

Volume 27
Number 3
July 1994

FLUORIDE

JOURNAL

of the

International

Society for

Fluoride

Research



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RECENT STUDIES ON ENDEMIC FLUOROSIS IN CHINA

Jianxue Li and Shouren Cao
Beijing, China

SUMMARY: The progress of recent research on the prevention and treatment of endemic fluorosis in China is reviewed. New types of endemic fluorosis areas have been discovered and surveyed. Regulations and standards for health management have been formulated. The biological effects of fluoride have been further investigated. The importance of fluoride research has gained greater recognition and international exchanges have increased.

Twenty nine of China's 30 provinces have endemic fluorosis areas. Areas of three types - water, coal-burning, and tea-drinking - have been found in 26, 14, and 5 provinces, respectively. Surveys in 1990 revealed that 300 million people live in such areas, of whom 3 million have skeletal fluorosis and 40 million have dental fluorosis.

Successes in the prevention and treatment of endemic fluorosis include:

- 1) gathering epidemiological data on prevalence and severity throughout the country;
- 2) implementing preventive measures that reduce drinking water and indoor air levels of fluoride, by changing water sources and types of stoves; and
- 3) developing methods of health regulation by early diagnosis and treatment.

Survey of new types of endemic fluorosis areas

Epidemiological investigations into coal-burning fluorosis have proceeded since 1988. For example, one project in the Sanxa area has been reported, on the extent of such pollution and its health effects. Twenty new types of stoves have been designed, which not only reduce fluoride pollution but save coal as well. Information has been published on new anti-fluoride medicaments, on the scientific and technical base for prevention and treatment, and on establishment of health regulations and standards.

In some areas of Xizang, Xinjiang and Nei Mongol provinces, the fluoride contents of tea infusion are over 2 mg/L. Prevalences of dental fluorosis in children and skeletal fluorosis in adults are over 40% and 20%, respectively. Some people have been exposed for several years to high fluoride intakes from both drinking water and tea infusion.

Endemic areas have been discovered where high levels of fluoride and other microelements coexist in the living environment. For example, it was found that coal-burning in some areas of Guizhou and Hubei provinces caused simultaneously high intakes of aluminium, selenium and arsenic as well as fluoride from the polluted indoor air, while in some areas of Xinjiang and Nei Mongol provinces the well water contained high levels of both arsenic and fluoride.

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Effective measurement of endemic fluorosis

Two advanced methods for determining the fluoride content of micro-samples are: a microdiffusive/combined electrode method and a microabrasive/opposite electrode method. The former is used for determining 5 ng ionic fluorine in a sample of 100 μ l, and the latter for determining at least 0.05 ng ionic fluorine in a sample of 1 μ l. Other improved electrode methods are extensively used for determining fluoride content in a variety of environmental and biological samples, such as: coal, water, air, corn and vegetables from endemic areas; teeth, nail, hair, saliva, blood, and urine from the human body; and bone, heart, liver, kidney, and brain - even of cells - from experimental animals.

In laboratory experiments, some variables affecting immunology, genetics, microelemental metabolism, and enzymology have been investigated, in order to discover early adverse effects of fluoride on human health. So far the tests, though having the advantage of being very sensitive, are unsuitable for early diagnosis of skeletal fluorosis, because of their low reliability and specificity. However, "a syndrome of articular fluorosis" and "an X-ray syndrome of bone transformation in skeletal fluorosis" are promising approaches which could facilitate early diagnosis of skeletal or articular fluorosis and provide fresh evidence for revising the standard for X-ray diagnosis of skeletal fluorosis.

Among over 10 recently developed medicaments for treating fluorosis, the one containing boron has the best understood mechanism for countering the action of fluoride. That is, boron integrates itself with fluoride in the body to form tetra-fluoroborate which can be excreted in urine, and reduces the permeability of fluoride through cellular membranes. All of these medicaments, however, are still in the stage of animal experiment or small clinical test.

More than 30,000 engineering projects, to reduce fluoride levels in drinking water by changing water sources, have been constructed in recent years, benefiting a population of 20 million. The 60% with skeletal fluorosis, for example, have already been on the mend since 1983, when the high fluoride levels in drinking water were lowered in Shandong and Shanxi provinces. The effects of electrodialysis and carbonized bone on the elimination of fluoride from drinking water are also being explored.

As for coal-burning fluorosis, the new types of stoves that save coal and reduce domestic air pollution are now widely used, by over 150,000 families. Levels of pollutants in indoor air, such as fluoride, sulphide, and carbon monoxide, have been decreased to 5-20% of original levels, complying with requirements of the national standard for atmospheric health in residential areas. Advanced farming techniques, adopted to shorten the time of stoving corn, are very helpful in reducing the chance of fluoride pollution of food by coal-burning.

Biological effects of fluoride and their mechanisms

More and more studies on the non-skeletal effects of fluoride, involving almost all systems and parts of the body, have been conducted by Chinese scientists since 1986. The fact that central and peripheral nerves are damaged directly by fluoride corrects an old point of view that the damaged function

of motor nerves should be imputed to osteoproliferation of vertebrae. Fluoride conjugates the phenolic hydroxyl of tyrosine by hydrogen bonding, interfering with the biological anabolism of thyrotropic hormone. Both non-specific and specific immune functions are inhibited by fluoride. The prevalence of tracheo-bronchitis is significantly higher in residents of endemic fluorosis areas. Fluoride can be transferred from the pregnant woman to the fetus and then interfere with the development of the central nervous system of the baby. Fluoride has some adverse effects on the biological functions of normal components in the blood, the electrocardiogram and the heart pump activity. The biotransformation of the liver and urinary function of the kidney are decreased in patients with fluorosis. Fluoride inhibits the activities of SOD and GSH-Px, resulting in a heavy cumulation of free radical and peroxide and then in various cell damage. By using the methods of biophysical analysis and quantum chemical calculation, it has been shown that fluoride has a very strong ability to form a hydrogen bond with the phenolic hydroxyl of tyrosine in proteins, even changing the normal hydrogen bonds of $O\cdots HO\langle\bigcirc\rangle R$ and $N\cdots HO\langle\bigcirc\rangle R$ into an unusual hydrogen bond of $F\cdots HO\langle\bigcirc\rangle R$, and then destroying the normal spatial conformations of various proteins - perhaps one of the most essential mechanisms of fluoride toxicity yet known.

Regulation and standards for health management of endemic fluorosis

The Handbook of Prevention and Treatment of Endemic Fluorosis, published in 1991 by the Department of Endemic Disease Control, Ministry of Public Health, describes almost all methods and standards in the field of fluoride research. Use of the handbook will make management of endemic fluorosis more scientific and beneficial.

A national surveillance network for endemic fluorosis control was set up in 1991, and each year a lot of data collected by the network have been referred to the Department of Endemic Disease Control.

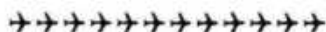
The regulation and standards of endemic fluorosis were formulated by the Endemic Diseases Branch of the National Committee for Health Standards and Techniques, which proposed a series of research projects for coming years. The aims included standards for diagnosing dental and skeletal fluorosis, recognition of endemic areas, evaluation of preventive effects and fluoride intakes, fluoride sampling and analysis, and assessment of therapeutic effects. Corn and fly ash reference materials, prepared and certified for fluoride composition to meet the need for quality analytical control when monitoring domestic soot fluoride pollution, have been approved by the National Bureau of Technical Supervision.

Increase of fluoride research status and international exchanges

More and more scientists are taking part in research projects on the prevention and treatment of endemic fluorosis, including postgraduates in some 20 medical colleges and universities who have finished or are preparing dissertations.

In recent years two national conferences on endemic fluorosis, three conferences of the Chinese Society for Fluoride Research, and ten specialist training

classes for the prevention and treatment of fluorosis have been held, in addition to several district symposia on fluorosis convened by local experts. At international conferences Chinese scientists have reported on their research, while Chinese and foreign experts have been invited to each other's countries to give special lectures. The increased quality and importance of fluoride research has been recognized by the World Health Organization and the Chinese government. Both will sponsor the 20th Conference of the International Society for Fluoride Research, to be held in Beijing from September 5 to 9, 1994.



A NOTE FROM THE EDITOR

Before the next issue of this journal appears, many of us will meet in September at our Society's 20th Conference in Beijing, China (see below). The co-sponsorship of this gathering by the Chinese Ministry of Public Health and the World Health Organization is a measure of its scientific importance. The International Society for Fluoride Research was founded in 1967 at a Conference in Frankfurt, Germany, following similar successful international conferences in Bern, Switzerland, in 1962 and in Detroit, USA, in 1966. Since those times, world-wide awareness of fluoride pollution and its effects has greatly increased. Our Society has played, and continues to play, a major role in that development.

John Colquhoun

THE 20th CONFERENCE OF THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH

will be held in Beijing, People's Republic of China, on September 5-9, 1994. The Conference is co-sponsored by the Consultant Committee on Endemic Fluorosis of the Ministry of Public Health of the People's Republic of China and the World Health Organization. The official language of the Conference will be English. All are welcome. In addition to the interesting scientific programme there will be local tours to places of historic interest. The registration fee is US\$200 - send to: ISFR '94 Scientific Secretariat, Dr Liang Chaoke, Institute of Environmental Health and Engineering, Chinese Academy of Preventive Medicine, 29 Nan wei Road, Beijing 100050, People's Republic of China. FAX: 0086-01-3011875. Phones: 0086-01-3013987 or 0086-01-3038761-Ext.261.

THE SIXTH FLUORINE SYMPOSIUM

will take place in Szczecin, Poland, on September 14-16, 1994. For further information contact Professor Zygmunt Machoy, Department of Biochemistry, Pomeranian Medical Academy, Al. Powstanców 72, 70-111 Szczecin, Poland.

ESR SPECTRUM STUDIES OF THE INFLUENCE OF FLUORIDE ON THE HUMAN ERYTHROCYTE MEMBRANE PROTEIN SH BINDING SITE PROPERTY

Yingyan Wang,* Xiaojie Li and Wenjuan Xin
Beijing, China

SUMMARY: Healthy human erythrocyte membrane was labeled with the various chain lengths of maleimide nitroxide I, II, III, IV, and V, and their ESR spectra showed that sodium fluoride (NaF) decreases W/S (weakly immobilized component/strongly immobilized component) ratio. Further experiments indicated that the reduction of W/S ratio due to fluoride is dependent upon the following three factors: 1) Dose. W/S ratio is decreased with increasing dose of fluoride. 2) Time. With increasing exposure, the time required to reach the maximal ratio is shortened. 3) Temperature. Between 0-50°C, the point of phase transition is altered from 28.5°C in the control to 25.0°C. However, fluoride did not induce a change of the basic ESR spectra of the membrane protein SH when labeled with maleimide. An occurrence of the Q peak is discussed.

Key words: Fluoride, Human erythrocyte, Membrane protein SH binding site property.

Introduction

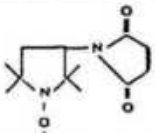
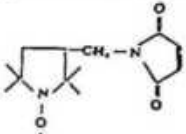

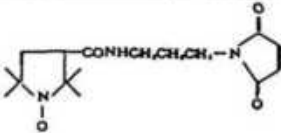

To analyse the alterations in the membrane fluidity due to fluoride exposure, it is important to understand the behavior of membrane proteins, as well as lipids. In a previous paper, we reported the influence of fluoride on the intact human erythrocyte membrane fluidity as monitored by electron spin resonance (ESR) with stearic acid spin labels (1). The ESR technique with maleimide-analogue sulphhydryl spin labels has been demonstrated to reflect the fluidity, *i.e.*, the conformation changes in membrane protein SH binding site properties (2). Under normal physiological conditions, the changes in conformation not only involve alterations in the permeability of the membrane (3), but can also distinguish the difference between normal and cancer cells (4). As a matter of principle, the typical spectrum of ESR labeled with maleimide molecule have shown the simultaneous existence of strongly and weakly immobilized components. The ratio of the signal amplitude of both components has been used as an index of protein conformation (5). In this work, therefore, we will employ the ratio of W/S measured by ESR technique with five chain lengths of maleimide nitroxide to further study the changes in the fluidity of the intact human erythrocyte protein SH binding site properties with fluoride exposure. It will offer new data at the sub-molecular level for elucidating the membrane toxicological behavior of fluoride.

Materials and Methods

Reagents: Tris-HCl GR., E.Merck. Spin labels as in Table 1 were purchased from Aldrich Co. USA. Sodium fluoride (NaF) and other reagents AR., were obtained from China.

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TABLE 1. Various chain lengths of maleimide nitroxide spin labels I, II, III, IV, and V

| No. | Compound | Molecular structure | $d(10^{-10})$ nm = A |
|-----|--|--|----------------------|
| I | 3-Maleimido-PROXYL |  | 4.57 |
| II | 3-Maleimidomethyl)-PROXYL |  | 6.11 |
| III | 3-(Maleimidomethyl)-carbamoyl)-PROXYL |  | 9.31 |
| IV | 3-(3-Maleimidopropyl)-carbamoyl)-PROXYL |  | 10.85 |
| V | 3-(2-(2-Maleimido-ethylethoxy)ethyl)-carbamoyl)-PROXYL |  | 15.33 |

Erythrocytes: Healthy human erythrocyte suspensions (ACD-B), containing 1×10^9 cells/mL, were provided by the Blood Centre of the Red Cross Society in Beijing. The original erythrocyte suspensions were diluted to 1×10^7 cells/mL with Tris-HCl glucose isotonic solution (pH 7.3).

Treatment with NaF: This experiment was divided into two groups. In the group treated with NaF, 10 μ L of 37.5 μ M NaF solutions were added. The mixture was supplemented to 5.0 mL with Tris isotonic solution giving a final NaF concentration of 0.075 μ M and, after shaking thoroughly, was incubated at 37°C for 25 min and immediately centrifuged at 3,000 rev/min (1000 g) for 8 min. After removing NaF-containing supernatant, the NaF-treated erythrocytes were obtained.

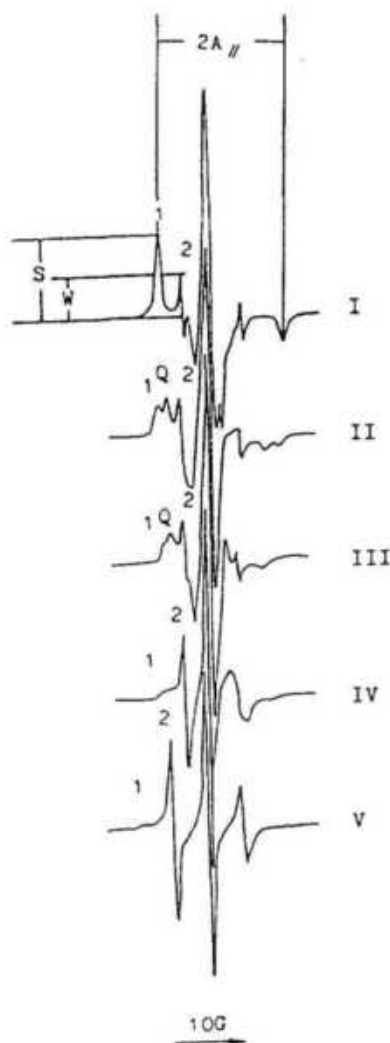
Spin labeling of NaF-erythrocytes: The samples of NaF-treated erythrocytes were added to a solution of maleimide spin labels to make a final concentration of 1.5×10^{-4} M. Then, after standing at 37°C for 2 h, the mixture was centrifuged at 3000 rev/min for 8 min. The labeled erythrocytes were rinsed with isotonic solution (5 mL each time) until a constant ESR spectrum was obtained. Finally, the total signals recorded were only contributed by the membranes labeled with 5 chain lengths of maleimide molecules which were inserted into the different layers in the membrane.

Measurement of ESR: ESR spectra were recorded using a Varian E 109-ESR spectrometer equipped with a variable temperature accessory. The measuring conditions were: microwave power 5 mW, X-band, 100 KHz field modulation, modulation amplitude 1.0 G, central magnetic field 3324, scan width 200, scan speed 25 G/min, time constant 0.128 s, temperature generally 37°C, except when recording temperature alterations, ranging from 0°C to 50°C.

Analysis of spectrum: Details of were given in the previous paper (1).

Calculation: The peaks of W and S (Figure 1) are weakly immobilized component (W), which is the amplitude of the second peak in the low field, and strongly immobilized component (S), which is the amplitude of the first peak in the low field, respectively. The signal amplitude was measured vertically from the horizontal base line to each peak. When their half amplitudes were divided, the quotient obtained was the ratio of W/S.

FIGURE 1. ESR spectra in human erythrocyte membrane labeled with various chain lengths of maleimide nitroxide I, II, III, IV, and V. 1 was strongly immobilized component (S); 2 was weakly immobilized component (W). The spectra were recorded at room temperature.



Results and Discussion

Typical ESR spectra of the healthy intact human erythrocyte membrane labeled with maleimide nitroxide spin labels of five chain lengths of I, II, III, IV, and V are shown Figure 1. Each spectrum consists of five lines folded from weakly immobilized components (W) and strongly immobilized components (S), indicating the label is bound to erythrocyte suspension. Analysis of the spectra demonstrated no change of 2 A, an increase of W/S ratio with the lengthening of the chain, and the appearance of Q peak in the labels II and III between two peaks of W and S.

TABLE 2. W/S ratio of the membrane protein SH labeled with 5 chain lengths of maleimide nitroxide with and without treatment with NaF

| Spin labels | | I | II | III | IV | V |
|-------------|------|-------|-------|-------|-------|--------|
| Control | Mean | 0.448 | 1.200 | 2.129 | 7.804 | 24.208 |
| | ±SE | 0.000 | 0.024 | 0.093 | 0.108 | 0.147 |
| NaF | Mean | 0.398 | 1.087 | 1.880 | 6.799 | 22.585 |
| | ±SE | 0.005 | 0.019 | 0.096 | 0.558 | 0.664 |

W/S ratios measured from the intact erythrocyte membrane with and without the treatment of NaF are presented in Table 2. The decrease in W/S ratio of the membrane labeled by 5 chain lengths of maleimide spin labels due to NaF indicated that fluoride has the same influence on the various layers in the erythrocyte membrane protein SH from the surface to the deeper interior.

As shown in Table 3, the results were confirmed by the fact that when the membrane was singly labeled with maleimide nitroxide I, the W/S ratio was decreased with the increasing dose of NaF. Thus, it was revealed that there is a relationship between the dose of fluoride and the alteration of the membrane protein SH binding site.

TABLE 3. The relationship between the dose of NaF and the W/S ratio of the membrane protein SH with maleimide nitroxide I

| Dose (μM) | | 0.0 | 5.0 | 12.5 | 25.0 | 37.5 |
|-----------|------|-------|-------|-------|-------|-------|
| W/S | Mean | 1.986 | 1.630 | 1.514 | 1.370 | 1.360 |
| | ±SE | 0.094 | 0.059 | 0.057 | 0.030 | 0.056 |

The effect of time on W/S ratio was studied over 1-6 h. The data in Table 4 reveal that W/S ratio of the membrane labeled only with maleimide nitroxide I, with or without the NaF treatment, had an optimum value in the range from 1 to 6 h. However, there are differences, namely: the optimum W/S ratio of 3.816 in the control group is at 1.0 h, but the ratio of 3.429 in the NaF-treated group is at 3.0 h. Thus, there is time dependence of fluoride on the membrane protein SH binding site.

The effect of temperature on the W/S ratio of the biomembrane preparation labeled only by maleimide nitroxide I is shown in Figure 2. The W/S ratio, with

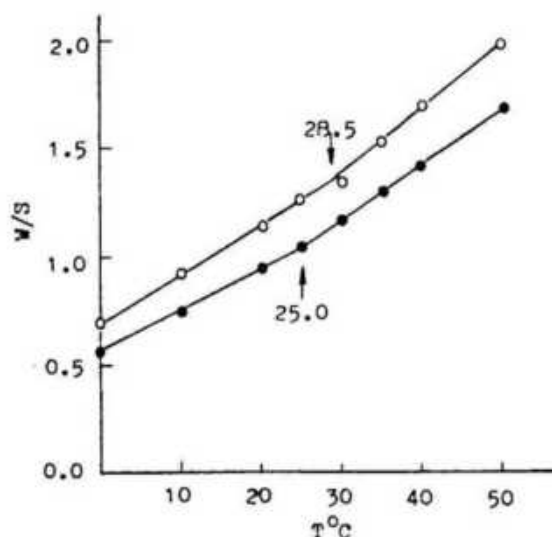
and without treatment with NaF, increased gradually between 0°C and 50°C, but rose abruptly at 28.5°C and 25.0°C. In addition, the results indicate that the W/S ratio also is decreased by fluoride treatment, though the increasing tendency is retained. Thus, there is a temperature dependence of the W/S ratio in the intact human erythrocyte membrane protein SH.

TABLE 4. The W/S-time effect of the membrane protein SH labeled with maleimide nitroxide I with and without NaF treatment

| Time (h) | 1 | 2 | 3 | 4 | 5 | 6 |
|----------|------------|-------|-------|-------|-------|-------|
| Control | Mean 3.161 | 3.251 | 3.429 | 3.341 | 3.309 | 3.180 |
| | ±SE 0.092 | 0.105 | 0.149 | 0.131 | 0.119 | 0.110 |
| NaF | Mean 3.816 | 3.538 | 3.377 | 3.274 | 3.244 | 2.996 |
| | ±SE 0.163 | 0.157 | 0.142 | 0.123 | 0.117 | 0.109 |

FIGURE 2. The temperature dependence of W/S ratio in the membrane protein SH labeled by maleimide nitroxide I with and without NaF treatment

—○— Control group —●— NaF-treated group



Q peak in Figure 1 occurred in ESR spectra of the binding sites between spin label II or III and membrane protein SH, in which Q of II was higher than that of III. However, Q was not found for the three other spin labels.

The following conclusions are reached from the above experimental results:

1) The parameters measured from ESR spectra of the intact human erythrocyte membrane, without NaF treatment, labeled by five chain lengths of maleimide nitroxide spin labels are:

- a) Mean \pm standard deviation of 2 A is 66.6 ± 1.85 G.
 - b) The shape of the membrane protein SH binding site with 5 chain lengths of maleimide molecule could be deduced to be conical and its depth is about 15.33Å.
 - c) Point of phase transition is 28.5°C. These parameters show that the basic ESR spectra properties of the human erythrocyte membrane protein SH site are not altered by fluoride.
- 2) The fact that the W/S ratio is decreased by NaF revealed that the fluidity of human erythrocyte membrane is reduced by fluoride. This means that the conformation of the membrane protein, to a certain extent, is changed. That is, the folding of protein is induced. It is the same conclusion as obtained from the experiment conducted by the ESR technique with stearic acid spin labels (1).
 - 3) Fluoride decreases the membrane fluidity by a time factor. The time-dependence relationship shows that fluoride markedly shortens the binding time of maleimide compounds with SH group in the erythrocyte membrane protein, implying that the decreasing fluidity makes it easier for the maleimide nitroxide free radical to attach to the membrane protein SH.
 - 4) The fluoride decrease in the membrane fluidity also affects the temperature factor. The temperature dependence curve shows that fluoride lowers the point of phase transition of 28.5°C in the membrane without the treatment of NaF to 25.0°C, indicating the SH binding site conformation of the erythrocyte membrane is changed by fluoride.

Maleimide nitroxide spin label which specifically binds to SH group of the membrane protein could induce the increase of splitting interval and line width from the original triple lines of equal intensity and distance, thus reflecting the folding of both weakly (W) and strongly (S) immobilized components. W and S correspond to attachment to two different sites of the membrane protein SH group, representing respectively, the specific binding of maleimide free radical with SH group located in the surface, having greater freedom; and in the interior of the three dimensional structure of the membrane, motion being restricted. The W/S ratio expresses the property of the binding site in the membrane protein SH group and suggests the conformations of the membrane.

On the basis of Fluid Mosaic Theory, an unsaturated fatty acid of phospholipids is one of the main components of the biomembrane and is essential for maintaining membrane fluidity (7). Fluoride is strongly electronegative and is an effective complexing agent (8). When an unsaturated fatty acid in the membrane is altered by fluoride, the modified fatty acid lessens the fluidity so that the W/S ratio is diminished. Therefore, the influence of fluoride on the intact human erythrocyte membrane is mainly the conformational changes transport process (7). In sum, these results provide microstructural data which elucidate the cyto-toxicological phenomenon in our work (3).

Compared with maleimide nitroxide spin labels I, IV, and V, the ESR spectra of II and III have a new peak Q (Figure 1). To address this peak, some experiments have been performed. The results are:

- 1) The aqueous solution of II or III exhibits only three sharp peaks, indicating that the Q peak is not produced by impurities.

- 2) The g value of Q is in the range of 2.016 to 2.023, obtained from the peaks of five different spin labels, which indicates that all of the peaks including Q originated from nitroxide free radicals.
- 3) $2A_{\parallel}$ value, being 52.1 G, is not in the range of 66.6 ± 2.58 G, which shows Q originates from other binding sites of the membrane SH group.
- 4) In the acid medium experiments, maleimide spin labels bind with the ϵ -amino group of lysine in the membrane protein, but with pH regulation (8.1 to 6.0), no other peaks occurred. Thus the Q peak originated from the binding sites of the membrane SH group.
- 5) When all the membrane protein SH groups are blocked by 10 mM $HgCl_2$ and the signals disappear, no peak is found. In addition:
- 6) W value changes while the S value is also altered, which indicates that Q is not involved with either W or S . According to the report of Rifkind *et al* (9), the Q peak, which occurred in the intact human erythrocyte membrane, may be related to the existence of a third class of SH binding site groups, which are formed when the maleimide molecule II and/or III insert into the membrane depth, where the bound membrane protein SH group is located within the protein structure, and the dipolar broadening vanishes.

Conclusion

From analysis of the results of this work obtained from ESR spectra labeled by stearic acid-nitroxide free radical (1), it is evident that fluoride could induce not only the conformation changes of the human erythrocyte membrane protein SH binding site, but also could decrease the fluidity in the biomembrane lipids. This conclusion elucidates the biomembrane behavior of fluoride.

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THE EFFECT OF NUTRITION SUPPLEMENTATION DURING THE ANNUAL DRY GRASS SEASON ON TOOTH WEAR IN INDUSTRY-FLUOROSED GOATS

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SUMMARY: The effect of nutrition on the teeth of goats consuming industrial fluoride-contaminated grass during the dry grass season was studied in three groups of ten goats each, pastured in the same area of fluoride pollution. The control group diet was not supplemented. The goats in the two treatment groups were supplemented either 100 g of mixed feed containing corn, wheat bran, CaCO_3 and trace of CuSO_4 (Ca:P=1.5:1) or 100 g of soybean meal per goat per day for six months of the first annual dry grass season. The results show that tooth wear decreased with increased protein supplementation. The heights of the first pairs of incisors, developed during the feed supplementation period and measured after a succeeding six month green grass season, were: 6.2 ± 0.8 mm (control), 8.9 ± 2.0 mm (mixed feed) and 11.1 ± 1.3 mm (soybean), respectively. This paper discusses the relationship between nutrition and tooth wear.

Key words: Goats, Industrial fluorosis, Nutrition supplementation, Tooth wear.

Introduction

In an area of severe fluoride pollution in Baotou in Inner Mongolia, sawteeth in goats is the main cause of early animal death. In recent years, researchers in veterinary medicine and environmental protection have sought to find elements that can slow the wear of teeth (1-3). Although all elements used had some biochemical effects, none lessened the severity of sawteeth. To study the mechanism of the formation of sawteeth, and to seek a practical method of prevention, the authors observed the development of each pair of teeth, examined the structure of sawteeth, and investigated some environmental factors (4). The findings revealed that high fluoride intake and nutrition deficiency during the annual dry grass season (November to April), in contrast to low fluoride intake and nutrition improvement in the green grass season (May to October) within three years after the birth of an animal, are direct causes of sawteeth, *i.e.* teeth developed in the dry grass season are very worn, while teeth developed in the green grass season have comparatively good hardness. Moreover, among the first three dry grass seasons after the birth of an animal, the first dry grass season has the greatest effect on animal life. This is because the first pair of incisors and the second pair of lower molars, which are needed for mastication, develop in this period. Therefore, supplementation of essential nutrition in the first dry grass season may be a key factor in lengthening animal life-span where fluoride pollution is high.

Materials and Methods

Experimental area and animals

The experimental area was about 3-5 km from a fluoride-emitting plant. In this area, the fluoride content of the grass varied seasonally. Soluble fluoride peaked at

80 ppm during the dry grass season, but was as low as 10 ppm during the period of green grass. All goats displayed sawteeth.

Thirty native goats, six months old, were divided into three groups. The goats were weighed and grouped into ten weight categories from light to heavy. From each of these weight categories a goat was assigned at random to each of the three groups. All goats were kept together on a fluoride polluted pasture during the day. In the evening, the first group (control) was not given additional feed. To each goat in the second group was given supplementation of 100 g of mixed feed containing 48 g corn, 50 g wheat bran, 2 g CaCO_3 , and 2 mg CuSO_4 (Ca:P=1.5:1, see Table 1). The third was provided with 100 g of soybean meal/goat/day. The period of feed supplementation was from November 1991 to April 1992 (the typical six-months dry season).

TABLE 1. Composition of mixed feed

| | | P | Ca |
|-----------------|-------|----------|----------|
| Corn | 48 g | 100.8 mg | 19.2 mg |
| Wheat bran | 50 g | 525 mg | 100.0 mg |
| CaCO_3 | 2 g | | 800.0 mg |
| Totals | 100 g | 625 mg | 919.2 mg |

Ca:P = 1.5:1

Experimental parameters

At the end of the feed supplementation, pregnancy numbers were recorded. In the blood haemoglobin, total protein, and albumin were determined. Bones were examined by radiography and scanning electron microscopy (SEM: Japan EMASIC-40) following Zhan's method (5). The heights of first incisors were measured in October 1992, when they had undergone wear for one green season of six months (May to October).

Results

Bone fluoride

At the end of supplementation, three rib bone samples from each group were surgically excised. Bone fluoride levels were 6450 ppm (control), 5854 ppm (mixed feed), and 5672 ppm (soybean).

Pregnancy numbers

Table 2 shows pregnancy numbers for the three groups.

TABLE 2. Pregnancy numbers of the three groups (Statistical comparison between groups by the method of direct probability calculation)

| Group | Pregnancy | No pregnancy | Total |
|---------|-----------|--------------|-------|
| Control | 1 | 5 | 6 |
| Mixed | 3 | 3 | 6 |
| Soybean | 7 | 0 | 7 |

Soybean-control: $P < 0.005$, Mixed-control: $P > 0.05$, Soybean-mixed feed: $P > 0.05$

Body weights

In the beginning of the experiment, average body weights were 16.5 ± 2.2 kg (control), 16.2 ± 1.6 kg (mixed feed), and 16.3 ± 1.8 kg (soybean). At the end of feed supplementation, all goats that gave birth showed decreased weights, wethers and other ewes with no pregnancy had a net weight increase of 0.3 kg (control, $n=9$), 1.8 kg (mixed, $n=7$), and 2.4 kg (soybean, $n=3$).

Blood levels of haemoglobin, total protein and albumin - See Table 3.

TABLE 3. Haemoglobin, total protein and albumin ($\bar{X} \pm \text{SD}$, g/L)

| Group | | Haemoglobin | Total protein | Albumin |
|---------|----|--------------------|---------------------|---------------------|
| Control | 10 | 82.3 ± 18.5 | 46.9 ± 2.6 | 20.9 ± 1.8 |
| Mixed | 10 | $106.4 \pm 21.4^*$ | $54.2 \pm 5.7^{**}$ | $25.6 \pm 2.1^{**}$ |
| Soybean | 10 | $108.5 \pm 19.4^*$ | $53.8 \pm 4.3^{**}$ | $24.8 \pm 3.5^{**}$ |

$p < 0.05$

$^{**}p < 0.01$

The heights of first pair of incisors

After the first pair of incisors, developed in first dry season, had been worn for a succeeding green season of six months, their heights were measured (Tables 4, 5 and Figure 1) in October 1992.

TABLE 4. The heights of first incisors of the three groups (mm)

| Group | n | Range | $\bar{X} \pm \text{SD}$ |
|---------|----|-------|-------------------------|
| Control | 10 | 5-8 | 6.2 ± 0.8 |
| Mixed | 10 | 6-12 | 8.9 ± 2.0 |
| Soybean | 10 | 9-13 | 11.1 ± 1.3 |

$p < 0.01$

TABLE 5. Comparison of the heights of the first incisors
(Comparison between groups by q test)

| Group | \bar{X} | $\bar{X}-6.2$ | $\bar{X}-8.9$ |
|---------|-----------|---------------|---------------|
| Soybean | 11.1 | 4.9^{**} | 2.2^* |
| Mixed | 8.9 | 2.7^{**} | |
| Control | 6.2 | | |

$^* p < 0.05$

$^{**} p < 0.01$

Figure 1. First pair of incisors
Left: control Right: soybean



Figure 2
Rib of soybean SEM X 400



Figure 3. Rib of control group
SEM X 100

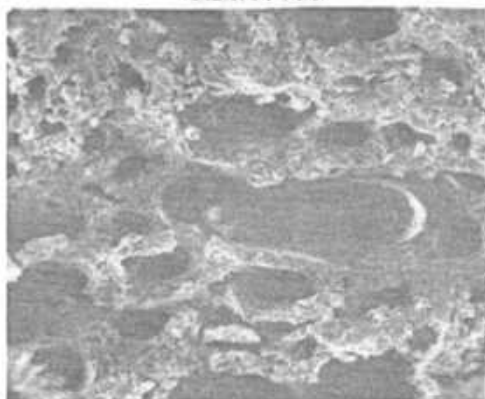


Figure 4. Mandible of control
SEM X 40



Radiographic and SEM observation of bone

Mandibles of the treatment groups showed thickened compact bone and relatively narrow marrow cavities. Figures 2,3, and 4 show SEM of rib and mandible.

Discussion

Effect of nutrition supplementation on general health

The goats experience their first dry grass season when they are six months old. Owing to stress from high fluoride intake and malnutrition, the animals develop slowly. In this study, haemoglobin, total protein, albumin, and weight gains increased with feed supplementation. Pregnancy rates increased significantly in the soybean group, indicating that essential nutrition supplementation in the first dry grass season is indispensable to growth, development, and reproductive efficiency.

Effect of nutrition on teeth

The height of first incisors of the treatment groups was significantly greater than that of the untreated control group. Comparison of tooth heights of the two

treatment groups revealed that the clinical effect of soybean feed was better than that of mixed feed. We suggest that nutrition supplementation played a role in decreasing the wear of teeth developed in the dry grass season under high fluoride conditions. As to the relationship between the nutrition supplementation and the wearing rate of teeth, we propose the following factors: First, protein in the supplemental feed can increase and improve the dental matrix, because protein is known to be important in its formation. Protein supplementation can also increase Ca absorption in the intestines (6). Second, the goats on supplemented diets might consume less abrasive material than the control goats in the period of feed supplementation, since nutrition for the controls was derived solely from pasture grass.

Effect of nutrition on bone

In 1978, Ericsson (7) reported that rats on adequate Ca and P supplementation showed significantly denser alveolar bone with increased protein. In our research, the supplemented goats revealed improved SEM and radiographic appearance of bone. In addition, during the SEM sampling, control bone was easily cut into pieces, but the supplemented bone was stronger. These findings support the view that protein is beneficial to bone formation.

Conclusion

Nutrition supplementation during the dry grass season can decrease the wearing rate of teeth. Therefore, it is a practical method of increasing pasture animal lifespan in fluoride-polluted areas.

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FLUORIDE IN WATER, URINE AND EGG SHELLS AS AN INDICATOR OF ENVIRONMENTAL CONTAMINATION

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SUMMARY: The fluoride content of drinking water, human urine, and hen egg shells was determined in the Gdansk region of Poland. A positive correlation was found between the concentration of fluoride in drinking water and urine of inhabitants. High fluoride levels were found in egg shells collected in the environs of a phosphate fertilizer plant.

Key words: Egg shells, Fluoride ; Environmental contamination; Urine; Water.

Introduction

The presence of high levels of fluorine compounds in the environment justifies the search for useful indicators of exposure. It is widely agreed that human urinary fluoride concentrations closely parallel intake levels in drinking water (1,2). It has also been shown that hair provides a useful indicator of environmental and occupational exposure (3, 4).

The aim of the present study was to investigate whether a correlation exists between the fluoride content in drinking water and in the urine of inhabitants of localities with different environmental pollution from fluorine compounds.

This work also includes an attempt to evaluate such environmental contamination by means of the fluoride content of egg shells. Such material is rich in calcium, an element to which fluorine has a strong affinity.

Materials and Methods

Collection of samples:

Urine and water samples were collected from three localities: 1) Malbork, a city with high water fluoride of natural origin; 2) Stogi, a quarter of Gdansk situated 3 km from a phosphate fertilizer plant with a high emission of volatile fluorine compounds; 3) the cities of Gdynia, Sopot and Gdansk, with a low water fluoride level, as the control region.

Similarly, hen egg shells were collected from individual farms located: 1) in an area with high water fluoride; 2) in the vicinity of a phosphate fertilizer plant; 3) in remote rural areas (controls).

Locations where samples have been collected are shown in Figure 1. The concentration of fluoride was determined in 21 samples of drinking water and 55 samples of human urine. Hen egg shells were collected from 34 individual farms.

Preparation of samples:

Fluoride content in samples of drinking water and urine was determined directly after dilution with equal volumes of TISAB buffer,

TISAB buffer: 57 mL of acetic acid, 58 g sodium chloride, and 0.3 g sodium citrate were diluted with water and adjusted with sodium hydroxide to pH 5.2 and made up to 1 liter.

Egg shells (5) were washed with redistilled water, dried at 100° for 3 hr and crushed. Specimens (0.2 g) placed in polyethylene tubes were extracted with 3 mL of 2 M perchloric acid for 1 hr at room temperature. The samples were then centrifuged and 0.5 mL of 0.5 M sodium citrate solution and 6.5 mL of redistilled water were added to the supernatant.

Determination of fluoride:

Fluoride concentrations were measured by a fluoride-specific electrode against a Ag/AgCl reference electrode with a double jacket (4,6). The coefficient of variation in water and urine samples was 4% and in egg shells 15%. Significance was determined by the Student's t-test.

Results

The concentrations of fluoride in urine and drinking water in different locations are presented in Table 1. The water and urinary fluoride in locations situated in the vicinity of a phosphate fertilizer plant (Stogi) and in a city with elevated fluoride level of natural origin (Malbork) were significantly ($p < 0.001$) higher compared to the control areas (Gdansk, Gdynia, Sopot).

A strong positive correlation between the concentration of fluoride in drinking water and urine of inhabitants was evident ($r = 0.973$). The regression equation was as follows:

$$y = 0.58x - 0.18$$

where y = fluoride concentration in urine, and x = fluoride concentration in water.

The fluoride levels in hen egg shells collected from different localities are presented in Table 2. The values differed from 90.4 $\mu\text{g/g}$ in a place situated 1 km from the fertilizer plant to 1.03 $\mu\text{g/kg}$ in a typical village situated about 70 km westwards from Gdansk. No correlation was found ($r = 0.29$) between the content of fluoride in water and egg shells.

Discussion

According to many authors (*e.g.* 1,7) the optimal fluoride concentration in drinking water is in the range 0.7-1.2 mg/L. However, lately there is growing concern about harmful effects of fluoride at levels around 1.0 mg/L or even 0.7 mg/L (8,9).

The water fluoride content in the municipal area of Gdansk, Gdynia and Sopot is distinctly lower and ranges from 0.16 to 0.48 mg/L. Significantly higher levels (1.85 mg/L) were found in the environs of the phosphate fertilizer plant and in a city with high fluoride level of natural origin (2.3 mg/L).

The human urine fluoride level closely reflects the concentration of fluoride in drinking water irrespective of whether the fluoride is of natural or anthropogenic origin.

The results of fluoride analysis of hen egg shells, however, do not allow such an univocal conclusion. The highest fluoride content was encountered near the phosphate fertilizer plant with a known high emission of volatile fluorine compounds. Similar results have been obtained by other authors (10,11) in the surroundings of another phosphate fertilizer plant located in the northwestern part of Poland.

FIGURE 1. Locations of sampling ○ Phosphate fertilizer plant at Stogi ⊙

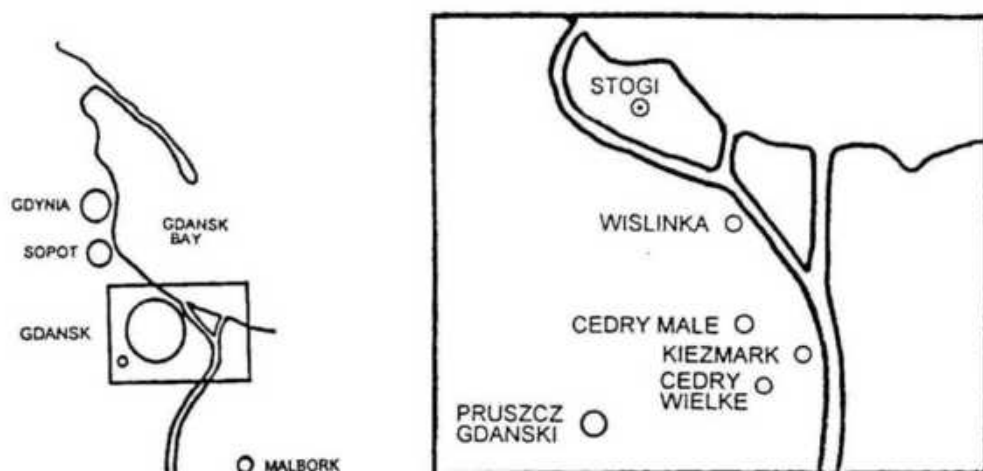


TABLE 1. Fluoride content in human urine and in drinking water

| | Urine (mg F ⁻ /g creatinine) | | | Water (mg F ⁻ /L) | | |
|-----------|---|----------|---------|------------------------------|-----------|---------|
| | Controls | Malbork* | Stogi** | Controls | Malbork** | Stogi** |
| n | 9 | 25 | 21 | 10 | 4 | 7 |
| \bar{x} | 0.95 | 3.82 | 3.84 | 0.37 | 2.30 | 1.85 |
| \pm SD | 0.41 | 2.03 | 2.03 | 0.34 | 0.39 | 0.06 |
| p | | 0.001 | 0.001 | | 0.001 | 0.001 |

* city with high fluoride level of natural origin

** suburb 3 km from phosphate fertilizer plant

n number of samples

 \bar{x} mean value

SD standard deviation

p level of significance

TABLE 2. Fluoride content in egg shells and in drinking water

| Locality | Egg shells (μ g F ⁻ /g) | Water (mg F ⁻ /L) |
|----------------------------|---|------------------------------|
| Cedry Male | 20.75 | 3.00 |
| Cedry Wielkie | 9.50 | 2.00 |
| Stogi I 1 km ^x | 90.40 | 1.87 |
| Stogi II 3 km ^x | 15.88 | 0.28 |
| Koszwały | 33.75 | 2.00 |
| Kieźmark | 25.50 | 0.50 |
| Pólczo | 1.03 | 0.33 |
| Pruszcz Gdański | 17.23 | 0.10 |
| Wislinka | 21.60 | 2.15 |

^x = distance from the phosphate fertilizer plant

However, we found also a considerable high fluoride content in egg shells in some locations without any known source of industrial or environmental pollution and with a rather low fluoride content in water,

Moreover, a negative correlation ($r = 0.289$) was found between the fluoride contents of the drinking water and the egg shells, which seems to indicate that the amount of fluoride in egg shells depends mainly on other sources, such as feed, soil, and emission of volatile fluorine compounds, than water fluoride.

Acknowledgement

The study was partially supported by grant W-76 from the Medical Academy in Gdansk.

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EFFECT OF CALCIUM ION CONTENT ON THE INTERACTION OF α -LACTALBUMIN WITH FLUORIDE IONS

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SUMMARY: The effect of calcium ions in α -lactalbumin on the binding of fluoride ions was investigated. It has been disclosed that at pH 4.6 both α -lactalbumin and apo- α -lactalbumin, when deprived of calcium ions, fail to bind fluoride ions. At pH 3.7 the α -lactalbumin molecule and its calcium-free form of apo- α -lactalbumin do bind fluoride ions, but the mechanism of this bindings is variable.

Key words: α -Lactalbumin; Apo- α -lactalbumin; Calcium; Fluoride.

Introduction

α -Lactalbumin is a globular protein present in mammary glands and whey. Its principal function is to facilitate lactose synthesis as a subunit of lactose synthetase. It may also take part in surface modification as well as in interaction and differentiation of cells (1).

From previous studies in our Department (2), it is known that this protein binds fluoride ions only at a pH below 4.0, thereby excluding the possibility of interaction with fluoride under physiological conditions. However, since α -lactalbumin is a protein that binds calcium (3,4), it is desirable to investigate whether the molecule deprived of calcium ions, *i.e.*, apo- α -lactalbumin, would be able to bind fluoride at pH above 4.0. Such a study takes on added significance in view of the fact that owing to very low concentration of Ca^{++} in the cell cytosol ($\sim 10^{-5}\text{M}$), α -lactalbumin appears there in the form of apo- (calcium free) only at the time of secretion when, passing through the Golgi apparatus, it binds calcium and is thereby transformed into typical structure (3). Interference with this process by reactions involving fluoride ions could have important biological effects because calcium binding is thought to be a signal for α -lactalbumin secretion from the Golgi apparatus and initiation of lactation (5).

The aim of the present work was to verify whether apo- α -lactalbumin binds to fluoride ions at pH above 4.0 and to investigate the stoichiometry of fluoride binding with α - and apo- α -lactalbumin.

Materials and Methods

Bovine α -lactalbumin type I and apo- α -lactalbumin type III (both SIGMA) were checked for calcium and fluoride content. For this purpose the samples of both proteins were dissolved in distilled, deionized water, and the fluoride concentration was measured by means of an ion selective electrode (2). In the case of apo- α -lactalbumin, its poor solubility was overcome by preparing a saturated solution and, after centrifugation, the concentration was measured in the supernatant. The concentration of proteins was determined, by measuring absorption at 280 nm, assuming the molar absorptivity for α -lactalbumin and apo- α -lactalbumin as $\epsilon_{280} = 28500 \text{ M}^{-1}\text{cm}^{-1}$ and $\epsilon_{280} = 27700 \text{ M}^{-1}\text{cm}^{-1}$, respectively (6).

Calcium concentration was measured by atomic absorption on a flame spectrophotometer (Philips PU 9100X). The measurement was made twice: first prior to and then after passing the protein samples through a column (0.8-70 cm) filled with Sephadex G-25 and equilibrated with doubly distilled deionized water.

Fluoride ions binding to α - and apo- α -lactalbumin were measured in 0.02 M acetate buffer at pH 4.6 and at pH 3.7 by the method of Hummel and Dreyer (7). Stoichiometry of fluoride ion binding was studied by Scatchard's method (8), with reference fluoride concentrations of 0.23, 0.56, 1.14, 2.95, 5.0 and 9.8 mM.

Results and Discussion

The fluoride and calcium contents in samples of α - and apo- α -lactalbumin are displayed in the Table. The results show that the protein preparations are not contaminated by fluoride and that each molecule of α -lactalbumin contains about 2 atoms of calcium, while 1 molecule of apo- α -lactalbumin claims 0.1 atom of calcium.

Lack of significant differences in calcium content in the molecules of proteins before and after the passage through the column of SEPHADEX G-25 indicates the possibility of using such proteins in studies without preliminary cleansing.

TABLE. Calcium and fluoride analysis of bovine α - and apo- α -lactalbumin

| | | Calcium (Ca^{2+}) content (M) | F^- (M) | Protein concentration (M) | No. of calcium atoms per molecule of the protein |
|--------------------------------|-------------------------------|---|---------------------|---------------------------------|--|
| α -lactalbumin | before column treatment | 1. 1.00×10^{-3} | $< 10^{-5}$ | 0.45×10^{-3} | 2.2 |
| | | 2. 2.18×10^{-3} | $< 10^{-5}$ | 1.04×10^{-3} | 2.1 |
| | | 3. 3.40×10^{-3} | $< 10^{-5}$ | 1.62×10^{-3} | 2.1 |
| | after column treatment | 1. 0.90×10^{-3} | - | 0.50×10^{-3} | 1.8 |
| | | 2. 0.50×10^{-3} | - | 0.25×10^{-3} | 2.0 |
| | | 3. 0.80×10^{-3} | - | 0.38×10^{-3} | 2.1 |
| apo- α - lactalbumin | before column treatment | 1. 0.40×10^{-5} | $< 10^{-5}$ | 0.40×10^{-4} | 0.10 |
| | | 2. 0.70×10^{-5} | $< 10^{-5}$ | 0.60×10^{-4} | 0.12 |
| | | 3. 1.00×10^{-5} | $< 10^{-5}$ | 1.00×10^{-4} | 0.10 |
| | after column treatment | 1. 0.25×10^{-5} | - | 0.25×10^{-4} | 0.10 |
| | | 2. 0.36×10^{-5} | - | 0.33×10^{-4} | 0.11 |
| | | 3. 0.45×10^{-5} | - | 0.45×10^{-4} | 0.10 |

Figure 1 presents the results of studies concerning the binding of fluoride ions to α - and apo- α -lactalbumin in 0.02 M acetate at pH 4.6. The characteristic horizontal straight line pattern of the fluoride elution profile indicates that at pH 4.6 there is no fluoride binding either to α - or apo- α -lactalbumin. Thus, essential conformational changes accompanying calcium dedissociation from α -lactalbumin (9-11) appear to exert no influence on its reaction with fluoride at pH above 4.0.

FIGURE 1. Effluent concentration profiles for F^- (\blacktriangle) from a column of G-25 Sephadex, when a sample (1.0 mL) of α -lactalbumin (O) (6.0×10^{-4} M, pH 4.6) and a sample (1.0 mL) of apo- α -lactalbumin (\bullet) (5.8×10^{-4} M, pH 4.6) were passed through separately. All solutions, including that used for column equilibration, contained 3.0×10^{-4} M NaF and 0.02 M sodium acetate buffer (pH 4.6); flow rate 8.0 mL/h, collected in 1 mL fractions.

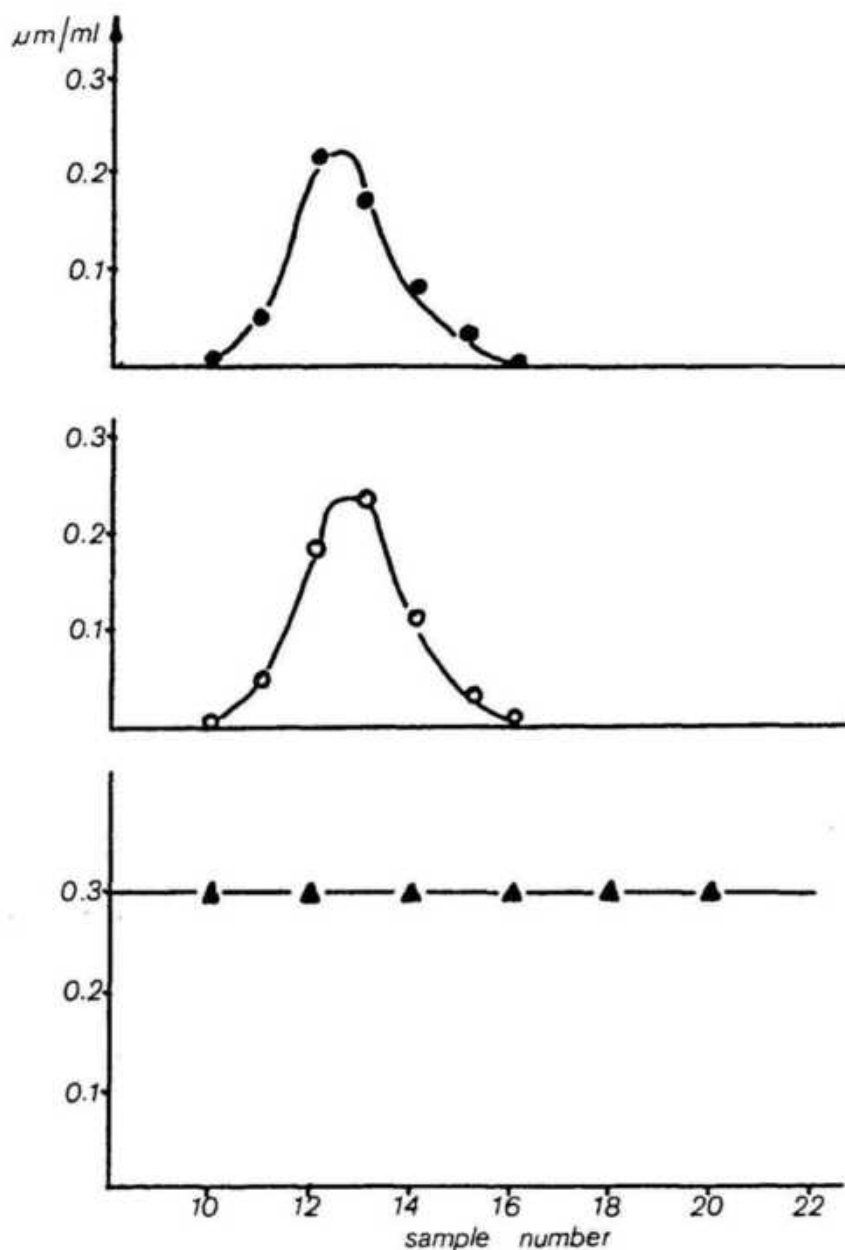


Figure 2 depicts the stoichiometry of fluoride binding by α - and apo- α -lactalbumin in 0.02 M acetate buffer at pH 3.7. Although the results indicate that in both cases 2 fluoride ions are bound to 1 protein molecule, the mechanism of binding is different. In apo- α -lactalbumin there are two binding sites with uniform affinity (straight line Scatchard plot). On the other hand, α -lactalbumin has two binding sites with different affinity.

These results are even more surprising in that, according to Kronman (4), at pH below 4.0, calcium dissociates from α -lactalbumin and transforms it into apo- α -lactalbumin. Hence, at pH 3.7 both molecules should behave in the same manner.

FIGURE 2. Data for the binding of F^- to α -lactalbumin (Δ) and apo- α -lactalbumin (\bullet) presented according to the graphical method of Scatchard as a plot of $V/[A]$ against V ; where V is ratio of bound F^- to total α -lactalbumin or apo- α -lactalbumin, and $[A]$ is concentration of F^- .

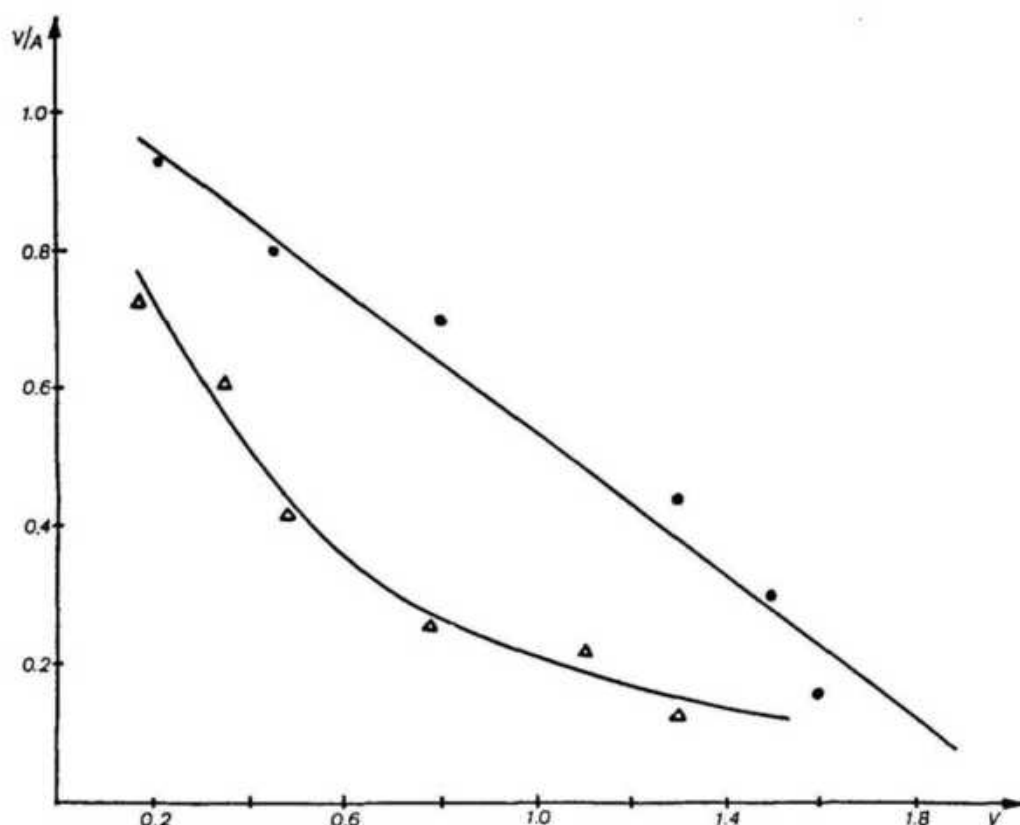
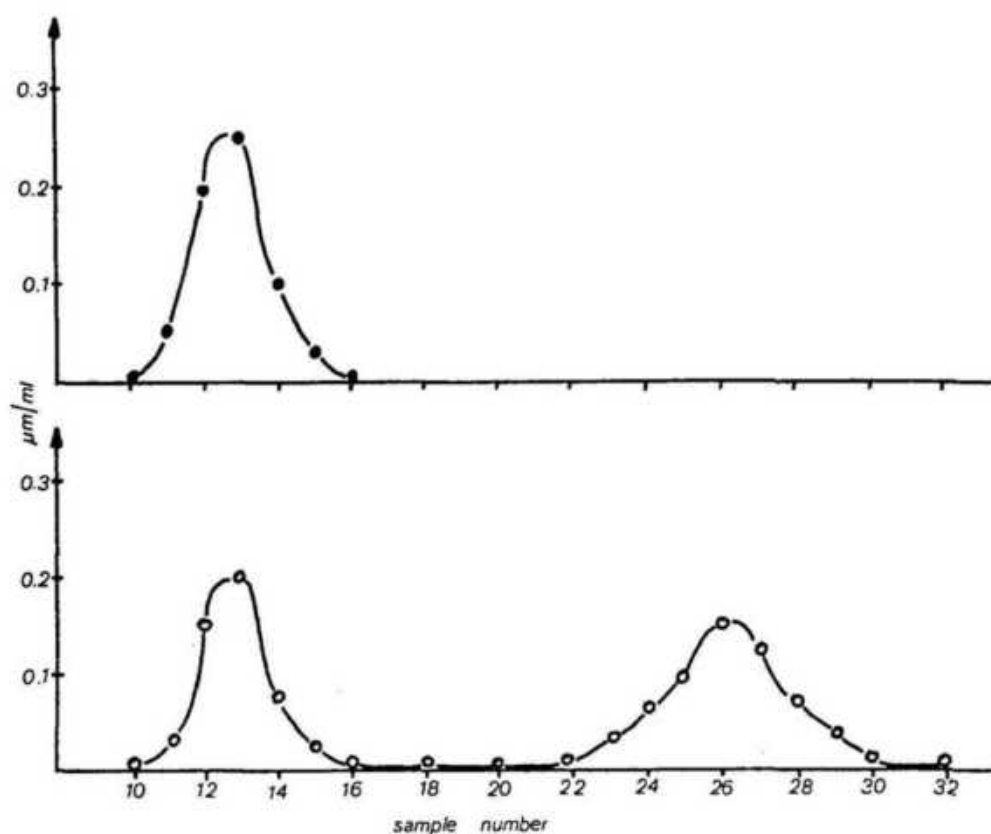


Figure 3 illustrates the elution profile of α -lactalbumin and calcium from the column filled with Sephadex G-25 in 0.02 M acetate buffer at pH 3.7. At this pH, α -lactalbumin continues to be bound to one calcium ion, thus substantiating the differences in the mode of binding of fluoride ions to α - and apo- α -lactalbumin at pH 3.7.

FIGURE 3. Effluent concentration profiles for Ca^{++} (○) from a column of G-25 Sephadex when a sample (1.0 mL) of α -lactalbumin (●) (6.0×10^{-4} M, pH 3.7) was passed through.



The above results emphasize the crucial role of pH in the interaction of fluoride with α - and apo- α -lactalbumin. Within the physiological values of pH, α -lactalbumin does not appear to bind fluoride, regardless of whether calcium ions are present in the molecule.

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FLUORIDE INCORPORATION INTO FETAL BONE

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SUMMARY: The mineral content (calcium, magnesium, phosphorus and fluorine) of fetuses, obtained from the Department of Anatomy after accidents and miscarriages, was determined. Higher contents of Ca, Mg, and P were disclosed in the diaphyseal (shaft) part, in which mineralization proceeds sooner than in the metaphyseal part. The behaviour of fluoride was different. Higher levels of fluoride were recorded in the metaphysis as compared to the shaft. The results provide some insight into the course of bone mineralization from 14 to 36 weeks of fetal life.

Key words: Fetal bone; Fluoride; Mineral composition.

Introduction

The process of bone mineralization arouses a well-founded interest. Owing to the large number of factors involved, it has been a difficult subject to understand. An especially complicating element in bone mineralization is fluorine. Previous studies on the influence of fluoride on bony tissues dealt primarily with the post-natal period, while that of fetal development has been relatively poorly investigated (1). The placental barrier controls, to a great extent, how much fluoride can penetrate into the fetus, and thus protects the fetus against intoxication by this element (2,3). One method for estimating the fluoride participation in the process of bone mineralization is to determine the correlation between the bone fluoride content and that of calcium as well as phosphorus - two principal elements in bone (4). Magnesium also is an important components of bone (4). The aim of the present work was to obtain appropriate answers to:

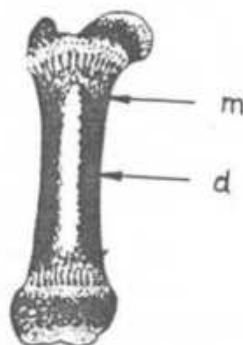
- a) Are there statistically significant differences between content of fluoride, calcium, magnesium, and phosphorus in the shaft and in the metaphysis of fetal bones?
- b) Are there statistically significant correlations between fetal age and individual elements, and among the elements themselves in these bones?

Materials and Methods

Calcium, magnesium, phosphorus, and fluoride contents of femoral bones of human fetuses, aged from 14 to 36 weeks of intra-uterine life, were measured. The material came from the Anatomy Department, after accidents, miscarriages, and other pathological states. It was not possible to define fluoride levels in mothers. All the mothers resided in the Szczecin region, where the fluoride level in drinking water is 0.2 ppm. After removal of soft tissues, the bones were weighed and subsequently dried at 105°C to become a solid mass. The bones were analyzed collectively and were then divided into 4 age groups, namely: group I from 14 to 19 weeks; group II from 20 to 24 weeks; group III from 25 to 29 weeks and group IV from 30 to 36 weeks of life. Samples for analysis were procured by drilling the defatted bone in shafts and metaphyseal parts with a dental drill (Figure 1). The study material was collected only from already mineralized bones, the non-mineralized chondral substance being discarded. (The term "metaphyseal part" means the mineralized part of the bone on the border of the shaft and the epiphysis).

The resulting powder for mineral assay was dissolved in nitric acid, and for fluoride determination in perchloric acid. Calcium and magnesium were determined by the atom absorptiometric method (4), phosphates by the colorimetric method with ammonium molybdate, and fluoride by means of an ion-specific electrode from the Radelkis Firm (4). Altogether, 66 femoral bones of human fetuses were analyzed.

Figure 1. Human fetal bone.
Sites of bone sample collection:
d = diaphyseal part (shaft)
m = metaphyseal part



Results and Discussion

The results are shown in Tables 1, 2 and 3. Table 1 gives mean values of calcium, magnesium, phosphorus, and fluoride - separately for the shaft (diaphyseal part) and metaphyseal parts of the bone (Figure 1). Statistically significant differences were found between the contents of respective elements in both the shaft and the metaphyseal part. The results disclose non-uniform depositing of mineral components in different parts of the femoral bones (5,6) throughout the whole set of samples studied. The different behaviour of fluoride as compared with Ca, Mg, and P is worth noting. More fluoride is accumulated in the metaphyseal parts and less in the shafts of the femoral bones. This pattern was confirmed in all four age groups (Table 2). Table 3 shows correlations of contents of the elements and the age of the fetus.

Statistically significant correlations appear here between:

- 1) fetal age and the content of calcium and phosphorus in the bones, both in the shaft and metaphyseal part (the older the bone, the more calcium and phosphorus it contains);
- 2) the calcium and phosphorus contents indicate that the higher the amount of calcium, the more phosphorus lodges in the shaft and metaphyseal part; (Equations of linear regression were derived for calcium and phosphorus. The formula for calcium is: $y = 1.763x + 127.37$ for the shaft, and $y = 2.227x + 94.667$ for the metaphyseal part. For phosphates it is: $y = 0.526x + 85.346$ for the shaft and $y = 0.643x + 71.100$ for the metaphyseal part.)
- 3) the magnesium content in the shaft and the magnesium content in the metaphyseal part; 4) the fluoride contents in the shaft and in the metaphyseal part. Mathematical presentation of fluoride accumulation in fetal bones was difficult, due to the relatively small number of analyses and the high values of the standard deviations.

In this study the measure of degree of mineralization was verified by correlation of the contents of calcium and phosphorus - the two basic elements present in fetal bones (Table 3). There is also no apparent influence of fluoride on the calcification

of the fetal bony tissue. The correlation concerned the variable fluoride contents in the shaft and in the metaphyseal part. The bones of younger individuals contained less fluoride, and those from older fetuses had more fluoride. However, after statistical analysis involving calculated mean values, a correlation with age cannot be

TABLE 1. Mean quantity (\bar{X}) of the contents of calcium, magnesium, phosphorus, and fluoride in the diaphyseal (d) and the metaphyseal (m) part of the femoral bones in human fetus aged from 14 to 36 weeks of fetal life. Ca, Mg and P in g/kg dry mass; F⁻ in ppm.

| | \bar{X} | SD | t-test | p |
|----------------|-----------|-------|--------|---------|
| Calcium (d) | 169.5 | 15.3 | 12.81 | < 0.001 |
| Calcium (m) | 148.4 | 19.9 | | |
| Magnesium (d) | 3,428 | 1,303 | 6.29 | < 0.001 |
| Magnesium (m) | 2,959 | 1,217 | | |
| Phosphorus (d) | 97.7 | 8.4 | 12.01 | < 0.001 |
| Phosphorus (m) | 86.2 | 9.4 | | |
| Fluoride (d) | 60.8 | 3.0 | -10.7 | < 0.001 |
| Fluoride (m) | 73.5 | 3.6 | | |

TABLE 2. Arithmetic means (\bar{X}) and standard deviation (SD) for the content of fluoride (ppm) in fetal bones with division into four age groups

| No. | Age groups (in weeks) | n | Diaphysis (shaft) | | Metaphyseal part | |
|-----|--------------------------|----|-------------------|-------|------------------|-------|
| | | | \bar{X} | SD | \bar{X} | SD |
| I | 14 - 19 | 16 | 54.1 | 28.32 | 61.8 | 31.18 |
| II | 20 - 24 | 20 | 58.1 | 17.79 | 70.8 | 19.30 |
| III | 25 - 29 | 22 | 65.4 | 23.52 | 80.0 | 27.05 |
| IV | 30 - 36 | 8 | 68.1 | 30.64 | 85.8 | 42.52 |

TABLE 3. Correlation coefficients and significance levels for the variables in Table 1

| | Age | Ca(d) | Ca(m) | Mg(d) | Mg(m) | P(d) | P(m) | F ⁻ (d) | F ⁻ (m) |
|--------------------|----------|----------|----------|----------|----------|----------|----------|--------------------|--------------------|
| Age | | 0.6488** | 0.6346** | | | 0.3479* | 0.3784** | | |
| Ca(d) | 0.6488** | | 0.7455** | | | 0.7682** | 0.5957** | | |
| Ca(m) | 0.6346** | 0.7455** | | | | 0.5545** | 0.6502** | | |
| Mg(d) | | | | | 0.8866** | | | | |
| Mg(m) | | | | 0.8866** | | | | | |
| P(d) | 0.3479* | 0.7682** | 0.5545** | | | | 0.6279** | | |
| P(m) | 0.3784** | 0.5957** | 0.6502** | | | | | | |
| F ⁻ (d) | | | | | | | | | 0.9545** |
| F ⁻ (m) | | | | | | | | 0.9545** | |

* 0.01

** 0.001

claimed. One reason may be too few cases in respective groups. Also small differences in age (a few weeks) do not decisively influence the level of components that construct the bone. The fluoride levels in fetal bones appear to be the result of several parameters, such as exposure to fluoride permeability across the placenta, and the ability of the organism to excrete fluoride. Fluoride penetrates the placental barrier, whereas the protective properties of the barrier may be differentiated and may depend on many external and internal systemic factors. In the case of frequently encountered calcifications in the placenta, a fluoride binding takes place, and by this virtue restriction of transfer in the direction of the fetus is imposed. With the progress of pregnancy, the villous membrane (barrier) becomes thinner and thinner, so that in full-term pregnancy an intensification of transport across the placenta may occur.

A markedly higher fluoride content in the metaphyseal part than in the shaft confirms the reports on the incorporation of fluoride into mineral structures that are sites of particularly intensive transformations of bony tissue (7). The metaphyseal part develops later than the shaft, the former being a site with better blood supply and with the highest intensity of metabolic activity and reconstruction of mineral structures. Maybe that is why the metaphyseal part is more abundant in fluoride, a constant component of bone.

Conclusions

1. The bones from younger fetuses contained less fluoride, and those from older fetuses more fluoride, but there was no clear correlation between fluoride content and age, unlike calcium and phosphorus contents where a definite age relationship exists.
2. In the process of fetal bone calcification, the accumulation of fluoride is non-uniform. It is slower in the shaft, but more rapid in the metaphyses, i.e., at the sites with increased metabolic activity.

Acknowledgement

The authors are grateful to Dr E Dabkowska for help with statistical calculations.

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EFFECT OF LONG-TERM ADMINISTRATION OF FLUORIDE ON LEVELS OF PROTEIN, FREE AMINO ACIDS AND RNA IN RABBIT BRAIN

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SUMMARY: Biochemical alterations in the brain produced during experimental fluorosis were studied. Albino rabbits of both sexes were administered sodium fluoride solutions in the concentrations of 5, 10, 20, and 50 mg/kg body wt/day by subcutaneous injection for 100 days. The control rabbits were given 1 cc distilled water/kg body weight/day for the same length of time. In fluoride treated rabbits the brain showed significant decline ($P < 0.001$) in soluble, basic, total protein and free amino acid levels. RNA content rapidly decreased ($P < 0.001$) in the brains of experimental animals compared to the controls. However, in male animals treated with 5 and 10 mg fluoride no statistically significant differences in RNA content of brain were observed. The depletion of proteins produced degenerative changes in purkinje cells of the cerebellar cortex. These changes in the brain lead to paralysis of limbs in fluoridated animals.

Key words: Basic protein; Brain; Experimental fluorosis; Free amino acid; RNA; Soluble protein.

Introduction

Endemic fluorosis is related to a high concentration of fluoride in water. The manifestations of the initial phase of fluorosis indicate injury to the central nervous system, *i.e.* the brain and the spinal cord (1). Involvement of the nervous system in skeletal fluorosis has been reported mainly from India. Singh and Jolly (2) studied 60 cases of chronic fluorosis in patients with skeletal fluorosis. The most important symptoms in these patients were muscular wasting, referred pain along the nerve roots and fibrillation and fasciculation of the muscles. Siddiqui (3) studied 53 advanced cases of the disease with neurological manifestations and recorded a patchy type of anaesthesia, spastic paraplegia, absence of vibration sense and loss of sphincter control. The neurological signs were due to pressure on the spinal roots and the cord by bony growths into the spinal canal which in the cervical region resembled the clinical picture of cervical spondylosis. Popov *et al* (4) detected neurological symptoms in 79% of patients with occupational fluorosis, thus also suggesting direct nerve involvement. Frank *et al* (5), while studying a fatal case of industrial fluorosis, recorded that fluoride ions damage nervous tissue without physical pressure upon the spinal cord. However, the effects of acute and chronic doses of fluoride on the brain in experimental animals are almost unknown. In the present investigation the following parameters were measured in order to evaluate such effects: soluble proteins and basic proteins, free amino acids, and RNA.

Materials and Methods

Animals: Albino rabbits of both sexes weighing 400-600 gm, procured from the Kaila Scientific Corporation, Agra, India, were divided into five groups of 12. All were fed a standard pellet diet, and water was supplied *ad libitum*.

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Experimental design: The animals were weighed before the experiment. One group given 1 cc distilled water/kg body weight/day was kept as control (Group C). The remaining four test groups (F_5 , F_{10} , F_{20} , and F_{50}) were administered subcutaneous injections of sodium fluoride in the dosages of 5, 10, 20, and 50 mg/kg body weight/day for 100 days, respectively. All the animals were sacrificed under ether anaesthesia, and the brains rapidly removed for quantitative analysis of proteins (soluble and basic), free amino acids, and RNA.

Analytic procedure: Protein in brain was assayed by the method of Lowry *et al* (6) using bovine serum albumin as standard. The free amino acids were estimated according to the method of Troll and Cannon (7) using KCN-pyridine solution and phenol reagent. The colour was developed by adding alcoholic ninhydrin, the intensity of which was read at 570 m μ on a spectronic-2 colorimeter. The nucleic acids were extracted accordingly to the method of Webb and Levy (8). The estimation of RNA was done as per the orcinol method described by Markham (9). The results were calculated as mg/g wet weight of tissue. However, to compare the effects of different doses of fluoride on the same biochemical parameters, the results are expressed as percent change relative to control.

Statistics: The statistical significance was assessed using student's t-test.

Results

During the course of the study the animals consumed normal amounts of food and had normal gains in weight. However, in the Control, F_5 and F_{10} groups the increase in body weight was more than in the F_{20} and F_{50} groups.

In the F_{10} and F_{20} groups some of the animals showed signs of paralysis at 35 days from the onset of the experiment. Most of the animals in the F_{50} group were attacked with paralysis. None of the rabbits completed the total duration of the experiment. The maximum survival was for 70 days at which stage the animals showed paralysis of both fore- and hind-limbs. The remaining animals showed signs of paralysis at 35, 40, 49, 59, 61 and 70 day intervals, and their weight fell suddenly during the experiment.

The metabolic responses of the brain from rabbits receiving different amounts of fluoride are remarkably different. The soluble and basic proteins are significantly decreased ($P < 0.001$) in brains of all fluoridated groups compared to the control (Table 1). The total proteins showed a higher percentage of degradation in males (94.5%) than in females (87.4%) of the F_{50} group.

The free amino acid (FAA) content of the brain (Table 2) suddenly decreased in test animals compared to controls. In female animals FAA dropped from 8.5 mg/g w.w. to 1.8 mg/g w.w., 1.2 mg/g w.w., 1.1 mg/g w.w. and 0.3 mg/g w.w. in F_5 , F_{10} , F_{20} and F_{50} respectively. The female rabbit brain showed a higher percent change in levels of FAA than the male (96.4% vs. 88.6%).

The concentration of RNA in male rabbit brain showed slight variations in two fluoridated groups (F_5 and F_{10}) which were not statistically significant (Table 2). However, RNA content of the brain in F_{20} group declined to almost half that of the control group (2.2 mg/g w.w. to 4.1 mg/g w.w.). In the F_{50} group RNA content in brain dropped significantly ($P < 0.001$) to 1.8 mg/g w.w. In female rabbit brains RNA declined in all the fluoridated groups. The greatest percent decrease (50%) was in the F_{50} group.

TABLE 1. Effect of different doses of fluoride on proteins of rabbit brain (Mean \pm SD)

| | Dose mg/kg/b.wt. | Soluble protein | Percent reduction | Basic protein | Percent reduction | Total protein | Percent reduction |
|--------|---------------------|-------------------------------|----------------------|-------------------------------|----------------------|------------------|----------------------|
| Male | 0 (Control) | 7.6 \pm 0.12 | | 3.5 \pm 0.09 | | 11.1 | |
| | 5 | 3.7 \pm 0.19 ^a | -51.3 | 2.0 \pm 0.41 ^a | -42.8 | 5.7 | -48.6 |
| | 10 | 1.6 \pm 0.09 ^{a,b} | -78.9 | 1.2 \pm 0.06 ^{a,b} | -65.7 | 2.8 | -74.7 |
| | 20 | 1.0 \pm 0.06 ^{a,b} | -86.8 | 0.5 \pm 0.06 ^{a,b} | -85.7 | 1.5 | -86.4 |
| | 50 | 0.4 \pm 0.07 ^{a,b} | -94.7 | 0.2 \pm 0.01 ^{a,b} | -94.2 | 0.6 | -94.5 |
| Female | 0 (Control) | 8.6 \pm 0.33 | | 4.1 \pm 0.10 | | 12.7 | |
| | 5 | 3.8 \pm 0.07 ^a | -55.8 | 2.0 \pm 0.03 ^a | -48.7 | 5.8 | -54.6 |
| | 10 | 1.2 \pm 0.04 ^{a,b} | -86.0 | 1.5 \pm 0.11 ^{a,b} | -63.4 | 2.7 | -78.7 |
| | 20 | 1.1 \pm 0.10 ^{a,d} | -87.2 | 1.3 \pm 0.14 ^{a,c} | -68.2 | 2.3 | -81.8 |
| | 50 | 1.0 \pm 0.12 ^a | -88.3 | 0.6 \pm 0.14 ^{a,b} | -85.3 | 1.6 | -87.4 |

Values are expressed in mg/g wet weight of tissue.

a = Difference between mean of experimental group and control was significant at $P < 0.001$.

b, c or d = Difference between the mean of the group and the one above it was significant at
b: $P < 0.001$, c: $P < 0.02$, d: $P < 0.05$.

TABLE 2. Effect of different doses of fluoride on free amino acids and ribonucleic acid content of rabbit brain (Mean \pm SD)

| | Dose mg/kg/b.wt. | Free amino acid | Percent reduction | Ribonucleic acid | Percent reduction |
|--------|---------------------|-------------------------------|----------------------|-------------------------------|----------------------|
| Male | 0 (Control) | 4.4 \pm 0.03 | | 4.1 \pm 1.06 | |
| | 5 | 2.1 \pm 0.26 ^a | -52.2 | 3.6 \pm 0.08 | -12.1 |
| | 10 | 1.8 \pm 0.01 ^{a,d} | -59.0 | 3.3 \pm 0.04 ^c | -19.5 |
| | 20 | 0.8 \pm 0.19 ^{a,c} | -81.8 | 2.2 \pm 0.01 ^{b,c} | -46.3 |
| | 50 | 0.5 \pm 0.02 ^{a,c} | -88.6 | 1.8 \pm 0.03 ^{a,c} | -56.0 |
| Female | 0 (Control) | 8.5 \pm 1.12 | | 3.2 \pm 0.04 | |
| | 5 | 1.8 \pm 0.04 ^a | -78.8 | 2.8 \pm 0.07 ^a | -12.5 |
| | 10 | 1.2 \pm 0.06 ^{a,c} | -85.8 | 2.3 \pm 0.02 ^{a,c} | -28.1 |
| | 20 | 1.1 \pm 0.01 ^a | -87.0 | 1.9 \pm 0.40 ^a | -40.6 |
| | 50 | 0.3 \pm 0.09 ^{a,c} | -96.4 | 1.6 \pm 0.01 ^a | -50.0 |

Values are expressed in mg/g wet weight of tissue.

a or b = Difference between mean of experimental group and control was significant at

a: $P < 0.001$, b: $P < 0.01$

c or d = Difference between the mean of the group and the one above it was significant at

c: $P < 0.001$, d: $P < 0.05$.

Discussion

Protein synthesis is affected in various ways by fluoride ions. They affect the rate of cellular protein synthesis (10,11) due to impairment of peptide chain initiation (12). The present experimental data show that in treated animals there is a decreased level of soluble, basic protein and a general decrease in total protein content. The observations agree with those of Kathpalia and Susheela (13) who recorded a 10 to 46 percent decrease in total protein content of different rabbit organs including kidney, liver, testis, and brain.

Administration of sodium fluoride inactivates certain enzymes of glycolysis and of the tricarboxylic acid cycle (14). Leonard (15) reported that NaF (50 μ g in 10 μ l) increases brain glycolysis in albino mice after one minute of intraventricular administration.

Shearer and Suttic (16) recorded that, in female rats ingesting 450 ppm fluoride for 3 days, the concentration of plasma free amino acids was decreased. During the present investigation, a reduction in the brain free amino acids of all fluoridated rabbits of both sexes was found. Such a reduction had already been reported by Schwartz *et al* (17) who observed that fluoride progressively lowered amino acid uptake and ATP content in incubated brain slices of mice as the concentration of fluoride increased.

Fluoride is also known to inhibit nucleic acid synthesis (18) and attachment of m-RNA to ribosomes. NaF retards initiation of globin chain synthesis and the free m-RNA are rendered inactive by fluoride (10). The decrease in RNA content of rabbit brain observed during acute and chronic fluoride intoxication seems to be due to fluoride-induced inhibition of protein synthesis.

Fluoride inhibits many enzymes *in vitro* (14). The action of fluoride on most of these enzymes is magnesium dependent. The degree to which fluoride complexes with magnesium ions varies in different cell compartments causing local changes in the concentration of the activator. This in turn affects the function of the RNA-polymerase system, the free energy of ATP hydrolysis and the conformational structure of some types of RNA and proteins (19). The biochemical changes in brain levels of proteins, amino acids, and ribonucleic acids are reflected in the morphological patterns of different cell structures of the brain.

The results reported here indicate that fluoride has a specific effect on the synthesis of proteins in the brain which may lead to degenerative changes in the form of ballooning degeneration of neurons, various degrees of loss of nissl substance, and changes in the purkinje cells of the cerebellar cortex. Such changes would provide a plausible explanation for some of the diverse neurological complaints in arms and legs such as numbness, muscle spasms and pains, tetani-form convulsions, and spastic paraplegia, encountered in patients afflicted with skeletal fluorosis (20).

Conclusion

Observed alterations in the brain protein synthesizing system in experimental fluorosis may offer a partial biochemical explanation for some of the diverse neurological complaints in patients with skeletal fluorosis.

Acknowledgement

The material in this paper is a part of the work undertaken for the PhD degree by the first author, Dr Shashi, who is grateful to the Indian Council of Medical Research for financial assistance.

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FLUOROSIS AND THE TEA-DRINKING HABIT AMONG KAZAKS IN XINJIANG

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Urumqi, Xinjiang, China

Abstracted from *Endemic Diseases Bulletin* 8 (3) 43-48 1993

This preliminary work investigated fluorosis in Kazak people, living in the pastured area of Xinjiang, who have a time-honored habit of drinking tea. Endemic fluorosis has been prevalent among them for a long time, but no reliable information was available before now. It was found that the fluoride levels in drinking water were less than 0.5 ppm, and that dental fluorosis prevalence in children aged 8-15 years was about 30%. Skeletal fluorosis prevalence in people aged over 16 years, in a typical pasture where the drinking water was only 0.15 ppm, was 16.67%. The method of tea infusion among Kazaks, from brick-teas commercially available in the endemic area, resulted in a mean fluoride content of 480 mg per kilogram. The mean tea consumption among Kazaks was 20.44 g, compared with only 3.65 g in Han people. The mean daily fluoride intake from drinking tea was 9.81 mg in Kazaks, which was 5.61 times that for Han people in the same locality. These findings suggest that fluorosis in Kazak people is caused by drinking too much tea.

Key words: Dental fluorosis; Fluorosis; Kazak people, Skeletal fluorosis; Tea.

EFFECTIVENESS OF ENDEMIC FLUOROSIS CONTROL FOR 8 YEARS
BY DEFLUORIDATION TO IMPROVE DRINKING WATER QUALITY IN
MARZHUANG VILLAGE, YANCHI COUNTY, NINGSIA, CHINA

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Yinchuan, Ningsia, China

Abstracted from *Endemic Diseases Bulletin* 8 (3) 49-52 1993

This study observed the effectiveness of an endemic fluorosis control program, by improving drinking water quality through defluoridation for 8 years, in Marzhuang village in Yanchi county, Ningsia Hui Autonomous Region, China. Assessment was based on the changes in fluoride levels in the water source, symptoms and signs of the cases of dental and skeletal fluorosis, including their urinary fluoride levels, and bone density and structures showed on roentgenograms. Fluoride concentration of the water source was 0.8 mg/L. Among adult cases, dental fluorosis did not vary significantly before and after improving water quality. Among the children, however, who were born after improvement of water quality, only a few cases of mild dental fluorosis were found. Obvious improvement of symptoms and signs occurred in 52.8% of cases of skeletal fluorosis. A follow-up survey by roentgenography among 26 selected cases of skeletal fluorosis revealed obvious improvement in the density and structure of their bones in 76.92% of the cases. Continuous determination, for 8 years, of fluoride concentrations in urinary samples from the cases, showed reductions in mean values from 15.61 mg/L to 5.80 mg/L in the first year, and then to 3.07 mg/L after 8 years. However, the mean urinary fluoride concentrations in the endemic area are still above the normal upper limit of 2.60 mg/L in non-endemic areas of Ningsia.

Key words: Dental fluorosis; Endemic fluorosis; Skeletal fluorosis.

ANALYSIS OF BONE MINERAL CONTENT IN CASES OF ENDEMIC SKELETAL FLUOROSIS OF DIFFERENT ROENTGEN RAY TYPES

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Zhangjiakou, Hebei, China

Abstracted from *Endemic Diseases Bulletin* 8 (3) 53-56 1993

Scans were made of left ulna and radius bones with a ^{125}I photon beam for determination of bone mineral contents among 128 cases of endemic skeletal fluorosis. The results showed that the bone mineral contents were lower than those of normal controls, and varied according to the four types of condition revealed by the roentgen rays (early osteopathic changes, osteosclerosis, osteoporosis, and mixed osteopathic changes). The order of mineral contents, from high to low, was: osteosclerosis > early osteopathic changes > mixed osteopathic changes > osteoporosis.

Key words: ^{125}I ; Analysis; Bones; Endemic skeletal fluorosis; Mineral contents; Roentgen rays.

AN INVESTIGATION INTO THE FLUORIDE LEVELS OF DRINKING WATER AND THE CONDITION OF FLUOROSIS IN SOME AREAS OF SOUTH XINJIANG

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Abstracted from *Endemic Diseases Bulletin* 8 (3) 57-60 1993

An investigation, carried out in parts of 13 counties or cities in 3 prefectures of Hotan, Kaxgar and Aksu in south Xinjiang in August 1991, showed that of 142 water samples collected from newly built water sources, 89 (62.7%) had fluoride levels exceeding 1 mg/L, whereas of 70 samples collected from previously used drinking water sources in the same places, only 13 (18.5%) exceeded 1 mg/L. Shallow ground water had the highest percentage (89.7%) of fluoride contents in excess of 1 mg/L, followed by deep ground water (52.4%) and surface water (18.5%). The mean urinary fluoride levels of children increased with higher drinking water fluoride levels. Dental fluorosis prevalences and severity among children in the vicinity of these water sources depended on the length of time the new sources had been used, being very prevalent and severe among children and adults after the longest periods of use. Because many new drinking water sources have been built in south Xinjiang, where natural climatic and geographical conditions along with some customs of minority groups aggravate the incidence of fluorosis, we are worried about the possible disastrous consequences in the future.

Key words: Drinking water; Endemic fluorosis; Epidemiology; Fluoride levels; South Region, Xinjiang.

Reprints: *Endemic Diseases Bulletin* is published by the Xinjiang Institute for Endemic Disease Control and Research, Urumqi 830002, China, and is distributed internationally by China National Publishing Corporation, PO Box 782, Beijing, China. Its contents are in Chinese, with English-language abstracts, tables, and figure captions.

ENDEMIC FLUOROSIS OF THE SKELETON: RADIOGRAPHIC FEATURES IN 127 PATIENTS

Y Z Wang, Y M Yin, L A Gilula and A J Wilson
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Abstract from *American Journal of Roentgenology* 162 (1) 93-98 1994

Objective: A wide range of radiographic appearances have been reported in skeletal fluorosis, but little has been written about the spectrum of radiographic features. We evaluated the spectrum of radiographic appearances in this disorder to help with its diagnosis and differentiation from other metabolic skeletal disorders.

Materials and Methods: One hundred twenty-seven patients with clinically proved endemic fluorosis had radiographs of the chest, spine, pelvis, elbow, forearm, and knee obtained. The radiographic findings were classified as osteosclerosis, osteopenia, intermittent growth lines, diaphyseal widening, or soft-tissue ossification. Two different osteopenic patterns were defined: an osteoporotic pattern with overall decreased bone density and an osteomalacic pattern that combines the features of osteoporosis with bone deformity. Soft-tissue ossification included involvement of ligaments, tendons, and interosseous membranes.

Results: Ninety-eight of the patients (89% of the adults) had some evidence of calcification and/or ossification of the attachments of ligaments, tendons, muscles, and interosseous membranes. Osteosclerosis was seen in 54 patients (43%), and osteopenia was seen in 51 patients (40%). Of the patients with osteopenia, the osteoporotic pattern was seen in 28 and the osteomalacic pattern in 23. Growth lines were found in 89 patients (70%). Metaphyseal osteomalacic zones were found in children. Diaphyseal widening was present in 35 patients (28%).

Conclusion: Endemic skeletal fluorosis can have a wide variety of radiographic appearances, including calcification and/or ossification of the attachments of soft-tissue structures to bone, osteosclerosis, osteopenia, growth lines, and metaphyseal osteomalacic zones.

Key words: Endemic fluorosis; Radiography; Skeletal fluorosis.

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* APPROPRIATE USES OF FLUORIDES FOR CHILDREN GUIDELINES FROM THE CANADIAN WORKSHOP ON THE EVALUATION OF CURRENT RECOMMENDATIONS CONCERNING FLUORIDES

D C Clark
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Abstract from *Canadian Medical Association Journal* 149 (12) 1787-1793 1993

Objective: To prevent fluorosis caused by excessive fluoride ingestion by revising recommendations for fluoride intake by children.

Options: Limiting fluoride ingestion from fluoridated water, fluoride supplements and fluoride dentifrices.

* For a more recent assessment, see *Fluoride* 27 (2) 1994 p 121. Also letters pp 184-185.

Outcomes: Reduction in the prevalence of dental fluorosis and continued prevention of dental caries.

Evidence: Before the workshop, experts prepared comprehensive literature reviews of fluoride therapies, fluoride ingestion and the prevalence and causes of dental fluorosis. The papers, which were peer-reviewed, revised and circulated to the workshop participants, formed the basis of the workshop discussions.

Values: Recommendations to limit fluoride intake were vigorously debated before being adopted as the consensus opinion of the workshop group.

Benefits, harms and costs: Decrease in the prevalence of dental fluorosis with continuing preventive effects of fluoride use. The only significant cost would be in preparing new, low-concentration fluoride products for distribution.

Recommendations: Fluoride supplementation should be limited to children 3 years of age and older in areas where there is less than 0.3 ppm of fluoride in the water supply. Children in all areas should use only a "pea-sized" amount of fluoride dentifrice no more than twice daily under the supervision of an adult.

Validation: These recommendations are almost identical to changes to recommendations for the use of fluoride supplements recently proposed by a group of European countries.

Sponsors: The workshop was organized by Dr D Christopher Clark, of the University of British Columbia, and Drs Hardy Limeback and Ralph C Burgess, of the University of Toronto, and funded by Proctor and Gamble Inc., Toronto, the Medical Research Council of Canada and Health Canada (formerly the Department of National Health and Welfare). The recommendations were formally adopted by the Canadian Dental Association in April 1993.

Key words: Dental fluorosis; Fluoride ingestion; Fluoride supplements.

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* ACUTE FLUORIDE POISONING FROM A PUBLIC WATER SYSTEM

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Abstract from *New England Journal of Medicine* 330 (2) 95-99 1994

Background: Acute fluoride poisoning produces a clinical syndrome characterized by nausea, vomiting, diarrhea, abdominal pain, and paresthesias. In May 1992, excess fluoride in one of two public water systems serving a village in Alaska caused an outbreak of acute fluoride poisoning.

Methods: We surveyed residents, measured their urinary fluoride concentrations, and analyzed their serum-chemistry profiles. A case of fluoride poisoning was defined as an illness consisting of nausea, vomiting, diarrhea, abdominal pain, or numbness or tingling of the face or extremities that began between May 21 and 23.

Results: Among 47 residents studied who drank water obtained on May 21, 22, or 23 from the implicated well, 43 (91 percent) had an illness that met the case

* For a critique of this report see *Fluoride* 27 (1) January 1994 pp 32-36.

definition, as compared with only 6 of 21 residents (29 percent) who drank water obtained from the implicated well at other times and 2 of 94 residents (2 percent) served by the other water system. We estimated that 296 people were poisoned; 1 person died. Four to five days after the outbreak, 10 of the 25 case patients who were tested, but none of the 15 control subjects, had elevated urinary fluoride concentrations. The case patients had elevated serum fluoride concentrations and other abnormalities consistent with fluoride poisoning, such as elevated serum lactate dehydrogenase and aspartate aminotransferase concentrations. The fluoride concentration of a water sample from the implicated well was 150 mg per liter, and that of a sample from the other system was 1.1 mg per liter. Failure to monitor and respond appropriately to elevated fluoride concentrations, an unreliable control system, and a mechanism that allowed fluoride concentrate to enter the well led to this outbreak.

Conclusions: Inspection of public water systems and monitoring of fluoride concentrations are needed to prevent outbreaks of fluoride poisoning.

Key words: Acute fluoride poisoning; Alaska; Public water supply.

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PSYCHOPHARMACOLOGY OF FLUORIDE: A REVIEW

B Spittle
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Abstract from *International Clinical Psychopharmacology* 9 79-82 1994

Although the blood-brain barrier is relatively impermeable to fluoride, it does not pose an absolute barrier and fluoride has the ability to enter the brain. The literature was examined to assess the quality of the evidence for cerebral impairment occurring due to exposure to fluoride from therapeutic or environmental sources. Several surveys of persons chronically exposed to industrial fluoride pollution reported symptoms related to impaired central nervous system functioning with impaired cognition and memory. Examination of individual case reports showed the evidence for aetiological relationships between symptoms and fluoride exposure to be of variable quality. The evidence was seen as being suggestive of a relationship rather than being definitive. The difficulties with concentration and memory described in relation to exposure to fluoride did not occur in isolation but were accompanied by other symptoms of which general malaise and fatigue were central. Possible mechanisms whereby fluoride could affect brain function include influencing calcium currents, altering enzyme configuration by forming strong hydrogen bonds with amide groups, inhibiting cortical adenylyl cyclase activity and increasing phosphoinositide hydrolysis.

Key words: Case reports; Chronic toxicity; Cognition; Cognitive impairment; Concentration; Fluoride; Memory; Psychopharmacology.

Reprints: Dr B Spittle, Department of Psychological Medicine, University of Otago Medical School, PO Box 913, Dunedin, New Zealand.

ISOLATION, CHARACTERIZATION AND EPISTASIS OF FLUORIDE-RESISTANT MUTANTS OF *CAENORHABDITIS ELEGANS*

I Katsura, K Kondo, T Amano, T Ishihara and M Kawakami
Mishima, Shizuoka, Japan

Abstract from *Genetics* 136 (1) 145-154 1994

We have isolated 13 fluoride-resistant mutants of the nematode *Caenorhabditis elegans*. All the Mutants in three of the genes (class 1 genes: flr-1 X, flr-3 IV, and flr-4 X) are resistant to 400 mug/ml NaF. Furthermore, they grow twice as slowly as and have smaller brood size than wild-type worms even in the absence of fluoride ion. In contrast, mutants in the other two genes (class 2 genes: flr-2 V and flr-5 V) are only partially resistant to 400 mug/ml NaF, and they have almost normal growth rates and brood sizes in the absence of fluoride ion. Studies on the phenotypes of double mutants showed that class 2 mutations are epistatic to class 1 mutations concerning growth rate and brood size but hypostatic with respect to fluoride resistance. We propose two models that can explain the epistasis. Since fluoride ion depletes calcium ion, inhibits some protein phosphatases and activates trimeric G-proteins, studies on these mutants may lead to discovery of a new signal transduction system that controls the growth of *C. elegans*.

Key words: *Caenorhabditis elegans*; Epistasis; Fluoride-resistant mutants.

Reprints: I Katsura, National Institute of Genetics, DNA Research Center, Mishima, Shizuoka 411, Japan.

EFFECT OF INTERMITTENT DELIVERY OF FLUORIDE TO SOLUTION ON ROOT HARD-TISSUE DE- AND REMINERALIZATION MEASURED BY I-125 ABSORPTIOMETRY

H Almqvist and F Lagerlof
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Abstract from *Journal of Dental Research* 72 (12) 1593-1598 1993

The effect of intermittent fluoride levels on root hard-tissue de- and remineralization was studied once daily for 21 days in a pH-cycling caries model with simulated fluoride clearance curves. Four root hard-tissue blocks, from each of 12 human teeth, were cut out parallel to the cementum surface. During a daily 15-hour period, the blocks were subjected 12 times to pH changes similar to those which occur in plaque after a carbohydrate intake. The fluoride was delivered immediately before a daily nine-hour remineralization period. Four experiments were independently carried out: One block from each tooth was subjected to pH-cycling without and with fluoride delivery, simulating a rinse with 0.025, 0.2, and 1.0% sodium fluoride (NaF), respectively. The mineral change in the blocks was monitored by I-125 absorptiometry and expressed as the change in transmission (Delta T). The surface between the data points (Delta T values) and the x axis (time points) was used as a summary measure, i.e., the area under the curve (AUC). When no fluoride was delivered, the Delta T increased over 21 days,

indicating loss of mineral. The AUC was, on average, 5.85 ± 0.68 (mean \pm S.E.) % day. In the 0.025% NaF-rinse experiment, there was a marked reduction in mineral loss, indicated by an average AUC of 1.66 ± 0.59 % day. In both the 0.2 and 1.0% NaF-rinse experiments, a decrease in Delta T, indicating gain of mineral, was observed from day 2. Negative Delta T values occurred after 7 and 3 days, respectively. The average AUCs for the 0.2 and 1.0% NaF-rinse experiments were calculated to -0.96 ± 0.84 and -4.32 ± 1.0 % day, respectively. Increasing the fluoride level decreased the Delta T significantly, and all fluoride levels had statistically different effects on the mineral content.

Key words: Absorptiometry; Demineralization; Remineralization; Root hard-tissue.

Reprints: H Almqvist, Karolinska Institute, School of Dentistry, Department of Cariology, Box 4064, S-14104 Huddinge, Sweden.

EXPERIMENTS ON THE INITIATION OF CALCIUM FLUORIDE FORMATION WITH REFERENCE TO THE SOLUBILITY OF DENTAL ENAMEL AND BRUSHITE

M J Larsen and S J Jensen
Aarhus, Denmark

Abstract from *Archives of Oral Biology* 39 (1) 23-27 1994

As calcium fluoride formation following topical application of fluoride may be responsible for at least some of the caries-reducing effect of fluoride, the concentrations of fluoride necessary to induce its formation were examined. The aim was to determine the degree of supersaturation with respect to calcium fluoride necessary for inducing its spontaneous precipitation, with close reference to its possible formation and retention on dental hard tissue when topical fluoride solutions are used clinically. Powdered enamel or brushite were suspended for 4 h in aqueous solutions buffered at pH 7.2 and 5.0. After the equilibration, ion concentrations were determined and degrees of saturation with respect to apatite and brushite were calculated. In aqueous solutions at pH 7.2 and 5.0 with similar concentrations as those found in the equilibrated suspensions the fluoride concentration was adjusted to from 5 to 500 parts/ 10^6 . After 2 h of gentle agitation the supernatant was analysed, the precipitate (if any) isolated and examined by X-ray diffraction. Initiation of spontaneous calcium fluoride formation required a calcium fluoride ion-activity product of $10^{-7.6}$ or more. 300 parts/ 10^6 fluoride were necessary to initiate calcium fluoride formation in neutral solutions saturated with enamel, and the increased solubility of enamel apatite at low pH allowed calcium fluoride formation from solutions with as low as 100 parts/ 10^6 fluoride. When phosphate was present in the solution a competing apatite formation could mask the calcium fluoride formation. In neutral solutions saturated with respect to brushite, spontaneous fluorapatite formation was initiated by 100 parts/ 10^6 fluoride. With 200 parts/ 10^6 fluoride a further competing formation of calcium fluoride occurred.

Key words: Calcium fluoride; Preventive measures; Dental enamel; Calcium phosphates.

Reprints: M J Larsen, University Aarhus, Faculty of Health Science, Royal Dental College, Bennelyst Blvd, DK-8000 Aarhus C, Denmark.

IONIC AND NONIONIC FLUORIDE LEVELS IN BLOOD OF DIALYZED AND UNDIALYZED PATIENTS WITH RENAL FAILURE AND KIDNEY TRANSPLANTED PATIENTS

T Kimura, G Yamamoto, K Yoshitake and T Ando
Shiga, Japan

Abstract from *Japanese Journal of Medical Science and Biology* 46 (3) 131-139 1993

The present study revealed that the total fluoride level in human whole blood is closely related to the renal function. For the undialyzed patients who had not undergone hemodialysis, the total fluoride level in whole blood linearly increased with the increase of creatinine (Cr) value. The increased fluoride was found to be nonionic in the other blood part than serum, while the nonionic fluoride level in serum was almost constant. That is, the hemodialysis treatment finally reduced the nonionic fluoride level in the other blood part than serum. On the other hand, one hemodialysis treatment could excrete ionic fluoride, but not nonionic fluoride. These results suggest that the ionic fluoride is transformed to nonionic fluoride to be accumulated in other blood part than serum and the nonionic fluoride is transformed to the ionic fluoride to be excreted. Thus the accumulation of nonionic fluoride in other blood part than serum plays a role of the buffer in preventing a too high serum fluoride level.

Key words: Blood fluoride levels; Hemodialysis; Ionic fluoride; Nonionic fluoride.

Reprints: T Kimura, Shiga University of Medical Science, Department of Chemistry, Otsu, Shiga 52021, Japan.

CLINICAL TRIAL OF FLUORIDE THERAPY IN POSTMENOPAUSAL OSTEOPOROTIC WOMEN - EXTENDED OBSERVATIONS AND ADDITIONAL ANALYSIS

B L Riggs, W M O'Fallon, A Lane, S F Hodgson,
H W Wahner, J Muhs, E Chao and L J Melton
Rochester, Minnesota, USA

Abstract from *Journal of Bone and Mineral Research* 9 (2) 265-275 1994

In a 4 year clinical trial in 202 postmenopausal osteoporotic women receiving NaF at 15 mg/day or placebo (both groups received supplementary calcium at 1500 mg/day), we found (*New England Journal of Medicine* 322 801, 1990) that NaF increased bone mineral density in the lumbar spine (LS-BMD) substantially but did not decrease vertebral fracture rate (VFR), and it increased the nonvertebral fracture rate. Additional analyses and extended observations are now available on 50 women from the NaF group followed for up to 6 years of treatment. In these women, LS-BMD increased linearly over the 6 years (median rate, 8.7%/year or 0.063 g/cm²/year). Because during the 4 year trial the NaF dosage was decreased (because of side effects) in 54 of the 101 women randomized to NaF, dose-response relationships could be evaluated. For the entire study population, serum F level correlated directly with increase in LS-BMD ($r = 0.61$, $P < 0.001$). When individual person-years of observation were grouped by deciles of LS-BMD, VFR

(per 100 person-years) decreased to a nadir of 24 as mean LS-BMD for the group increased from 0.6 to 1.2 g/cm² and then doubled to 52 in the group with mean LS-BMD of 1.6 g/cm². Multivariate analyses and inspection of three-dimensional plots revealed a complex pattern in which VFR was influenced by interaction of several variables. When the effects of LS-BMD, changes in LS-BMD, and serum F were assessed simultaneously, VFR was seen to decrease with increasing LS-BMD except when the higher LS-BMD was associated with rapid rate of increase in LS-BMD or a large increase from baseline serum F. For some patients (non-compliers or nonresponders), serum F or LS-BMD failed to increase. Thus, it is possible that lower dosages of NaF produce moderate decreases in VFR.

Key words: Fluoride therapy; Osteoporosis.

Reprints: B L Riggs, Mayo Clinic and Mayo Foundation, Division of Endocrinology, Metabolism and Internal Medicine, Endocrine Research Unit, Rochester, MN 55905 USA.

FLUORIDE PHARMACOKINETICS IN INFANCY

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Iowa City, Iowa, USA

Abstract from *Pediatric Research* 35 (2) 157-163 1994

Fluoride pharmacokinetic data are presented for infants given a fluoride supplement. Seventeen infants participated in a total of 20 studies. On one day, 0.013 mmol (0.25 mg) fluoride was given as a supplement (fluoride supplement study), and on another day a placebo was given (control study). Samples of plasma and urine were collected for 5 h and analyzed for fluoride. During control studies fluoride intake averaged 0.15 $\mu\text{mol/kg}$ (2.9 $\mu\text{g/kg}$), and plasma fluoride concentrations ranged from 0.05 to 0.11 $\mu\text{mol/L}$ (10 to 20 $\mu\text{g/L}$). In nine instances, the quantity of fluoride excreted in the urine was more than twice that consumed. When the fluoride supplement was given, total fluoride intake averaged 1.93 $\mu\text{mol/kg}$ (36.6 $\mu\text{g/kg}$). Plasma peak concentration was reached by 30 min in 14 studies and by 60 min in six studies. Mean plasma peak fluoride concentration was 3.3 $\mu\text{mol/L}$ (63 ng/mL). Area under the plasma concentration curve averaged 236 $\text{nmol}\cdot\text{m}^{-1}\cdot\text{min}$ (4479 $\text{ng}\cdot\text{mL}^{-1}\cdot\text{min}$) and was not related to the dose of fluoride. The rate of urinary excretion was significantly correlated with rate of urinary flow. When the dose of fluoride was expressed per unit of body weight, fluoride retention was strongly related to the dose. Retention of the fluoride absorbed from the fluoride dose ranged from 75.4 to 87.6%. Plasma clearance averaged 6.8 $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and decreased significantly with age. Net fractional clearance (renal clearance of the fluoride dose/GFR) averaged 56.7%, which was significantly greater than the 29% observed during the control studies. The greater percentage retention of fluoride by infants than by adults is probably explained by a greater capacity of the infant to deposit fluoride in hard tissues.

Key words: Fluoride pharmacokinetics; Fluoride supplements; Infancy; Plasma fluoride; Urinary fluoride.

Reprints: S J Fomon, University of Iowa, College of Medicine, Department of Pediatrics, Iowa City, IA 52242 USA.

DISTRIBUTION PROFILES OF FLUORIDE IN 3 DIFFERENT KINDS OF RAT BONES

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Abstract from *Bone* 14 (6) 835-841 1993

The study was performed to reveal the detailed distribution profiles of fluoride in three different kinds of rat bone-humerus, vertebral arch, and parietal bone-and to compare this with the histological appearance of each bone type. Two groups of Wistar rats were provided water ad libitum containing 0 and 100 ppm fluoride, respectively, for 24 weeks. The fluoride distribution profiles across the bone of the three different bones from the outer to the inner surface were determined by means of an abrasive micro-sampling technique. In control animals, both humerus and parietal bones showed higher concentrations at the periosteal and endosteal surfaces, while the vertebral arch showed additional high levels in the middle (containing trabecular bone) of the tissue. In exposed animals, fluoride levels increased greatly in all three bone types. The vertebral and parietal fluoride distribution profiles were relatively unchanged, although humerus fluoride increased from periosteum to endosteum. The differences in fluoride distribution profiles were apparently related to the histological appearances of these bones. The surface area of bone available and the extent of vascularity appear to affect fluoride uptake.

Key words: Bone; Fluoride; Rat.

Reprints: J Li, Aichi Gakuin University, School of Dentistry, Department of Preventive Dentistry and Dental Public Health, 1-100 Kusumoto CHO, Chikusa Ku, Nagoya 464, Japan.

EFFECT OF MOUTHWASHES OF VARIABLE NaF CONCENTRATION BUT CONSTANT NaF CONTENT ON ORAL FLUORIDE RETENTION

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Wirral, Merseyside, England

Abstract from *Caries Research* 28 (1) 43-47 1994

Previous work showed that oral fluoride levels increased with increasing applied F dose for both mouthwashes and dentifrices. This study aimed to determine whether the above dependence was related to applied fluoride concentration or applied fluoride amount. Ten adults mouthrinsed with aqueous NaF solutions of 1-10 ml, each of which contained 2.5 mg F i.e. in the concentration range 250-2,500 ppm F. Subjects rinsed for 1 min and then spat out. Samples of mixed saliva were collected for 3 h afterwards, which were analysed for fluoride. Salivary fluoride clearance curves were obtained which could be fitted to a pharmacokinetic model involving processes ascribed to loss of fluoride from saliva by swallowing and to exchange of fluoride between saliva and an oral reservoir. Mean salivary fluoride concentrations increased significantly with increasing applied F concentration both within the first 3 h after single use and up to at least 18 h after regular

daily use. These findings suggest that applied F concentration is a more important factor than applied F amount *per se* in determining the elevation of oral fluoride levels following topical fluoride use. This implies that application of a given F dose, in a smaller volume at higher concentration than the current norm, may increase efficacy without increasing the risk of adverse effects.

Key words: Fluoride; Mouthwash; Reservoir; Salivary clearance.

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FLUORIDE-INDUCED DEVELOPMENTAL CHANGES IN ENAMEL AND
DENTINE OF EUROPEAN ROE DEER (*CAPREOLUS CAPREOLUS* L)
AS A RESULT OF ENVIRONMENTAL POLLUTION

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Abstract from *Archives of Oral Biology* 38 (12) 1071-1081 1993

Using macroscopic, microradiographic and scanning electron-microscopic methods, the effects of increased fluoride exposure on enamel and dentine formation were studied in fluorosed mandibular premolars and molars of roe deer from the heavily industrialized Ruhr area, Germany. Macroscopically, fluorosed teeth were characterized by opaque and stained enamel and in more severe cases also by enamel surface lesions, reduction or loss of enamel ridges on their occlusal surfaces and increased wear. Microradiographically, fluorosed enamel exhibited different degrees of subsurface hypomineralization, in part apparently indicating a fluoride effect during enamel maturation. In some specimens, a pronounced but varying enhancement of the pattern of Retzius lines was observed throughout the enamel, denoting strongly intermittent fluoride exposure during enamel matrix secretion. This variation in exposure was also reflected histologically in dentine, by bands of interglobular dentine and marked accentuation of incremental lines. Microradiography of sections through enamel surface hypoplastic lesions showed the enamel forming the bottom and partly also the walls of the lesions to be highly mineralized. Scanning electron microscopy showed that the outer enamel along the more pronounced hypoplastic lesions consisted of stacked, thin layers of 'aprismatic' enamel, indicating that the ameloblasts in these areas had lost the distal (rod-forming) regions of their Tomes' processes. These observations demonstrate that the origin of enamel hypoplasias in deer clearly differs from that in rodents, where fluoride induces the formation of subameloblastic cysts. The differences in the degree of fluorotic alteration between the teeth of a single tooth row could be related to the developmental sequence of the dentition in roe deer. The roe deer is thus considered to be a very sensitive and useful bioindicator of environmental pollution by fluorides.

Key words: Roe deer; Biomonitoring; Dental fluorosis; Intermittent fluoride exposure; Enamel and dentine hypomineralization; Enamel hypoplasias.

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SITE-SPECIFIC DIFFERENCES IN THE SALIVARY CONCENTRATIONS
OF SUBSTANCES IN THE ORAL CAVITY - IMPLICATIONS FOR THE
AETIOLOGY OF ORAL DISEASE AND LOCAL DRUG DELIVERY

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Abstract from *Advanced Drug Delivery Reviews* 13 (1-2) 23-42 1994

There have been many attempts to administer drugs locally from devices placed in the oral cavity. The ability to reach a target site following release will be influenced by the ease with which a drug can move around the oral cavity. The rate at which a drug is cleared from that site may influence the magnitude and duration of its effect. Studies suggest that the movement and clearance of substances dissolved or suspended in saliva are complex. In this review we forward explanations for these site-specific patterns and discuss the significance such regional variations may have with respect to the aetiology of oral disease, the placement of delivery systems for optimisation of delivery and in the design and formulation of oral mucosal drug delivery systems used for local delivery of bioactive materials to the oral cavity.

Key words: Absorption; Buccal; Fluoride distribution; Glucose distribution; Oral clearance; Saliva.

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THE MECHANISM OF FLUORIDE-INDUCED HYPOCALCAEMIA

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Abstract from *Human and Experimental Toxicology* 13 (3) 149-155 1994

1. Fluoride intoxication leads to sudden cardiac death which has been assumed to result from the accompanying severe hypocalcaemia. The aim of this study has been to investigate the suggestion that fluorapatite formation rather than CaF_2 precipitation is responsible for this low calcium.

2. Measurements of free Ca^{2+} and F^- ion concentrations in HEPES buffered solutions containing F^- , Ca^{2+} , and phosphate ions at different concentrations in the absence and presence of hydroxyapatite showed that the presence of hydroxyapatite enhanced the decrease of Ca^{2+} and F^- concentration.

3. The ratio of $\text{Ca}^{2+}:\text{F}^-$ clearance was 5:1 which is consistent with formation of fluorapatite. These results support the hypothesis that hydroxyapatite acts as a nucleation catalyst for fluorapatite formation and this process is responsible for the hypocalcaemia induced by fluoride intoxication.

4. The proposed mechanism explains also the metabolic acidosis which is frequently seen in cases of fluoride intoxication.

Key words: Fluorapatite; Fluoride intoxication; Hypocalcaemia.

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THE EFFECT OF HONEY ON HUMAN TOOTH ENAMEL *INVITRO*
OBSERVED BY ELECTRON MICROSCOPY AND
MICROHARDNESS MEASUREMENTS

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Abstract from *Archives of Oral Biology* 39 (2) 147-153 1994

Various fruit juices with relatively low pH are known to have erosive effects on human tooth enamel in a reasonably short time. Honey, also with a relatively low pH, could do the same, but scanning electron microscopy showed no erosion of enamel by honey over a period of 30 min, neither did Knoop microhardness tests show any deterioration of enamel structure. The absence of any effect could be only partially attributed to the calcium, phosphorus and fluoride levels in honey.

Key words: Analysis; Erosivity; Honey; Microhardness; Scanning electron microscopy.
Reprints: S R Grobler, University of Stellenbosch, Faculty of Dentistry, Private Bag X1, Tygerberg 7505, South Africa.

SLOW-RELEASE SODIUM FLUORIDE IN THE MANAGEMENT
OF POSTMENOPAUSAL OSTEOPOROSIS
A RANDOMIZED CONTROLLED TRIAL

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B Adamshuet and J S Reisch
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Abstract from *Annals of Internal Medicine* 120 (8) 625-632 1994

Objective: To test whether intermittent treatment with slow-release sodium fluoride and continuous calcium citrate supplementation inhibits vertebral fractures without causing fluoride complications.

Design: A placebo-controlled, randomized trial.

Setting: Outpatient setting of specialty clinics in Dallas and Temple, Texas.

Interventions: Slow-release sodium fluoride (25 mg twice daily) in repeated 14-month cycles (12 months on treatment followed by 2 months off treatment) compared with placebo. Both groups took calcium citrate (400 mg calcium twice daily) continuously.

Patients: 110 patients with postmenopausal osteoporosis were randomly assigned to two groups. In the slow-release sodium fluoride group, 48 of 54 patients completed more than 1 cycle of treatment (mean, 2.44 cycles/patient), whereas 51 of 56 patients in the placebo group completed at least 1 cycle (mean, 2.14 cycles/patient) in this interim analysis.

Measurements: Vertebral fracture rate and lumbar bone mineral content. Vertebral fractures were quantified from yearly radiographs. Bone mass was determined annually by densitometry.

Results: In the sodium fluoride group, the mean L2 to L4 bone mineral content increased by 4% to 6% in each cycle and the mean femoral neck bone density

increased by 4.1% and 2.1% during the first two cycles, but the radial bone density did not change. The placebo group showed no statistical change in bone mass at any site. Compared with the placebo group, the sodium fluoride group had, a lower individual new vertebral fracture rate (0.057/patient cycle compared with 0.204/patient cycle, $P = 0.017$), a higher fracture-free rate (83.3% compared with 64.7%, $P = 0.042$), and a lower group fracture rate (0.085/patient cycle compared with 0.239/patient cycle, $P = 0.006$). The side-effect profile was similar for the two groups; no patient developed microfractures, hip fractures, or blood loss anemia. *Conclusions:* Intermittent slow-release sodium fluoride plus continuous calcium citrate, administered for about 2.5 years, inhibits new vertebral fractures, increases the mean spinal bone mass without decreasing the radial shaft bone density, and is safe to use.

Key words: Osteoporosis; Slow release sodium fluoride.

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NORMAL AGE-RELATED CHANGES IN FLUORIDE CONTENT OF VERTEBRAL TRABECULAR BONE - RELATION TO BONE QUALITY

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Abstract from *Bone* 15 (1) 21-26 1994

In several clinical osteoporosis studies, fluoride treatment has been shown to have a positive effect on bone mass but without a concomitant decrease in vertebral fracture rate. In contrast, some studies have shown that increases in spinal BMD are also paralleled by decreased vertebral fracture incidence. We have previously demonstrated, in a pig model, that 6 month treatment with fluoride increased bone mass but decreased bone quality. The aim of the present study was to elucidate whether normal age-related fluoride accumulation in human bone *per se* influences bone quality. From 73 normal individuals, aged 20-91 years (36 females, 37 males) two trabecular bone cylinders were obtained from the central part of L3. Biomechanical competence, ash density, and fluoride content were assessed in one cylinder, and trabecular bone volume was determined in the other. The results showed an age-related decrease in bone mass for both men and women. Bone strength normalized for bone mass (bone quality also identical with bone material strength) also showed an age related decrease in men and women. Bone fluoride concentration increased significantly in both sexes (range 463-4000 ppm). Multiple regression analyses disclosed that fluoride by itself had no influence on bone quality, in this study with a limited number of cases, when the influence of sex and age were taken into account. It is concluded that normal age-related accumulation of fluoride in vertebral trabecular bone does not seem to affect the quality of bone. Whether this is also the case during fluoride therapy has to be assessed.

Key words: Aging; Bone quality; Fluoride; Vertebrae.

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COMMENTS ON DR COLQUHOUN'S REPLY TO MY CRITIQUE

G Neil Jenkins
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The 21 City Survey

Colquhoun (1) states that Dean from 'the survey forms used' had caries data on hundreds of communities and asks why did he publish data from only 21 in his classical two papers demonstrating the fluoride/caries relationship. It would be tedious to analyse in detail the six papers (2-7) on which Colquhoun bases his discussion but the main points are as follows. Only in one paper (2) are hundreds of communities mentioned. This paper includes the text of a questionnaire sent to dental societies and individual dentists requesting information about the incidence of mottling in their locality and reports that 207 and 632 replies were received respectively. Caries is not mentioned in the questionnaire or in the text or tables of the paper nor is information sought on the fluoride of the water (this would not be known in the 1930s but the severity of mottling would give an indication). The next paper (3) describes mottling in 66 communities in Texas, again with no mention of caries. In reference (4) a different questionnaire is given (reproduced by Colquhoun (1)) described as 'a special card designed for mottled enamel surveys' although it did request information on caries but included no results. References (5) and (6) refer only to mottling but are the first of this series to include fluoride concentrations in water. Reference (7) is in a publication not available to me and is not listed in the Dental Periodical Literature for 1936: its title mentions a dental survey among children in 26 States. Unless an average of eight communities per State were studied the total could not reach 'hundreds'. Dean (8) discloses that it shows 'a lower amount of dental caries in the mottled enamel areas than in non-endemic areas in the same states'.

The answer to Colquhoun's question would seem to be that Dean preferred to publish results collected under his own supervision by two named examiners who had received special training and calibration (9) unlike the unpublished data collected by a large number of dentists working in 29 States without supervision and remote from research centres. Dean also had reliable information on the fluoride history of the 21 cities and was aware of the need for numerous samples spread out over the year (4).

Colquhoun gives no reason why my description of how the fluoride effect was discovered in England is unconvincing evidence of its validity. I can only repeat that the difference in caries was found first by examiners who, like almost everybody else in the 1940s, were unaware of the effect of fluoride: only later was it discovered that South Shields water contained 1.4 ppm of fluoride. Colquhoun states that I did not mention that there was 'little or no difference in tooth decay among older children and adults' in the high and low fluoride areas. The DMF values for groups of 48 16-year-olds in the non-F area and 38 in the F area were 6.9 and 4.7 respectively (10) (the 15 and 17 age groups were too small to give reliable results but showed the same trend). DMF values in adults are notoriously unreliable as so many teeth are lost for reasons other than for caries and the numbers studied were small but all showed a trend supporting an effect of fluoride. Weaver (10) discussed in some detail the differences in infantile mortality and gave his reasons for thinking that fluoride was not a factor.

DMF scoring of caries

Remineralization of early lesions may lead to the postponement or prevention of frank cavities because the remineralized enamel is more resistant to acid than is normal enamel (11). Thus it could influence later DMF scores.

Colquhoun quotes Sutton (12) who 'devastatingly analysed the tabulation' of fluoridation studies of Murray and Rugg-Gunn (13) now updated (14). Sutton's criticisms were: 1) that 23 surveys that gave data on both deciduous and permanent teeth were counted as 46 surveys; 2) one result referred to the fluoridation of salt and not water; 3) two were anonymous, three were personal communications and eight were progress reports; 4) a column headed 'Non-fluoridated community caries experience' gave the impression that all trials had a control group. These points can readily be answered: 1) Although the term 'sets of data' might have been more accurate than 'surveys' the results on deciduous and permanent teeth do represent individual pieces of evidence. 2) The salt fluoridation scheme had four groups: NaF in salt, CaF_2 in salt, NaF in water and no F at all. Only the last two groups were in the table as in most other studies. 3) While, admittedly, these reports are less authoritative than refereed papers in a journal they are acceptable as supporting data. As Colquhoun himself stated (15) one has to use whatever data are available. 4) many of the studies did have control populations without fluoride. In others, the results some years after fluoridation were compared with those of the same area before fluoridation - they were non-fluoride at that time. In the later up-date of this table (14) those schemes with a separate control are plotted separately from those using 'self' or 'retrospective' controls and the results are very similar with a slight tendency for higher reductions in the latter.

The Anglesey 'blind' study

Colquhoun states that I have 'not answered at all' his criticism of this study. I did point out that the two areas were not entirely "rural versus urban," that the evidence on whether caries is higher in urban areas is contradictory (16) and when there are differences they are seldom as large as that produced by fluoridation. While, admittedly, pre-fluoridation data would have strengthened the case, it is a gross exaggeration to call the study 'worthless' because of its absence.

Social studies

Colquhoun's studies may have covered large numbers of children but I repeat that the social status was not assessed for every child but was based on the status of the community served by the clinic. Also, the caries was not all scored by the same observers under standardised conditions.

The recent decline in caries

I am pleased to note that Colquhoun does not dispute that fluoride toothpastes 'may have' contributed to the recent decline although he does not say why he has doubts about it. The reason that fluoridation of water has a smaller effect now (17) areas has lowered caries to levels similar to those with fluoride in the water than 30 or more years ago (9, 14) is that the use of fluoride toothpastes in control

Defluoridation

The results tabulated by Murray *et al* (14) clearly show the complete falsity of Colquhoun's statement that the effect of fluoridation 'has disappeared by the time

the children are 12 or 13 years old'. The data on 11,659 children in The Netherlands (18) refer almost entirely to the fall in caries in that country and states that 'the number of groups in regions with water fluoridation was small'. A rise from defluoridation would not therefore be expected. Only one short paragraph involving the numbers I mentioned (93 and 158) refers to defluoridation.

Uniformity of the early results

By a normal distribution, I meant, of course, a normal distribution about the mean figure.

Ziegelbecker's graph

Colquhoun (17) reproduced Ziegelbecker's graph in which data were superimposed from many studies of the fluoride-caries relationship carried out over 25 years by many operators using different diagnostic standards and which he claimed showed 'no correlation'. When these results are examined separately there is in almost every case a lower caries score with higher levels of fluoride (19).

Conclusion

My conclusion can be summed up in the words of Lord Jauncey, the judge in the Strathclyde law case '... in a perfect world each study might have been carried out in a more perfect manner in one or more details ...' nevertheless '... the message is loud and clear from all parts of the world. Water fluoridation reduces the incidence of caries.'

(For References see pages 178-179.)

REPLY TO PROFESSOR JENKINS COMMENTS

John Colquhoun
Auckland, New Zealand

Jenkins has not refuted my main observation: that comprehensive surveys without preselection, from several countries (16,20-26), do not support the claims for a dental benefit from fluoridation, which rely on studies of samples of children from selected communities. Since this discussion began another comprehensive survey, of some 300,000 children from all parts of India, has been published (20). It reports more, not less, tooth decay at higher water fluoride levels - as did others, from Tucson, Arizona (21) and New Zealand (22,23), which were of whole child populations. Jenkins' other points are easily answered:

The 21 City Survey

Hundreds of US communities *were* surveyed for child dental decay in the years before Dean presented his famous study of 21 selected "cities" (8 of them were suburbs of Chicago) purporting to prove an inverse water fluoride/dental caries relationship. Five of the early studies (2-6) were about dental fluorosis so did not report on caries rates. But the examination forms (not the questionnaires) used in them show that caries data were also collected, though not published, in the hundreds of communities examined for dental fluorosis. In the most comprehensive of these studies (of which Jenkins claims "caries is not mentioned"), Dean actually wrote, explaining surveys he himself carried out in four states: "the clinical findings are first recorded, and then the individual water history is noted on a card provided for that purpose." (2) The card is reproduced in the paper and, like the

one reproduced in my earlier reply to Jenkins, shows clearly that caries data as well as water histories were collected. The sixth early study (7), which Jenkins admits he has not seen, was not about dental fluorosis. Co-authored by Dean, it reported the results of dental caries examinations of around 1½ million children in 500 communities in 26 states.

Admittedly much of the earlier information could have lacked precision - but if so, why were the early studies satisfactory for the extent and severity of fluorosis? It is clear that information was available to Dean for many more than the 21 communities, in the whole of USA, that he later chose to include in his famous 1942 graph, which is featured in most dental textbooks, including one by Jenkins (27).

Concerning the early caries/fluoride studies by Weaver in Shields, Jenkins' interpretation differs from that of Weaver, who in his second 1944 paper actually stated of his examinations of older pupils and mothers: "evidence was obtained which suggests that F is a caries-postponing rather than a caries-preventing factor" (10). In 1950 Weaver wrote that his dental examinations of mothers: "show that the protection afforded by fluorine is not lasting" (28). Concerning the higher infant mortality in fluoridated South Shields, Weaver expressed no strong opinion, though he discussed the possibility of "some unidentified factor or factors" (10).

DMF scoring of caries

Jenkins misses the point. Only "frank cavities" were counted in the fluoridation trials. There is no way fluoridated water could make such cavities disappear, in the same group of children, after one year.

Nor does Jenkins really answer Sutton's exposure of the claim that fluoridation was verified by over a hundred studies. Jenkins tries to blow up the number from the 20 odd which can today be found, but the fact remains that none of them meet acceptable standards of research design. Quality, not quantity, decides the value of research.

The Anglesey "blind" study

I am pleased Jenkins puts "blind" in parentheses. When studies, whether blind or not, compare only two communities, not randomly chosen, they are worthless if their results are contradicted by more comprehensive studies involving all available, or randomly chosen, communities.

Social Studies

Jenkins' criticism applies to many comprehensive studies which compared disease prevalences with socio-economic status. In the case of my studies, the New Zealand School Dental Service operators who did the caries scoring did work under standardized conditions.

The Recent Decline in Decay

The claim that fluoride toothpastes have made decay declines similar in fluoridated and nonfluoridated areas was disputed by Jenkins' fellow fluoridationist, the late Professor D Jackson (author of the Anglesey study). Jackson pointed out that the declines could not be due to fluoride toothpastes, because during the declines the toothpastes reached only a minority of homes. The thesis that such toothpastes were responsible, he wrote, "must be viewed with some scepticism. ... If fluoride toothpastes were solely responsible, one might have expected the rate of decline to have increased as sales increased, but there is no evidence of this." (30)

Defluoridation

Jenkins claims that his and his Newcastle colleagues' review of fluoridation studies establishes the "complete falsity" of my results, which are based on data collected from the whole population of New Zealand children and are supported by other recent comprehensive studies. He then goes on to dispute my interpretation of Dutch data on 11,659 children, falling back on the dubious data on 93 and 158 Tiel/Culemborg children to support his claims about defluoridation. Dr Moolenburg commented on the worth of the Tiel / Culemborg comparison (30). The designers knew before the study commenced that the diet in nonfluoridated Culemborg was more cariogenic than in fluoridated Tiel. However, many more Dutch children than those in Tiel had been subjected to fluoridation. Professor König told me in 1980 that 25% of Holland was fluoridated by the time that country's parliament banned fluoridation. There is no evidence that the affected children suffered more tooth decay as a result of defluoridation.

Uniformity of the Early Results

Because recent comprehensive and population surveys report no benefit from water fluoridation, one could expect a collection of random comparisons of fluoridated with nonfluoridated communities to form a normal curve, with sometimes decreases and sometimes increases in tooth decay in the fluoridated places. Jenkins claims that the differences reported in the early fluoridation studies (with always decreases in fluoridated places) were a normal distribution. The main point Diesendorf had made was that in those early studies the same large decreases (usually 50-60%) were claimed whether the difference was between test and control groups at a fixed time, or between a test group at different times (before and after fluoridation). The claims were absurd because the independent variables could not have always been the same. Jenkins' "normal distribution" is hardly relevant.

Ziegelbecker's graph

Jenkins simply repeats the argument which was answered in my original article. For a summary see pages 16-17 in *Fluoride* 27 (1) 1994.

Conclusion

Jenkins concludes by quoting a Scottish judge's 1982 opinion, reached from the weight (quantity) of evidence at that time. The comprehensive surveys which refute that opinion have been made since then, making the message much less "loud and clear".

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For well over ten years it has been apparent that fluoride supplements are unwise for young children. Indeed, a Rand Corporation literature review in 1981 found that supplements are "simply not warranted" by the results (RAND report N 1732-RWJF Dec 1981). Furthermore, their use clearly increases the risk of dental fluorosis, which is now at an all time high in the US. It is equally clear that there is little or no evidence of any dental benefits from pre-eruptive supplementation. For these reasons, fluoride supplements are banned or strictly regulated in Africa, Europe, Canada, Japan, and India.

It is heartening, therefore, to read that a long time fluoride advocate agrees that "fluoride supplements should no longer be used for young children in North America" (Abstract in *Fluoride* 27 121 1994). The question that Dr. Burt should now address is: Given the uselessness and risk of fluoride supplements, why should the practice of giving young children the same fluoride dissolved in water (fluoridation) be continued?

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Professor Burt responds:

The American Dental Association (ADA) conducted a workshop to consider its schedule for dietary fluoride supplementation on Jan 31-Feb 1, 1994. Dr. Lee has circulated some inferences from my paper at that workshop which stray far from the sense of my presentation, and which could be interpreted to imply that I am opposed to water fluoridation as a public health measure in the United States. That is not the case, and this response is to comment briefly on Dr. Lee's inferences.

Dr. Lee has quoted accurately from the abstract, but it seems that he has not read the full paper. (Along with other papers from the workshop, this is to be published in the *Journal of Public Health Dentistry* in due course). My conclusion, correctly quoted by Dr. Lee, was that the risks of using fluoride supplements in young children outweigh the benefits. "Risk" was described as the likely development of the mildest forms of fluorosis from regular use of supplements by infants and young children, while "benefits" were the minor (at best) cariostatic effects likely to result from this use. Dr. Lee then goes on to extrapolate that line of thinking to the use of water fluoridation, but this does not follow because with water fluoridation the benefits outweigh the risks.

Dr. Lee seems to be suggesting that the relative lack of cariostatic effect of systemic fluoride is a new finding, but this is not true. The first review of literature that I know of to point this out was in 1976 (Levine RS. The action of fluoride in caries prevention: a review of current concepts. *British Dental Journal* 140 9-14 1976). The evidence for a primarily topical cariostatic effect of fluoride has grown and has been cited many times since then, culminating with the 1989 Georgia conference (proceedings in *Journal of Dental Research* 69 special issue 1990).

There is considerable evidence that vehicles like fluoridated water and table salt have powerful topical cariostatic effects. These methods are highly effective public health approaches to caries control, though they are accompanied by about 12% prevalence of the mildest forms of fluorosis. I consider the benefits of water fluoridation exceed the risks associated with its use, and my argument about eliminating supplements for young children in fact depends partly upon the

effectiveness of fluoride in water and toothpaste. Supplements are unlikely to add much to this existing effectiveness, but do increase the risk of fluorosis.

Dr. Lee may not have read the following part of my discussion, which I think helps put the issue in perspective:

The exposure to fluoride from multiple sources, a fact of life in the United States today, is a prime reason why dental caries experience has been reduced to its current low levels. The caries decline is a major public health achievement which must be preserved in those who have benefited from it, and extended to those remaining segments of society which need it most.

I would like to finish by summarizing my philosophy on fluoride use, which I believe is well-based on published evidence. I hope that this will counter any wrong impression that Dr. Lee's inferences may have produced.

- Fluoride most effectively controls caries when a low concentration can be maintained consistently in the oral environment. While any method of using fluoride which helps achieve this state will be effective, fluoridated water and fluoride toothpaste rank first as public health measures in the United States.
- Fluoride continues to be a major reason why the oral health of Americans is today better than ever and continues to improve.
- Fluoridation of water to appropriate levels, and the regular use of fluoride toothpaste, as the two most effective public health means of controlling dental caries. The majority of Americans need little or no extra fluoride to maintain oral health.
- Public health uses of fluoride mean that some systemic absorption of fluoride is inevitable. In the amounts associated with water fluoridation, there will be some dental fluorosis of the mildest varieties. This condition is minor when compared to the accompanying benefits of reduced tooth decay.
- It is incumbent on dentistry to reduce the risk of fluorosis as far as possible while not compromising the benefits of fluoride. My view is that eliminating supplements use for infants and young children will be a step toward achieving this goal.

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Dr Lee replies:

I wish to thank Dr Burt for his acknowledgement of the accuracy of my use of the quotations from his published summary (despite his objections to my inferences of them) and for his further comments defending the continued practice of public water fluoridation. Further, I appreciate his acknowledgement that water fluoridation has little or no systemic dental benefit but, rather, supposedly works solely by its topical effects. It is good that this aspect of the argument be addressed.

- Common sense would dictate that the amount of fluoride touching the teeth during the act of swallowing fluoridated water is extremely small. In fact, I doubt that it would even be measurable. It is certainly uncommon for children to swish their drinking water back and forth through their teeth while imbibing a drink. One would think that brushing with fluoridated toothpaste would be more effective in bringing fluoride into contact with the teeth.

- The argument that fluoride in public drinking water is responsible for the observed decline in children's dental caries is contrary to numerous studies in the US and world-wide which find that the same decline occurred also in unfluoridated communities. (See references 1 - 11 in the list that follows below.) In fact, I have continuously challenged any dental authority to provide one valid study of the past two decades justifying the presumption of fluoridation's dental benefits and none has been forthcoming.
- Dr. Burt's statement that fluoridation results in only "12 % prevalence of the mildest forms of fluorosis" is contradicted by the US Public Health Service (the Hoover report) indicating a fluorosis prevalence of 22.3% in fluoridated communities and by other authoritative reports in which dental fluorosis prevalence was variously found to be 30-60 % in communities with supposedly "optimal" fluoridation. (See references 4 and 12-15 in list below.)
- Dr. Burt is seemingly unaware that dental fluorosis connotes fluoride toxicity far more important than mere dental disfigurement. Dental fluorosis is a visible indicator in developing teeth of generalized fluoride toxicity throughout the body, including damage to connective tissue, bone tissue, immune functions, and enzyme functions. As such, any rise in the prevalence of dental fluorosis is cause for concern.

I shall look forward to continuing this discussion when Dr. Burt's full paper is published (in due course) in the *Journal of Public Health Dentistry*.

John R Lee MD

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[Editor: *Fluoride* 23 (3) 1990 page 106 listed 7 studies which reported dental fluorosis prevalences in 12 fluoridated communities. The average prevalence was 29%. One of the studies, co-authored by Dr Burt, reported dental fluorosis prevalences in 3 Michigan fluoridated communities of 32%, 49% and 51% (*Journal of Dental Research* 67 802-806 1988).]

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The International Standard Serial Number (ISSN) is 0015-4725

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