Volume 26
Number 4
October 1993

FLUORIDE

JOURNAL

of the

International

Society for

Fluoride

Research

KAJ ROHOLM

Vol.26 No.4 October 1993 Pages 237 - 298

FLUORIDE

QUARTERLY REPORTS

ISSUED BY THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH

CONTENTS

ANOTHER LOOK AT THE INTERACTIONS OF FLUORINE WITH CALCIUM

A Machoy-Mokrzynska and Z Machoy
Szczecin, Poland

Calcium has always been considered the basic element in vital processes. The significance of the phosphorus-calcium balance and of fluorine in the processes of mineralization of bones and teeth has also been long known. These processes depend, among other things, on appropriate amounts of calcium, phosphorus, vitamins and hormones. The literature on this subject is abundant, and has been dwelt upon by numerous researchers. The participation of fluorine compounds in these processes is also unquestionable. However, more precise observations have revealed that the interactions of fluorine with calcium take place not only in bones and teeth. There are a number of other less known processes that are dependent upon their mutual interaction.

Due to the strong affinity of fluoride toward calcium, the latter is an antidote for fluoride poisoning (1). The administration of calcium compounds is recommended in the treatment of intoxication by fluorine compounds as well as in prophylaxis. In such situations, the simplest procedure is to consume milk, which is rich in calcium and proteins. The CaF_2 which is formed is only slightly soluble in aqueous solution and hence is less toxic. Besides the processes of mineralizing bones and teeth, fluoride may be expected to interact with calcium at any site reached by fluoride in the presence of calcium, primarily in the form of fluoride ions.

In the late G L Waldbott's book *Fluoridation: The Great Dilemma* (2), Figure 11-1 presents the silhouette of a man showing the highest reported levels of fluoride in various organs and soft tissues. For example, the highest reported fluoride content in the aorta (8,400 ppm) greatly exceeds even the normal fluoride content in teeth and bones. It is likely that one of the reasons may be calcium or disorders in its metabolism. Calcium, present in each cell, is the factor that attracts fluoride, which results in CaF_2 formation. The element is deposited in various forms in cells and tissues. Waldbott found earlier that fluoride accumulation in the aorta is not dependent on the calcium content (3). Therefore it is very worthwhile to discuss certain other examples of calcium and fluorine interactions that have been reported.

Increased blood fluoride levels are observed in people living in endemic regions, and in persons contaminated by industrial emissions as well as in individuals suffering from severe poisonings. If that fluoride level in blood is more than double the amount in the control group, hypocalcemia sets in, and symptoms of tetany may be observed (4).

The placenta does not form a barrier to fluoride which, to a limited extent, penetrates the fetus (5). Some placentas we examined showed disseminated foci of calcification, and it is unknown whether they were primary or secondary features. It is postulated that calcification may bind excess fluoride reaching the fetus (6).

Pomeranian Medical Academy, 70-111 Szczecin, Al.Powstanców 72, Poland.

In general, calcium ions have relatively poor affinity toward proteins (7). Calcium binding by certain proteins fails to influence fluoride binding, since protein-bound calcium is short of free valencies to form new chemical bonds. The main sources of calcium binding in proteins are dicarboxylic aminoacids. However, the tendency to bind fluoride is exhibited by proteins that contain alkaline aminoacids (lysine, arginine) (8), which would point to their electrostatic interaction.

Calcium ions are generally not specific activators of enzymatic reactions. However, some enzymes (peptidases, alpha-amylases, phosphatases, ATP-ases) are activated by calcium ions and are inhibited by added fluoride. One of the possible mechanisms of enzyme inhibition in these reactions may be calcium binding to fluoride in the catalytic center (7).

The consumption of adequate amounts of calcium (*e.g.* in milk or dairy products) during childhood or adolescence is decisive for producing maximal bony mass and for providing adequate protection against osteoporosis (9). Fluoride hampers the escape of calcium from bony tissue by increasing the mineralization of bones and activation of osteoblasts, and in that way slows the pace of osteoporositic processes. This fails, however, to improve the physical properties of bone, *e.g.*, strength (10,11). Thus there is no evidence that water fluoridation can help prevent osteoporosis (11).

Calcium is also the principal inorganic component of fingernails (1600 ppm) and may be what binds fluoride in them (12). Fingernails easily adsorb fluoride from the environment, even up to hundreds of parts per million and, with the lapse of time, fluoride is desorbed. One of the factors accelerating the process of superficial desorption is certainly frequent hand washing with the use of alkaline agents.

All urinary calculi, even those constructed of organic compounds, contain fluoride (13). In areas where the water and foodstuffs are low in fluoride, it is always present in the urinary stones. Quantitatively, more fluoride is present in calculi with a high calcium content (1). Similarly, a somewhat less intense reaction is demonstrated by phosphorus and still less by magnesium. The role of calcium in fluoride binding in urinary calculi has been stressed earlier by other authors (14).

Dental calculi are 70-80% inorganic substances, wherein calcium makes up 40%. Some of the compounds in tartar appear in the form of $Ca_{10}(PO_4)_6F_2$ and CaF_2. From a chemical viewpoint the formation of such salts is understandable. Electropositive calcium reacts with electronegative anions, such as fluoride and phosphate. Fluoride activity is so great that it may evict oxygen from a phosphate group, forming the anion PO_3F^{2-}. These changes are not ionic binding, since both compounds present reticular structure with durable stability.

The mechanism of dental calculus formation may be depicted in the following manner: cytotoxic fluoride exerts, in the oral cavity, influence on degeneration and decomposition of many bacterial cells, speeding up their calcification, thereby creating conditions for progressive calcification involving dental calculus. The possibility for CaF_2 to form is based on the existence of calcium ions as well as fluoride ions in saliva and dental plaque (15). Old, earlier formed, tartar is richer in fluorine compounds than recently formed calculi.

The tartar-forming mechanism resembles the primary origin, in nature, of apatite and phosphate mineral deposits in seas that are not too deep. These minerals are of sedimentary origin, whose composition includes sparingly soluble compounds of formula CaF_2 and $Ca_3(PO_4)_2CaF_2$. Sea waters are quite well stocked with fluoride ions, and the materials facilitating the formation of minerals are organic debris containing calcium. Today, large sedimentary deposits of apatites and phosphates provide significant industrial raw materials.

Finally, it should be mentioned that there are new observations concerning the behaviour of calcium and fluoride ions in metabolic processes, independently of the formation involving various types of connections and compounds. This idea is confirmed by the work of Bober, who studied the effect of fluoride on oxygen metabolism of neutrophilic granulocytes (16). In the normal state the oxygen metabolism of neutrophilic granulocytes is minimal. The addition of fluoride triggers a slight rise in oxygen uptake, which increases dramatically after calcium ions are added.

Clearly, further studies will widen the scope of our present knowledge and views on the mutual biological interaction of fluoride and calcium.

References

1 Editorial. Antidotes for fluoride intoxication. *Fluoride 13* 96-99 1980.
2 Waldbott GL, Burgstahler AW, McKinney HL. *Fluoridation: The Great Dilemma.* Coronado Press, Lawrence Kansas 1978 pp148-174.
3 Waldbott GL. Fluoride and calcium levels in the aorta. *Experientia 22* 835-841 1966.
4 Kierst W. Fluorine poisoning. *Biuletyn Instytutu Medycyny Morskiej, Gdansk 15* 87-96 1964.
5 Forestier F, Daffos F, Said R *et al.* The passage of fluoride across the placenta. An intrauterine study. *Journal de Gynecologie, Obstetrique et Biologie de la Reproduction (Paris) 19* 171-175 1990.
6 Chlubek D, Machoy Z. Role of placenta in fluoride metabolism. *Ginekologia Polska 62* 568-572 1991.
7 Machoy Z. Biochemical mechanisms of fluorine compounds action. *Folia Medica Cracoviensia 28* 61-81 1987.
8 Wieczorek P, Samujlo D, Chlubek D, Machoy Z. Interaction of fluoride ions with milk proteins studied by gel filtration. *Fluoride 25* 171-174 1992.
9 Renner E, Knie G, Stracke H. Effect of calcium intake on the bone mineral content and incidence of osteoporosis. *Metabolism of Fluorine '92.* Szczecin 1992 pp 25-29.
10 Editorial. Fluoride therapy and osteoporosis. *Fluoride 16* 1-4 1983.
11 Editorial. Water fluoridation and osteoporosis. *Fluoride 20* 1-3 1987.
12 Machoy-Mokrzynska A. Adsorption and desorption of fluoride in nails. *Environmental Sciences 1* 137-142 1992.
13 Machoy P, Bober J. Fluorine - constant component of urinary calculi. *Environmental Sciences 2* 45-49 1993.
14 Editorial. Fluoride and kidney stones. *Fluoride 13* 1-3 1980.
15 Borysewicz-Lewicka M. *Studies on the effect of fluoride on mineralization at early stages of dental calculus formation.* MD hab thesis, School of Medicine, Poznan 1989.
16 Bober J. *Effect of fluoride ion on oxygen metabolism and functions of human and rabbit neutrophil granulocytes.* PhD thesis, Pomeranian Medical Academy, Szczecin 1992.

Published by the International Society for Fluoride Research
Editorial Office: 216 Atkinson Road, Titirangi, Auckland 7, New Zealand

MORE "AUTHORITATIVE REVIEWS"

Yet further reviews claiming to confirm the safety and effectiveness of water fluoridation have been published (1,2). The US one is reported on pages 278-281. One of their findings, which repeats assessments in earlier reviews, is examined in Dr Spittle's review article. Another is examined in Dr Lee's article.

The New Zealand review ignored the recent evidence that the increase in bone cancer in young males is much greater in fluoridated areas of the USA than in non-fluoridated areas (commented upon and cited in *Fluoride 26* 66 67 79-96 1993, as well as in the *New Zealand Medical Journal 105* 436 1992 and *106* 111-112 1993).

It also dismissed the recent evidence, from comprehensive surveys, of an association between fluoridation and hip fractures (see *Fluoride 25* 1-4 and 45-50 1992), by quoting an opinion, at a 1991 USA workshop, that "most of the studies have important limitations". These limitations, plus the existence of conflicting epidemiological evidence, are held to justify the view that the fluoride/fracture relationship is "unresolved", and that fluoridation should therefore be continued. Two recent hip fracture reports (see abstracts on page 287), one comparing two Canadian cities and the other examining a small population in a single US city over a limited time (see Lee review), are being cited to support that view.

It is maintained that the studies reporting a fluoridation-fracture association can be disregarded because they are "ecologic" (*i.e.*, did not study individual cases) and did not establish a causative fluoride/fracture link. But that view disregards compelling supporting evidence of a causative link (cited in *Fluoride 25* 1-4 162-164 1992 and in *New Zealand Medical Journal 103* 593 1990 and *104* 343 454-455 1991) - in particular: the measurement of fluoride content of bones of Finnish *individuals* who had lived in a fluoridated city. Those individuals had very high bone fluoride levels, as high as or higher than levels which have been reported in patients receiving fluoride therapy for osteoporosis, which is now acknowledged to make bones more liable to fracture. It is undisputed that only around half of ingested fluoride is excreted, the rest accumulating gradually in bones. So even without the new epidemiological evidence, and whether it is "only ecologic" or not, there is evidence that fluoridation has contributed to the undisputed increase in old people's bone fragility.

The New Zealand review repeats the old claim that "fluoride at 1 part per million in water has been shown to reduce tooth decay by 40-60%." Several pages are devoted to the numerous past and recent studies (with no mention of their limitations) which examined samples of children from selected communities - while School Dental Service data collected for *all* New Zealand children, which reveal no advantage from fluoridation (*Fluoride 26* 125-134 1993), are ignored. Other published peer-reviewed studies, based on large scale surveys and whole populations of children, which report little or no benefit to teeth from fluoridation, are simply omitted from this new "review of evidence."

"Authoritative reviews" in defence of fluoridation seem prone to omit and select scientific evidence.

John Colquhoun

1 Treasure ET, Drummond BK, Buchan HA *et al. Fluoridation of Water Supplies: Draft Policy Statement.* Public Health Commission. Wellington. New Zealand 1993.

2 Wagner BM, Burt BA, Cantor KP *et al. Health Effects of Ingested Fluoride.* National Research Council. Washington DC August 17 1993.

PLASMA BIOCHEMISTRY OF ADULT GOATS WITH CHRONIC FLUORIDE POISONING IN MOROCCO

A Bennis,[1] M Kessabi,[2] A Hamliri,[2] F de La Farge[3] and J P Braun[1]
Toulouse, France and Rabat, Morocco

SUMMARY: Biochemical screening of goat plasma of the Darmous zone of Morocco where fluorosis is endemic showed mainly increases of potassium, phosphates, urea, bilirubin and GGT, while decreases of glucose, cholesterol, phospholipids, total proteins and γ-globulins were observed. These changes are similar but less intense than those previously reported in cattle or sheep.

Key words: Darmous; Fluoride; Fluorosis; Goat; Morocco; Plasma biochemistry.

Introduction

In the center of Morocco, around the cities of Khouribga and Youssoufia, lies a vast zone in which intensive phosphate mining is done. These phosphates contain up to 5% fluorine, which contaminates natural waters and vegetables, and is ingested by animals and humans causing a very severe chronic fluorosis, named Darmous from the Berber word "DrGhmas" meaning early tooth decay.

Animals are contaminated by ingestion of water containing from 0.4 to 1.9 ppm fluoride, vegetables such as straw (50 to 150 ppm) or barley (10 to 43 ppm) and soil dust (up to 12 000 ppm) (1). In this zone, where cattle, sheep, goats and horses are bred, total daily intake of fluoride was estimated to range from 2.8 to 4.6 mg F^-/kg bodyweight, while it was 0.39 mg F^-/kg bodyweight for controls (2). Dental signs and exostoses of mandibles and ribs were reported; cattle were more severely affected than small ruminants but dental mottling and exostoses of mandible were observed in ewes, which had no other bone signs (2). Effects of fluoride intoxication on serum biochemistry were reported in cattle and sheep in the Darmous area. Main disturbances were increases of potassium, urea, lactate dehydrogenase, alkaline phosphatase and aspartate aminotransferase, while decreases of calcium, proteins and albumin were reported.

As the breeding of goats for milk and meat is currently increasing in Morocco, we investigated the effects of natural chronic fluoride poisoning in this species in order to determine if the effects were similar to those observed in sheep.

Materials and Methods

Animals were adult female goats: 42 in the Darmous area and 45 in a control zone, near the Darmous zone but in which soil, water and food fluoride contents were previously reported to be within normal ranges (1). Breeding conditions, nutrition and water supply were identical in both groups with the exception of fluoride levels.

[1] Department of Physiopathology, Ecole Nationale Vétérinaire, 23 Chemin des Capelles, 31076 Toulouse, France. [2] Department of Pharmacy-Toxicology, Institut Agronomique et Vétérinaire Hassan II, Rabat, Morocco. [3] Department of Biochemistry, Rangueil Hospital, Toulouse, France. Correspondence to Professor J P Braun.

A single 10 ml blood sample was taken from the jugular vein into a Li-heparin tube. It was centrifuged within 2 hours and plasma was separated and frozen at -20°C until analysis within 2 months.

Plasma fluoride was measured with a selective electrode and a digital pHmeter (Orion 96-09 and Beckman 4500) according to Hall et al (3).

The following constituents were analyzed by dry-chemistry procedures with a Kodak Ektachem XR700®: sodium, potassium, chloride, bicarbonates, calcium, phosphates, urea, creatinine, glucose, bilirubin, cholesterol, triglycerides, proteins, alkaline phosphatase (ALP, EC 3.1.3.1), gamma-glutamyl transferase (GGT, EC 2.3.2.2), aspartate aminotransferase (ASAT, EC 2.6.1.1), alanine aminotransferase (ALAT, EC 2.6.1.2), lactate dehydrogenase (LDH, EC 1.1.1.27). Phospholipids were determined by an enzymatic procedure with a Cobas Bio Roche analyzer. Proteins were fractionated by electrophoresis on cellulose acetate in Veronal buffer, pH = 8.6, μ = 0.05; after staining by Ponceau S, fractions were quantified with a Sebia® densitometer. Lipoproteins were separated by agarose gel electrophoresis in a Trisbarbital buffer, pH = 9.2, μ = 0.05. Gels were stained with Sudan black, then strips were scanned with a densitometer (Sebia®, Issy-lès-Moulineaux, France).

Quality control was based on commercially available control sera (Wellcontrol, Clinical chemistry quality assessment programme, Wellcome).

Results were analyzed by Mann and Whitney's test as the normality of distributions was not assessed and as variances of samples differed (4).

Results

Plasma fluoride concentration in goats of the Darmous area was: 0.31 ± 0.15 mg/L (range: 0.10 to 0.58). In Tables 1 and 2, it can be observed that only a few parameters did not differ significantly between both groups: sodium, calcium, triglycerides, phospholipids, ALP, ALAT and some protein and lipoprotein fractions. Most significant alterations observed in animals of the Darmous area were: 1) decreases of glucose, proteins, especially of (β + γ)-globulins; 2) an increase of urea; and 3) a modification of the relative proportions of lipoproteins, with an increase of chylomicrons and (VLDL + LDL) and a decrease of HDL (see Figure).

Discussion

The concentration of plasma fluoride is not the best indicator of fluoride poisoning but it was not possible to kill the animals used in this experiment to determine fluoride concentration in bone, kidney or liver. It was reported that "normal" plasma fluoride concentration in ruminants could range from 0.19 to 0.30 mg/L (2). In control sheep and goats, it was reported to be about 0.10 mg/L (5,6). In fluoride intoxication, plasma fluoride concentration was reported to be as high as 1.3 mg/L (6). So, in goats of this study, plasma fluoride was only mildly elevated, although it was reported that soil, water and straw fluoride mean concentrations were 3232 ppm, 1.2 ppm and 104 ppp respectively (1). This probably reflects a moderate fluoride intake of the animals at the period of sample collection. Nevertheless, loss of body weight indicated that the disease was moderately severe in these animals.

Main clinical signs of chronic fluorosis are osteo-dental. Exostoses cause painful locomotion, thus limiting the movement of animals foraging for food. This point is

Table 1. Plasma minerals, substrates and enzyme activities of adult goats in Darmous area (n = 42) and control zone (n = 45). Results as mean ± SD; comparison between groups by Mann and Whitney Test

Parameter	Unit	Control	Darmous	P<
Sodium	mmol/L	144 ± 2	143 ± 3	N.S.
Potassium	mmol/L	4.6 ± 0.4	5.5 ± 0.6	0.001
Chloride	mmol/L	109 ± 3	105 ± 3	0.001
Bicarbonates	mmol/L	24 ± 2	26 ± 3	0.001
Calcium	mmol/L	2.29 ± 1.15	2.27 ± 0.13	N.S.
Phosphates	mmol/L	1.8 ± 1.3	2.2 ± 0.4	0.05
Urea	mmol/L	3.7 ± 1.5	9.8 ± 1.5	0.001
Creatinine	µmol/L	48 ± 7	35 ± 5	0.001
Glucose	mmol/L	2.35 ± 0.52	1.07 ± 0.22	0.001
Bilirubin	µmol/L	4 ± 2	7 ± 4	0.001
Triglycerides	mmol/L	0.34 ± 0.27	0.33 ± 0.19	N.S.
Cholesterol	mmol/L	1.88 ± 0.36	1.63 ± 0.31	0.001
Phospholipids	mmol/L	1.42 ± 0.21	1.31 ± 0.25	0.05
ALP	U/L	325 ± 391	267 ± 363	N.S.
GGT	U/L	91 ± 21	126 ± 45	0.001
ASAT	U/L	174 ± 19	147 ± 19	0.001
ALAT	U/L	36 ± 7	34 ± 7	N.S.
LDH	U/L	1253 ± 185	1356 ± 189	0.01

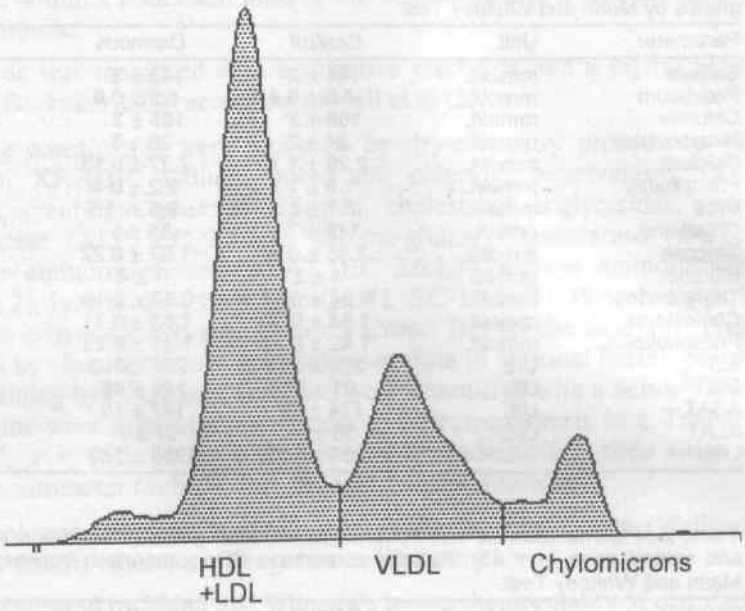
Table 2. Plasma proteins and lipoproteins of adult goats in Darmous area (n = 42) and control zone (n = 45). Results as mean ± SD; comparison between groups by Mann and Whitney Test.

Parameter	Unit	Control	Darmous	P<
Proteins	g/L	77 ± 5	70 ± 6	0.001
Albumin	g/L	35 ± 3	38 ± 5	N.S.
α1-Globulins	g/L	4 ± 1	3 ± 1	N.S.
α2-Globulins	g/L	7 ± 1	7 ± 1	N.S.
β-Globulins	g/L	11 ± 2	6 ± 1	0.001
γ-Globulins	g/L	19 ± 4	16 ± 6	N.S.
Chylomicrons	%	9 ± 2	13 ± 3	0.001
VLDL+LDL	%	23 ± 4	30 ± 8	0.01
HDL	%	68 ± 4	57 ± 8	0.001

Table 3. Serum biochemical disturbances reported in previous studies of fluorosis in cattle and sheep of the Darmous area; comparison to results of this study in goats. Results are variation as percent of difference with values obtained in control animals (= not significantly different from controls; n.d. not determined)

Parameter	Cattle	Sheep	Goats
Sodium	=	=	=
Potassium	+34%	+35%	+20%
Chloride	n.d.	n.d.	-4%
Bicarbonates	n.d.	n.d.	+8%
Calcium	-15%	-21%	=
Phosphates	=	=	+22%
Urea	+55%	+24%	+165%
Creatinine	=	+36%	-27%
Glucose	-13%	-21%	-54%
Bilirubin	n.d.	n.d.	+75%
Cholesterol	-24%	-31%	-13%
Triglycerides	n.d.	n.d.	+20%
Proteins	-5%	-8%	-9%
ALP	+389%	+145%	-18%
GGT	+10%	+30%	+38%
ASAT	+158%	+72%	+15%
ALAT	=	+71%	=
LDH	+106%	-9%	+8%

Fluorosis-free zone:-



Darmous zone:-

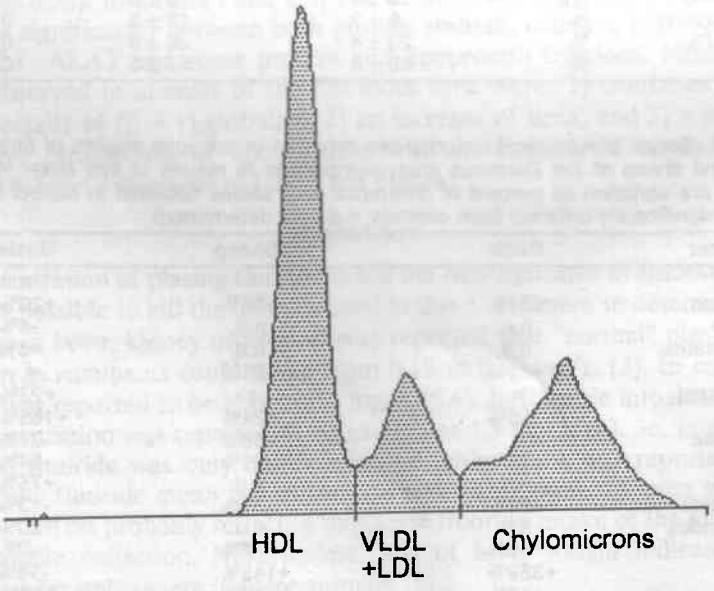


FIGURE. Typical agarose gel electrophoretic profiles of plasma lipoproteins in goats of a fluorosis-free zone (above) and of the Darmous area (below)

especially important for goats, which are left grazing poor pastures in semi-arid zones and have to move long distances to feed, while cattle are allowed access to better pastures. Moreover, premature crown wear and gingivitis hinder food intake and mastication. These restrictions limit the availability and utilization of food by the animals, thus explaining the decrease of their productions and their progressive evolution to cachexy (2). Moreover, fluorosis is reported to be aggravated by undernutrition (7-9).

Changes of serum biochemistry could be observed for about half the parameters analyzed, but it had been previously reported that experimental poisoning of goats with 2.2 to 3.6 mg F^-/kg/day for up to 3 years did not alter serum biochemistry (5). Some of the results obtained in this study, which are compared to results previously obtained in cattle and sheep in Table 3, can be related to global undernutrition of goats. The main effect, which had already been observed, is a very significant lowering of glycemia. Although it is not the most significant parameter of energy metabolism in ruminants, glucose is the easiest to measure, especially in field conditions, when samples for ketone bodies determination are more difficult to handle. In control goats, plasma glucose was lower than in Saharian goats (3.3 ± 1.0 mml/L, $n = 58$) living in similar conditions but in which the alimentary supply was better (15). Goats of the Darmous area had a very low glycemia, even lower than in cattle of the same zone (1.9 ± 1.0 mmol/L, $n = 100$) (10).

Lipoprotein patterns were also indicative of some kind of nutritional imbalance. In normal goats, the proportion of chylomicrons and (VLDL+LDL) is low. In goats of the Darmous area, they accounted respectively for 13% and 30% of total lipoproteins, indicating that the metabolism of triglycerides was more intense than in normal goats, maybe due to the lowered availability of glucose.

Similarly, higher urea concentration could be related to restricted food intake, which determines an increase of protein breakdown, and thus of urea excretion. But the lower concentration of proteins in goats of the Darmous area resulted mainly from a lowering of ($\beta + \gamma$) globulins: it could indicate that animals were weakened and less able to react to infectious challenges.

Fluoride was reported to be a potent nephrotoxic compound, in both acute and chronic studies in sheep (11). Some degree of renal insufficiency could also account for the increase of plasma urea observed in goats, but this can be ruled out here as creatinine concentration was lower in goats of the Darmous zone. This lower plasma creatinine concentration could result from muscle thinning as it has been reported to be lowered in humans with reduced muscle mass (12, 13).

Although statistically significant, variations of lactate dehydrogenase and asparte aminotransferase, which are mainly in skeletal muscles of goats (14), were very limited and opposite; thus cytolysis of muscles, which could be expected because of general weakness of animals, is very unlikely.

Elevations of plasma GGT activity and of bilirubin in goats of the Darmous area were intense. In sheep and cattle, similar increases of GGT activity have already been reported (2, 6, 13). This is indicative of hepato-biliary disturbances, which usually also determine increases of plasma ALP activity, but in ruminants this enzyme is a poorly sensitive marker of hepato-biliary disturbance (16). This liver disturbance might also be partially responsible for the higher concentration of chylomicrons, which are normally removed from the circulation by the liver.

Alterations of phospho-calcic and bone metabolism have been reported in ruminants. In this study, only very limited effects were observed with no alteration of plasma calcium and ALP activity and only a moderate increase of inorganic phosphates. In sheep and cattle, very significant and intense increases of plasma ALP activity were reported in hydro-telluric or industrial fluorosis. This could be consistent with the lower incidence of bone disturbances in goats than in cattle or sheep; for instance around the city of Tadla where 89% of cattle and 53% of sheep had exostoses of the mandible, only 31% of goats had the same signs (2).

In goats, chronic fluoride poisoning mainly resulted in metabolic disturbances of energy metabolism and in liver disorders.

References

1 Kessabi M, Amouzigh M. Fluoride levels in soil, water, plants and cattle in the Darmous zone of Morocco. *Fluoride 14* 169-171 1981.

2 Kessabi M. *Contribution à l'étude expérimentale et clinique de la fluorose des ruminants.* Thèse Doctorat ès-Sciences, Toulouse, 1984.

3 Hall LL, Smith FA, Lopez OH, Gardner DE. Direct potentiometric determination of total ionic fluoride in biological fluids. *Clinical Chemistry 18* 1455-1458 1972.

4 Snedecor GW, Cochran WG. *Méthodes statistiques.* Association de Coordination Agricole, Paris 1971.

5 Crombet M. *Etude expérimentale de la fluorose caprine.* Thèse Doctorat Vétérinaire, Créteil 1980.

6 Kessabi M, Hamliri A, Braun JP. Experimental fluorosis in sheep: fluoride kinetics and alleviating effects of aluminium sulfate. *Fluoride 21* 193-200 1982.

7 Ferrando R, Zundel G. Les signes dentaires dans l'évolution spécifique de la fluorose chez les animaux. Influence du régime alimentaire. *Bulletin, Académie Nationale Médecine 162* 119-123 1978.

8 Héraud G, Rox E. Métabolisme du fluor. *Médecine et Nutrition 9* 65-71 1973.

9 Takamori T. Recent studies on fluorosis. *Fluoride 4* 154-166 1974.

10 Kessabi M, Khouzaimi M, Braun JP, Hamliri A. Serum biochemical effects of fluoride of cattle in Darmous area. *Veterinary and Human Toxicology 25* 403-406 1983.

11 Kessabi M, Hamliri A, Braun JP, Rico AG. Experimental acute sodium fluoride poisoning in sheep: renal, hepatic and metabolic effects. *Fundamental and Applied Toxicology 5* 1025-1033 1985.

12 Jacobs DS, Kasten BL, Dermott WR, Wolfson WL. *Laboratory Test Handbook.* 2nd edition. Williams and Wilkins, Baltimore 1990 p 171.

13 Murray RL. Creatinine. In: Pesce AJ, Kaplan LA (Eds). *Methods in Clinical Chemistry.* Mosby, St Louis 1987 pp 10-17.

14 Braun JP, Bezille P, Raviart I, Rico AG. Distribution de l'alanine et de l'aspartate aminotransférases, de la gamma-glutamyl transférase, de la lactate deshydrogénase, des phosphatases alcalines et de la créatine kinase dans les principaux organes de chèvres adultes et de chevreaux. *Annales de Recherches Vétérinaires 18* 389-392 1978.

15 Bennis A, de La Farge F, Kessabi M *et al.* Valeurs usuelles des constituants biochimiques sériques de chèvres sahariennes au Maroc (Serum biochemical references for Saharian goats in Morocco). *Revue de Médecine Vétérinaire 142* 831-837 1991.

16 Braun JP, Bezille P, Rico AG. Sémiologie biochimique du foie chez les ruminants. *Reproduction Nutrition Développement 26* 227-243 1986.

Published by the International Society for Fluoride Research
Editorial Office: 216 Atkinson Road, Titirangi, Auckland 7, New Zealand

THE EXCRETION OF URINARY GLYCOSAMINOGLYCANS BY FLUOROTIC RATS

J B Stanbury[1] and G Embery[2]
Liverpool, England and Cardiff, Wales

SUMMARY: This study describes differences in excretion of sulphated glycoproteins, including glycosaminoglycans and possibly Tam-Horsfall like proteins, in the urine of fluorotic and control rats. Changes in molecular size of protein and covalently bound radiosulphate profiles together with differences in Alcian blue and Ponceau S staining of the electrophoretically separated molecular size fractions were evident in fluorotic urines. The findings highlight changes in connective tissues in fluorosis which manifest themselves as urinary metabolites.

Key words: Excretion; Fluorosis; Glycosaminoglycans; Rats; Urine.

Introduction

Glycosaminoglycans (GAG) are high molecular weight linear heteropolysaccharides which are covalently linked to a specific core protein to form proteoglycans (PG). These in turn interact with collagen and in mineralised tissues are arranged in such a manner to function as templates for the deposition of calcium phosphate crystals during mineralisation of dentine and bone.

Changes in the organic components of mineralised tissues are evident during fluorosis. Embery and Smalley (1) showed that the PG and GAG of rat dentine underwent an alteration in molecular size during fluorosis leading to the appearance of low molecular weight products. Smalley and Embery (2) showed that fluoride inhibited the incorporation of radiosulphate into chondroitin 4 sulphate by isolated rat incisor odontoblasts. Changes in the GAG composition of mineralised tissues have also been noted with the appearance of dermatan sulphate in fluorotic cancellous bone (3,4).

GAG have been detected in low amounts in the urine of normal human subjects (5) but are particularly evident in the genetically enzyme-deficient disorders termed the 'mucopolysaccharidoses' such as Hurler's Syndrome and Hurler-Scheie Syndrome where excess amounts of heparan sulphate and dermatan are excreted due to inability of the tissues to fully metabolise the native GAG (5).

This report examines the notion that a reduction in molecular size and change in composition of PG and GAG in fluorotic mineralised tissues may be reflected in their appearance in the urine and thus feature as markers of fluorosis. The study was facilitated by the use of radiosulphate as a tracer label which in addition to acting as a marker of GAG metabolism is also incorporated into Tamm-Horsfall and other proteins which may also reflect the fluorotic state.

[1] Department of Clinical Dental Science, University of Liverpool, England.
[2] Department of Basic Dental Science, Dental School, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XY, Wales, United Kingdom. Correspondence to Professor Embery.

Materials and Methods

Animals

Male albino Wistar Harvard rats aged 6-8 weeks and average weight 150 g were used throughout this study. Six animals were divided randomly into two groups. For 4 weeks, one group received deionized water fluoridated at 45 ppm *ad libitum*, while the control group received non-fluoridated deionized water *ad libitum*.

Urine collection

Prior to urine collection, the rats were acclimatised overnight in the metabolism cages. These were designed to allow the separate collection of urine and faeces. Each animal was injected intraperitoneally with (^{35}S) sulphate (Amersham International plc, Little Chalfont, Bucks., UK), in sterile isotonic saline, at a dose of 0.185 MBq (5 μCi) g^{-1} body weight. Urine was collected during the next two consecutive 24h periods. After each 24h period pressure was applied to the animal's bladder to ensure complete voiding of urine over the funnel before the sample was removed. The urine volume was measured and recorded, and the sample subsequently stored at -20°C to avoid bacterial breakdown of the GAG. At the end of the 48h period, the animals were killed by exposure to rising comcentrations of carbon dioxide.

Preparation of urine samples

In order to minimise the effect of individual variation on the investigation, the samples within each 24h group of the two regimes were pooled. Firstly the individual samples were thawed, and then for each 24h group insoluble material present in the samples was removed by passing the urine through a glass wool filter into a common vessel. The urine was concentrated by freeze-drying the four pooled samples.

Gel filtration chromatography

The urinary constituents were resolved using gel filtration on Sephadex G-150 column. The column had a V_t of 630 ml and a V_o of 155 ml, as determined by the complete exclusion of Blue Dextran.

The lyophilised samples were each dissolved in 5 ml of distilled water. Solubilised samples were then applied to the column and eluted with 0.1 M NaCl, at a flow rate of 8 ml h^{-1}, at 40°C. The solute was collected in 5 ml fractions, and the protein monitored by 280 nm absorption. Aliquots (0.5 ml) were taken for determination of the total (^{35}S) sulphate activity. Based on the profiles obtained, the effluent from each sample was pooled into 5 fractions. These were then exhaustively dialysed against distilled water, which allowed the removal of inorganic (^{35}S) sulphate, and the non-diffusible material was recovered by lyphilisation.

Cellulose acetate electrophoresis

In order to give an accurate representation of the polyanionic material present in the effluent, the 5 fractions obtained following gel filtration were redissolved in distilled water in proportion to the original total volumes of the fractions (30 μl ml^{-1} effluent). 5 μl aliquots were then subjected to cellulose acetate electrophoresis followed by Alcian blue staining, after the method of Stanbury and Embery (7). This study also briefly examined protein constituents of the fractions. 5 μl aliquots were again subject to the electrophoretic conditions as above, following which the cellulose acetate sheets were stained with 0.2% (w/v) Ponceau S in 3% (w/v) trichloroacetic

acid for 15 min. Excess dye was then removed by washing in 1% (v/v) acetic acid, with gentle agitation, until the background cleared. Finally, they were rinsed in distilled water and allowed to dry at room temperature.

Results

Gel filtration

The elution profiles obtained from the separation on Sephadex G-150 of the urine samples from the two regimes, collected during the 48h period, are depicted in Figures 1 and 2. Total (^{35}S) sulphate activity, determined as cpm (^{35}S), together with protein, monitored by 280 nm absorbance, are shown. Also depicted is the column V_o of 155 ml determined by complete exclusion of Blue Dextran.

Comparison of the molecular weight elution profiles from the 0-24h collection of the two regimes (Figure 1) disclosed distinct alterations resulting from the F^- ingestion of the (^{35}S) profile. Peak 1 (V_o) of the F^- regime showed a distinct increase in this (^{35}S) peak while the protein peak showed no clear sign of change.

The most dramatic alterations in the profiles were observed in the material eluting just after the V_o (fraction II). Comparison of the (^{35}S) profiles revealed that a small narrow molecular weight range peak detected in the control had increased considerably in that from the F^- regime. A relatively small protein peak from the control regime also underwent an obvious increase.

The intermediate molecular weight material (fractions III and IV) from both regimes revealed a broad peak of incorporated (^{35}S) sulphate. Again, the peak from the F^- regime was greater than that from the control. In contrast, the two protein peaks from the control regime that were eluted in this region were of remarkably similar profile to those of the F^- regime.

Since inorganic (^{35}S) sulphate is of smaller molecular weight than the fractionation range of the gel filtration medium utilized in this study, it will elute at the V_t. The high cpm of (^{35}S) at the V_t of both profiles is, therefore, likely to have occurred largely from radioactive inorganic sulphate. Also eluted at the V_t was the pigment (urochrome) that, in the main, gives urine its normal yellow colour.

Examination of the second 24h collections (Figure 2) disclosed that during the 24-48h period after the injection of radioactive inorganic sulphate only small amounts of incorporated (^{35}S) sulphate were excreted in the urine. The excreted inorganic (^{35}S) sulphate was also considerably reduced. This reduction in the levels of (^{35}S) sulphate incorporated into the high molecular weight fractions made an assessment of the differences between the fluoride and control regimes difficult to interpret. On the other hand the protein profiles of the second 24h collection displayed differences in the proportions of fractions of I, II and IV between the control and fluoride regimes which in terms of molecular size distribution were considered of significance.

Cellulose acetate electrophoresis

Following cellulose acetate electrophoresis of the 5 gel filtration fractions, of each of the 4 samples, and staining with either Alcian blue or Ponceau S, the sheets were examined by transillumination. Comparison of the electrophoretic profiles of the 0-24h and 24-48h urine collections did not reveal obvious differences. Only one collection, 0-24h, is therefore shown.

FIGURE 1. Gel filtration profiles of ^{35}S-sulphate labelled urinary glycosaminoglycans (0 - 24h) from control and fluorotic rats (protein ····· and ^{35}S ——)

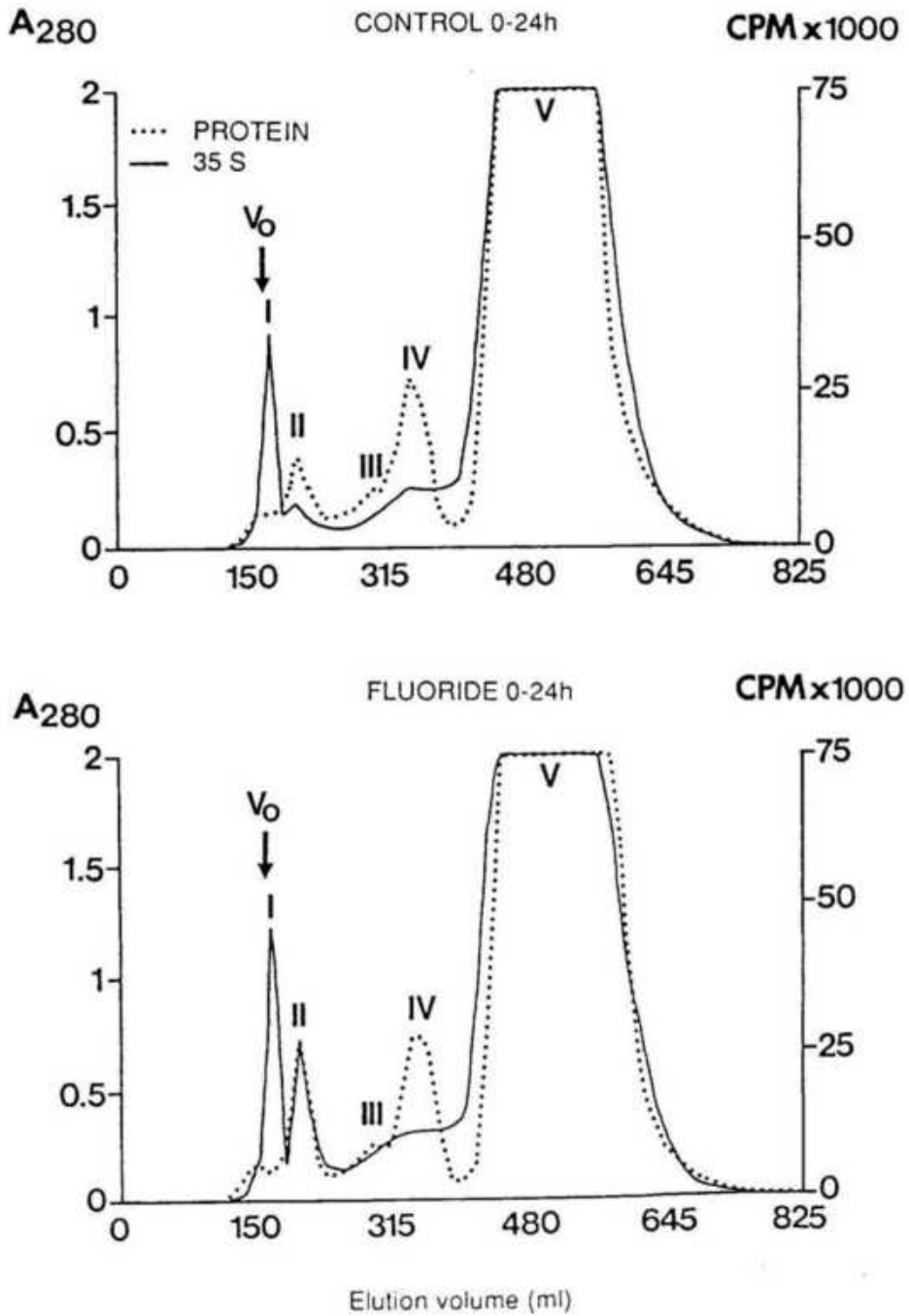


FIGURE 2. Gel filtration profiles of ^{35}S-sulphate labelled urinary glycosaminoglycans (24 -48h) from control and fluorotic rats (protein ····· and ^{35}S ——)

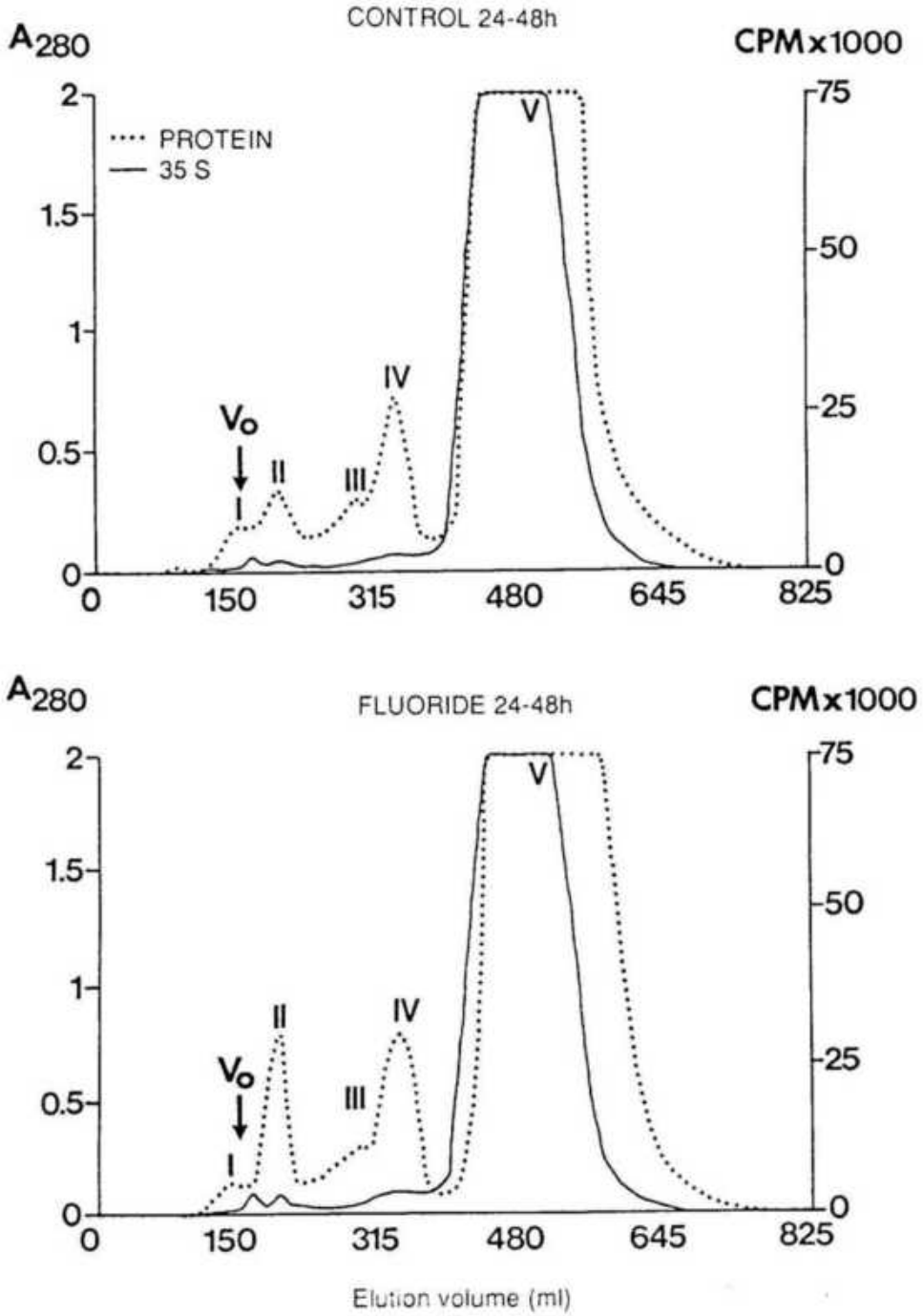


FIGURE 3. Electrophoretic profiles obtained from gel filtration fractions visualised with Alcian blue. Comparisons are shown for control and fluorotic rats. (HA - hyaluronic acid; HS - heparin sulphate; DS - dermatan sulphate; C4S - chondroitin 4 sulphate and C6S - chondroitin 6 sulphate) Degree of hatching reflects the intensity of staining

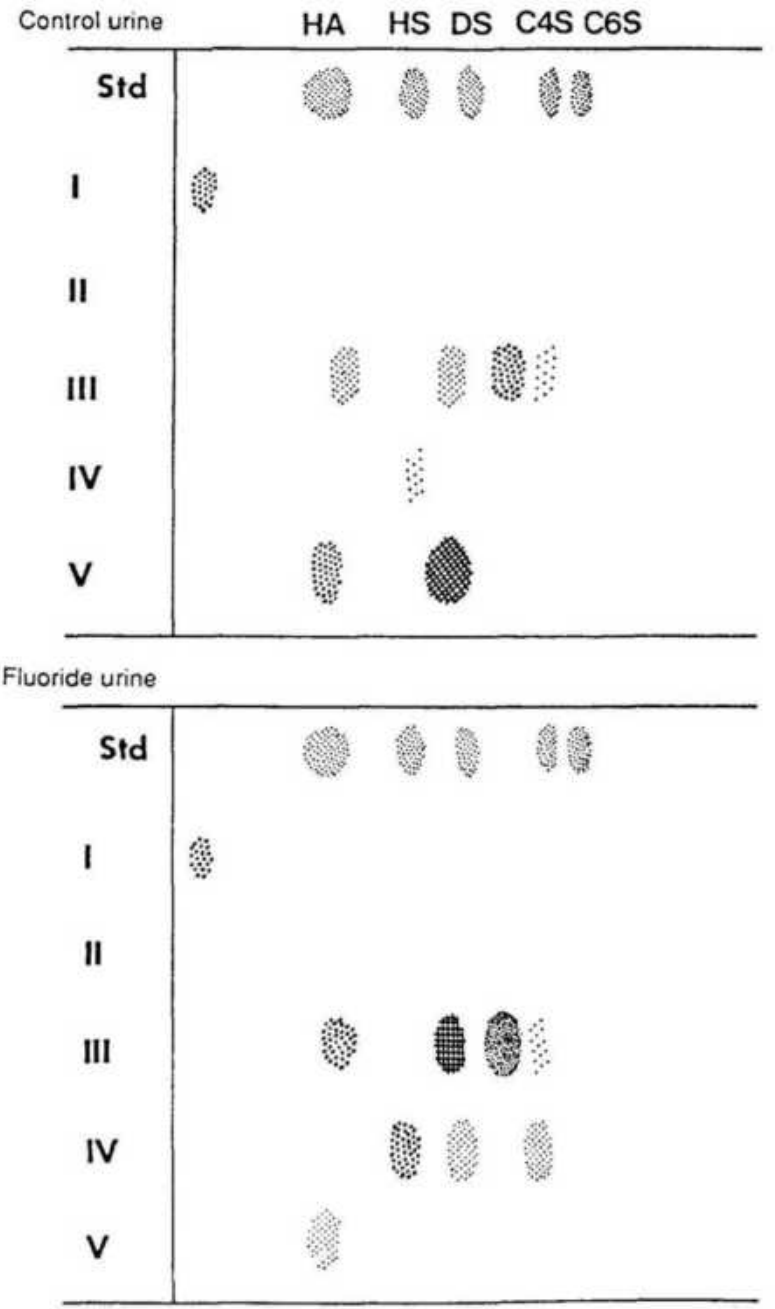
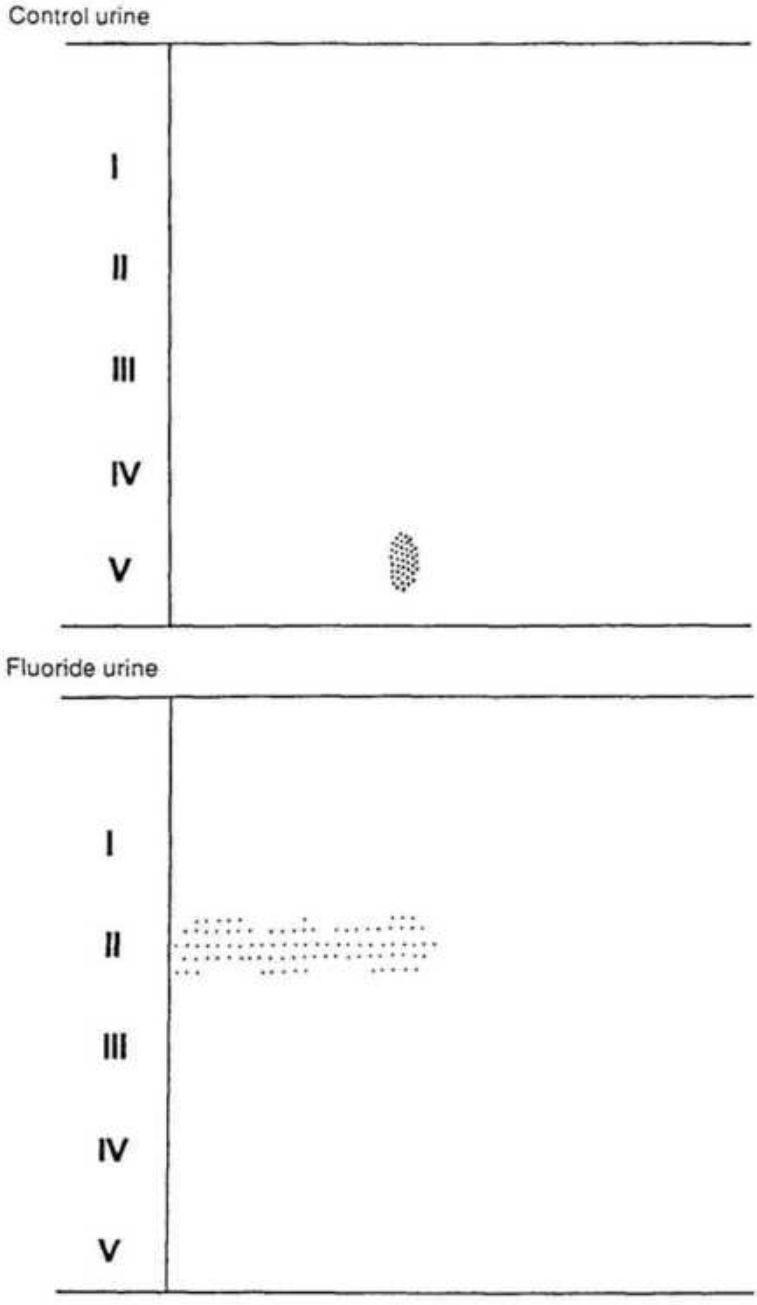


FIGURE 4. Electrophoretic profiles described in Figure 3 stained with the protein reagent Ponceau S



Examination of the Alcian blue-stained sheets (Figure 3) showed that the high molecular weight material (fractions I and II) from both regimes contained no detectable band of electrophoretic mobility similar to the GAG standards. Both V_o fractions contained an Alcian blue band, but its mobility was considerably less than that of the hyaluronic acid standard. The V_o bands were of similar staining intensity. No Alcian blue bands could be detected in fractions II.

Comparison of the intermediate molecular weight material from the regimes revealed that the F^- intake had an effect on the electrophoretic profiles of fractions III and IV. Fraction III of the control produced an Alcian blue band of electrophoretic mobility coincident to the HA standard, and one of mobility intermediate to dermatan sulphate and chondroitin 4 sulphate. Also present was a faint band of mobility approaching that of dermatan sulphate. Fraction III of the F^- regime produced bands of increased staining intensity. The band of mobility approaching that of dermatan sulphate became the most intense; the remaining bands showed only slight increases. The most obvious increase in Alcian blue intensity occurring as a result of F^- intake was observed in fraction IV. The control fraction produced only a trace amount of band in the region of the heparan sulphate standard. In contrast, the F^- regime produced a band of similar mobility but of considerably greater staining intensity, together with faint dermatan sulphate and chondroitin 4 sulphate migrating bands.

The most dramatic changes in the Alcian blue profile occurred at the V_t. The control produced a distinct hyaluronic acid coincident band, together with an intensely-stained band of mobility approaching that of dermatan sulphate. This contrasted greatly with the fraction from the F^- regime which showed only a faint hyaluronic acid coincident band; the faster migrating band failed to be detected.

The inclusion of Ponceau S staining in this study enabled an investigation of possible involvement of protein in the changes, brought about by F^- intake, in the polyanionic constituents of urine. Attention was therefore directed towards the Ponceau S-staining material that migrated towards the anode (Figure 4). Such material was detected in only two of the fractions. In fraction II from the F^- regime broad diffuse Ponceau S banding was shown, which contrasted with its failure to be detected in that from the control. In fraction V from the control a discrete band was observed that could not be detected in the F^- fraction.

Discussion

The findings of this novel investigation have tentatively identified a variety of constituent GAG. Enzymic degradation has been used in the characterisation of urinary GAG in the mucopolysaccharidoses (8).

Amorphous deposits commonly form in urines on standing. Since insoluble material was filtered from the urine prior to gel filtration, there could be concern that during this process constituent GAG may have been removed. It has been demonstrated by uronic acid analysis (5), however, that these precipitates do not contain GAG .

It is well documented (9) that the major portion of administered radioactive inorganic sulphate that is incorporated appears as ester sulphate in GAG. To a small

extent it is also incorporated into protein, in the sulphur-containing amino acids (10). The constituents present in normal urine are largely derived from the glomerular filtrate; components are also added from the urinary tract. Tamm-Horsfall protein (11) makes an important contribution to the latter. This urinary constituent is a glycoprotein of molecular weight 7×10^6, which can be degraded into components of 180 000. Since the glycoprotein is weakly sulphated and anionic, it is conceivable that the slow-migrating Alcian blue band of the V_0 fraction is Tamm-Horsfall protein.

Some plasma proteins pass into the glomerular filtrate and are present in normal urine (12). Work in our laboratories (13), however, has shown that plasma proteins, under the electrophoretic conditions utilised migrate towards the cathode. The dramatic increase of protein in fraction II as a result of F^- intake, shown by the 280 nm profiles, was reflected in the detection of the diffuse Ponceau S-staining band migrating towards the anode. The failure to detect Alcian blue-staining material in this fraction suggests that the increase in bound sulphate in fraction II from the F^- regime is associated with protein. Determination of the exact nature of the component involved would be of interest in future investigations. It is, however, beyond the design of this present study of urinary GAG.

The findings of fractions I and II show the absence of detectable high molecular weight GAG and/or PG in rat urine from both the control and F^- regimes. Attention was therefore centred on the intermediate and low molecular weight fractions.

In both intermediate molecular weight fractions F^- intake resulted in increases of incorporated (^{35}S) sulphate, the increase being more obvious in fraction IV than Fraction III. These increases were reflected in the intensity of Alcian blue staining of the GAG. Again, the increase as a result of F^- intake was more obvious in fraction IV than III. Since there was no sign of protein staining in either of the fractions, it appears that the (^{35}S) sulphate is incorporated in the GAG.

Examination of the V_t is difficult since the presence of radioactive inorganic sulphate would be expected to dominate any radioactive bound sulphate present. A further complication arises from the elution of urinary pigment in this fraction which would cause colour quenching of the radioactivity. Attention was, therefore, focused on the electrophoretic separation of fraction V. Dialysis of the fractions prior to electrophoresis will have removed molecules of molecular weight less than 12 000 along with the inorganic sulphate. Despite this removal, the most dramatic changes in both Alcian blue and Ponceau S staining were observed in this fraction. Of particular interest was the intensely-staining Alcian blue band of similar mobility to dermatan sulphate from the control. The failure to detect this band in the fraction from the F^- regime results in a greater overall loss of urinary GAG from the control than from the F^- regime. This finding is consistent with that of Susheela and her co-workers (3) who investigated total GAG in rabbit urine and reported a decrease in urinary GAG following F^- ingestion.

Comparison of the Alcian blue and Ponceau S staining of the V_t fraction from the control revealed that the DS band had migrated similarly to the protein band. Since the electrophoretic separation was performed under identical conditions, it appears that the dermatan sulphate has some protein bound to it. Clearly, the protein is of relatively low molecular weight, as judged by its eluting position. The recent availability of

benzylated dialysis tubing with a lower molecular weight cut-off (2 000 rather than 12 000) would allow a more complete examination of the V_t fraction in future work.

To the authors' knowledge, this study remains the sole report to date of the effect of F^- administration on the molecular weight distribution of urinary GAG. The use of radioactive inorganic sulphate in conjunction with cellulose acetate electrophoresis proved useful in detecting changes in the metabolism of the connective tissue ground substance, in particular the reduced excretion of dermatan sulphate at the early stages of fluorosis. Also notable is the presence in fluorotic urine of an unidentified protein band (fraction II). It is contended that studies along these lines would be of value as diagnostic tests in the assessment of fluorosis in addition to shedding light on the overall metabolism of connective tissue in response to the presence of F^-.

References

1 Smalley JW, Embery G. The influence of fluoride administration on the structure of proteoglycans in the developing rat incisor. *Biochemical Journal 190* 263-272 1980.

2 Embery G, Smalley JW. The influence of fluoride on the uptake of radiosulphate by rat incisor odontoblasts *in vitro*. *Archives of Oral Biology 25* 659-662 1980.

3 Jha M, Susheela AK. *In vivo* chondrogenesis and histochemical appearance of dermatan sulphate in rabbit cancellous bone. *Differentiation 22* 235-236 1982.

4 Prince CW, Navia JM. Glycosaminoglycan alterations in rat bone due to growth and fluorosis. *Journal of Nutrition 113* 1576-1582 1983.

5 Scott JE, Newton DJ. The recovery and characterisation of acid glycosaminoglycans in human urine. *Connective Tissue Research 3* 157-164 1975.

6 McKusick VA, Neufield EF, Kelley TE. In: Wyngaarden JB, Fredrickson DS (Eds). *The Metabolic Basis of Inherited Disease*. McGraw-Hill, New York 1978 pp 1281-1307.

7 Stanbury JB, Embery G. An improved method for the electrophoretic separation of acidic glycosaminoglycans (mucopolysaccharide). *Medical Laboratory Science 34* (3) 266-271 1977.

8 Taniguchi N, Roizuni S, Masaki K, Kobayash YI. Diagnosis of genetic mucopolysaccharidoses: Electrophoretic and enzyme characterisation of urinary glycosaminoglycans. *Biochemical Methodology 14* 241-249 1975.

9 Lucus PA, Ophang RH, Singer L. The effect of vitamin A deficiency and fluoride on glycosaminoglycan metabolism in bone. *Connective Tissue Research 13* 17-26 1984.

10 Dziewiatkowski DD. Utilisation of sulphate sulfur in the rat for the synthesis of cystine. *Journal of Biological Chemistry 207* 181-186 1954.

11 Tamm E, Horsfall FL. Characterisation and separation of an inhibitor of viral haemaglutination present in urine. *Proceedings of the Society for Experimental Biology and Medicine 74* 108-114 1950.

12 Harrison JF, Nartham BE. Low molecular weight urine protein investigated by gel filtration. *Clinica Chimica Acta 14* 679-688 1966.

13 Last KS, Stanbury JB, Embery G. Glycosaminoglycans in human gingival crevicular fluid as indicators of active periodontal disease. *Archives of Oral Biology 30* 275-281 1985.

Published by the International Society for Fluoride Research
Editorial Office: 216 Atkinson Road, Titirangi, Auckland 7, New Zealand

FACTORS OF INDIVIDUAL PREDISPOSITION TO OCCUPATIONAL FLUOROSIS

E V Polzik, G A Valova, S V Shcherbakov and M Yu Yakusheva
Ekaterinburg, Russia

SUMMARY: Fourteen different risk factors for occupational fluorosis were studied comprehensively among workers engaged in cryolite production. For multifactorial analysis use was made of mathematical methods of pattern recognition. The results of recognition of the "exam" samples are as follows: the group of workers who did not fall ill with fluorosis - 81.4%; the group of workers who fell ill with fluorosis - 66.6%. Each factor was estimated for its degree and direction of influence. For estimating genetic predisposition to fluorosis dermatoglyphics were used as markers. The elimination of the effect of basic non-genetic risk factors and comprehensive analysis using the pattern recognition methods show that genetic predisposition to fluorosis does exist.

Key words: Dermatoglyphics; Occupational fluorosis; Predisposition.

Introduction

Researchers have long been interested in the fact that, in the same workplace, only a few of the employees who are in contact with harmful agents develop chronic occupational diseases. The majority do not show any marked pathological changes. This situation is true of fluorosis as well. The ability to detect persons susceptible to fluorosis would make possible medical recommendations to avoid hiring them, or even prohibition of their employment in fluorosis hazardous industries. The importance of medical selection increases in those cases where it is impossible to ensure safe labour conditions by technical means. At the present time, however, there are no scientifically grounded criteria for such selection.

Material and Methods

Earlier we developed a general methodology for conducting studies on the criteria of individual predisposition to occupational diseases, and succesfully tested it for different pneumoconioses (5,9,10). The methodology has three successive stages. This paper presents results from the first two stages, which consisted of complex estimates for non-genetic risk factors and for genetic predisposition to fluorosis.

The studies were carried out among the workers of two large cryolite plants located in the Urals region, Russia. In accordance with stage 1 of the methodology, on January 1, 1970, we formed a cohort of 376 employees from the main production shops, who had no signs of pathological changes characteristic of fluorosis. Each of the workers in the cohort was assessed for 14 potential risk factors, as listed in Table 1.

The cohort was divided into 2 classes: 1) those who did not fall ill with fluorosis in the period 1971-1990 (245 persons); 2) those who fell ill with fluorosis over the same period (131 persons).

Ecological Safety Centre, Ural Division of the Russian Academy of Sciences, Ekaterinburg 620219, Russia.

Multifactorial analysis was carried out, using the mathematical methods of pattern recognition, in order to:
1) obtain a reliable description of the differences between the two classes of workers;
2) estimate the degree of influence (informativity) produced by each factor;
3) determine the direction of influence of each factor.

The first objective was met using the variant of discriminant analysis known as "training with a teacher". Essentially it consists of selecting a random sample of 10-12% of the entire set of observations of both classes for an "exam". The other observations are used to "teach" the computer, which then searches for corresponding discriminating rules. The criterion of quality is the percentage of correctly recognized observations of the "exam" sample.

The degree of influence (informativity) of each factor was estimated by measuring the Eukledean distance between the centres of the classes under consideration. The direction of influence was determined by noting the rate of occurence of the of the factors.

All the above problems were solved using the KVAZAR package of programs (4) developed at the Institute of Mathematics and Mechanics of the Ural Division of the Russian Academy of Sciences.

At the second stage, we studied the role of the genotype in the development of fluorosis. Dermatoglyphic patterns were used as a genetic marker. This choice was determined by both our own experience gained in the studies of genetic predisposition to silicosis, coronary heart disease and cancer (10,11,12) and the data in the literature on the use of dermatoglyphics for estimating predisposition to endemic fluorosis (1,2). However, our methodology of dermatoglyphic study was different from the commonly used one.

First, we eliminated the effect of four non-genetic factors: three factors found to have the greatest effect during the first stage of work - the duration of exposure to fluorine, time of life in the region of fluorine pollution, occupation (see Table 1); and the factor "nationality" as directly connected with the genotype. To this end, we selected out of the cohort 60 pairs matched on the basis of the above four factors.

Secondly, we analyzed the set of 59 dermatoglyphic features that we determined for each individual in accordance with the method's nomenclature (8). For this multifactorial analysis we also used by the pattern recognition method mentioned above.

Results and Discussion

First stage:

The best results were obtained when all the 14 factors were taken into account using an algorithm based on the principle of seniority committees (7) and majority committees (6). The "exam" results were: the class "not ill with fluorosis" - 81.4% of correct answers; the class "ill with fluorosis" - 86.6%. Attempts to reduce the numbers of factors for analysis impaired the quality of recognition.

The results suggest that the chosen set of factors contains sufficient information to produce a satisfactory description of the patterns of predisposition to fluorosis. These results required the use of all the selected factors. This confirms the hypothesis of multifactorial dependance of predisposition to fluorosis. At the same time, the impossibility of obtaining better results of recognition, with all the available information on the risk factors, indicated that the set under study did not contain some important factors. The lacking link was assumed to be directly related to the genotype.

Table 1 shows the data on the informativity of the factors under study. The factors characterizing "fluorine exposure" are of the greatest importance (ranks 1,2,4,7). Analysis of the direction of influence of the factor "duration of occupational exposure to fluorine" showed that the dependance has a two-phase character. At the first stage, from 1 to 15 years, the risk of contracting fluorosis increases, and then it begins to reduce. This can be explained by the fact that at the first stage fluorine is accumulated in the organism, thus increasing the probability of falling ill with fluorosis. At the second stage, the most susceptible individuals leave the industry, while others remain healthy, *i.e.* show more resistance despite the continuing exposure. Thus, the risk of developing fluorosis goes down. We had discovered the same dependance earlier when estimating risk factors for pneumoconioses (5,9).

We observed direct dependance of the disease on the time of life in a fluorine polluted region, which can be explained by additional exposure to fluorine and is in agreement with the results of earlier studies (13). All the jobs typical of cryolite pruduction were divided into two groups: 1) workers who are in contact with both hydrofluoric acid and fluorine salts; 2) workers exposed predominantly to HF. Workers of the first group were found to have a higher probability of falling ill with fluorosis due to a greater fluorine exposure and to the specific toxicokinetic features of gaseous and aerosol fluorine compounds that account for their retention in the organism.

Breaks in exposure to fluorine reduced the risk of contracting fluorosis. This fact is in agreement with findings of other researchers (3,14).

Table 1 shows that social factors play a great part in predisposition to fluorosis (ranks 3,5,6,10). Thus, smoking, alcohol abuse, unsatisfactory housing conditions, and availability of personal small-holding promote fluorosis, which is shown by the direction of influence of these factors. The last factor is due, in our opinion, to the additional supply of fluorine compounds from food produced in personal small-holdings located in the zone of environmental pollution.

Biological factors were found to have an additional effect. Thus, those who exposed themselves to fluorine at the age of 18-26 ran a higher risk of developing fluorosis than those who began to work in fluorosis-hazardous plants at the age of 30-40. Higher predisposition of young people to the effect of fluorine compounds seems to be due to the functional immaturity of the protective mechanisms.

Of the two basic nationalities (Russians and Turkish peoples) inhabiting the Urals region, Russians run a higher risk of contracting fluorosis.

TABLE 1
The degree of influence of different factors on the formation of predisposition to occupational fluorosis

Rank	Name of factor	Informativity in relative units
1	Duration of life in region polluted with fluorine compounds before occupational exposure	1.00
2	Duration of occupational exposure to fluorine	0.75
3	Housing conditions	0.60
4	Occupation	0.50
5	Smoking	0.32
6	Personal small-holding	0.27
7	Continuity of exposure to fluorine	0.25
8	Nationality	0.15
9	Locomotor diseases in personal history	0.14
10	Alcohol abuse	0.14
11	Liver diseases in personal history	0.14
12	Age at beginning of exposure	0.09
13	Kidney diseases in personal history	0.05
14	Endocrine diseases in personal history	0.01

TABLE 2
Informativity of dermatoglyphic features

Rank	Name of feature	Informativity in relative units
1	Width of palm lines on right hand	1.000
2	Direction of main palm line D on left hand	0.550
3	Ball pattern of finger I of right hand	0.533
4	Palm ridge count ab of right hand	0.500
5	Ball pattern of finger IV of right hand	0.475
6	Pattern in zone between fingers IV and V of left hand	0.467
7	Palm ridge count cd of left hand	0.458
8	Ball pattern of finger I of left hand	0.450
9	Direction of main palm line B on left hand	0.367
10	Palm ridge count ab on left hand	0.355

Concerning the presence of various diseases before falling ill with fluorosis: only diseases of the locomotor system were observed to increase the probability of fluorosis Our results suggest that diseases of the liver, kidneys and endocrine system do not play any substantial role in the formation of susceptibility to fluorosis.

Second stage:

We then studied the question whether genetic predisposition to occupational fluorosis exists at all. When processing the data mathematically we estimated first the informativity of each of the dermatoglyphic features. Then we checked the information about 59 features for their adequacy in describing the differences between the classes of those who fell ill with fluorosis and those who did not. We obtained good results of recognition of the exam sample (83.3% of correct answers in both classes) when the seven most informative features were taken into account. On the whole, we obtained a great number of reliable discriminating rules when we used from 7 to 10 such features presented in Table 2.

Estimates of the direction of influence for each of the dermatoglyphic features showed that the risk of fluorosis is higher when the palm ridge count ab and cd exceeds 33, the width of palm lines is more than 1 mm, palm line D is directed towards fields 9 and 13 while line B is towards the 7th field, there is no pattern in the zone between fingers IV and V, and there is a "whorl" or "double loop" on fingers I and IV.

The results of the study show that our methodology, which we had applied earlier to study individual predisposition to pneumoconiosis, is also effective for fluorosis. First of all, we estabished that individual predisposition to this occupational disease depends on the industrial and social environment as well as the biological factors affecting the organism. We estimated the degree and the direction of influence for each of the 14 factors studied (the data on whose effect on the development of occupational fluorosis were scarce and disparate), but the results show that the probability of this disease is high only when they are taken all together as a set. At the same time, we established that the selected set of factors lacked some important information for estimating predisposition to fluorosis. This might be information about the specific features of the genotype.

At the 2nd stage, we obtained evidence of the existence of genetic predisposition to occupational fluorosis, and found that dermatoglyphic patterns present a sufficiently reliable marker. The fact that individual predisposition to fluorosis can be estimated using a relatively small number (seven) of dermatoglyphic features indicates a considerable difference in the dermatoglyphic pattern between people with high and low predisposition to fluorosis. Attemps to compare these results with the data obtained for endemic fluorosis were not successful, since the Indian reseachers used different methods of analysis and confined themselves to estimating finger patterns only. The discriminating rules worked out in the course of the study may be used for estimating genetic predisposition to occupational fluorosis. On the whole, the results are very promising taking into account the relative simplicity and low cost of the dermatoglyphic method as well as its complete safety and conveniance. These qualities permit it to be used for mass examinations for detecting persons with high genetic predisposition.

Further on we plan to conduct a study similar to that performed at the 1st stage, but in this case the set of factors will include "genetic predisposition to fluorosis", whose importance will be based on a multifactorial estimate of the dermatoglyphic pattern in accordance with the established criteria. This study should permit us to examine the relationship between fluorosis and the set of genetic and non-genetic risk factors, as well as to estimate their relative contributions to the formation of individual predisposition.

References

1 Batish KL, Sidhu SS, Makhni SS *et al.* Fluorosis and finger ball dermatoglyphics: A comparative study. *Fluoride 16* 198-208 1983.

2 Bhatnagar DP, Sidhu SS, Batish KL, Batish MK. Ecological study of dermatoglyphic diversity in fluorosis belt of Punjab, India. *Fluoride 17* 168-172 1984.

3 Grekhova TO, Yelnichnykh LN. Experimental study on prophylactic efficiency of intermittent inhalation exposure to hydrogen fluoride. *Gigiena Truda 12* 64 1982.

4 Kazantsev VS. *Classification Problems and Software (the KVAZAR package).* Nauka, Moscow 1990.

5 Katsnelson BA, Polzik EV, Morozova KI *et al.* Trends and perspectives of the biological prophylaxis of silicosis. *Environmental Health Perspectives 82* 311-321 1989.

6 Mazurov VD. On construction of a committee of convex inequalities. *Kibernetica 2* 64-67 1967.

7 Osborne NL. The seniority logic - a logic for committee machine. *Institute of Electrical and Electronic Engineers. Transactions on Computers C-26* 1302-1306 1977.

8 Penrose LS. Memorandum on dermatoglyphic nomenclature. Birth defects. *Original Articles Series 4* 1-13 1968.

9 Polzik EV, Kogan FM, Kochneva MY, Potanin NI. Risk factors for asbestosis in workers of asbestos mills. *La Medicina del Lavoro 80* 363-370 1989.

10 Polzik EV, Katsnelson BA, Kochneva MY, Kazantsev VS. The principle of predicting the individual risk of silicosis and silicotuberculosis. *La Medicina del Lavoro 81* 87-95 1990.

11 Polzik EV, Sidorovich SB. Dermatoglyphic features of coronary heart disease patients. *Tsitologiya i Genetika 25* 31-35 1991.

12 Polzik EV, Katsnelson BA, Lezhnin VL *et al.* On the problem of search for genetic markers of individual predisposition to lung cancer. *Tsitologiya i Genetika 26* 36-41 1992.

13 Shcherbakov SV, Plotko EG, Lyubashevsky NM. Hygienic aspects of technogeneous fluorine pollution of environment. *Vestnik AMN SSSR 1* 54-59 1991.

14 Symansky H. Fluor - ein problem von allgemaine hygienischer und arbeitsmedizinischer Bedeutung. *Verhandlung der Deutsche Gesellschaft für Arbeitsschutz 1* 13-23 1953.

Published by the International Society for Fluoride Research
Editorial Office: 216 Atkinson Road, Titirangi, Auckland 7, New Zealand

WHO DATA ON DENTAL CARIES AND NATURAL WATER FLUORIDE LEVELS

R Ziegelbecker[1] and R C Ziegelbecker[2]
Graz, Austria

SUMMARY: Data collected in 1987 from World Health Organization data banks contradict earlier reports of an inverse relationship between dental caries prevalence and drinking water fluoride levels.

Key words: Dental caries; Natural water fluoride; World Health Organization.

Introduction

Before 1987 data on the relationship between natural water fluoride levels and the prevalence of dental caries, which were free of the suspicion of being selected in some way, were not available to R Ziegelbecker. In earlier studies (1,2) he had demonstrated that the well-known 21 city study of Dean *et al* (3) was based on highly selected data. When the data from that study, and the 23 or so others which followed it, were combined, no caries/fluoride relationship was apparent. That exercise was criticized by Busse *et al* (4) who could not, however, prove that the data within each fluoride/caries study were not selected. Such selection prevents conclusions from a single study.

Materials and Methods

In 1987 the authors had the opportunity to visit Geneva and search the WHO Global Oral Health Data Bank (containing dental caries prevalence data expressed as "DMFT" - *i.e.*, mean number of decayed, missing and filled teeth) and the National Oral Health Pathfinder Survey of WHO (containing some locations and towns with the values of natural fluoride content of their drinking water).

We were able to gather the data of those countries where at least 4 locations appeared in both data banks, and where a range of fluoride concentrations occurred. When there was more than one measurement of water fluoride level at a location, the arithmetic mean was used. Only caries data for 12-year-old children were recorded, since this is the prevalent age group in fluoridation statistics. At the time of our Geneva visit, only five countries (Malta, Sri Lanka, Spain, Greece and Hungary) provided the above data, which are set out in the Table and Figures. No data from these countries were excluded.

Results and Discussion

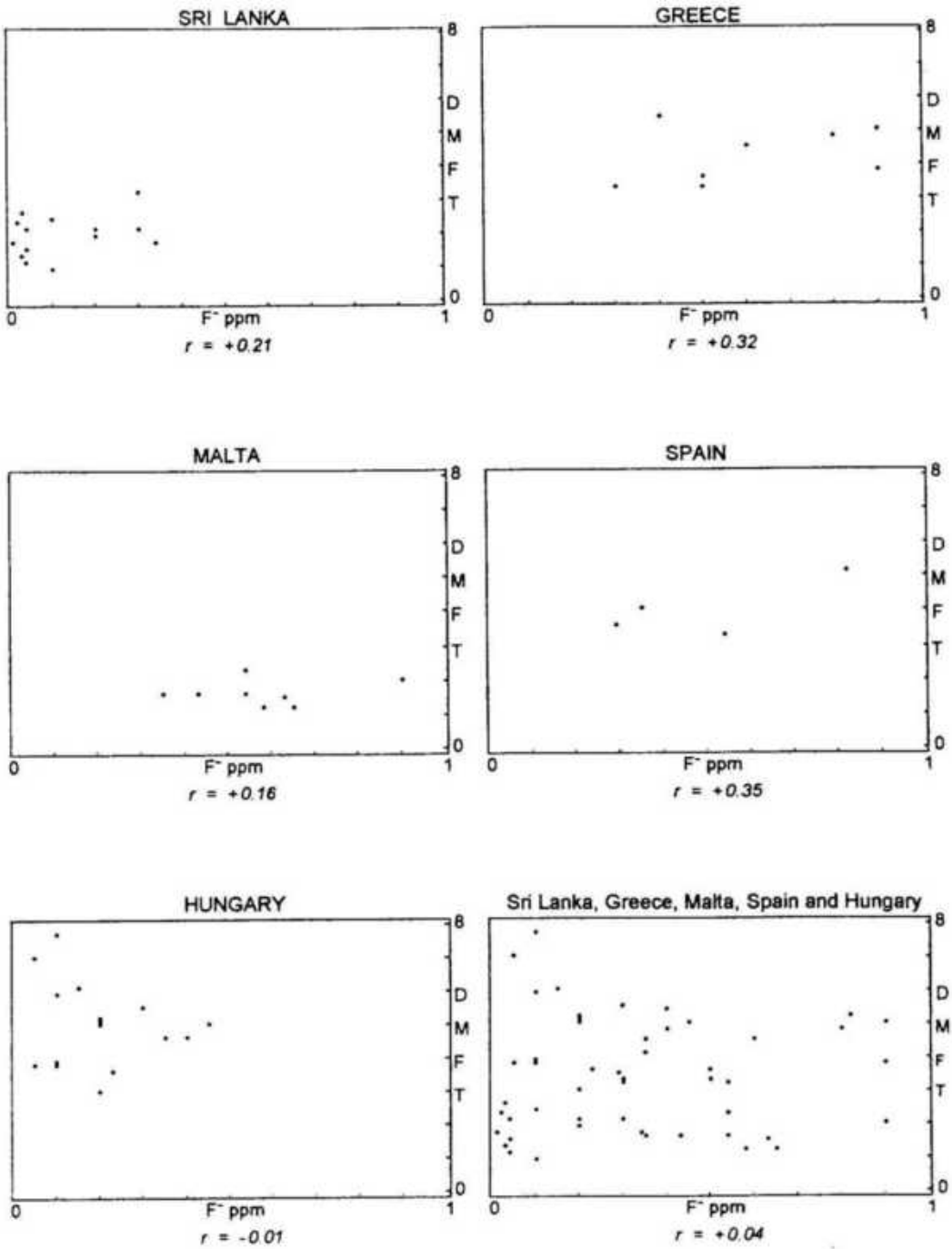
It can be seen that dental caries prevalence does not change significantly with variation in water fluoride content. In most of the countries the relationship tends to be direct rather than inverse: dental caries increases as water fluoride increases. That finding conflicts with the widespread belief, based on the highly selected data in the early studies of Dean and others, that drinking water fluoride reduces dental caries as the fluoride concentration increases toward the claimed "optimum" of one part per million (ppm). But our finding is in accord with some other studies (5-7). The belief in an inverse caries-fluoride relationship was reinforced by the numerous "fluoridation trials." But they, too, have been shown to be based upon highly selected data (8-10).

[1] Peterstalstrasse 29, A-8042 Graz, Austria. [2] Franckstrasse 24, A-8010, Graz, Austria..

TABLE

Country	Code	Year	Province/Town	Children (N)	F(ppm)	DMFT
Sri Lanka	539	1983/84	Colombo Prov.	351	.04	2.1
		1983/84	Kegalla rural	112	.03	1.3
		1984	Ratnapura Prov.	74	.01	1.7
		1984	Kalutara Prov. (0.57/0.02;0.295)	118	.30	3.2
		1984	Galle Prov.	79	.02	2.3
		1984	Badulla Prov.	80	.04	1.5
		1984	Hambantota Prov.	40	.1	.9
		1984	Kandy Prov.	155	.2	1.9
		1984	Batticaloa Prov.	30	.03	2.6
		1984	Polonaruwa Prov.	40	.34	1.7
		1984	Jaffna Prov.	158	.3	2.1
		1984	Anuradhapu Prov.	78	.04	1.1
		1984	Puttalam Prov.	40	.1	2.4
		1981	Kandy urban	186	.2	2.1
Greece	648	1985	Athen urban	50	.4	5.4
		1985	Sparta urban	47	.3	3.3
		1985	Salonica urban	49	.5	3.3
		1985	Salonica rural	49	.5	3.6
		1985	Jannena urban	49	.8	4.8
		1985	Larissa urban	49	.9	5
		1985	Naxos urban	50	.9	3.8
		1985	Salonica urban	51	.6	4.5
Malta	661	1966	Malta (0.52/0.56)	84	.54	2.3
		1986	Total (0.52/0.56)	405	.54	1.6
		1986	Floriana & Valette (0.3/0.4/0.6)	41	.43	1.6
		1986	Sliema (0.7/0.6)	40	.65	1.2
		1986	St. Paul B. & Mellicha (0.60/0.65)	80	.63	1.5
		1986	Robert and Dingli (1.0/0.8)	86	.90	2
		1986	Zurrieq, Grendi, MQA (0.3/0.4)	78	.35	1.6
		1986	Victoria, Zebbug, NAD (0.45/0.7)	80	.58	1.2
Spain	667	1985	Madrid urban	149	.29	3.5
		1985	Valencia urban	99	.54	3.2
		1985	Sevilla urban	97	.35	4.1
		1985	Zaragoza urban	50	.82	5.2
Hungary	650	1985	Budapest urban	100	.23	3.6
		1985	Budapest rural	50	.45	5
		1985	Gyor urban	50	.1	3.8
		1985	Gyor rural	44	.15	6.1
		1985	Pecs urban	49	.35	4.5
		1985	Pecs rural	49	.2	3
		1985	Szombathel urban	50	.05	3.8
		1985	Szombathel rural	50	.2	5
		1985	Szeged urban	50	.1	3.9
		1985	Szeged rural	50	.2	5.1
		1985	Miscolc urban	50	.1	5.9
		1985	Miskolc rural	51	.4	4.8
		1985	Debrecen urban	50	.2	5.2
		1985	Debrecen rural	50	.3	5.5
		1985	Nyiregyhaz urban	50	.05	7
		1985	Nyiregyhaz rural	50	.1	7.7

FIGURES
(correlation coefficient: r)



Acknowledgement

The authors are indebted to Professor G N Pakhomov and Dr David Barmes, Dental Officers of the World Health Organization, for offering them access to the WHO Global Oral Health Data Bank.

References

1 Ziegelbecker R. Fluoridated water and teeth. *Fluoride 14* 123-128 1981.
2 Ziegelbecker R. Natürlicher fluoridgehalt des trinkwassers und karies. *gwf-Wasser/Abwasser 122* 495-497 1981.
3 Dean HT, Arnold FA, Elvove E. Additional studies of the relation of fluoride domestic waters to dental caries experience in 4,425 white children aged 12 to 14 years, of 13 cities in 4 States. *Public Health Reports 57* 1155-1179 1942.
4 Busse H, Bergmann E, Bergmann K. Fluoride and dental caries: two different statistical approaches to the same data source. *Statistics in Medicine 6* 823-842 1987.
5 Ray SK, Ghosh S, Tiwari TC *et al.* An epidemiological study of caries and its relationship to fluoride content of drinking water in rural communities near Varanasi. *Indian Journal of Preventive and Social Medicine 12* 154-158 1981.
6 Chibole O. Dental caries among children in high fluoride regions of Kenya. *Journal of the Royal Society of Health 108* 32-3 1988.
7 Teotia M, Teotia SPS. Dental caries: A disorder of high fluoride and low calcium interactions. (Paper to XIXth Conference, International Society for Fluoride Research, Kyoto, Japan, 1992.)
8 Exner FB, Waldbott GL. *The American Fluoridation Experiment.* Deven-Adair, New York 1957.
9 Sutton PRN. *Fluoridation: Errors and Omissions in Experimental trials.* Melbourne University Press, Melbourne 1960.
10 Ziegelbecker R. A critical review of the fluorine-caries problem. *Fluoride 3* 71-79 1970.

Published by the International Society for Fluoride Research
Editorial Office: 216 Atkinson Road, Titirangi, Auckland 7, New Zealand

ALLERGY AND HYPERSENSITIVITY TO FLUORIDE

Bruce Spittle
Dunedin, New Zealand

SUMMARY: A review of the literature was undertaken in response to four recent reviews which found that the evidence that fluoride was an allergen was unconvincing. Reports were found of urticaria, contact dermatitis and stomatitis occurring in response to fluoride, settling on the withdrawal of fluoride and recurring with appropriate challenges. It is concluded that the four reviews were seriously incomplete in their coverage of the literature, and that when a more complete examination is made there are reasonable grounds for concluding that there are individuals in whom allergy or hypersensitivity to fluoride has been demonstrated. The sources of fluoride included those used in the fluoridation of community water supplies.

Keywords: Allergy; Contact dermatitis; Fluoridation; Fluoride; Hypersensitivity; Stomatitis; Urticaria.

Introduction

Four recent reviews, from the United States of America (1,2), Australia (3) and New Zealand (4), have concluded that claims that fluoride is an allergen could not be supported from studies undertaken to date, and that the weight of evidence shows that fluoride is unlikely to produce hypersensitivity and other immunological effects. Although the two US subcommittees involved were different, the sections dealing with the effects of fluoride on hypersensitivity and the immune system are almost the same. Thus although all four reports reached a similar conclusion that fluoride was unlikely to produce allergic or hypersensitivity effects, the 1993 reports (2,4) refer to those published in 1991 (1,3) and are not completely independent. The present review was undertaken to see if the same conclusion was reached.

Literature Review

In dismissing the occurrence of allergic reactions to fluoride, the New Zealand report (4) refers to the earlier United States (1) and Australian (3) reviews both of which in turn cite a statement by Austen *et al* (5) on behalf of the American Academy of Allergy. The Academy reviewed reports of fluoride allergy and found no evidence of allergy or intolerance to fluorides as used in the fluoridation of community water supplies (5).

Waldbott made a rebuttal of the findings of Austen *et al* in 1971 (6) and noted that in 1978 this was still unrefuted (7). He observed that the statement by Austen *et al* cited only seven references, of which only five referred to fluoride (6). He commented that the committee had referred to a book of his, *A Struggle with Titans* (8), which was written for lay persons, but had apparently not given attention to 19 articles of his in scientific journals (6).

Dr Bruce Spittle, Department of Psychological Medicine, School of Medicine, University of Otago, PO Box 913, Dunedin, New Zealand.

Austen *et al* conclude that in the review of the cases reported there was insufficient evidence to state that true syndromes of fluoride allergy or intolerance existed (5). This included the cases reported by Feltman and Kosel (9). They had reported that 1% of their cases reacted adversely to fluoride tablets (9). Atopic dermatitis and urticaria occurred with the use of fluoride tablets, disappeared with the use of placebo tablets, and recurred when the fluoride tablets were, unknowingly to the patient, given again (9). Kaplan (10) notes that when an urticarial drug reaction is suspected, this diagnosis may be tested by eliminating the agent. If it is correct, gradual resolution of the urticaria is anticipated. He notes that all medications should be considered a potential cause of urticaria. Except for penicillin, it is stated that no routine tests are available that can reliably confirm or refute the diagnosis of drug-induced urticaria or angioedema, and an empirical approach is therefore indicated (10). The empirical approach adopted by Feltman and Kosel of withdrawal of the fluoride tablets, substitution with placebo tablets and later a blind challenge with fluoride tablets (9) appears to be in keeping with the guidelines of Kaplan (10). Contrary to the view of Austen *et al*, the results suggest that there is clinical evidence that a syndrome of fluoride allergy exists.

Another paper reviewed by Austen *et al*, by Shea, Gillespie and Waldbott (11), reported allergy to fluoride in toothpaste and drops. In one case, involving a 48-year-old man with giant urticaria, double-blind testing was used to confirm the ætiologic relationship with fluoride (11). The lesions had involved mainly the hands and feet but sometimes the entire body surface. They usually occurred about one hour after breakfast. He had been using a fluoridated toothpaste at the time. Six days after discontinuing this he was completely free of symptoms. Three years later he experienced another episode of generalized urticaria. This occurred within an hour of his inadvertently brushing his teeth with a fluoridated toothpaste. The double-blind testing involved taking a tablespoonful of water each morning from three bottles labelled 1, 2 and 3 with each bottle being used in turn for a week at a time. Bottle 2 contained 1 mg of fluoride per tablespoonful, this code being known only by the pharmacist who prepared the bottles. On the fourth day on bottle 2 he developed generalized pruritis and oedema in the distal joints of his extremities. Nevertheless he continued taking the water from bottle 2 for another three days during which time he developed hives on the right elbow and pains in the lumbo-sacral area followed by an outbreak of generalized urticaria. These symptoms disappeared 2 days after the patient discontinued the use of bottle 2 (11).

In a second case the ætiological role of fluoride was confirmed using a patch test (11). The patient, a 9-year-old female, had frequent urticaria, allergic conjunctivitis and minor asthmatic attacks. There had been constant episodes of ulcers distributed throughout the oral cavity. Slight abdominal tenderness was present. A fluoridated toothpaste had been used since the onset of the oral lesions. A patch test gave a two plus reaction to the fluoride toothpaste but not to chewing gum, Lifesavers, or a non-fluoride toothpaste. During the development of the positive patch test reaction the patient experienced a flare-up of the oral lesions associated with severe abdominal pain. After changing to a non-fluoride toothpaste the oral lesions as well as the

abdominal pains subsided completely. One year later a recurrence of the stomatitis occurred within 15 minutes of inadvertently brushing her teeth with a fluoridated toothpaste. Severe abdominal pain also occurred (11). Again in this case the guidelines of Kaplan (10) appear to have been followed and indicate that there is clinical evidence to show that a syndrome of fluoride allergy exists. Although the above cases refer to the use of fluoride tablets and toothpaste in contrast to the mention in the statement by Austen *et al* of fluorides as used in the fluoridation of community water supplies, this qualification is not mentioned earlier in the article by Austen *et al* (5). There it is stated that there is not sufficient clinical evidence to state that a true syndrome of fluoride allergy exists (5).

Urticaria is characterized by the appearance of pruritic, erythematous, cutaneous elevations that blanch with pressure, indicating the presence of dilated blood vessels and oedema (10). Urticaria, both local and generalized, was described with acute sodium fluoride poisoning by Lidbeck, Hill and Beeman (13). In 1959 Waldbott described six cases of urticaria due to fluoridated water (13). In one case, Mrs PO aged 40 years, the relation of the urticaria to fluoride in water was substantiated by a double-blind test (14). The patient was required to take a tablespoonful of water daily from three bottles labelled 1, 2 and 3, using each for a week at a time. One bottle contained 1 mg of fluoride per tablespoonful but neither the patient nor her attending physician knew which one it was. The urticaria reappeared on the third day of using the fluoride solution. Another patient, Mrs HP aged 48 years, had generalized urticaria which began three weeks after moving to a fluoridated area. On using water with a low amount of fluoride in hospital (0.1 ppm) the urticaria subsided. Within 24 hours of resuming using fluoridated water the urticaria recurred. An intradermal skin test with a 1:100 dilution of a 1% aqueous solution of sodium fluoride gave a 3-plus wheal reaction. This was followed by a generalized outbreak of urticaria within ten minutes. Control tests with a 1% solution of sodium bromide and sodium iodide were negative. With double-blind testing involving three bottles of water only one of which contained fluoride, urticaria recurred within two days of taking the water from the fluoride-containing bottle (14).

Contact dermatitis is a term used to describe any rash resulting from a substance touching the skin and as a synonym for allergic contact dermatitis (15). Allergic contact dermatitis is the result of a substance contacting skin that has undergone an acquired specific alteration in its reactivity (15). This altered reactivity is the result of prior exposure of the skin to the material eliciting the dermatitis or a chemically closely related substance (15). The patch test, whereby the suspected substance is applied to the skin under an occlusive dressing for one to two days and the test site observed after removal, remains the only practical test for demonstrating contact dermatitis (15). In 1948 Abelson reported a typical contact dermatitis with vesiculopapular pruritic lesions on the hand of a dentist occurring immediately upon application of a 2% solution of sodium fluoride to a patient's teeth (16). Waldbott reports observing repeatedly the same pattern of dermatitis in dentists with confirmation by patch testing (17). Waldbott (14) also described a scaly erythematous pruritic lesion on the thighs of a woman aged 20 years which subsided after moving for observation

to a nonfluoridated area. After she had been symptom-free the dermatitis recurred at the same site with papulous, vesicular lesions and intense pruritis within an hour of receiving a test dose of 6.8 mg of fluoride in 300 ml of water. A placebo test with 300 ml of distilled water produced no ill effect (14).

Aphthous stomatitis and ulcers of the mouth have been described as being not uncommon in persons using fluoride toothpaste and in children who have had topical fluoride applications applied to their teeth (14). Douglas (18) has described 133 cases of stomatitis from fluoride containing toothpaste. All the lesions were refractory to antibiotic therapy and local medication. The lesions cleared up with changing to a nonfluoride toothpaste. In 32 patients the stomatitis was reproduced by applying the fluoride toothpaste, in some as often as six times (18). Waldbott (14) records the case of Mrs LCH aged 62 years who developed a mouth ulcer within three days of starting the use of a fluoride toothpaste. Elimination of the fluoride toothpaste caused the condition to gradually disappear. Application of a saline solution with a cotton swab beneath her tongue produced no ill effect. When a 1% aqueous solution of sodium fluoride was applied, there developed, within five minutes, a hyperæmic œdematous intensely pruritic lesion in the test area which extended into a large portion of the oral mucosa. A smear of the mucus from the area showed marked eosinophilia (14).

Waldbott (19) also reported the case of Mrs WEA aged 62 years who developed the allergic symptoms of rhinitis, allergic sinus disease and urticaria within hours of using fluoridated water with an intake of 1 to 2 mg a day. A typical allergic appearance of the nasal mucosa, eosinophilia and an allergic wheal followed the intradermal injection of 0.1 mg of sodium fluoride. Control injections with horse serum, saline solution and weaker aqueous dilutions of sodium fluoride had no adverse effect (19). Zanfagna (20) has reported on Mrs MET aged 48 years who developed acute generalized urticaria after drinking fluoridated water. A further attack was also traced to fluoridated water. It was stated that sensitivity to fluoride was confirmed by positive challenge tests (20).

Discussion

Currently allergy is considered to be synonymous with hypersensitivity in meaning (21). They usually refer to type 1 immediate hypersensitivity, mediated by specific IgE antibodies in genetically predisposed individuals and resulting in symptoms characteristic of eczema, urticaria, rhinitis, asthma and anaphylaxis, although it is noted that several types of allergic states encompass all the mechanisms described by Gell and Coombs (21).

Waldbott (14) saw a difference between reactions to fluoride due to the toxic action of the fluoride ion and allergic sensitivity. He pointed out that the degree of tissue damage from the toxic action of the fluoride ion has been seen to depend on numerous factors including the dose of the fluoride ion, the duration of the contact with the involved tissue, the pH of the intracellular and extracellular fluids, and the presence of calcium, magnesium and other metals. When in contact with fluids in an acid medium such as gastric juice, fluoride compounds tend to induce undissociated hydrofluoric acid which has a corrosive action. True allergic reactions, on the other

hand, can result from relatively insignificant doses and from short exposures. The presence of such allergic symptoms as urticaria, vasomotor rhinitis, dermatitis and eosinophilia, a prompt response to adrenaline, and occasionally positive skin and patch test reactions, point to allergy (14). As an example of the difference between allergy or hypersensitivity to a drug and intolerance to it, reactions to aspirin can be considered (7). Intolerance to aspirin is characterized by hæmorrhages in the stomach whereas allergy to aspirin results in such symptoms as hives, asthma, allergic nasal and sinus disease or even anaphylactic shock (7).

To establish the existence of allergy to fluoride, community studies which are prone to the ecological fallacy (22) are insufficient and stronger evidence based on the studies of individuals is required. Although in the above discussion reference is made to cases of allergy related to fluoride tablets and toothpaste, there are included cases (Mrs PO, Mrs HP, Mrs WEA, Mrs MET) in which the reaction of allergy has been to fluorides as used in the fluoridation of community water supplies.

Although Waldbott found that allergic reactions to fluoride could occur, it was not considered that this was the only mechanism whereby adverse reactions to fluoride were experienced (7). Intolerance to fluoride was seen to occur for example through the formation of corrosive undissociated hydrofluoric acid when fluoride ions were in contact with acidic gastric secretions.

This potential mechanism for fluoride damaging the gastroduodenal mucosa has been supported by Susheela *et al* (23) along with other potential mechanisms such as enzyme system inhibition. By studying patients intensively, including by endoscopy and biopsy for histopathological and scanning electron microscope examination, they found that the gastroduodenal mucosa could be severely damaged by the toxic effects of fluoride resulting in dyspeptic symptoms. The changes found included surface abrasions with loss of microvilli in the gastric antrum and duodenum, and a 'cracked-clay' appearance of the duodenal mucosa. Gastrointestinal discomfort, in the form of dyspeptic symptoms was thus seen to be an important diagnostic feature in identifying persons affected by fluoride and it was considered that such symptoms should not be dismissed as non-specific (23).

Moolenburgh (24) described abdominal discomfort occurring on a double-blind basis with exposure to fluoride. He found in his Dutch general practice patients with illnesses similar to those described by Waldbott. He considered that far from having exaggerated the side-effects, Waldbott had, on the contrary, been inclined to understatement. Although Moolenburgh expected to find an allergic basis for the adverse effects associated with fluoride, he considered that the symptoms represented poisoning with inhibition of the immune system by a toxic substance in sensitive persons. Where an exacerbation of illnesses with an allergic component such as eczema and asthma occurred, his view was that immune system inhibition by fluoride had resulted in a loss of the ability to cope with the allergy (24). The work by Moolenburgh and his colleagues has been described by Grimbergen (25). By double-blind testing with 60 patients he showed that certain individuals were intolerant to fluoride and that exposure to this could reproduce gastrointestinal symptoms,

stomatitis, joint pains, polydipsia, headaches and visual disturbances. Grimbergen noted that Young had found that intracutaneous injections of sodium fluoride gave positive reactions in four persons with urticaria associated with the use of fluoridated water but no such reactions in four persons without urticaria (25).

Petraborg (26,27) similarly described a wide spectrum of symptoms in 27 persons exposed to fluoridated water. He considered that since none of the persons were aware that their drinking water was fluoridated or were familiar with the manifestations of fluoride toxicity, that the accounts of their illnesses were equivalent in validity to those associated with double-blind procedures. He noted that several patients were not convinced that something in their drinking water was causing their illness and resumed drinking fluoridated water. Relapses of their illnesses followed. The symptoms included extreme chronic fatigue, polydipsia, general pruritis, headaches and gastrointestinal symptoms (26,27).

Another adverse effect of fluoride, described by Lee (28), involved an elevation of the serum bilirubin level in six patients with Gilbert's disease. Long-term testing and studying the effect of fluoride tablets in one patient gave evidence that the hyperbilirubinæmia was due solely to fluoride and not to some other ingredient of the water supply. An enzyme-inhibiting action by fluoride was considered to be the most likely mechanism involved (28).

It is concluded, on the basis of the above examination, that the recent North American, Australian and New Zealand reviews (1-4) were seriously incomplete in their coverage of the literature. There are some individuals in whom allergy or hypersensitivity to fluoride has been demonstrated by appropriate challenge tests. This is seen to be just one of a number of mechanisms whereby adverse reactions to fluoride occur. It is considered that intolerance to fluoride may also follow the formation of corrosive hydrofluoric acid or through enzyme inhibition.

References

1 Subcommittee on fluoride of the committee to coordinate environmental health and related programs. *Review of Fluoride: Benefits and Risks*. Department of Health and Human Services, Public Health Service Washington 1991.

2 Wagner BM, Burt BA, Cantor KP *et al* (Subcommittee on health effects of ingested fluoride, Committee on Toxicology, Board on Environmental Studies and Toxicology, Commission on Life Sciences, National Research Council). *Health Effects of Ingested Fluoride*. National Academy Press, Washington DC 1993 pp 8-9.

3 National Health and Medical Research Council. *The Effectiveness of Water Fluoridation*. Australian Government Publishing Service, Canberra 1991.

4 Public Health Commission. Fluoridation of water supplies: draft policy statement. Public Health Commission, Wellington 1993.

5 Austen KF, Dworetzky M, Farr RS *et al*. A statement on the question of allergy to fluoride as used in the fluoridation of community water supplies [editorial]. *Journal of Allergy* 47 347-348 1971.

6 Waldbott GL. Fluoridation of community water supplies [letter]. *Journal of Allergy and Clinical Immunology* 48 253-254 1971.

7 Waldbott GL, Burgstahler AW, McKinney HL. *Fluoridation: the great dilemma.* Coronado Press, Lawrence, Kansas 1978.
8 Waldbott GL. *A Struggle With Titans.* Carlton Press, New York 1965.
9 Feltman R, Kosel G. Prenatal and postnatal ingestion of fluoride: fourteen years of investigation; final report. *Journal of Dental Medicine 16* 190-198 1961.
10 Kaplan AP. Urticaria and angioedema. In: Middleton E, Reed CF, Ellis EF (Eds). *Allergy: Principles and Practice.* vol 2 2nd ed CV Moseby, St. Louis 1983 pp 1341-1360. 11 Shea JJ, Gillespie SM, Waldbott GL. Allergy to fluoride. *Annals of Allergy 25* 388-391 1967.
12 Lidbeck WL, Hill IB, Beeman JA. Acute sodium fluoride poisoning. *Journal of the American Medical Association 121* 826-827 1943.
13. Waldbott GL. Urticaria due to fluoride. *Acta Allergologica 13* 456-468 1959.
14. Waldbott GL. Allergic reactions to fluoride. *Journal of Asthma Research 2* 51-64 1964.
15. Maibach HI, Epstein E. Contact dermatitis. In: Middleton E, Reed CF, Ellis EF (Eds). *Allergy: Principles and Practice.* vol 2 2nd ed CV Moseby, St. Louis 1983 pp 1313-1339.
16. Abelson JH. Case of hypersensitivity to sodium fluoride in a dentist. *Chicago Dental Society Fortnightly Review 16* 6 1948.
17 Waldbott GL. *Contact dermatitis.* CC Thomas, Springfield 1953.
18 Douglas TE. Fluoride dentifrice and stomatitis. *Northwestern Medicine 56* 107-139 1957.
19 Waldbott GL. Fluoride in clinical medicine. *International Archives of Allergy and Applied Immunology 20* (Suppl 1) 1-60 1962.
20 Zanfagna PE. Allergy to fluoride. *Fluoride 9* 36-41 1976.
21 Fadal RG, Nalebuff DJ, Ali M. The importance of total and allergen-specific IgE measurements. In: Johnson F, Spencer JT (Eds). *Allergy: Immunology and Medical Treatment.* Symposia Specialists, Miami 1980 pp 15-28.
22 Morgenstern H. Uses of ecologic analysis in epidemiologic research. *American Journal of Public Health 72* 1336-1344 1982.
23. Susheela AK, Das TK, Gupta IP *et al.* Fluoride ingestion and its correlation with gastro-intestinal discomfort. *Fluoride 25* 5-22 1992.
24. Moolenburgh H. *Fluoride: The Freedom Fight.* Mainstream Publishing, Edinburgh 1987 pp 65-66, 146-147.
25. Grimbergen GW. A double blind test for determination of intolerance to fluoridated water (preliminary report). *Fluoride 7* 146-152 1974.
26. Petraborg HT. Chronic fluoride intoxication from drinking water (preliminary report). *Fluoride 7* 47-52 1974.
27. Petraborg HT. Hydrofluorosis in the fluoridated Milwaukee area. *Fluoride 10* 165-169 1977.
28. Lee JR. Gilbert's disease and fluoride intake. *Fluoride 16* 139-145 1983.

Published by the International Society for Fluoride Research
Editorial Office: 216 Atkinson Road, Titirangi, Auckland 7, New Zealand

 Fluoride Vol.26 No.4 274-277 1993

FLUORIDATION AND HIP FRACTURE

ACCORDING TO THE NATIONAL RESEARCH COUNCIL REPORT: "Health Effects of Ingested Fluoride" *

John R Lee
Sebastopol, California, USA

Fluoride's bone effect is considered by most authorities world-wide to be toxic: bone density may increase but histomorphologic studies reveal areas of bone destruction, and fracture rates, especially of the hip, increase. The NRC* reviewed three US studies (1-3) and one European study (4) of osteoporosis "therapy" using sodium fluoride (NaF) in doses of 50-80 mg (20-32 mg of fluoride) daily, equivalent to 5-10 times the daily fluoride intake of persons living in fluoridated communities. They report that in all three US studies hip fracture rates increased significantly compared to controls and that, in all the studies, periarticular joint pain and gastrointestinal side effects afflicted the fluoride-treated groups. The NRC stated no conclusion from these findings. However, in an independent review of "therapeutic" fluoride treatments, Dr Avioli, Shoenberg Professor of Medicine and Director, Division of Endocrinology and Mineral Metabolism, at the Washington University School of Medicine, concluded that "sodium fluoride is accompanied by so many medical complications and side effects that it is hardly worth exploring in depth as a therapeutic mode for post-menopausal osteoporosis" (5). Not mentioned by the NRC was the Hedlund and Gallagher study (6) which likewise found a fluoride-induced increase in hip fracture.

The NRC also reviewed 10 studies comparing fracture incidence relative to fluoridation status. *Seven* (8,10-13,16,17) found a positive correlation (*i.e.*, increased hip fracture incidence) of fluoridation and hip fracture while *three* (7,14,15) reportedly did not. *None* reported any fluoride bone benefit. Two of the seven positive studies were presentations made at the Workshop on Drinking Water Fluoride Influence on Hip Fractures and Bone Health (April 10 1991, Bethesda MD) and all of the remaining five were published in major peer-reviewed journals. In spite of this array of evidence linking fluoridation to hip fracture, the NRC concluded that the three negative studies were of sufficient relevance to out-weigh the findings of the seven positive studies. Let us look at the three studies that reported no fluoride effect.

The first, by Cauley *et al* and referenced only by an abstract in the *American Journal of Epidemiology*, looked at 1,878 white women aged 65-93 years (mean age: 70.9) only 73% of whom had exposure to public drinking water, with a mean exposure time of only 6.0 years. Since bone turnover (remodelling) rate is relatively rapid before menopause and slow after menopause, fluoride's major effect on bone is most likely to occur during the years before menopause (*i.e.*, before age 45-50), as was clearly shown in Danielson's study. Thus, *none* of the women in this study were exposed to fluoride during the time in their life when fluoride would be expected to affect bone. The study, therefore, is useless and should be discarded.

Dr John R Lee, 9620 Bodega Highway, Sebastopol, California 95472, USA.

* For summary of contents of NRC report see Press Release on pages 278-279.

The second, the last of the Jacobsen studies reviewed by the NRC, and reported as a Public Health Brief in the *American Journal of Public Health*, suffers the same problem, *i.e.*, few if any of the women studied were exposed to fluoridated water prior to menopause. For reason unknown, he reviewed data only through 1969, the ten years after fluoridation of Rochester MN, the site of the Mayo Clinic. Where were the data through 1979 or 1989? The Mayo Clinic is good at keeping such data. By using those, he could have observed the hip fracture incidence of women who had been premenopausal prior to being fluoridated. Even worse is the fact that the population studied (< 50,000) was just too small. During the 20 years covered by the study, there were only 383 hip fractures in women and 268 in men; *i.e.*, less than 20 per year among women and only about 1 per month among men. The annual variation in the incidence of hip fracture prior to fluoridation was over 100% and the range of standard error (95% confidence interval) was approximately 300%. If one were looking for, say, a 40% fluoride-induced increase in fracture incidence, it could not have been found. The authors' own conclusions state that fluoridation in these age groups was "not associated with an *immediate increase* in rates of hip fractures" (emphasis added) and that "further studies .... are clearly required before public policy decisions can be made." It has been reported that, when Jacobsen himself was asked if his study could be used to prove whether or not fluoride had a deleterious effect on fracture rates, his answer was "No". This study, too, must be discarded.

The third, a 28-year-old study by Goggin *et al*, looked at the hip fracture rates of women over 60 years of age for the 5 years before and after fluoridation of Elmira NY, in 1960 when the population was only 46,517 (similar to Rochester MN). Again we see that 1) the women had all passed through menopause prior to fluoridation, and 2) the number of women in the study is too small for the purpose of the study. Therefore, this study, too, is useless and must be discarded.

Interestingly, the *Lancet* of July 24 1993, in their section Facts, Figures, and Fallacies, carries a timely article entitled "Is the study worth doing?". It points out the elementary statistical fact that *the degree of uncertainty increases with decreasing sample size* and states: "studies that are too small may fail to detect medically important effects or produce estimates too imprecise to be useful". It is apparent that NRC failed to employ the services of any competent statistician in even including these three useless studies for their consideration.

With seven studies showing a positive correlation of fluoridation with increased hip fracture incidence and not one acceptable study showing the contrary, on what basis does the NRC conclude that the fluoride MCL (maximum contaminant level) be left at 4 mg/L? They argue, without the least evidence, that there exists a "publication bias" in favor of ecologic studies showing positive results. Even if true, what does it matter? A study can either stand on its merits or it can't. They do not mention that public health reports are routinely published as a "public service" by legitimate medical journals *without any independent peer review*. Their second argument is that, because of un-named "limitations of ecologic analyses" and "the potential in all studies for confounding factors", the positive studies "offer only limited support for a hypothesis of a weak association between fluoridated water and hip fracture." They do admit, however, that "when results from a number

of such studies converge to indicate an exposure-disease relation, confidence in collected findings is bolstered." In a remarkable feat of denial, they then conclude "there is no basis at this time to recommend that EPA lower the current MCL of fluoride of 4 mg/L."

Further, they call for additional studies that "should use information from individuals rather than population groups," stating that "it is important that individual information be collected about fluoride intake from drinking water and all other sources" plus hormone status, dietary factors, and other factors that "might influence the risk." If the NRC truly believed that, they should be calling for the throwing out of all the previous dental caries studies as well since the same argument applies.

This obvious *selective reasoning* does not speak well of the NRC report. Such deviousness is employed not for truth finding but for political ends and, as such, is a disservice not only to the cause of science but to the people of the US. Given the data available, it is impossible to conclude that fluoride is safe. When the NRC concludes otherwise, one must question whether they are competent to evaluate scientific studies, or whether they are guilty of a deliberate lie. If merely the data of this one chapter of the report (chapter 3) is properly analyzed, the only responsible conclusion is that 1) total daily fluoride exposure should be reduced, 2) the MCL for fluoride should be lowered, and 3) the addition of fluoride to public drinking water should be ended.

References

1 Dambacher MA, Ittner J, Ruegsegger P. Long-term fluoride therapy of postmenopausal osteoporosis. *Bone 7* 199-205 1986.

2 Riggs BL, Hodgson SF, O'Fallon WM *et al.* Effect of fluoride treatment on the fracture rate in postmenopausal women with osteoporosis. *New England Journal of Medicine 322* 802-809 1990.

3 Kleerekoper ME, Peterson E, Phillips E *et al.* Continuous sodium fluoride therapy does not reduce vertebral fracture rate in postmenopausal osteoporosis (Abstract). *Journal of Bone and Mineral Research 4* (Suppl 1) S376 1989.

4 Mamelle N, Dusan R, Martin JL *et al.* Risk-benefit ratio of sodium fluoride treatment in primary vertebral osteoporosis. *Lancet 2* 361-365 1988.

5 Avioli LV. Fluoride treatment of osteoporosis. *Postgraduate Medicine: special report* 26-27 14 Sept 1987.

6 Hedlund LR, Gallagher JC. Increased incidence of hip fracture in osteoporotic women treated with sodium fluoride. *Journal of Bone and Mineral Research 4* 223-225 1989.

7 Cauley JA, Murphy PA, Riley T, Black D. Public health bonus of water fluoridation: Does fluoridation prevent osteoporosis and its related fractures? (Abstract) *American Journal of Epidemiology 134* 768 1991.

8 Sowers MFR, Clark MK, Jannausch ML, Wallace RB. A prospective study of bone mineral content and fracture in communities with differential fluoride exposure. *American Journal of Epidemiology 133* 649-660 1991.

9 Sowers MFR, Wallace RB, Lemke JH. The relationship of bone mass and fracture history to fluoride and calcium intake: a study of three communities. *American Journal of Clinical Nutrition 44* 889-898 1986.

10 Keller C. Fluorides in drinking water. Paper presented at the Workshop on Drinking Water Fluoride Influence on Hip Fractures and Bone Health. Bethesda MD April 10 1991.
11 May DS, Wilson MG. Hip fractures in relation to water fluoridation: an ecologic analysis. Paper presented at the Workshop on Drinking Water Fluoride Influence on Hip Fractures and Bone Health. Bethesda MD April 10 1991.
12 Jacobsen SJ, Goldberg J, Miles TP *et al.* Regional variation in the incidence of hip fracture among white women aged 65 years and older. *Journal of the American Medical Association 264* 500-502 1990.
13 Jacobsen SJ, Goldberg J, Cooper C, Lockwood SA. The association between water fluoridation and hip fracture among white women and men aged 65 years and older. A national ecologic study. *Annals of Epidemiology 2* 617-626 1992.
14 Jacobsen SJ, O'Fallon WM, Melton III IJ. Hip fracture incidence before and after fluoridation of the public water supply, Rochester, Minnesota. *American Journal of Public Health 83* 743-745 1993.
15 Goggin JE, Haddon W, Hambly GS, Hoveland JR. Incidence of femoral fractures in postmenopausal women. *Public Health Reports 80* 1005-1012 1965.
16 Danielson C, Lyon JL, Egger M, Goodenough GK. Hip fractures and fluoridation in Utah's elderly population. *Journal of the American Medical Association 268* 746-774 1992.
17 Cooper C, Wickham CAC, Barker DJR, Jacobsen SJ. Water fluoridation and hip fracture. (Letter) *Journal of the American Medical Association 266* 513-514 1991.

Published by the International Society for Fluoride Research
Editorial Office: 216 Atkinson Road, Titirangi, Auckland 7, New Zealand

HEALTH EFFECTS OF INGESTED FLUORIDE

Bernard M Wagner, Brian A Burt, Kenneth P Cantor, Daniel Krewski, Steven M Levy, Ernest Eugene McConnell and Gary M Whitford (Subcommittee)*

Press Release issued August 17 1993 by **National Research Council**
(National Academy of Science, National Academy of Engineering, Institute of Medicine)
Washington DC, USA

Currently allowed fluoride levels in drinking water do not pose a risk of health problems such as cancer, kidney failure, or bone disease, concludes a report* released today by the National Research Council. Based on a review of available data on fluoride toxicity, the subcommittee that wrote the report concluded that the Environmental Protection Agency's (EPA) ceiling of 4 ppm (parts per million) for fluoride in drinking water is "appropriate as an interim standard."

"More research is needed on patterns of fluoride exposure from other sources such as dental products and foods, as well as fluoride's effects on bone strength and tooth enamel," said subcommittee chair Bernard M Wagner, research professor of pathology at New York University School of Medicine. "The current standard should then be reviewed and changed, if necessary, based on the results of that research. "

Despite conflicting results from other studies and limited data on possible links between fluoride and the risk of hip or other bone fractures, the subcommittee concluded that there is "no basis" to recommend that EPA lower the current standard for fluoride in drinking water.

At the current EPA ceiling for fluoride in drinking water, a small percentage of the population will have staining or pitting of tooth enamel, known as dental fluorosis. The question of whether to consider this a cosmetic effect or an adverse health effect "is one for the regulatory agencies to decide," Wagner said.

But the report finds that scientific evidence does not link the currently permitted level of fluoride to the following health problems in humans:

» Cancer. Available laboratory data do not demonstrate a carcinogenic effect of fluoride in animals, the subcommittee said. Moreover, "the weight of the evidence from more than 50 epidemiologic studies does not support the hypothesis of an association between fluoride exposure and increased cancer risk in humans."

» Kidney disease. The threshold dose of fluoride in drinking water that produces kidney effects in animals is approximately 50 ppm - more than 12 times the maximum level allowed by EPA. The subcommittee therefore concluded that "ingestion of fluoride at currently recommended concentrations will not produce kidney toxicity in humans."

» Stomach and intestinal problems. Adverse gastrointestinal effects are "not likely" to result from the concentrations of fluoride found in drinking water in the United States, the subcommittee concluded.

---

* The subcommittee's report, *Health Effects of Ingested Fluoride*, is available from: Office of News and Public Information, 2101 Constitution Avenue NW, Washington DC, USA. The cost of the report is US$35 plus $4 shipping for the first copy and $.50 for each additional copy.

» Infertility and birth defects. Drinking fluoridated water at current concentrations should have "no adverse effects" on human reproduction, the subcommittee said. Fluoride concentrations associated with infertility and birth defects in animals are "far higher."

» Genetic mutations. In several laboratory tests, fluoride has caused mutations and chromosomal damage in animal cells. But the smallest amount that produced these effects was more than 100 times greater than the average amount found in most people in the United States, offering a "large margin of safety," the subcommittee said.

Fluoride was first added to U.S. drinking water during the 1940s in Grand Rapids, Michigan. According to the U.S. Centers for Disease Control and Prevention, approximately 132 million Americans now receive drinking water that contains fluoride at concentrations higher than 0.7 ppm, the lowest concentration thought to be effective in preventing tooth decay.

Since 1962, the optimal concentration of fluoride in drinking water for U.S. communities has been set at 0.7 to 1.2 ppm depending on the average local temperature, based on the assumption that in warmer climates people drink more water. That range provides a balance between effective cavity prevention and the incidence of dental fluorosis.

In addition to ingesting fluoride in water, people receive fluoride from a large number of other sources such as toothpaste, mouth rinse, soft drinks, tea, processed foods, and vegetables. Fluoride is also consumed in food when fluoridated water is used during cooking. The report notes that fluoride intake from sources other than drinking water can be significant for some individuals, especially young children who frequently swallow toothpaste or mouth rinse containing fluoride.

The project was supported by the U.S. Environmental Protection Agency.

The National Research Council is the principal operating agency of the National Academy of Sciences and the National Academy of Engineering. It is a private, nonprofit institution that provides science and technology advice under a congressional charter.

IMMEDIATE COMMENT ON ABOVE PRESS RELEASE

Press Release issued August 18 1993 by William Hirzy, EPA scientist, on behalf of EPA scientists, lawyers and other professionals*

The union representing EPA professionals has had an interest in EPA's rulemaking activities regarding fluoride since 1985. At that time, a scientist came to the Union complaining that he was being forced to write things he did not believe were ethical in order to keep his job.

Since that time the Union has fought against the institutional distortions of science that have characterized the federal government's actions on hazards associated with exposure to fluoride ion. The report issued yesterday by the National Academy of Sciences, *Health Effects of Ingested Fluoride*, does little to change the Union's opinion about the improper use of science in this matter. (continued next page)

* Dr William Hirzy, President, National Federation of Federal Employees, Local 2050. For more information telephone 202-260-2383.

The failure of NAS to include on the assessment group such active researchers in the field as Drs A K Susheela, John Lee, John Colquhoun, Mark Diesendorf and Edward Czerwinski, in favor of a long list of National Institute of Dental Research grantees, makes the NAS/NRC report suspect from the outset. NIDR is a major promoter of water fluoridation in the government.

Since NAS refused to give me a copy of the report yesterday, I am unable to comment on it in detail at the moment. However, based on the oral summary of it given by NAS to the Drinking Water Committee of EPS's Science Advisory Board and the press release that accompanied the report, I can draw some conclusions about it.

First, the fear I expressed last April to the SAB - during a NAS progress report on its assessment - that the assessment would not be objective, seems to have been well founded. During the April meeting of the EPA's Science Advisory Board I expressed concern that the cycle of producing only politically acceptable science on fluoride was continuing. That seems to have been just what happened.

One small, but significant example, discernable even without having had a chance to read the entire document, is how the press release and oral presentation yesterday used media spin control to deal with the issue of dental fluorosis. (Dental fluorosis is a condition in which teeth become progressively more damaged, beginning with slight mottling of the enamel and progressing to staining, pitting, cracking and breaking of the tooth.)

Yesterday, NAS stated that the EPA standard of 4 ppm was "appropriate" and would probably result in a small fraction of the population incurring the more severe forms of dental fluorosis. NAS spokesmen and the press release stated that the question of whether dental fluorosis is an adverse health effect, "is one for the regulatory agencies to decide", implying - for media consumption - that no conclusions had been reached on this issue, and that it wasn't very important. But in a May 1992 meeting of the NAS assessment committee which I attended, the members stated a consensus that severe dental fluorosis (the staining, pitting, and cracking stage) ***is*** an adverse health effect. I asked the NAS representatives after their presentation yesterday what had changed to alter their consensus of May, 1992, and I was told by Committee Chairman Dr Bernard Wagner, that it hadn't changed at all! Dr Wagner then showed me, buried deep in the report, an equivocal statement to the effect that dental fluorosis severe enough to affect food choices, etc. was a real problem.

In this way, the NAS Committee keeps its backside covered with some allusion to the adverse nature of an effect *expected to occur* in some people at the "appropriate" level of fluoride in drinking water.

This logical and ethical conundrum appears to have caused little problem for the NAS Committee. It probably will cause very large problems for the maintenance of the "appropriate" 4 ppm standard by EPA. The Safe Drinking Water Act requires EPA to set enforceable standards that protect against adverse health effects with an adequate margin of safety. If NAS ***expects*** the 4 ppm standard to result in induction of severe dental fluorosis in some people, then the standard must be abandoned.

This one example points out - and I am sure that after studying the report in detail others will surface - why EPA chose to once again contract out the job of assessing fluoride risks, rather than give the job to sworn-to-duty Civil Service scientists. An honest assessment of risks might lead to publicity that could damage the Public Health Service's long-standing program of trying to convince Americans to fluoridate all public water supplies.

This situation exacerbates the sense of frustration among EPA scientists, who are offended by the Agency's buying science, which implies that EPA employees are unworthy of performing such an important piece of work.

FURTHER COMMENT

Press Release issued August 19 1993 by Robert Carton
former EPA scientist, now Editor of *The Fluoride Report*•

The "Clean Bill of Health" Report on Fluoride, released by a selected Panel of the National Research Council of the National Academy of Sciences (NAS) on August 17, proclaiming fluoride in drinking water to be safe and not linked to cancer, diseases and other ill effects, is propaganda masquerading as science. The NAS undermined its own claims of safety of the current EPA standard by admitting to shocking new evidence that fluoride causes bone fractures in the elderly and bone cancer in young males, that severe dental fluorosis is on the rise in young children, and by admitting that they are completely baffled about how much fluoride people are exposed to from all sources: their diet, mouthrinses, tooth paste, *etc.* Instead of calling for an immediate end to the practice of fluoridation *and more research*, the National Research Council recommends continuing the massive human experiment of fluoridation, while conducting research to see if the practice is causing harm. This recommendation amounts to human experimentation without informed consent, and should be condemned.

The EPA, which has jurisdiction over fluoride in drinking water, was required by law to review the levels of fluoride allowed in public water supplies three years after its drastic raising of the maximum contaminant level of fluoride in 1985. Instead, they have been delaying their investigation and delegating their duties to others. Money was given to NAS to conduct a review of the latest fluoride studies and give their recommendation to EPA. Fluoridation critics questioned from the beginning the validity of the outcome, based on the biased panel that NAS formed. Many of the panel members have received grant money from the National Institute of Dental Research, and *none* of the scientists who are concerned about fluoride's health effects were permitted on the panel. NAS and NRC are included on lists of endorsements of fluoridation for many years, while purporting to be "independent entities."

•Dr Robert Carton, Editor of *The Fluoride Report*, PO Box 219, Buckeystown MD 21717, USA (Annual Subscription US$10).

RADIOLOGICAL SPECTRUM OF ENDEMIC FLUOROSIS: RELATIONSHIP WITH CALCIUM INTAKE

A Mithal, N Trivedi, S K Gupta, S Kumar and R K Gupta
Lucknow, India

Abstract from *Skeletal Radiology* 22 257-261 1993

Skeletal fluorosis continues to be endemic in many parts of India. Osteosclerosis and interosseous membrane calcification have long been regarded as hallmarks of this disease. Our study showed in addition a wide variety of radiological patterns: course trabecular pattern, axial osteosclerosis with distal osteopenia and diffuse osteopenia. Subjects with osteopenic changes had a significantly lower dietary intake of calcium than those groups having normal radiological findings, predominant osteosclerosis or course trabecular pattern ($p<0.001$, $p<0.01$, and $p<0.01$ respectively). This suggests the role of calcium intake in determining the skeletal changes in endemic fluorosis.

Key words: Calcium; Endemic fluorosis; Fluoride; Osteopenia; Osteosclerosis; Protein.
Reprints: Ambrosia Mithal MD, Department of Medical Endocrinology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, PO Box 375, Lucknow 226001, India.

SKELETAL SCINTIGRAPHIC FINDINGS IN ENDEMIC SKELETAL FLUOROSIS

S K Gupta, S Gambhir, A Mithal and B K Das
Lucknow, India

Abstract from *Nuclear Medicine Communications 14* 384-390 1993

Endemic skeletal fluorosis is characterized by bone, joint and muscle pain, progressive ankylosis of various joints and crippling deformities. Whole body skeletal scintigraphy with $^{99}Tc^{m}$-methylene diphosphate was performed for 17 symptomatic subjects suffering from this disorder. The fluoride content of drinking water ranged from 4.1 to 12.9 mg l^{-1} (normal <1 mgl^{-1}). Urinary and serum fluoride levels were markedly elevated. Serum calcium (total and ionized), inorganic phosphorus, creatinine and albumin were essentially normal while serum alkaline phosphatase was elevated in six subjects (mean ± S.D. 206 ± 106; range 22-1072 IU l^{-1}). Skeletal radiology revealed a wide spectrum of bony abnormalities. Skeletal scintigraphy revealed a picture similar to metabolic 'superscan' in all subjects, *i.e.* increased tracer uptake in axial and appendicular skeleton, reduced soft tissue uptake, poor or absent renal images, prominent costochondral junction and 'tie' sign in sternum. Increased uptake was present in all subjects irrespective of age, water fluoride content, serum alkaline phosphatase level and radiological abnormalities. Our findings suggest the presence of a high bone turnover state in endemic skeletal fluorosis irrespective of other variables.

Key words: Endemic fluorosis; Radiology; Scintigraphy; Skeletal fluorosis.
Reprints: Ambrish Mithal MD, Department of Medical Endocrinology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, PO Box 375, Lucknow 226001, India.

SELECTIVE INHIBITION OF COLLAGEN SYNTHESIS BY FLUORIDE IN HUMAN PULP FIBROBLASTS *INVITRO*

M H Veron, M L Couble and H Magloire
Lyon, France

Abstract from *Calcified Tissue International 53* (1) 38-44 1993

Human dental pulp cells were cultured in fluoridated mediums (0, 1, 10, 25 ppm) in order to study the biological effect of the ion regarding the cellular metabolism: cell growth, alkaline phosphatase (ALP) activity, and protein synthesis. The results indicated a decrease of the cell growth at 25 ppm and a dose-dependent decrease of the ALP activity. Type I collagen immunoperoxidase staining, radioimmunoassay quantisation, and analysis of type I and III collagens mRNA levels showed an inhibition of collagen production and gene expression. In contrast, fibronectin production and gene expression were not affected by fluoride. The treatment did not influence the qualitative pattern of the different mRNA species. Of the three collagen chains, the alpha1(I) was the most affected. These data suggest that fluoride does not exert a general depletive effect on human dental pulp cells but rather a selective inhibition on collagen production.

Key words: Collagen; Fibronectin; Fluoride; Human dental pulp cell.
Reprints: M H Veron, Faculté Odontologie Lyon, CNRS, Upr 412, Rue Guillaume Paradin, F-69372 Lyon 8, France

DIETARY MODULATION OF THE SYMPTOMS OF CADMIUM TOXICITY IN RATS - EFFECTS OF VITAMIN-A, VITAMIN-C, VITAMIN-D, VITAMIN-D HORMONE, AND FLUORIDE

E W Pleasants, C Waslien and B A Naughton
Leonia, New Jersey, USA

Abstract from *Nutrition Research 13* (7) 839-850 1993

The effects of vitamins A, C, D, and D hormone (1, 25-dihydroxyvitamin D3), and fluoride and combinations of these micronutrients on the toxic symptoms of young rats exposed to 80 ppm cadmium (Cd^{2+}) in the drinking water and fed a modified AIN-76 diet were studied for 14 weeks. Cd^{2+} treated rats displayed smaller body weight gains and larger relative kidney and testis weights than controls. Treatment of Cd^{2+} exposed rats with vitamins A or D ameliorated these symptoms. When combined, these two vitamins were synergistic in this protection as was the combination of vitamin A and 1,25-dihydroxyvitamin D3. Animals exposed to Cd^{2+} and treated with vitamin A or a combination of A and D had lower femur dry weights than rats exposed to Cd^{2+} alone but this effect was not seen with the combination of vitamin A and 1,25-dihydroxyvitamin D. Vitamin C had no protective action on the kidneys or testes but caused an increase in the dry weight of the femurs of the exposed rats. Fluoride partially reduced the weight depression of the Cd^{2+} exposed rats at certain time periods and lowered the relative weights of the testes in these animals. Fluoride, in combination with 1,25-dihydroxyvitamin D3, significantly increased the dry and

mineral (ash) weights of the femurs of the Cd^{2+} exposed rats above that of the controls, a finding which may be relevant to the treatment of osteoporosis. Exposure of rats to Cd^{2+} significantly depressed the hematocrits and erythrocyte counts and altered the ratio of granuloid to mononuclear peripheral leukocytes. Treatment of Cd^{2+} exposed rats with either vitamin A and 1,25-dihydroxyvitamin D3 or vitamins A and 1,25-dihydroxyvitamin D3 and fluoride appeared to lessen the hematotoxicity associated with Cd^{2+} exposure.

Key words: Cadmium; Fluoride; Osteoporosis; Vitamin-A; Vitamin-C; Vitamin-D(3).
Reprints: E W Pleasants, 27 Linden Terrace, Leonia, NJ 07605, USA.

PLASMA INORGANIC FLUORIDE LEVELS WITH SEVOFLURANE ANESTHESIA IN MORBIDLY OBESE AND NONOBESE PATIENTS

E J Frink, T P Malan, E A Brown, S Morgan and B.R Brown
Tucson, Arizona, USA

Abstract from *Anesthesia and Analgesia 76* (6) 1333-1337 1993

Administration of several of the inhaled anesthetics result in plasma inorganic fluoride concentrations that are higher in obese compared to nonobese patients. Sevoflurane, a new inhaled anesthetic, is metabolized to inorganic fluoride; however, plasma inorganic fluoride levels with sevoflurane anesthesia in obese subjects have not been evaluated. We studied plasma inorganic fluoride concentrations during and after sevoflurane surgical anesthesia in morbidly obese (n = 13, body mass index > 35) and nonobese (n = 10) patients. Sevoflurane anesthesia in 60% nitrous oxide/40% oxygen was administered with a semiclosed circle absorption system. Mean anesthetic duration was 1.4 minimum alveolar anesthetic concentration (MAC) hours (sevoflurane MAC = 2.05%) for both groups. Pre- and postoperative blood urea nitrogen, creatinine, and liver function tests were evaluated. Venous blood samples were obtained during and after anesthesia for plasma inorganic fluoride analysis. In six morbidly obese and nonobese patients arterial blood samples were obtained during and after sevoflurane anesthesia for determining sevoflurane blood concentration. Plasma fluoride concentrations during and after anesthesia did not differ between morbidly obese and nonobese groups. Peak plasma inorganic fluoride ion concentrations were 30 ± 2 μmol/L (mean ± SEM) in obese and 28 ± 2 μmol/L in nonobese patients 1 h after discontinuing anesthesia. The hourly rate of change of fluoride ion concentration in plasma during anesthesia was similar between the groups. The maximal recorded plasma fluoride concentrations were 49 μmol/L in an obese patient and 42 μmol/L in a nonobese patient. Pre-and postoperative hepatic and renal tests did not differ significantly in either group. Sevoflurane biotransformation and plasma fluoride levels seem to be similar in morbidly obese and nonobese surgical patients. Therefore, obese patients seem to be at no greater risk for possible fluoride-induced renal injury than nonobese patients.

Key words: Halothane anesthesia; Plasma fluoride; Sevoflurane.
Reprints: E J Frink, Arizona Health Sciences Center, Department of Anesthesiology, University of Arizona, 1501 N Campbell Ave, Tucson, AZ 85724, USA.

EVIDENCE THAT FLUORIDE THERAPY INCREASES TRABECULAR BONE DENSITY IN A PERIPHERAL SKELETAL SITE

H Resch, C Libanati, S Farley, P Bettica, E Schulz and D J Baylink
Loma Linda, California, USA

Abstract from *Journal of Clinical Endocrinology and Metabolism 76* (6) 1622-1624 1993

We measured the spinal bone density (SBD) and femora con bone density (FCD) in normal and osteoporotic females (n = 219) both before and during fluoride therapy. SBD and FCD in untreated osteoporotics were significantly lower ($P < 0.05$) than those in the age-matched controls. SBD and FCD were correlated in the untreated ($r = 0.62$; $P < 0.0001$) as well as in the fluoride-treated osteoporotics ($r = 0.42$; $P < 0.0001$). SBD and FCD were significantly increased ($P < 0.05$) in response to fluoride therapy. The average rates of increase in FCD and SBD were similar (1.3 ± 1.3 vs. 1.24 ± 1.4 mg/cc-month). We conclude that the osteogenic action of fluoride is not limited to the axial skeleton. An increase in trabecular bone density also occurs at peripheral weight-bearing sites such as the femoral condyle.

Key words: Fluoride therapy; Osteoporosis; Trabecular bone density.
Reprints: H Resch, Jerry L Pettis Medical Center, 11201 Benton St, Loma Linda, CA 92357, USA.

BIOAVAILABILITY AND PHARMACOKINETICS OF FLUORIDE FROM 2 GLUTAMINE MONOFLUOROPHOSPHATE PREPARATIONS

G Warneke and I Setnikar
Monza, Italy

Abstract from *Arzneimittel-Forschung/Drug Research 43-1* (5) 584-590 1993

A two-way cross-over study was conducted on 12 Caucasian male healthy volunteers aged between 25 and 38 years in order to determine the bioavailability and pharmacokinetics of fluoride after single oral administration in fasting conditions of two products (tablets and powder for oral use) of L-glutamine monofluorophosphate (G-MFP CAS 116420-36-1). The two products contained the equivalent of 10 mg F and the equivalent of 300 mg CA as calcium gluconate and calcium citrate. The two products were found bioequivalent with regard to the release of fluoride, both on the basis of the AUC and C(max) of fluoride in plasma and of the urinary excretion of fluoride during the 48 h following the administration. The pharmacokinetics of fluoride in plasma is characterized by a short lag time (< 6 min), a rapid absorption, a peak which is reached 0.5-1.0 h after administration, followed by a biphasic elimination. The first phase with a k(alpha) of 1.8 h-1 is followed by a slower phase with a K(beta) of 0.14 h-1. Probably the terminal elimination rate is slower, about 0.05 h-1. The urinary excretion of fluoride during the 48 h after administration accounted for 40-50% of the administered dose of fluoride. The results are consistent with those found in previous studies after administration in fasting conditions of sodium fluoride or sodium monofluorophosphate alone or in combination with calcium salts.

Key words: Antiosteoporotic drugs; CAS-116420-36-1; Clinical pharmacokinetics; Fluoride; Glutamine monofluorophosphate, Tridin(R).
Reprints: I Setnikar, Via Valosa Sopra 7, I-20052 Monza, Italy

EFFECTS OF MEAL ON THE PHARMACOKINETICS OF FLUORIDE FROM ORAL MONOFLUOROPHOSPHATE

G Warneke, I Setnikar
Monza, Italy

Abstract from *Arzneimittel-Forschung/Drug Research 43-1* (5) 590-595 1993

A two-treatment cross-over study was conducted on 8 Caucasian male healthy volunteers aged between 26 and 32 years in order to determine the influence of a standard meal on the bioavailability and pharmacokinetics of fluoride after a single oral administration of tablets containing the equivalent of 10 mg fluoride as sodium monofluorophosphate (Na-MFP, CAS 10163-15-2) in a fixed combination with 300 mg Ca as calcium gluconate and calcium citrate. The meal provoked a significant delay of the appearance of fluoride in blood (from 4 to 11 min), a slowing of the absorption rate, a prolongation of t(max) (from 34 to 146 min), a decrease of C(max) (from 369 to 122 ng/ml) and a prolongation of the Mean Residence Time (from 7.1 to 9.1 h). However, the amount of fluoride entering in the systemic circulation was not affected by the meal, as shown by the ANOVA on the AUC and on the cumulated urinary excretion of fluoride in the 48 h following the administration of Na-MFP It is concluded that appropriate meals do not influence the amount of absorbed fluoride from Na-MFP, and that they modify the rate of absorption with pattern that may improve the safety and possibly also the efficacy of Na-MFP.

Key words: Antiosteoporotic drugs; CAS-10163-15-2; Fluoride; Sodium monofluorophosphate, Clinical Pharmacokinetics

Reprints: I Setnikar, Via Valosa Sopra 7, I-20052 Monza, Italy.

HIP FRACTURE RATES IN HONG KONG AND THE UNITED STATES, 1988 THROUGH 1989

Suzanne C Ho, Edward Bacon, Tamara Harris, Anne Looker and Stefania Maggi
Hong Kong and Hyattsville/Bethesda Md USA

Abstract from *American Journal of Public Health 83* 694-697

Objectives. Prior studies have suggested that hip fracture rates are substantially lower in Asian countries than in the United States. However, comparisons have been limited by unavailability of recent data, differences in case definition, lack of data from similar time periods, and small sample sizes. This study sought to examine trends by age and sex, with separate statistics for those aged 85 or older.

Methods. Hospital discharge data were used to obtain hip fracture incidence in Hong Kong and the United States from 1988 through 1989.

Results. Within each population, women had higher hip fracture rates than men. Fracture rates in the United Staes were significantly higher for both sexes than rates in Hong Kong. For persons over the age of 80, rates of hip fracture among White US males exceeded those for Hong Kong women. Inclusion of transferred cases in hip fracture rates minimized differences between the countries.

Conclusions. Despite increasing hip fracture rates in Hong Kong, those rates are still substantially lower than the rates in the United States. Identifying factors responsible for this variation may prove useful in the search for preventive strategies.

Key words: Hip fracture; Hong Kong; United States.

Reprints: Dr W Edward Bacon, Division of Health Care Statistics, National Center for Health Statistics, Room 952, 6525 Belcrest Rd, Hyattsville Md 20782, USA.

THE FLUORIDATION OF DRINKING WATER AND HIP FRACTURE HOSPITALIZATION RATES IN 2 CANADIAN COMMUNITIES

M E Suarezalmazor, G Flowerdew, L D Saunders, C L Soskolne and A S Russell
Edmonton, Alberta, Canada

Abstract from *American Journal of Public Health 83* (5) 689-693 1993

Objectives. The purpose of this study was to compare hip fracture hospitalization rates between a fluoridated and a non-fluoridated community in Alberta, Canada: Edmonton, which has had fluoridated drinking water since 1967, and Calgary, which considered fluoridation in 1991 but is currently revising this decision.

Methods. Case subjects were all individuals aged 45 years or older residing in Edmonton or Calgary who were admitted to hospitals in Alberta between January 1, 1981, and December 31, 1987, and who had a discharge diagnosis of hip fracture. Edmonton rates were compared with Calgary rates, with adjustment for age and sex using the Edmonton population as a standard.

Results. The hip fracture hospitalization rate for Edmonton from 1981 through 1987 was 2.77 per 1000 person-years. The age-sex standardized rate for Calgary was 2.78 per 1000 person-years. No statistically significant difference was observed in the overall rate, and only minor differences were observed within age and sex subgroups, with the Edmonton rates being higher in males.

Conclusions. These findings suggest that fluoridation of drinking water has no impact, neither beneficial nor deleterious, on the risk of hip fracture.

Key words: Calgary; Edmonton; Fluoridation; Hip fractures.
Reprints: Dr M E Suarez-Almazor, University of Alberta, Department of Medicine, 562 Heritage Medical Research Center, Edmonton T6G 2S2 Alberta, Canada.

HIP FRACTURE INCIDENCE BEFORE AND AFTER THE FLUORIDATION OF THE PUBLIC WATER SUPPLY, ROCHESTER, MINNESOTA *

S J Jacobsen, W M O'Fallon and L J Melton
Rochester, Minnesota, USA

Abstract from *American Journal of Public Health 83* (5) 743-745 1993

Recent ecological comparison studies have suggested a positive association between fluoridation and hip fracture. Using data from the Rochester Epidemiology Project, we found the incidence of hip fracture for the 10 years before the fluoridation of the Rochester, Minnesota, public water supply was 484 per 100 000, compared with 450 per 100 000 in the following 10 years. When the effects of calendar time and age were controlled for, the relative risk association with fluoridation was 0.63. These ecologic trend data suggest that the fluoridation of public water supplies is not associated with an immediate increase in rates of hip fracture. Further studies of this association at the individual level are clearly required before public policy decisions can be made.

Key words: Fluoridation; Hip fractures; Minnesota; Rochester.
Reprints: Dr S J Jacobsen, Mayo Clinic and Foundation, Department of Health Science Research, Biostatistics Section, 200 1st St SW, Rochester MN 55905, USA.

* See Comment by John R Lee, pages 274-277.

The following are abstracts from the dental research literature. As can be noted, it is now widely accepted that the caries-reducing effect of fluoride is mainly a local ("topical") action rather than a systemic one. It is also now acknowledged that this topical action is a re-mineralizing of early carious lesions, and has little if any effect on sound tooth enamel - *i.e.* it is early treatment, rather than prevention, of dental caries. While some, like Wei and Yiu below, now wisely caution against unnecessary ingestion of fluoride during such treatment, others do not. It is especially disturbing to note the continuing advocacy of "slow release" fluoride compounds which, while intended to act topically, must inevitably result in increased fluoride ingestion. - J.C.

EVALUATION OF THE USE OF TOPICAL FLUORIDE GEL

S H Y Wei and C K Y Yiu
Hong Kong

Abstract from *Caries Research* 27 29-34 1993

Professionally applied topical fluoride should be used judiciously and is indicated only in patients with moderate or high caries activity. While the anticaries effectiveness of acidulated phosphate fluoride gels has been clinically documented, the 2% neutral NaF gel has not been adequately tested, and further clinical verification is needed. Daily use of a self-applied fluoride gel is recommended for patients undergoing orthodontic treatment or to those affected with xerostomia as a result of disease or head and neck radiation therapy. The dental profession and the public should be well informed on the proper use of clinically proven products to achieve optimum effectiveness and minimize the risk of fluoride ingestion.

Key words: Fluoride gel; Fluoride ingestion; Topical fluoride, efficacy, safety.

Reprints: S H Y Wei, University of Hong Kong, Department of Childrens Dentistry and Orthodontics, 34 Hospital Road, Hong Kong.

SYSTEMIC FLUORIDES - DROPS AND TABLETS

K W Stephen
Glasgow, Scotland

Abstract from *Caries Research* 27 (Suppl 1) 9-15 1993

Fluoride drops and tablets are effective caries-inhibiting agents which exercise their benefit through mainly topical means. Results of caries trials in which drops or tablets were used in the home vary from excellent to poor, depending on the compliance rates of both parents and offspring. Delivery in schools has also produced reductions of >80%, and as poor as 20%, again possibly dependent on teacher vigilance. The pre-natal controversy re fluoride tablet benefits has only recently been tested under a true double-blind protocol and resulted in a non-significant effect, although the trend was in favour of those whose mothers had taken F- tablets during pregnancy. Hence, while at the individual level, the slow intra-oral dissolution of fluoride tablets can be of great benefit for coronal caries in children, adolescents,and possibly medically compromised adults (with or without root caries), their contribution on a community basis cannot readily be compared with that of water or salt fluoridation. Although fluoride tablets have been held responsible for an increase in fluorosis prevalence, data which were assessed blind now exist to show that a daily dosage of 0.25 mg fluoride from birth is not asssociated with dental fluorosis, if additional fluoride products are not used injudiciously over the same period.

Key words: Fluoride Drops, Fluoride supplements; Fluoride tablets; Fluorosis.

Reprints: K W Stephen, Department of Oral Medicine and Pathology, University of Glasgow School of Dentistry, 378 Sauchiehall St, Glasgow G2 3JZ, Scotland.

ROLE OF FLUORIDE TOOTHPASTES IN A CARIES-PREVENTIVE STRATEGY

K G Konig
Nijmegen, Netherlands

Abstract from *Caries Research 27* (Suppl 1) 23-28 1993

While the clinical relevance of fluoride toothpastes per se is firmly established, the multifactorial processes involved in causation and prevention of caries render it difficult to define their exact role in an overall caries-preventive strategy. Therefore, an indirect pragmatic approach was chosen: the interpretation of secular trends in caries prevalence and other parameters is used to estimate the relative importance of this method of topical fluoride administration and to compare it with the contributions by other methods of prevention. All methods of caries prevention, with the exception of population-administered measures such as fluoridation of drinking water or domestic salt, can only work when applied regularly and conscientously. For this reason behavioural aspects such as compliance and the chances for widespread (if not universal) application have to be considered. There is indirect evidence that toothbrushing with fluoride toothpastes is a highly probable explanation for the decline of caries prevalence in developed countries since the 70s; moreover, toothbrushing, and the use of therapeutic dentrifices, is considered to meet spreading compliance because a 'healthy mouth' becomes more and more socially desirable in growing numbers of subpopulations.

Key words: Caries epidemiology; Caries prevention; Fluoride toothpastes; Topical fluoride.
Reprints: K G Konig, Catholic University of Nijmegen, Faculty of Medicine, POB 9101, 6500 HB Nijmegen, Netherlands.

FLUORIDE MOUTHRINSES AND FLUORIDE VARNISHES

L G Petersson
Basel, Switzerland

Abstract from *Caries Research 27* (Suppl 1) 35-42 1993

The cariostatic efficacy of rinsing with a 0.05-0.2% neutral sodium fluoride solution has been clearly demonstrated, especially in supervised school-based programmes in moderate and high caries risk children. The cost-benefit effect, however, is questionable in populations with low caries prevalence, and fluoride rinsing programmes are gradually being replaced by more individual fluoride therapy comprising combinations of fluoride toothpastes, tablets, or varnishes. Fluoride varnishes were developed as individual alternatives to conventional topical fluoride application and are today gaining acceptance for clinical application. Two varnishes, Duraphat(R) containing 5%wt NaF and Fluor Protector(R) with 0.9% wt fluor silane, are available commercially. The clinical effects seem to depend mainly on application frequency, especially in high caries risk groups. The cost-benefit effect is high, but can be increased by delegating application to auxiliary personnel in conjunction with regular dental visits. Toxicologically both fluoride mouthrinses and fluoride varnishes are safe if used as directed.

Key words: Fluoride mouthrinses; Fluoride varnishes; Topical fluorides.
Reprints: L G Petersson, Department of Preventive Dentistry, Medical and Dental Center, S-30185 Halmstad, Sweden.

INSITU REMINERALIZATION OF SUBSURFACE ENAMEL LESION AFTER THE USE OF A FLUORIDE CHEWING GUM

W J Lamb, R E Corpron, F G More, E D Beltran, D S Strachan and C J Kowalski
Ann Arbor MI, USA

Abstract from *Caries Research 27* 111-116 1993

In situ remineralization of early enamel lesions by a fluoride chewing gum was studied. Human enamel specimens with subsurface lesions were mounted in removable lower appliances for 6 adults. Subjects used a F-free dentifrice 3 x /day and chewed five sticks/day for the F gum group (0. 1 mg F/stick) or five sticks of sugarless gum. No gum was chewed for controls. Surface microhardness was performed on: 1) sound enamel; 2) lesions; 3) after intraoral exposure, and 4) after acid-resistance testing (ART). Separate specimens were etched and measured for F uptake and image analyses on microradiographs were performed for all regimens. DELTAZ values were calculated and converted to percent of mineralization. Values for F gum were significantly higher ($p > 0.05$) than non-F gum and controls for ART, percent remineralization, and F uptake up to 70 µm depth.

Key words: Enamel caries; Fluoride gum; Remineralization
Reprints: R E Corpron, University of Michigan School of Dentistry, Ann Arbor MI 48109, USA.

CALCIUM FLUORIDE FORMATION IN ENAMEL FROM SEMI-CONCENTRATED OR LOW-CONCENTRATED-F AGENTS *INVITRO*

C Bruun and H Givskov
Copenhagen, Denmark

Abstract from *Caries Research 27* 96-99 1993

The formation of CaF_2 was measured on sound enamel and in artificial, standardized (acidified gelatine, pH 4.5) caries-like enamel lesions after exposure to (a) dentifrice/saliva slurries adjusted to relevant F- concentrations of approximately 100 or 8 ppm by appropriate addition of 1,000 ppm F NaF or Na monofluorophosphate (MFP) dentifrice, respectively, or (b) a mixture of saliva with a 0.2% NaF solution obtained by a usual 1-min rinse procedure or an aqueous solution of 0.2% NaF CaF_2 was determined after extraction with KOH and fluoride analysis by gas chromatography. Only negligible amounts of CaF_2 were produced on sound enamel ranging from (mean ± SEM) 0. 76 ± 0.14 µg F/cm^2 with the 0.2% NaF solution to as little as 0.04 ± 0.06 with the MFP dentifrice slurry. In caries-like enamel lesions, the CaF_2 production with the 0.2% NaF solution/saliva mixture corresponded to 3.7 ± 0.4 µg F/cm^2 and corresponding amounts obtained with the dentifrice/saliva slurries were 15 ± 0.19 µg F/cM^2 with NaF, but only 0.19 ± 0.04 with MFP. It was suggested that the deposition of CaF_2 in the micropores of early lesions can be expected to be an important mechanism with F rinses, probably to some extent with NaF dentifrices, but barely with MFP dentifrices. The formation of CaF_2 on sound enamel is unlikely to play a significant role in the caries-reducing effect of F rinses and F dentifrices.

Key words: Artificial caries lesions; CaF_2 formation; Topical fluoride, Sound enamel.
Reprints: C Bruun, University of Copenhagen School of Dentistry, 20 Norre Allee, DK-2200 Copenhagen, Denmark.

INFLUENCE OF CONSTANT FLUORIDE LEVELS IN SOLUTION ON ROOT HARD TISSUE DEMINERALIZATION AND REMINERALIZATION MEASURED BY I-125 ABSORPTIOMETRY

H Almqvist and F Lagerlof
Huddinge, Sweden

Abstract from *Caries Research* 27100-105 1993

To study the effect of fluoride on de- and remineralization of root hard tissue, an automatic pH-cycling caries model, simulating Stephan curves, was used for 21 days. From each of 13 unexposed human roots, four cementum/dentin blocks were prepared. Four experiments were carried out: one block from each tooth as subjected to pH cycling without and with fluoride at the concentrations of 0.02 ppm (1.0 µmol/l), 0.20 ppm (10.5 µmol/l) and 2.00 ppm (105.3 µmol/l) in the de- and remineralizing solutions, respectively. Mineral change in the specimens was monitored by I-125 absorptiometry. When no fluoride was added to the solutions the change in transmission (DELTAT) increased continuously over 21 days, indicating loss of mineral. In the 0.02-ppm F experiment, there was a marked decrease in DELTAT, but almost no change in the 0.20-ppm F experiment. pH cycling with 2.00 ppm F in the solutions resulted in a gain of mineral in or most likely on the surface of the cementum/dentin blocks, indicated by a continuously decreasing DELTAT. The fluoride level in the solution significantly influenced the change in the mineral content of the specimens. Microradiographs of the sectioned blocks showed radiodense surface zones, varying degrees of subsurface demineralization and signs of remineralization or mineral deposition.

Key words: Artificial caries; Demineralization; Fluoride; I-125 absorptiometry; Lesions; Microradiography; pH; pH cycling; Remineralization; Root surface.

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

FLUORIDE AND MINERAL CONTENT IN HYPER-REMINERALIZED CORONAL BOVINE DENTINE *INVITRO* AFTER AN ACID CHALLENGE

Y Iijima, J L Ruben, T G M Zuidgeest and J Arends
Nagasaki, Japan

Abstract from *Caries Research* 27 106-110 1993

In this paper the acid resistance of hyper-remineralized dentine was quantified by means of fluoride and mineral measurements. Hyper-remineralization was achieved by demineralization of dentine in an acidic gel system (pH 5) for 3 weeks, followed by remineralization in a solution containing 1.5 mM Ca, 0.9 mM phosphate and 10 ppm F at pH 7 and 37-degrees-C for 8 days. The samples were subsequently again demineralized in the gel system mentioned for 1, 2 and 3 weeks. Analysis for fluoride was done by means of the microdrill biopsy technique and to obtain information on the fluoride distribution by secondary ion mass spectrometry (SIMS); mineral was assessed by microradiography. The results showed that in hyper-remineralized dentine the original fluoride content was approximately 30,000 ppm F. This value was still in the same order after the acid challenge of 3 weeks at pH 5. These values were substantially higher than the baseline values in sound or in demineralized dentine being about 900 ppm F. The main microradiographic result was that there was no significant mineral change in the hyper-remineralized dentine due to the acid challenges. There was, however, a tendency for mineral redistribution deeper into the dentine lesion leading to lamination phenomena. The SIMS experiments on some of the samples showed a fluoride

distribution in agreement with the microdrill fluoride data. Furthermore a fluoride redistribution took place in deeper parts of the lesion due to the acid challenges. The combined fluoride concentration and microradiographical data indicate that fluoride-enriched and highly mineralized hyper-remineralized dentine is more acid resistant than sound or demineralized dentine.

Key words: Acid resistance; Dentine; Fluoride; Mineral; Remineralization.
Reprints: Y Iijima, Nagasaki University, Nagasaki 852, Japan.

LONG-TERM FLUORIDE RELEASE OF VISIBLE LIGHT-ACTIVATED COMPOSITES *INVITRO* - A CORRELATION WITH *INSITU* DEMINERALISATION DATA

G E H M Dijkman, J Devries, A Lodding and J Arends
Groningen, Netherlands

Abstract from *Caries Research 27* 117-123 1993

Fluoridated composites are able to reduce or inhibit secondary caries around fillings. The aims of this study were firstly to investigate the amount of F released *in vitro* from composites with a F content between 0 and 26 vol % as a function of time over a 1-year period. A second aim was to correlate the *in vitro* data on F release with enamel demineralisation data *in situ* after a 1-month period. The results show that all fluoridated composites release sizable amounts of F in solution, the total amounts of F released from three composites were proportional to log t over at least 1 year. In one case proportionality of the total amount of F released with t was observed. Possible mechanisms of F release are considered. The results presented also show a linear relation between the log of *in vitro* F release data and the effects on enamel demineralisation *in situ* next to the composite. Extrapolation of the data reveals that a F release of about 200-300 µg/CM2 over a 1-month period from a fluoridated composite would completely inhibit secondary caries under plaque conditions.

Key words: Demineralisation; Dental materials; Fluoridated composites; Fluoride release.
Reprints: G E H M Dijkman, University of Groningen, Ant Deusinglaan 1, 9713 AV Groningen, Netherlands

SLOW-RELEASE FLUORIDE

K J Toumba and M E J Curzon
Leeds, England

Abstract from *Caries Research 27* (Suppl 1) 43-46 1993

The latest evidence supports the concept of frequent applications of relatively low concentrations of fluoride for the elimination of caries, even in situations of high caries challenge. Dental materials exhibit a 'burst effect', and the fluoride release is short-lived. Intral-oral devices in the form of copolymer membranes or glass devices cause an elevation of salivary F levels for up to 2 years in animals and humans and have led to increased F uptake in enamel. Caries reduction has been achieved in rats using the copolymer device, and, at present, human caries trials using the copolymer and glass devices are under way in the USA and Leeds, respectively. These intra-oral devices hold great promise to target financial resources for prevention in groups of the population with high caries levels, in particular low socio-economic groups including ethnic minorities and the handicapped.

Key words: Copolymer devices; Fluoride; Intra-oral release devices; Glass devices.
Reprints: K J Toumba, Leeds Dental Institute, Department of Paediatric Dentistry, Clarendon Way, Leeds LS2 9LU, England

BEIJING CONFERENCE REMINDER

Members amd readers are reminded that the XXth Conference of the International Society for Fluoride Research will be held in Beijing, China, on October 10-13 1994.

The registration fee will be US$200. Abstracts of papers for the conference, and other enquiries, should be directed to:

ISFR '94 Secretariat, c/- 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 Telephone 0086-01-3038761-2611)

FLUORIDE, official journal of the International Society for Fluoride Research (ISFR), publishes quarterly reports on biological, chemical, ecological, industrial, toxicological and clinical aspects of inorganic and organic fluoride compounds.

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

SUBSCRIPTION: US$50 (or equivalent) per year in advance. Send to the Treasurer, ISFR, 216 Atkinson Road, Titirangi, Auckland 7, New Zealand.

MANUSCRIPTS, including papers presented at ISFR conferences, are accepted for publication after appropriate evaluation and recommendation by qualified reviewers. Send to Editor, *Fluoride*, 216 Atkinson Road, Titirangi, Auckland 7, New Zealand.

INSTRUCTIONS TO AUTHORS

1. **General.** The submitted paper, with a copy, should be written concisely in English. Either American or British spelling is accepted. Double space with generous margins. Measures should be in metric system.
2. **Title.** A concise but informative title should be followed by the name(s) of the author(s). The address where the research was carried out should appear at the bottom of the first page.
3. **Summary.** Begin with a brief factual summary.
4. **Key words.** List the major themes or subjects.
5. **Introduction.** State the reason for the work with a brief review of previous work on the subject.
6. **Materials and Methods.** Condense. However, if the methodology is new or developed by the author(s) it can be more detailed.
7. **Results.** List the direct conclusions of the work.
8. **Discussion.** Deal with general conclusions, referring to other work on the subject. In short papers Results and Discussion may be combined.
9. **Abbreviations or Acronyms.** Define, either in brackets or in footnotes, when they first appear.
10. **Acknowledgements.** Keep brief. They may include funding source, technical assistance, text editing and useful comments.
11. **References.** See current issues of journal for usual style, which identifies references by bracketed numbers in the order in which the references first occur. Other styles may be accepted, provided they are accurate and consistent.

MEMBERSHIP. Researchers are invited to join ISFR. Applications for membership should be sent to the Secretary, Professor Gene W Miller, Biology Department, Utah State University, Logan, Utah 84322-5305, USA. Application forms are available from either the Secretary or Treasurer.