	0.022-4.20	0.200 ±0.574	0.872	> 0.05

Discussion and Summary

The natural fluoride level in ground water depends on such factors as geographical location, chemical and physical characteristics, consistency of the soil, porosity of rock, pH and temperature, complexing action of other elements and depth of wells (1). According to analysis of water samples, the fluoride content of water in most parts of the province is low. Accumulation of fluoride on the earth's surface is not anticipated because of abundance of rainfall; precipitation is greater than evapotranspiration; due to many rivers within boundaries, the discharge of water is large. According to Li Ribang et al (2) the fluoride content in shallow ground water and total fluoride content in cultivated soil cannot be significantly correlated. There is, however, a positive significant correlation between the fluoride content in shallow ground water and that dissolved in water from cultivated soil. In the tropics and sub-

tropics fluoride is dissolved in water from red and yellow soil; the latter is neutral to acid (2). Many factors, which determine fluoride in ground water, conform to practical conditions of the province.

In our provinoe, rich in terrestrial heat resources, more than 90 hot spring areas determine 25 hot springs; samples containing above standard fluoride are as high as 24 which is identical with data reported by Zheng Buoshan (3). In some areas, high-fluoride in hot spring water causes the fluoride level in river water to rise. The fluoride content of hot spring is 6 ppm in He-Keng village of Guan Xia commune; in Shi-Cheng county nearby the fluoride content of river water is 1.8 ppm; it is 3.2 ppm in Xing Teng commune; in An-Yuan county, nearby, it is 1.6 ppm.

Our province is rich in mineral resources. Because some rock has a high-fluoride level, the high-fluoride water which is close to the rock, causes endemic fluorosis. For example, the mining areas in Ruo-Tang village of Ning-Du county and in De-An county belong to this kind of fluorosis area.

In Japan and Beijing, reports indicate a lack of relationship between fluoride content in drinking water and incidence of dental fluorosis (4,5). The same situation exists here. Further research is needed to determine whether or not the high-fluoride in food and air is the cause of dental fluorosis. Fluorosis caused by smoke pollution from burning coal has been recorded in our country. Whereas the fluoride content of drinking water does not exceed the standard in Xing-Mei and Lao Fushi villages in the city of Pin-Xiang, and in Men-Qian village in the city of Yi-Cheng where coal is found, dental fluorosis in these areas is marked. Possibly fluorosis results from smoke pollution. The cause of fluoride pollution requires further research.

References

1. WHO: Environmental Health Criteria 36: In Fluorine and Fluorides, Geneva Switzerland, 1984, pp. 25.
2. Li Ribang, Tan Jianan, Wang Lizhen, et al: The Fluoride Content in Cultivated Soil Under Different Geographical Conditions in China and its Relation to Endemic Fluorosis. Geographical Research, Vol. 4, March, 1985, pp. 36.
3. Zhen Baushan: The Geochemistry of Fluorosis in Southern China. Guiyang Institute of Geochemistry, Academia Sinica, December, 1982, pp. 1-2.
4. Song, Pu, Xin, Zhi, Zhu et al: Medicine and Fluoride, External Medicine: Section on Medicine and Geography, No. 2, 1983, pp. 85.
5. Dai Ruiting: The Endemic Fluorosis and the Control, Beijing Anti-Epidemic Station, May, 1983, pp. 23-25.

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URINARY FLUORIDE IN WORKERS AND RATS EXPOSED TO PHOSPHORITES

by
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SUMMARY: Absorbed phosporites may release free fluorides into the human organism. Urinary fluorides of workers employed in transport of phosphorites was significantly higher than in controls. In rats given single doses of phosphorite dust orally and intratracheally urinary fluoride increased distinctly for several days. Pharmacokinetic parameters indicate that occupational exposure to phosphorite dust may lead to accumulation of fluorides in humans.

KEY WORDS: Humans; Phosphorites; Rats; Urinary fluorides.

Introduction

The output and processing of fluorine-bearing sedimentary phosphorite rocks increases with the growing demand for fertilizers. Workers employed in transport of phosphorites are often exposed to high concentrations of dust.

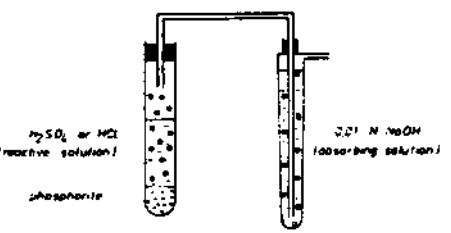
Although the biologic activity of phosphorites is rather low (1-3), considering their fluoride content it cannot be excluded that, under certain conditions, they may be harmful for humans. Increased uptake of fluorine compounds may cause several serious acute and chronic effects (4,5).

Phosphorites contain several percent fluoride; fluoroapatite contains even more. However, since only a small part of the fluoride, that in free form, exerts an effect on the organism, release of free fluoride may depend on various conditions.

In the present study this process has been investigated in workers and rats exposed to phosphorite dusts by measuring urinary fluoride.

Figure 1

Apparatus for Release and Absorption of HF.



Materials and Methods

Release of fluoride from phosphorites. To establish the fluoride content three brands of phosphorites were treated with water, sulphuric acid and hydrochloric acid. Dissolution of phosphorites in acids occurs with release of gaseous hydrogen fluoride which was absorbed in alkaline solution (Figure 1).

The fluoride content was measured in combined absorbing and reactive solutions after neutralization.

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Urinary fluoride in humans: Urinary fluoride was determined in 49 workers employed in phosphorite transport. During the experiment in spring 1986, phosphorites of brands Alger, Jordan, and Sfax were reloaded. Specimens of urine were collected after the work shift in the middle of the week. The control group consisted of 20 healthy adults who had no professional contact with fluorides and fertilizers.

Urinary fluoride in rats. Male Wistar rats weighing 180 ±20 g were given Jordan phosphorite —the brand which contains the highest amount of fluoride. Only the fraction containing particles 0.07 mm or less in diameter was used. The phosphorite was administered in single doses: a] orally as a suspension of 500 mg in 1 mL of water per rat; b] intratracheally as a suspension of 50 mg in 0.5 mL of saline per rat. Intratracheal insufflation was performed in rats anesthetized with ethyl ether by insertion of a rigid tube into the trachea.

After administration of a single dose of phosphorite dust, animals in groups of two were placed in metabolic cages and 24 h portions of urine were collected. The animals were given water ad libitum. To avoid contamination of urine, rats were fed outside the cages (2 h every day). The experiment was terminated as soon as the fluoride concentration of treated animals reached the pre-exposure level.

Determination of fluoride. Fluoride in water solution and urine was determined by means of a fluoride-specific electrode (Aquajon, model B 002) and Ag/AgCl reference electrode with a double jacket. The ion potential was measured by a N-512 Elpo pH meter five minutes after immersion of the electrodes. Before measurement, samples were diluted with equal volumes of pH 7.0 citric buffer (6). Calculations were based on a response factor from a standard curve prepared daily.

Student's t-test was applied to determine significance.

Results

The results of fluoride release from phosphorites imported to Poland are presented in Table 1.

A complete dissolution of phosphorites did not occur in any medium. The

Table 1

Fluoride Released from Phosphorites in Different Media (g F^-/100 g)*

Brand of Phosphorite	Medium		
	water	10 N H_2SO_4	10 N HCl
Jordan	0.0018	1.57	0.029
Algier	0.0023	1.21	0.022
Sfax	0.0011	0.77	0.034

*Values represent the mean of three determinations

greatest amount of fluoride was released from phosphorites by 10 N sulphuric acid, namely close to the total fluoride content in phosphorites (7).

Urinary fluoride in rats after administration of a single oral and intratracheal dose is presented in Figure 2 and in Tables 2 and 3.

Figure 2

Excretion of Fluoride in Urine of Rats Treated with Jordan Phosphorite

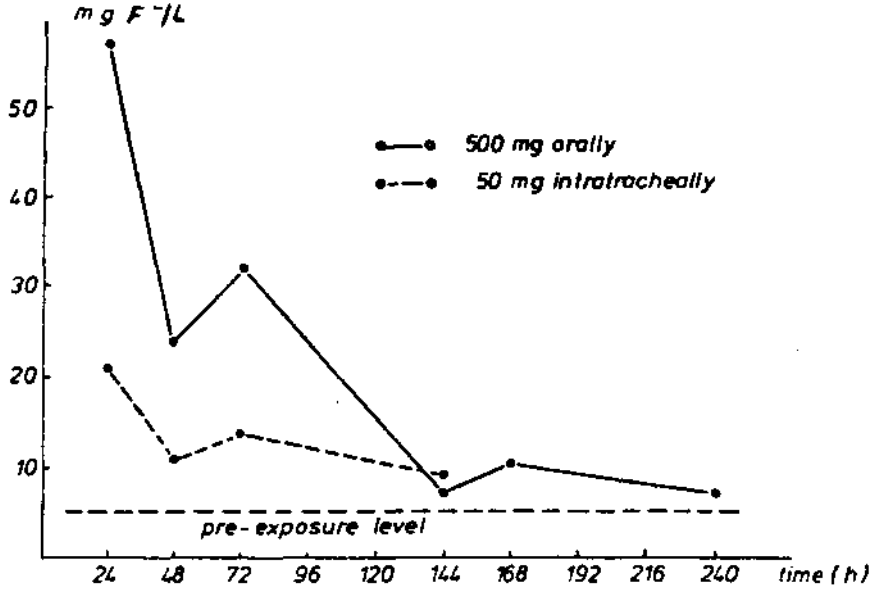

Table 2

Urinary Fluoride in Rats Given Orally 500 mg of Phosphorites

F^-(mg/L)	Time after treatment (hr)					
	24	48	72	144	168	240
mean	57.7	24.2	31.8	7.1	10.3	7.5
±S.D.	18.1	9.0	9.6	1.7	2.7	1.5
n	10	10	10	10	10	10

S.D. = standard deviation n = number of animals

Tables 4 and 5 present pharmacokinetic parameters on fluoride excretion in rats treated with phosphorites and urinary fluoride concentrations in workers employed in transport of phosphorites respectively.

Discussion

The mean urinary fluoride level in untreated rats determined in 30 pairs of animals was 6.05 ±1.78 mg/L.

Table 3

Urinary Fluoride in Rats Given Intratracheally 50 mg of Phosphorites

F^-(mg/L)	Time after administration (hr)			
	24	48	72	144
mean	20.8	10.7	13.5	8.3
±S.D.	9.3	3.4	4.2	2.4
n	10	10	10	10

(Abbreviations as in Table 2)

Table 4

Pharmacokinetic Parameters

Time interval	Route of Administration			
	oral		intratracheal	
	k_e	$t_{0.5}$	k_e	$t_{0.5}$
0-48 hr	0.034	20.1	0.026	26.6
72-168 hr	0.012	58.7	0.005	136.1

k_e = elimination rate constant (h^-) = $\frac{\log c_1 - \log c_2}{0.4343\ (t_2 - t_1)}$

c_1, c_2 = fluoride concentrations in times t_1, t_2

$t_{0.5}$ = elimination half-life (hr) = $t_{0.5} = \frac{0.693}{k_e}$

Table 5

Urinary Fluoride in Workers Employed in Transport of Phosphorites

F^-(mg/L)	Workers	Controls
mean	1.70*	1.05
±S.D.	1.20	0.38
n	49	20

Abreviations as in Table 2

*Significant at $p < 0.01$

After administration of phosphorites both orally and intratracheally urinary fluoride increased considerably for several days.

Excretion of fluoride is biphasic; the rapid phase occurs between 0-48 h and a slower phase between 72-168 h. At the third day (48-72 h) after administration of phosphorites by both routes urinary fluoride increased distinctly,

possibly the result of liberation of free fluoride in the body or release from primary depots – erythrocytes and serum proteins (9).

The result of experiments on rats (elimination half-life) indicates that occupational exposure to high levels of phosphorite dusts may lead to accumulation of fluoride in the body. Whereas phosphorites were transported ashore from ships and from wharfs to freight cars by band conveyors, some operations are performed manually. About 60 workers were employed in phosphorite transport. The exposure depends on the intensity of work and the supply of material. Usually they do not use any respiratory protective devices. The concentration of dust in air on work-stand ranged from 1-250 mg/m^3 (in Poland the MAK value is 10 mg/m^3). Microscopic examination of phosphorite dust demonstrated that 40-80% of the particles belong to the respirable fraction (below 5 μm in diameter) which easily penetrates to respiratory bronchioles and pulmonary alveoli.

Nevertheless, no significant changes in health of workers with long-term exposure was ascertained. Only a few cases of upper airways irritation and one case of moderate pneumoconiosis were found in this group.

Conclusion

The mean urinary fluoride level of workers employed in transport of phosphorites was significantly higher than in controls ($p < 0.01$). The highest result found in workers was 8.2 mg/L. Values obtained by others in unexposed persons are in the range of 0.18-1.14 mg/L; in exposed persons 2.41-43.41 mg/L (3,6,8-10).

Fluctuations in urinary fluorosis observed on different days may depend on intensity of work and meterological conditions.

References

1. Więcek, E., Szymczykiewicz, K., Gościcki, J., Szendzikowski, S., and Woźniak, H.: Experimental Silicosis: Evaluation of Silicogenic Properties of Apatites and Phosphorites. (Polish), Med. Pracy, 29:33-44, 1978.
2. Aitembetov, B.N.: Some Parameters of Fat Metabolism in Normal Subjects Engaged in Phosphorous Manufacture. (Russian), Gig. Trud. Prof. Zabol., 5:8-9, 1984.
3. Patty, F.A.: Industrial Hygiene and Toxicology, vol. 2. Interscience, New York-London-Sydney, 1965, p. 832-844.
4. Franke, J., Rath, F., Runge, H., Fengler, F., Auermann, E. and Lenart, G.: Industrial Fluorosis. Fluoride, 8:61-84, 1975.
5. Groth, III, E.: An Evaluation of the Potential for Ecological Damage by Chronic Low-Level Environmental Pollution by Fluoride. Fluoride, 8:224-240, 1975.
6. Sunshine, J.: Manual of Analytical Toxicology. Chemical Rubber, Cleveland, 1971, p. 165-167.
7. Cross, Jr., F.L., and Ross, R.W.: Fluoride Emission from Phosphate Processing Plants. Fluoride, 2:97-105, 1969.
8. Krechniak, J.: Fluoride Hazard Among Welders. Fluoride, 2:13-24, 1969.
9. Jacyszyn, K. and Marut, A.: Fluoride in Blood and Urine in Humans Administered Fluoride and Exposed to Fluoride-Polluted Air. Fluoride, 19:26-34, 1986.
10. Rosenberg, E.E., Shcherbakov, S.V., Lobarev, V.N., Golub, I.I. and Galperin, G.B.: On the Use of Fluoride Excretion Indices for the Assessment of the Efficiency of Individual Protective Devices for Respiratory Organs, (Russian). Gig. Sanit., 11:89-91, 1985.

CHANGES IN METABOLITES AND PHYSIOLOGICAL ACTIVITIES IN A FRESHWATER MUSSEL, INDONAIA CAERULEUS (PRASHAD) DUE TO SHORT-TERM EXPOSURE TO FLUORIDE

by

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SUMMARY: Exposure of freshwater bivalve Indonaia caeruleus to 0.5, 2.0 and 5.0 ppm fluoride for 12 hr, produced changes in rate of heart beat and oxygen consumption. Fluoride affected protein, glycogen and lipid content of various tissues. When the animals exposed to fluoride were maintained in fluoride-free medium for 12 hr, the rate of heart beat and oxygen consumption almost returned to normal. In this medium changes in metabolites varied considerably. Toxicity of fluoride is specific to different tissues and doses/concentration.

KEY WORDS: Fluoride toxicity; Freshwater bivalve; Glycogen; Heart beat; Lipid; Oxygen consumption; Protein.

Introduction

Studies on the toxicity of members of the aquatic ecosystem to trace elements is gaining considerable attention from biologists. One of the trace elements, fluoride, plays a major role in deteriorating the aquatic ecosystem (1). In an assessment of the toxic components of the Illinois River water (USA), fluoride was one of 6 major contributors to toxicity in blue gills (Lepomis machrochirus) (2). Few reports are available on the toxicity of fluoride to aquatic vertebrates, such as fishes (3,4), invertebrates like crabs (5,6) and bivalve molluscs (7,8). The fluoride ion is a protoplasmic poison and a living cell can only tolerate a small amount of it (9). Studies (in vitro) have shown that incubation of liver and kidney cells of Wistar rats in various fluoride concentrations (1.5-12 mM NaF) for 60 min adversely affected protein synthesis (10). However, in vitro effects of fluoride on vertebrate tissues may differ from in vivo effects since the major part of ingested fluoride is incorporated into the mineralized tissues (11). Hence in the present study the bivalve mollusc, an important link in the aquatic ecosystem at the lower level of the food chain, was selected as the test animal.

This study, aimed at understanding the changes, if any, in physiological activities such as oxygen consumption, heart beat and protein, glycogen and lipid content in different tissues (foot, mantle, gill, hepatopancreas and gonad) of the fluoride-exposed bivalve molluscs, I. caeruleus.

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This study, aimed at understanding the changes, if any, in physiological activities such as oxygen consumption, heart beat and protein, glycogen and lipid content in different tissues (foot, mantle, gill, hepatopancreas and gonad) of the fluoride-exposed bivalve molluscs, I. caeruleus.

Materials and Methods

The animals (I. caeruleus), collected from Godavari River at Paithan in Dist. Aurangabad, Maharashtra State, India, were brought to the laboratory, the shells cleaned and stocked in water from a reservoir for 24 hr. Our previous studies have shown that I. caeruleus can easily acclimatize to this water (12). Fifty animals, 40-45 mm in length, were maintained in distilled water (19°C). At the end of 24 hr the rate of oxygen consumption, heart beat and protein, glycogen and lipid contents of different tissues namely foot, mantle, gills, hepatopancreas and gonad of the animals were estimated. Similar estimations were also conducted on the animals maintained in reservoir water. The initial determination of these, prior to treatment, was to note changes (if any) due to maintenance in distilled water. The study was designed to understand the changes in physiological activities (rate of heart beat and oxygen consumption) and metabolites (protein, glycogen and lipid) in I. caeruleus, when fluoride was added to the exposure medium (experiment I) and when fluoride was withdrawn from the medium (experiment II).

Experiment I: Ten animals in each treatment were exposed to 0.0 (controls), 0.5, 2, and 5 ppm fluoride (sodium). At the end of 1, 6 and 12 hr, rate of heart beat and oxygen consumption were measured from animals exposed to the various treatments. After measurement the animals were returned to the respective exposure media. Protein, glycogen and lipid content in different tissues of animals from the fluoride treatment as well as controls were estimated at the end of 12 hrs.

Experiment II: After 12 hrs animals exposed to different fluoride concentrations were separately maintained in distilled fluoride-free water. At the end of 12 hrs, biochemical estimations were conducted on these animals as well as on controls.

To count the heart beat, the heart of the animal was exposed by removing a portion of the shell at the umbo region. The shell was cut to a triangular shape using a hacksaw and the cut portion carefully removed. The rate of

heart beat was expressed as the number of diastole beats/min. The oxygen consumed by the animals was measured according to Golterman (3) and expressed as mg oxygen consumed per liter solu in 1 hr by 1 g of animal (excluding the weight of the shells) (mg/L/h/g). The protein content in tissues was estimated by the biuret method (14) using bovine serum albumin as the standard. Glycogen content was measured according to de Zwaan and Zandee (15). Lipid content was estimated gravimetrically as given by Blig and Dyer (16). The results are expressed on 100 mg wet weight of tissue.

Determinations of physiological activities and estimation of metabolites were done from 3 randomly selected animals. Analysis of variance (two way, with replicates), followed by Student-Newman-Keuls test for comparison of means (17) was used to determine significant differences within the treatments in experiments I and II.

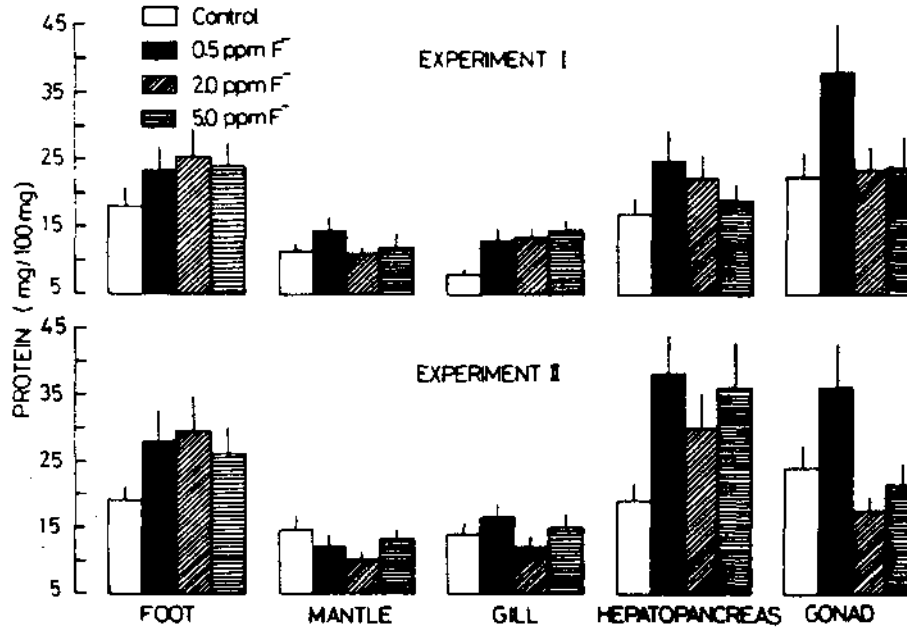
Results

Initial estimations, prior to fluoride exposure, did not show any significant difference between animals from reservoir water and those maintained in distilled water.

In experiment I, (Figure 1) protein content in foot, hepatopancreas and gills of the animals exposed to the fluoride treatments (0.5, 2.0 and 5.0 ppm)

Figure 1

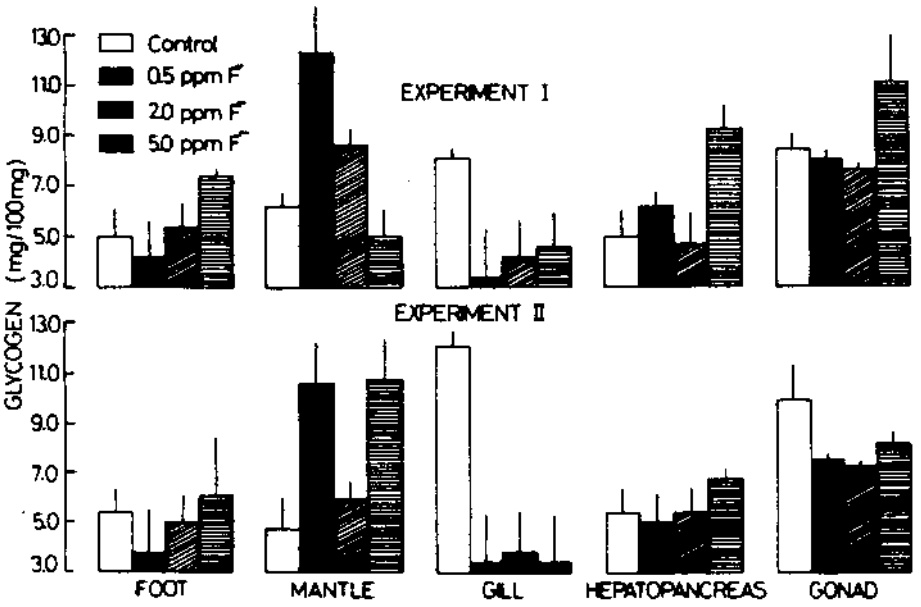
Protein content in different tissues in I. caeruleus. Experiment I, animals exposed to different fluoride media for 12 hr; Experiment II, fluoride-treated animals maintained in fluoride-free medium for 12 hr. Values represent means ±S.D. of 3 animals.



significantly increased compared to the control ($p < 0.5$). In mantle and gonad of animals exposed to 0.5 ppm fluoride, protein content increased significantly compared to the control ($p < 0.05$). In experiment II animals exposed to different fluoride media showed high protein content ($p < 0.05$) in foot and hepatopancreas, compared to the control, as in experiment I. Protein content in gills did not change significantly ($p > 0.05$). In mantle and gonad, protein content decreased significantly ($p < 0.05$) in animals exposed to 2.0 ppm fluoride. Comparing experiment II with experiment I, the increase in protein content was significant only in hepatopancreas (Figure 1).

Figure 2

Glycogen content in different tissues of I. caeruleus. Experiment I, animals exposed to different fluoride media for 12 hr; Experiment II, fluoride-treated animals maintained in fluoride-free medium for 12 hr. Values represent means ±S.D. of 3 animals.

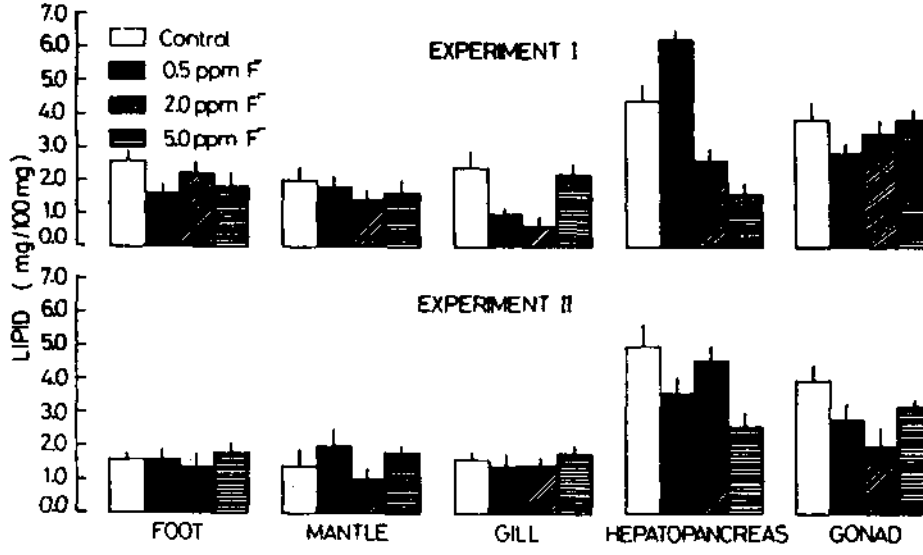


In experiment I, glycogen content (Figure 2) in foot did not change significantly compared to the control ($p > 0.05$). It increased significantly in gonads and in hepatopancreas of the animals exposed to 5.0 ppm fluoride. In mantle, glycogen content increased significantly in animals exposed to 0.5 ppm fluoride ($p < 0.05$) and decreased in those exposed to 5.0 ppm fluoride. Gills showed a significant decline in glycogen content in all exposure media ($p < 0.05$). In experiment II, changes in glycogen content in foot and gills compared to the control remained the same as in experiment I. In mantle it increased significantly, compared to controls in animals exposed to 0.5 and 5.0 ppm fluoride ($p < 0.05$). Hepatopancreas did not show any significant change in glycogen content ($p > 0.05$). It decreased significantly in gonads ($p < 0.05$) of animals exposed to all fluoride media. Comparing experiment II with experiment I changes in glycogen content in different tissues was insignificant ($p > 0.05$) (Figure 2).

In experiment I, compared to the control, lipid content did not change significantly ($p > 0.05$) in foot, mantle, gills and hepatopancreas of the animals exposed to different fluoride media (Figure 3). However, it decreased significantly ($p < 0.05$) in gonads of animals exposed to 0.5 ppm fluoride. In experiment II, as in experiment I, no significant change was observed in lipid content in foot, mantle, gill and hepatopancreas of the animals compared to controls. Compared to the control, lipid content in gonad significantly declined ($p < 0.05$). When experiment II was compared with experiment I, changes in lipid content were insignificant in tissues, except in gonad, where it had significantly decreased ($p < 0.05$) (Figure 3).

Figure 3

Lipid content in different tissues of I. caeruleus. Experiment I, animals exposed to different fluoride media for 12 hr; Experiment II, fluoride-treated animals maintained in fluoride-free medium for 12 hr. Values represent means ±S.D. of 3 animals.



Compared to the control, a significant, dose-dependent decrease in rate of heart beat was observed in animals exposed to different fluoride media ($p > 0.05$) (Figure 4). In animals maintained in distilled water for 12 hr (experiment II), the rate of heart beat did not change significantly, compared to controls ($p > 0.05$) (Figure 4). Rate of oxygen consumption, compared to the controls, increased significantly in the animals at the end of 1 and 6 hr exposure to different fluoride media ($p < 0.05$) (Figure 5). However, at the end of 12 hr it declined significantly in higher concentrations of fluoride ($p < 0.05$). In experiment II, the animals exposed to 0.5 and 2 ppm fluoride did not show a significant change in rate of oxygen consumption ($p > 0.05$). However, animals exposed to 5.0 ppm fluoride showed a significantly higher rate of oxygen consumption than controls ($p < 0.05$) (Figure 5).

Figure 4

Rate of heart beat of I. caruleus. Experiment I, animals exposed to different fluoride media; Experiment II, fluoride-treated animals maintained in fluoride-free medium. Values represent means ±S.D. of 3 animals.

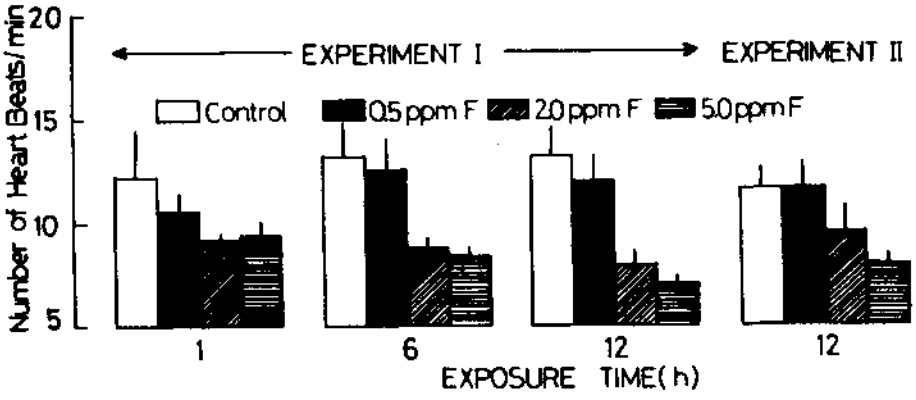
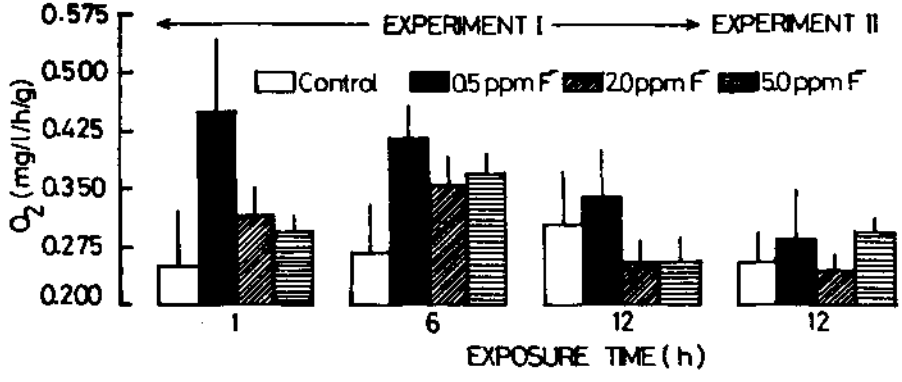


Figure 5

Rate of oxygen consumption of I. caeruleus. Experiment I, animals exposed to different fluoride media; Experiment II, fluoride-treated animals maintained in fluoride free medium. Values represent means ±S.D. of 3 animals.



Discussion

Abnormal fluoride content in river water can be a major threat to animal life (6). Fingernail clams (Musculium tranversum) exposed to 0.75 ppm fluoride showed 50% reduction in cilia beating at the end of 10 min (18). These clams were more sensitive to sub-lethal than to lethal concentrations of fluoride. In the present study, protein content in tissues of the bivalve molluscs was

altered more by 0.5 ppm than by 2.0 and 5.0 ppm fluoride. This alteration was more pronounced in gonad, followed by hepatopancreas. In 0.5 ppm fluoride concentration, lipid content in gonad declined, whereas glycogen content remained unchanged; Gills showed an increase in protein content, a decrease in glycogen content. Mantle in 0.5 ppm increased in protein and glycogen content whereas in 5.0 ppm glycogen content decreased. Different tissues of this bivalve mollusc show differential sensitivity to fluoride toxicity. Whereas several in vitro studies (19,20) emphasize that the cytotoxic effect of fluoride is inhibition of protein synthesis according to Hongslo et al. (10). There may be some other mechanism of cytotoxic effect of fluoride depending on cell types.

In Gonads the primary target for fluoride toxicity seems to be a lipid content decrease which may be due to increases in oxygen consumption. In barnacles oxygen consumption is directly related to biochemical resources (21). A steady increase in oxygen consumption coupled with a decrease in lipid store was observed in starving carnivorous prosobranch, Thais lamellosa (22). In the present study, rate of heart beat was negatively related to oxygen consumption. It has been shown that rate of heart beat in Arctica islandica is directly related to oxygen tension of the blood (23). Bradycardia in I. caeruleus exposed to fluoride media may be due to the increased oxygen tention of blood. Increase in oxygen consumption caused peroxidative destruction of lipid (24). In the present study some of the tissues of I. caeruleus, particularly gonad and hepatopancreas are profoundly affected by fluoride toxicity. Certain cells are able to grow in the presence of toxic fluoride concentrations by keeping intracellular fluoride concentrations low (25). Hepatopancreas and gonads of I. caeruleus seem to have less capacity to maintain a low intracellular fluoride concentration.

Conclusion

Fluoride toxicity to freshwater bivalve molluscs is many-fold and tissue specific. Certain tissues are more sensitive to lower fluoride concentrations than to higher ones. Decrease in lipid content coupled with increase in oxygen consumption suggests the possibility of lipid perodixation which, however, requires further investigation to substantiate.

References

1. Rose, D. and Marier, J.R.: Environmental Fluoride, 1977. National Research Council (Canada), NRCC No. 16081, 1977, p. 38-39.
2. Lubinski, K.S. and Sparks, R.E.: The Use of Toxicity Indices to Assess the Quality of the Illinois River. Assoc. South East Biol. Bull., 22:64, 1975.
3. Wright, D.A.: Toxicity of Fluoride to Brown Trout Fry (Salmo trutt). Environ. Pollut., 12:57-62, 1977.
4. Pillai, K.S. and Mane, U.H.: Effect of F^- Effluent on Some Metabolites and Minerals in Fry of Catla catla (Hamilton). Fluoride, 17:224-233, 1984.
5. Moore, D.J.: The Uptake and Concentration of Fluoride by the Blue Crab, Callinectes sapidus. Chesapeake Sci., 12:1-13, 1971.
6. Wright, D.A. and Davison, A.W.: The Accumulation of Fluoride by Marine and Intertidal Animals. Environ. Pollut., 8:1-13, 1975.
7. Hemens, J. and Warwick, R.J.: The Effects of Fluoride on Estuarine Organisms. Wat. Res., 6:1301-1308, 1972.

8. Barbaro, A., Francescon, A. and Polo, B.: Fluoride Accumulation in Aquatic Organisms in the Lagoon of Venice, Italy. Fluoride, 14:102-107, 1981.
9. Pack, M.R.: Effects of Hydrogen Fluoride on Production and Organic Reserves of Bean Seeds. Environ. Sci. Technol., 5:1128-1132, 1971.
10. Hongslo, C.F., Hongslo, J.K. and Holland, R.I.: Fluoride Sensitivity of Cells from Different Organs. Acta Pharmacol. et Toxicol., 46:73-77, 1980.
11. Eanes, E.D. and Posner, A.S.: Structure and Chemistry of Bone Mineral. in H. Schraer (Ed.) Biological Calcification — Cellular and Molecular Aspects, Appleton-Century-Crofts, 1970, p. 1-26.
12. Mane, U.H., Akarte, S.R. and Muley, D.V.: Effect of Cythionmalathion on Respiration in Three Freshwater Bivalve Molluscs from Godavari River near Paithan. J. Environ. Biol., 5:71-80, 1984.
13. Golterman, H.L.: Methods of Chemical Analysis of Freshwaters. IBP, Blackwell Scientific Publication, Oxford and Edinburgh, 1969, p. 127-133.
14. Gornall, A.G., Bradwill, C.J. and David, M.M.: Determination of Serum Protein by Means of Biuret Reaction. J. Biol. Chem., 177:751-766, 1949.
15. de Zwaan, A., and Zandee, D.I.: Body Distribution and Seasonal Changes in the Glycogen Content of the Common Sea Mussel, Mytilus edulis. Comp. Biochem. Physiol., 43:53-55, 1972.
16. Blig, E.G. and Dyer, W.J.: A Rapid Method for Lipid Extraction and Purification. Can. J. Biochem. Physiol., 37:911-927, 1959.
17. Woolf, C.M.: Principles of Biometry. D. Van Nostrand Co., Inc., Princeton, 1968, p. 101-109.
18. Sparks, R.E., Sandusry, M.J. and Paparo, A.A.: Identification of the Water Quality Factors Which Prevent Fingernail Clams (Musculium traversum) from Recolonising the Illinois River (USA): Phase 3. Univ. Ill. Urbana-Champaign Wat. Resour. Cent. Res. Rep., 0:1-55, 1983.
19. Mankovitz, R., Kisilevsky, R. and Florian, M.: Chinese Hamster Cell Lines Resistant to the Cytotoxic Action of Fluoride, Can. J. Genet. Cytol., 20:71-84, 1978.
20. Holland, R.I.: Fluoride Inhibition of Protein Synthesis, Cell Biol. International Rep., 3:701-704, 1979.
21. Barnes, H., Barnes, M. and Finlayson, D.M.: The Seasonal Changes in Body Weight, Biochemical Composition and Oxygen Uptake of Two Common Boreo-artic Cirripedes, Balanus balanoides and B. balanus. J. Mar. Biol. Assoc. U.K., 43:182-211, 1963.
22. Stickle, W.B. and Duerr, F.G.: The Effect of Starvation on the Respiration and Major Nutrient Stores of Thais lamellosa. Comp. Biochem. Physiol., 33:689-695, 1970.
23. Taylor, A.: Aspects of the Respiratory Physiology of the Bivalve, Arctica islandica (L.). Ph.D. Thesis, Liverpool Univ., (1974).
24. Dixit, R., Mukhtar, H. and Bricks, D.R.: Studies on the Role of Reactive Oxygen Species Mediating Lipid Peroxide Formation in Epidermal Microsomes of Rat Skin. J. Invest. Dermatol., 81:369-375, 1983.
25. Holland, R.I. and Hongslo, J.K.: Cellular Resistance to Fluoride. Cell Biol. International Rep., 2:551-560, 1978.

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FLUORIDE SUPPLEMENTATION

by

Committee on Nutrition* 1985-1986

(Abstracted from Pediatrics 77:758-761, 1986)

The narrow therapeutic range of fluoride and the danger of excess fluoride ingestion which results in dental mottling (fluorosis) is increasingly being recognized. In view of the fact that mineralization of permanent teeth continues up to 6 years of age, the systemic dental effects of fluoride are exerted during this period. Excess fluoride ingestion at this time can cause fluorosis. The narrowness of the therapeutic range is emphasized by the fact that mild fluorosis has been seen with oral intakes greater than 0.1 mg/kg/d. For the permanent teeth, the most critical period of vulnerability to excess fluoride occurs at approximately 2 years of age. The effectiveness of fluoride supplementation in pregnancy is still in dispute.

Fluoride intake of 6-year-old infants in the United States has been estimated to vary from 0.207 to 0.541 mg/d (0.03 to 0.07 mg/kg/d). Thus the total amount of fluoride added to the diet may be appreciable.

Since toothpaste contains 1 mg fluoride per gram it is essential that parents teach their children to avoid swallowing toothpaste.

Laurence Finberg, M.D., Chairman
Signed by six members of the American Academy of Pediatrics

KEY WORDS: Dental fluorosis, Fluoride supplements

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OCCURRENCE AND DISTRIBUTION OF FLUORIDE IN GROUNDWATERS OF KENYA

by

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Nairobi, Kenya

(Abstracted from East African Medical Journal 61:503-512, 1984)

Water samples from 1286 boreholes and wells from different parts of Kenya were analyzed for their fluoride ion concentration. In the majority of the samples (61.4%) the fluoride ion level was above 1.0 ppm; 19.5% were above 5.0 ppm. The highest concentration recorded was 57.0 ppm. Excess levels of fluoride were found in most parts of the country, especially in the Nairobi, Rift Valley, East and Central Provinces, where approximately 59.5% of Kenya's population is located.

In all, 1286 samples of water were analyzed; 183 from Nairobi Province, 13 from Western Province, 93 from Coast Province; 31 from Nyanza Province, 181 from Eastern Province, 76 from North Eastern Province, 313 from Rift Valley Province, and 396 from Central Province. Of samples analyzed, in 248 (19.3%) the fluoride ion concentration was between 0.0 and 0.4 ppm; in 247 (19.2%) between 0.5 and 1.0 ppm; in 411 (31.9%) between 1.1 and 3.0 ppm; in 129 (10.0%) between 3.1 and 5.0 ppm; in 99 (7.7%) between 5.1 and 8.0 ppm; and in 152 (11.8%) 8.1 and over.

The distribution of high fluoride in groundwaters was found to approximate the distribution of volcanic soils in Kenya.

Fluorosis has been reported from both Ethiopia in the north, and from Tanzania in the south. The high prevalence of dental fluorosis reported to be endemic may possibly indicate that groundwaters form an important source of fluoride in the diet.

Clearly, if the waters examined here are being drunk by humans then the majority of groundwater sources in Kenya are in need of defluoridation. Research to investigate economic and cost-effective methods of partial defluoridation is urgently needed.

KEY WORDS: Defluoridation needed; Fluorosis; High F in groundwater; Kenya

REPRINTS: K.R. Nair, Department of Dental Surgery, University of Nairobi, Nairobi, Kenya.

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RESPIRATORY SURVEY OF NORTH AMERICAN INDIAN CHILDREN LIVING IN PROXIMITY TO AN ALUMINUM SMELTER

by

P. Ernst, D. Thomas, and M.R. Becklake
Montreal, Quebec, Canada

(Abstracted from Am. Rev. Respir. Dis. 133:307-312, 1986)

To determine whether respiratory abnormality and lung function were related to intensity (level, duration) of exposure to industrial fluoride emissions consisting of particulate and gaseous fluoride, 253 North American Indian children 11 to 17 years of age — living on the Akvasasne Reserve, adjacent to an aluminum smelter — were studied. Subjects were evenly distributed among the differing ages that constitute adolescence, and a similar number of boys and girls were examined. The overall prevalence of cigarette smoking (18% in boys, 33.3% in girls) were similar to that previously reported in other Canadian adolescents. Among boys, closing volume versus vital capacity (CV/VC%) was increased in those raised closest to the smelter as opposed to those living most of their lives farthest from this source of air pollution. In both sexes, there was a significant linear relationship between increasing CV/VC% and the amount of fluoride contained in a spot urine sample.

The authors concluded that exposure to fluoride air pollution in the community may be associated with abnormalities in small airways. The implication of these abnormalities for future respiratory health is unknown.

KEY WORDS: Air pollution (fluoride); Aluminum smelter; Canada; Indians (North American); Respiratory abnormalities.

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HYDROGEN FLUORIDE PERMEABILITY OF ASTOMATOUS AND STOMATOUS PLANT CUTICLES

Influence of Stomata and Comparison with Water Permeability

by

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Champenoux and Grenoble, France

(Abstracted from *Environmental and Experimental Botany* 26:299-308, 1986)

Cuticles from the adaxial surface (with few stomata) and the abaxial surface (with numerous stomata) of Monstera deliciosa (Philodendron) have been isolated enzymatically to determine HF and H_2O permeability and the influence of stomata on these permeabilities. The results indicate:

1. The permeability coefficients, higher for HF than for H_2O, are of the order 2.06×10^{-5} ms^{-1} for HF and 2.8×10^{-6} ms^{-1} for H_2O.

2. Extraction of soluble lipids from the cuticle increases the permeability of both HF and H_2O. Soluble lipids are demonstrated to be the main resistance for H_2O permeability in cuticles, but for HF, both the cutin matrix and soluble lipids determine total resistance.

3. The HF and H_2O permeabilities of the cutin matrix are of the same magnitude, and the results suggest that HF permeates the cuticle mainly via the lipidic components.

In abaxial cuticles, the presence of stomata increases their HF and H_2O permeability, and the permeability coefficient of the closed stomata are of the same magnitude for HF and H_2O. In these stomatous cuticles, H_2O permeates the cuticles mainly via the stomata, whereas HF permeates them via both the stomata and cuticular membrane.

KEY WORDS: Cuticles; HF permeability; Influence of stomata.

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INFLUENCE OF DITHIOCARB, (+)-CATECHIN AND SYLBINE ON HALOTHANE HEPATOTOXICITY IN THE HYPOXIC RAT MODEL

by

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Lübeck, W. Germany

(Abstracted from Acta Pharmacol. et Toxicol., 53:125-129, 1983)

In phenobarbital (phenemalum NFN)-pretreated male rats exposed to 1% halotane for 2 hrs under hypoxic conditions (10% O_2) significant increases in serum enzyme activities of alanine aminotransferase and sorbitol dehydrogenase were observed 24 and 48 hrs later indicating liver damage. In this known model of halothane hepatotoxicity, pretreatment with (+)-catechin (200 mg/kg orally) or silybine (150 mg/kg orally) protected against halothane-induced liver injury, whereas diethyldithiocarbamate (200 mg/kg orally) failed to be effective. Halothane decreased the concentration of reduced glutathione in liver only under hypoxic conditions indicating that glutathione might be involved in the non-oxidative metabolic pathways of halothane. Free fluoride in plasma was used as a measure of non-oxidative defluorination of halothane. Higher plasma fluoride levels were observed under conditions which led to hepatotoxicity but did not correlate with the protective effects of the antidotes. This further supports the assumption that 2-chloro-1,1,1-trifluoroethane might be the radical intermediate responsible for halothane hepatotoxicity.

KEY WORDS: (+)-Catechin; Diethyldithiocarbamate; Halothane; Hepatotoxicity; Silybine; Rats.

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Author's Abstract

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FLUORIDE IN MIXED HUMAN SALIVA AFTER DIFFERENT TOPICAL FLUORIDE TREATMENTS AND POSSIBLE RELATIONS TO CARIES INHIBITION

by

C. Bruun, D. Lambrou, M.J. Larsen, O. Fejerskov, and A. Thylstrup
Copenhagen, Denmark

(Abstracted from Community Dent. Oral Epidemiol. 10:124-129, 1982)

The present study attempts to relate measurements of fluoride concentrations in mixed saliva, following various forms of topical treatments, with available findings from corresponding clinical trials. It is estimated that a caries reduction of about 30% might be obtained from any of these treatments.

No simple relationship between fluoride levels in saliva and caries reduction was observed. The potential of remineralizing solutions like saliva is markedly increased by the presence of fluoride ions in concentrations as low as about 1 ppm or even lower. The higher caries reductions obtained with supervised toothbrushing as compared with unsupervised brushing may in part be related to the controlled removal of plaque, which may facilitate the action of fluoride.

In conclusion, caries inhibition obtained from any of these treatments can be ascribed to the capacity of fluoride in the local environment to reduce caries progression at clinical and subclinical levels.

KEY WORDS: Caries inhibition; Salivary fluoride; Topical fluorides.

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****

EFFECTS OF FLUORIDE ON MEMBRANE PERMEABILITY AND BRUSH BORDER ENZYMES OF RAT INTESTINE IN SITU

by

R.M. Shayiq, H. Raza, and A.M. Kidwai
Srinagar, Kashmir, India

(Abstracted from Food Chem. Toxicol. 24:33-6, 1986)

When 12 mM sodium fluoride was instilled into the ligated intestine of anesthetized rats for 30 min., concentration-dependent change in permeability was observed; there was in increase in the volume of luminal fluid and altered net transport of Na^+ and K^+ ions. The change in permeability was accompanied by increased protein, sialic acid and nucleic acid accumulation in luminal fluid. A striking loss of brush border alkaline phosphatase (41%) sucrase (59%) and gamma-glutamyl transpeptidase (73%) activities was observed at 96 mM fluoride with a corresponding increase in the activity of these enzymes in luminal fluid; 12 mM fluoride on the other hand did not produce any significant effect.

This loss was probably not due to an inhibition of the enzymes by fluoride since in vitro experiments did not produce any such effect over a 0-32 mM range of NaF concentrations except on alkaline phosphatase activity at the 32 mM NaF concentration. The studies, therefore, suggest that loss of brush-border enzyme activities observed in situ was most probably due to membrane damage caused by the high fluoride concentration.

KEY WORDS: Enzyme inhibition; Membrane damage by NaF; Rats.

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****

EFFECT OF FLUORIDE ON THE PHOSPHODIESTERASE OF BOVINE PHOTORECEPTORS

by

Enrico Bignetti, Robert T. Sorbi and Roberto Tirindelli
Parma, Italy

(Abstracted from Vision Res. 26:383-389, 1986)

In the absence of the specific hormone, fluoride is able to activate the adenylate cyclase because it interacts with the GTP binding protein. It has been reported that fluoride activates also the phosphodiesterase of the light-sensitive enzymatic cascade in dark-adapted retinal rod outer segments, but there is no indication whether or not the GTP-binding protein is involved in this process. We show here that, also in the photoreceptor system, fluoride does interact with GTP-binding protein in order to activate the phosphodiesterase in the dark. Further, we show evidence that fluoride solubilizes the GTP-binding protein in the dark and that the resulting complex activates the phosphodiesterase in dark-adapted rod outer segment membranes.

KEY WORDS: Fluoride; GTP-binding protein; Phosphodiesterase; Photoreceptors; Rod outer segments.

REPRINTS: Robert T. Sorbi, Instituto di Fisiologia Umana, Università di Parma, 43100 Parma, Italy

****

ULTRASTRUCTURE OF FLUORIDE-INDUCED CYSTS IN THE RAT MOLAR ENAMEL ORGAN

by

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Fredrik Henell and Lars Hammarstrom
Stockholm, Sweden

(Abstracted from Scand. J. Dent. Res. 94:327-337, 1986)

Ultrastructure of ameloblasts forming the cystic wall together with cells within the cystic lumina was studied by means of transmission electron microscopy. Twenty-four hours after the injection of fluoride the ameloblasts of the cystic wall showed varying degrees of cytoplasmic and nuclear alterations. Some cells displayed signs of necrosis as indicated by condensation of the chromatin. The cytoplasmic changes ranged from altered organelle morphology to fragmentation and almost complete shedding of the whole cytoplasm. In the ameloblasts of the cystic wall, secretory products accumulated intracellularly in distended rough endoplasmatic reticulum, in vesicles of the Golgi region and extracellularly between ameloblasts as well as between cells in the stratum intermedium, indicating an altered matrix secretion. Electron lucent material, cell and cell fragments were found in the cystic lumina, the two latter

apparently originating from the ameloblastic layer. The degenerative changes seemed to follow the normal pattern of cell degeneration.

Light microscopy showed cystic lesions of the ameloblastic layer following a single high dose of fluoride and hypoplasias of the developing enamel, associated with these cysts. According to ultrastructural studies atrophy of the distal part of the ameloblasts followed both chronic and acute fluoride administration. Secretory products accumulated in the rough endoplasmic reticulum. Since morphologic changes of the ameloblasts in the cystic area have not been described in detail, the present investigation was undertaken to study the ultrastructure of these cells, with special attention to the distal part of the ameloblasts facing the developing enamel and to the content of the cystic lumen.

The fluoride-induced alterations in the molar enamel organ consisted of subameloblastic cysts separated from each other by regions of ameloblasts that displayed no morphologic signs of degeneration.

KEY WORDS: Ameloblasts; Fluoride; Tooth development.

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FLUORIDE ABSORPTION: INDEPENDENCE FROM PLASMA FLUORIDE LEVELS

by

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Augusta, Georgia, USA

(Abstracted from the Society for Experimental Biology and Medicine 181:550-554, 1986)

Four different methods were used to evaluate the effect of plasma fluoride levels on the absorption of the ion in rats: (1) the percentage of daily fluoride intake that was excreted in the urine; (2) the concentration of fluoride in femur epiphyses; (3) the net areas under time-plasma fluoride concentration curves after intragastric fluoride doses; and (4) the residual amounts of fluoride in gastrointestinal tracts after the intragastric fluoride doses. These methods failed to indicate that plasma fluoride levels influence the rate or degree of fluoride absorption. Unless extremely high plasma fluoride levels are involved (pharmacologic or toxic doses) it was concluded that absorption of the fluoride ion is independent of plasma levels. These results provide further evidence that plasma fluoride concentrations are not homeostatically regulated.

KEY WORDS: Intragastric F^- doses; Plasma F^-; Rats.

REPRINTS: Departments of Oral Biology and Physiology, Medical College of Georgia, Augusta, Georgia 30912, USA.

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Studies in Environmental Science 27: FLUORIDE RESEARCH 1985 (Elsevier, Amsterdam-New York-Oxford-Tokyo, 1986)

This 435-page volume contains 49 selected papers presented at the 14th Conference of the International Society for Fluoride Research, Morioka, Japan, June 12-15, 1985. Clearly printed and sturdily bound, it is capably edited by Humio Tsunoda, Department of Hygiene and Public Health, School of Medicine, Iwate Medical University, Morioka, Japan, and Ming-Ho Yu, Huxley College of Environmental Studies, Western Washington University, Bellingham, Washington, USA.

Fittingly, the book is dedicated to the memory of the late Dr. Noriko Tsunoda, former Director of Neshi Matzuzono Clinic and wife of Professor Tsunoda. She "not only gave substantial financial assistance to the conference, but was also its hostess and organized all of the social programs." Her untimely death occurred on July 18, about a month after the conference.

Divided into four parts, the volume contains many excellent photographs, drawings and numerous tables, a key word as well as an author index. Part 1. Analytical Methods for Fluoride (9 papers); Part 2. Environmental Fluoride Pollution (13 papers); Part 3. Biological Effects of Fluoride (15 papers); Part 4. Effects of Fluoride on Humans (12 papers). The approximately 200 participants came from eleven countries and represented such diverse disciplines as biology, chemistry, dentistry, environmental science, medicine, pharmacology, toxicology, and veterinary science. Altogether, nearly 100 papers were presented at the conference at either oral or poster sessions.

Among the excellent papers on analytical methods, especially noteworthy are those on new developments in the determination of submicrogram quantities of fluoride (K. Itai and H. Tsunoda), spectrochemical analysis of F by aluminum monofluoride (K. Tsunoda et al.), and gas-chomatographic microanalysis of fluoride (Y. Zaima and S. Goto).

In the section on environmental fluoride pollution, some of the outstanding contributions are concerned with fluoride transport around industrial areas (F. Murray), Fluoride absorption and excretion from F-contaminated food (H. Tsunoda and N. Tsunoda), dental lesions in cattle and sheep caused by coal combustion (F. Riet-Correa et al.), long-term retention of F in bones of former aluminum workers (C.A. Baud et al.), and symptomatology of fluoride-exposed workers (V.K. Desai et al.).

In connection with biological effects of fluoride, valuable contributions covered a broad range of topics, e.g., changes in glucose and calcium metabolism (Y. Suketa et al.), erythrocyte membrane abnormality (A.K. Susheela and S.K. Jain), DNA and RNA synthesis (Y. Li and H. Ma), induction of protein in HeLa cells (T. Imai et al.), thyroid function in rats (M. Tsuchida et al.), and gastrointestinal absorption of fluoride (T. Sato et al.).

The final section, entitled "Effects of Fluoride on Humans," which unavoidably overlaps a number of papers in the other three areas, also deals with a remarkably wide range of important topics, namely ossifications and calcifications of muscle and tendon insertions (J. Franke), topographical localization of fluoride in bone tissue (S. Bang and C.A. Baud), bone histomorphometry in endemic skeletal fluorosis (S.P.S. Teotia et al.), dental fluorosis in relation to

enamel development (T. Ishii and H. Nakagaki), fluoride clearance in the aging kidney (K. Kono et al.), fluoride excretion in human saliva (Y. Yoshida et al.), and comparison of trace amounts of fluoride in human hair (Y. Takagi et al.).

Since fluoride is now being detected in increasing amounts in areas around many industrial operations throughout the world, the publication of this impressive volume is a most valuable and welcome contribution to the scientific community. Few other sources provide such convenient access to and detailed information concerning recent developments in such a wide array of areas of fluoride research.

The book may be ordered from Elsevier Science Publishers, P.O. Box 211, 1000 AE Amsterdam, The Netherlands, or from P.O. Box 1663, Grand Central Station, New York, NY 10163. The price is U.S. $103.75/280.00 Dutch guilders. (U.S. prices are subject to exchange rate fluctuations.)

E.M.W. & A.W.B.

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INSTRUCTIONS TO AUTHORS

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

PREPARATION OF PAPERS

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

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

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

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

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

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

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

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

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

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

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

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