Volume 27
Number 1
January 1994

FLUORIDE

JOURNAL

of the

International

Society for

Fluoride

Research

KAJ ROHOLM

Vol.27 No.1 January 1994 Pages 1 - 58

FLUORIDE

QUARTERLY REPORTS

ISSUED BY THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH

CONTENTS

continued next page

ABSTRACTS

THE MANNERS OF SCIENCE

It was stated in an earlier Editorial: "... The ISFR is not a political society and does not endorse or oppose public views on fluoride. It can, however, provide the forum for scientific exchange and publication of sound research. ..." (*Fluoride 25* page 54 April 1992).

With a desire to achieve balance in our presentations, I invited Professor G Neil Jenkins, a long-term member of our Editorial Board and a proponent of artificial water fluoridation, to submit an article in support of that procedure. Professor Jenkins responded by submitting his critique, published in this issue.

He should be commended for adopting the proper method for commenting on a colleague's research - that is, to send any criticisms to a scientific journal for publication - preferably the journal which published the research being criticized, for the information of those who read the criticized papers originally, but anyway to one which provides a forum for such discussions. The authors being criticized can then respond, others can contribute to the discussion, and the arguments can be fairly compared and assessed.

A reply to Jenkins' critique is also in this issue. He has been invited to submit a rejoinder. This did not arrive in time for this printing, but we hope it will appear in our next, April, issue. Further contributions to the discussion, from our readers, are invited.

Unfortunately, the above method has not usually been followed in the long and continuing scientific controversy over water fluoridation. Other methods, ably analyzed in sociologist Brian Martin's book *Scientific Knowledge in Controversy: The Social Dynamics of the Fluoridation Debate* (State University of New York Press, 1991), have too often been employed. These included the circulation of criticisms, often unpublished, of the work of scientific opponents of fluoridation, without their knowledge or opportunity to respond.

We prefer the manners of science: publication and open discussion.

J.C.

ISFR CONFERENCE

The XXth Conference of the International Society for Fluoride Research will be held in Beijing, Peopls's Republic of China, on September 5-9, 1994. The Conference is sponsored by the Consultant Committee on Endemic Fluorosis of the Chinese Ministry of Public Health, and is co-sponsored by the World Health Organization.

The official language of the Conference will be English. The registration fee is US$200.

In China, fluorosis is a severe endemic disease. At the Conference, Chinese scientists will report on their recent research achievements. As usual at ISFR Conferences, research reports will be also be presented from the many lands where members of the Society live and work.

The Conference program includes tours to the Great Wall, the Ming tombs, the Palace Museum and the Summer Palace.

Main topics will be: environmental fluoride pollution; biological effects of fluoride; influence of fluoride on health; analytical methods and monitoring of fluoride; and methods of defluoridation.

Abstracts of papers for the Conference should reach the Conference Secretariat by March 31, 1994. Information on form of abstracts and other Conference matters can be obtained from the Secretariat:

ISFR '94 Scientific Secretariat, Dr Liang Chaoke
Institute of Environmental Health and Engineering
Chinese Academy of Preventive Medicine
29 Nan wei Road
Beijing 100050
People's Republic of China

EFFECT OF FLUORINE INGESTION ON CELL-MEDIATED IMMUNE RESPONSE IN CALVES

V Kapoor[1] and T Prasad[2]
Hisar and Karnal, India

SUMMARY: To investigate the effect of dietary fluorine (F) on cell-mediated immune (CMI) response, twenty male crossbred calves of 6-8 months of age, divided into four equal groups, were fed on diets of concentrate mixture and green corn for 20 weeks (50:50 for first 3 months and 40:60 during the later phase). The four dietary treatments differed only with respect to the F content of the mineral mixture. Treatments 1,2 and 3,4 contained dicalcium phosphate and rock phosphate, respectively. Treatments 2 and 4 in addition were supplemented with NaF so as to provide an additional 80 mg F/Kg diet. The resultant dietary F levels were 7, 79, 132 and 191 ppm in treatment group 1 to 4, respectively. The CMI response of lymphocytes determined by ^{3}H thymidine uptake by lymphocytic cultures during blastogenesis at O' day and after 5 months of experimental feeding declined significantly with increasing level of dietary F thus indicating immunity breakdown at higher F intake.

Key words: Blastogenesis; Cell-mediated immunity; Lymphocytes; Phosphatic supplements.

Introduction

In addition to other sources, mineral supplements are important sources of dietary fluorine (F) intake in animals, affecting their health and performance. The F content of rock phosphate varies from 1.5 to 4% depending upon its origin. Defluoridation of rock phosphate is an expensive process, and the high F content has always been a caution in use of rock phosphate for animal feeding as a phosphate supplement. Studies with farm animals, planned and controlled administration of F, case reports of accidents of acute poisoning, and epidemiological studies have provided information about the toxicological properties of F. However, the consequences of F intake on immunogenic response in animals have not been fully elucidated. This study investigates the effect of dietary F upon cell-mediated immunity (CMI) in calves.

Materials and Methods

Twenty Karan Fries (Tharparkar x Holstein Friesian) male calves, of 6 to 8 months of age, were randomly divided into four groups on body weight basis. They were fed on concentrate mixture and green corn (50:50 for first 3 months and 40:60 during the later phase) as per NRC (1) recommendations. The concentrate mixture consisted of Barley 40, ground-nut cake 40, wheat bran 17 and mineral mixture 3 parts (CP 22% and TDN 75%). Mineral mixtures of groups 1 and 2 contained dicalcium phosphate as phosphorus supplement, whereas in groups 3 and 4,

[1] Department of Animal Nutrition, Haryana Agricultural University, Hisar 725 004, India.
[2] Division of Dairy Cattle Nutrition, National Dairy Research Institute, Karnal 132 001, India.

dicalcium phosphate was replaced by rock phosphate. Fluorine as NaF was added to the mineral mixtures of groups 2 and 4 (Table 1). In addition, mineral mixtures of the four groups were supplemented with required amounts of major (Ca, Mg, Na, Cl) and trace (Fe, Zn, Cu, Mn, Co and I) elements. The dietary F levels were 7, 79, 132 and 191 ppm in the four treatment groups.

For measuring the activity of lymphocytic cultures, derived from blood samples of experimental calves under different treatment groups at O' day and at the end of 5 months experimental period, the procedure described by Waithe and Hirachhorn (2) was followed.

Table 1. Composition of mineral mixtures (per 3 Kg)

Treatment group	Ingredients			
	Dicalcium* phosphate (Kg)	Rock* phosphate (Kg)	'F' added** (g)	Other*** minerals
1	1.65	-	-	+
2	1.65	-	16	+
3	-	1.65	-	+
4	-	1.65	16	+

* Basal F levels in dicalcium phosphate and rock phosphate were 0.018% and 1.5%, respectively.

** Varied time to time depending upon DM requirement so as to supply 80 ppm additional F in the diet.

*** Chalk powder 0.3312 Kg, $MgCO_3$ 0.09 Kg, NaCl 0.90 Kg and trace elements 0.0288 Kg.

Blood collection. Blood samples were collected by jugular vein puncture at O' day and after 5 months of experimental feeding into 10 ml evacuated heparanized glass tubes. A laminar flow cabinet was used for all subsequent handling of samples.

Isolation of lymphocytes. Within 2 to 4 hours after collection of blood, lymphocytes were isolated from heparanized blood by density gradient centrifugation. Ten ml of blood was diluted with 14 ml of phosphate buffer saline (PBS-0.12 M, pH 7.2). Five ml of diluted blood was overlaid with density gradient media (Histopaque, Sigma, USA) and centrifuged at 400 x g for 30 minutes at 20°C. Lymphocytes were harvested from the interface and washed thrice with PBS followed by two washes in culture media (Gibco, Dulbecoo's modification of Eagles minimum essential media-DMEM, USA) containing 25MM HEPES buffer and fetal bovine serum (15% of culture media). All washes were followed by centrifugation at 1200 rpm for 10 minutes. The total counts of isolated lymphocytes were taken on haemocytometer. Viability was determined by dye exclusion method with 1% trypan blue (3).

Lymphocyte cultures. Lymphocytes were resuspended at 2 x 10^6 cells/ml in culture medium at pH 7.1 to 7.4 fortified with 100 units of penicillin and 125 µg of streptomycin. Triplicate cell samples were cultured in flat bottom wells at 37°C in a 5% CO_2 atmosphere. To stimulate the blastogenic response 1 µg poke weed mitogen (PWM)/ml of culture (Sigma,USA) was added.

Methyl ^{3}H thymidine incorporation. Cultured wells were harvested on the day of maximal blastogenic response - *i.e.*, at 72 hr after incubation. Cells were pulsed with: 1.0 µci/well methyl ^{3}H thymidine (1.8000 mci/mM specific activity) during the final 4 hr of culture.

Cells were then harvested by adding 2 ml of cold phosphate buffer saline with azide (PBSA-0.12 M pH 7.4) to each tube. The cell pellet obtained after centrifugation was solubalized with 0.1 ml IN NaOH at 50-60°C. It was reprecipitated by cold TCA and resolubalized in NaOH. The pellet obtained after centrifugation was then dissolved by adding 0.2 ml of soluene at 50-60°C. To this was added 0.5 ml methanol. Sample was transferred to scintillation vial contaning 6 ml of modified Bray's solution. Beta emission was counted in Packard liquid scintillation counter. The stimulation index (SI) was counted as follows :-

$$SI = \frac{\text{Counts/minute of PWM stimulated sample}}{\text{CPM of control cultures}}$$

Statistical analysis. Data were subjected to two way classification of variance with interaction to statistically evaluate between groups. Differences were considered significant when $P < 0.05$.

Results

Stimulation index (SI) values ± SEM are presented in Table 2. The SI at the start of the experiment were 1.24 ± 0.07, 2.09 ± 0.52, 1.45 ± 0.14 and 2.61 ± 0.59 in treatment groups 1 to 4, respectively. The initial values were not similar in the four groups but the intergroup variabilities were not large. The differences being marginally low represent only the initial immune status of animals before resorting to experimental feeding. At the end of 5 months experimental feeding, SI values in the four treatment groups were 5.96 ± 1.42, 4.80 ±1.89, 2.38 ± 0.21 and 1.70 ± 0.16, respectively.

Change in SI over a period of 5 months of experimental feeding indicated that there was sufficient gain in the values of SI in group 1, lower gain in group 2, followed by group 3 and loss in group 4. It is evident that as the dietary F load increased there was fall in the SI values from groups 1 to 4 so much so that in case of group 4, the change in SI values became negative suggesting decrease in the CMI response of calves at higher levels of dietary F intake.

Table 2. Blastogenic response of lymphocytes in calves as measured by incorporation of (^{3}H) thymidine

Treatment group	SI (initial value)	SI (after experimental period of 5 months)	Change in SI*
1	1.24 ± 0.07	5.96 ± 1.42	+ 4.72 ± 1.42
2	2.09 ± 0.52	4.80 ± 1.89	+ 2.71 ± 1.44
3	1.45 ± 0.14	2.38 ± 0.21	+ 0.93 ± 0.26
4	2.61 ± 0.59	1.70 ± 0.16	- 0.91 ± 0.27

* Means differ significantly at $P < 0.05$

Discussion

Lymphocyte transformation provides a simple reproducible semiquantative *in vitro* correlate for CMI which is used as a general screen (4). *In vitro* transformation of lymphocytes by use of PWM is one of the methods used for assessing the CMI of animals. PWM is generally considered a B cell stimulant, although it also stimulates the proliferation of T cells. Therefore, ^{3}H-thymidine incorporation in PWM stimulated cultures reflect both T and B cell response. Further, determination of SI is an improved procedure for studying the lymphocytic responses (5,6). The hypothesis that long-term exposure of animals to subtoxic levels of F induces metabolic alterations in mouse fibroblasts (cells commonly found in repairing or developing tissues and concerned with protein and collagen synthesis) which resulted in F-resistant fibroblast cells (7) invited attention to whether dietary F levels caused any alterations in cellular immunity. The results indicate that there is immunity breakdown at higher dietary F levels. The mechanism is not fully understood. In an earlier study (8) it has been reported that F (NaF), although an effective inhibitor of glycolysis, did not exert any effect on phagocytosis by mouse macrophage *in vitro*. Since no method is available for studying this parameter in intact animals, direct evidence of this type of response is lacking in the literature.

References

1 *National Research Council: Nutrients Requirements of Dairy Cattle,* 5th Edition. National Academy of Sciences, Washington DC 1978.
2 Waithe WI, Hirachhorn K. The lymphocyte response to activators. In: Weir DM (Ed). *Handbook of Experimental Immunology.* 1973 Chapter 25.
3 Boyum A. In: Weir DM (Ed). *Handbook of Experimental Immunology.* 1973 Chapter 25.
4 Catalona WJ, Tarpley JL, Potwin C, Chnetien PB. Correlations among cutaneous reactivity of DNCB, PHA-induced lympocyte blastogenesis and peripheral blood E rosettes. *Clinical and Experimental Immunology 19* 327-333 1975.
5 Prasad SK, Prasad T. Influence of mitogen on blastogenic response on ruminant lymphocytes. *Journal of Nuclear Agriculture and Biology 14* 114-116 1985.
6 *International Atomic Energy Agency: Laboratory Training Manual on the Use of Radionuclides and Radiations in Animal Research.* 3rd Ed Technical Reports Series No. 60. International Atomic Energy Agency, Vienna 1972.
7 Holland RI, Hongslo JK. Cellular resistance to fluoride. *Cell Biology International Reports 2* 551-559 1978.
8 Cifarelli A, Pepe G, Paradisi F, Piccolo D. The influence of some metabolic inhibitors on phagocytic activity of mouse macrophages *in vitro. Research in Experimental Medicine 114* 197-240, 1979.

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

EFFECT OF FLUORIDE ON RAT TESTICULAR STEROIDOGENESIS

M V Narayana and N J Chinoy
Ahmenabad, Gujarat, India

SUMMARY: In view of reports of infertility among human populations in fluorosis prevailing regions, we investigated the effect of fluoride ingestion on testicular steroidogenesis in rats. Sodium fluoride (NaF) was administered to the rats orally at a daily dose of 10 mg/kg bodyweight for 50 days. The treatment did not cause significant change in testicular cholesterol levels, indicating that metabolism was not altered and that there was no hypo/hypercholesterolemic effect. In addition, activities of the intermediary enzymes in androgenesis, *viz.*, 3β- and 17β-hydroxysteroid dehydrogenase were only modestly decreased by NaF ingestion. Subsequently, the determination of circulating androgen levels was similar in NaF-treated rats showed a downward trend compared to those of the control group, suggesting alteration in testosterone concentration. The histomorphometric studies revealed significant change in the Leydig cell diameter in correlation with the androgen levels. These results indicate that fluoride does interfere with steroidogenesis in short-term low-dose exposures in rats.

Key words: Androgen levels; Fluoride, Leydig cells; Rats, Steroidogenesis, Testis.

Introduction

In recent years the effects of fluoride on reproductive functions have been investigated. Schulz and Lamb (1) and Udall and Kellers (2) reported a positive relationship between fluoride toxicity and reduction in fertility in animals. Vogel (3) observed a slight decrease in fertility of *Drosophila melanogaster* exposed to sodium fluoride. Tokar and Savchenko (4) obtained low testosterone levels with high FSH and LH in individuals afflicted with fluorosis. On the other hand, Li *et al* (5) reported that fluoride does not have adverse effects on spermatogenesis, indicating that fluoride plays no mutagenic role. However, recent experimental studies performed in mice and rats revealed arrest of spermatogenesis due to extensive damage to the seminiferous tubules leading to denudation of cells, vacuolization in their cytoplasm, and nuclear pyknosis (6-7). Furthermore, the target organ functions (epididymides, vas deferens, and accessory sex organs) were impaired adversely, despite the fact that the serum testosterone levels were close to normal after NaF treatment for 30 days at a dose of 10 mg/kg body weight (8-9). Thus, the impact of fluoride on rat testicular steroidogenesis, along with the impairment of target organ function, is not clearly understood, a situation which led to the present investigation in experimental rats.

Material and Methods

Mature, healthy, pathogen-free albino rats (*Rattus norvegicus*) of Charles Foster strain, 50-55 days old and weighing 200-250 g, were obtained from the National Institute of Occupational Health, Ahmedabad. The animals were acclimatized to laboratory conditions for one week.

Zoology Department, School of Sciences, Gujarat University, Ahmedabad 380 009, Gujarat, India. Correspondence to Professor N J Chinoy, Head of Department.

The rats were divided into two groups and caged separately. Those in the first group (the controls) were given food and water *ad libitum*. Those in the second group were given NaF just before feeding so as to avoid contact with food.

Sodium fluoride (Loba Chemie, Bombay; 99% purity) was dissolved in distilled water and administered daily to the second group of animals at a dose of 10 mg/kg body weight orally for 50 days. The dose used was mainly based on the LD 50 value for male rats, which is 250 mg/kg body weight (10); the present dose was 1/25 of the LD 50 value. The duration was selected on the basis of the kinetics of spermatogenesis in rat. The experimental protocol is summarized in Table 1

TABLE 1. Summary of Treatment

Group	Treatment	Duration (Days)	Day of autopsy	Number of animals used
I	Control	–	sacrificed along with treated	10
II	NaF (10mg/kg body weight).	50	51	10

At the end of the treatment period, control and treated groups of rats were sacrificed by cervical dislocation. The testes were dissected out, blotted free of blood, and used freshly for carrying out different parameters, *i.e.*, cholesterol levels and 3β- and 17-β-hydroxysteroid dehydrogenase (HSD) activities. Blood was collected by cardiac puncture and allowed to clot; serum was separated by centrifugation for testosterone assay.

Histocytometric studies of Leydig cells and their nuclear diameter were also carried out. Testes of control and treated rats were excised and fixed in Bouin's fixative for 18 hours, then transferred to 70% alcohol and processed until the colour of the fixative was removed by adding lithium carbonate. Sections of 5 μ thickness were cut on a microtome and stained with haematoxylin-eosin. The morphometric analyses of Leydig cells and their nuclear diameter were determined by means of an ocular eye-piece and micrometer scale.

Cholesterol: The testicular cholesterol levels in the control and treated rats were estimated by the method of Pearson *et al* (11) and expressed as μg/mg fresh tissue weight.

3β- *and* 17β- *hydroxysteroid dehydrogenase* (HSD): The activities of testicular 3β- and 17β-HSDs were determined by the method of Talalay (12) and expressed as 5α-diol formed/mg protein/30 minutes.

Testosterone: The serum testosterone levels of control and treated rats were assayed by the double antibody technique of Peterson and Swerdloff (13) and the concentrations were expressed as ng/ml serum.

Statistics: For each biochemical parameter a minimum of 10 replicates were taken, and the data were statistically analysed by the Student's 't' test.

Results

Cholesterol: The cholesterol levels in testes of NaF-treated rats showed an insignificant increase compared to the controls (Table 2).

3β- *and* 17β-HSD: The activity of both 3β- and 17β-HSDs were lowered after 50 days of treatment compared to controls (Table 2).

Testosterone: The serum testosterone levels also showed a downward trend ($P < 0.02$) with NaF treament (Table 2).

Histocytochemistry: The Leydig cell diameter of NaF-treated rats showed a significant ($P < 0.01$) decline compared to control (Table 3). The Leydig cell nuclear diameter also showed a significant ($P < 0.01$) change after NaF treatment (Table 3).

TABLE 2. Testicular cholesterol, 3β- and 17β-hydroxysteroid dehydrogenase (HSD) and serum testosterone levels of control and after 50 days in NaF-treated rats

Parameter	Control	NaF Treatment
Cholesterol (μg/mg fresh tissue wt.)	0.46 ± 0.01	0.48 ± 0.01
3β- HSD (5∝-diol formed/mg protein/30 minutes)	5.8 ± 0.92	4.78 ± 0.83
17β- HSD (5∝-diol formed/mg protein/30 minutes)	5.5 ± 0.63	4.65 ± 0.38
Testosterone	0.46 ± 0.01	0.42 ± 0.01*

Values are mean ± S.E. * $P < 0.02$

TABLE 3. Histocytometry: Leydig cell and nuclear diameter (μm) of control and after 50 days in NaF-treated rats

Parameter	Control	NaF Treatment
Leydig cell diameter	8.4 ± 0.28	6.7 ± 0.12*
Leydig cell nuclear diameter	4.9 ± 0.25	3.1 ± 0.08*

Values are mean ± S.E. * $P < 0.01$

Discussion

The present study was undertaken to explore the effect of fluoride on testicular steroidogenesis in rats because that effect is not clearly understood. Also in view was the wide prevalence of infertility in the fluorosis-afflicted human population in India and other parts of the globe (4,14).

The sodium fluoride treatment, a daily dose of 10 mg/kg body weight administered orally for 50 days, caused no significant alterations in testicular cholesterol levels. Saralakumari *et al* (15) also reported normal serum cholesterol concentration in rats that were fluoride intoxicated for two months by a dose of 100 ppm. In corroboration, Chinoy and Sequeira (8) also obtained unaltered cholesterol in testes of mice fed with 10 mg fluoride/kg body weight for 30 days. A single microdose vasal injection of sodium fluoride (50 µg/50 µL) also caused no change in cholesterol levels (9). Our subsequent studies in human populations afflicted with fluorosis found cholesterol levels to be within the normal range (16). These results indicate that fluoride does not interfere with cholesterol metabolism in mammals and imply no hypo/hypercholesterolemia leading to atherosclerosis - at least after short-term low-dose exposure.

However, fluoride treatment of rabbits with 100 mg/kg body weight for 100 days resulted in a 2- to 3-fold increase in cholesterol and triglycerides (17). Ectopic calcification of rabbit aorta after chronic fluoride intoxication has also been reported (18). Therefore, effects of chronic exposure to fluoride on human populations in endemic areas should be examined closely because of the possibility of cardiovascular problems.

The treatment investigated here brought about a small change in serum testosterone levels indicating that there is a downward trend of circulating androgen levels caused by fluoride. In accordance with these results, Chinoy *et al* (16) found that the circulating testosterone levels of human populations in endemic areas of Gujarat, India, exposed to 3-4 ppm water fluoride for about 5-7 years, were only slightly affected. However, Tokar and Savchenko (4) reported low serum testosterone levels with high FSH and LH in fluoride-afflicted patients. This discrepancy is difficult to interpret as the duration and concentration of fluoride exposure were not known in their investigations.

In the present investigation, further exploration of intermediary enzymes in androgenesis showed, in testes after fluoride ingestion for 50 days, small reductions in 3β-hydroxysteroid dehydrogenase (HSD), which converts dehydroepiandrosterone into androstenedione, and in 17β-HSD, which converts androstenedione into testosterone. Furthermore, the histomorphometric studies revealed a significant change in Leydig cell diameter and its nuclear diameter. In support of these findings, Zahvoronkov and Strochkova (19) reported a decrease in dry weight of Leydig cells together with cytochemical alterations as evident by disturbances in protein synthesizing system. Hence, hormonal imbalance would occur as observed in the present study.

In conclusion, fluoride seems to interfere with androgenesis. The target organ structures and functions (epididymis and other accessory sex organs), which are dependent on circulating androgen levels, were adversely impaired after fluoride intoxication, leading to failure of fertility in experimental animals. This failure can affect endemic populations, as mentioned elsewhere (4,8,9,14). The possible impact

of fluoride may be on the receptor sites, *viz.*, by altering the concentration or configuration of the receptor, thus inhibiting the action of testosterone on target organs. This interpretation was further supported by alterations in phospholipid concentration, especially reduction in phosphatidyl inositol in the sperm membrane after fluoride treatment, which is important for hormone receptor transduction (19). Furthermore, the conversion of testosterone into its potent metabolite, dihydrotestosterone, is probably impaired by fluoride. Hence, it is important to investigate these aspects in order to understand the exact mechanism of action of fluoride at the receptor level, especially in the target sites.

Acknowledgement

The financial support provided by the Council of Scientific and Industrial Research to one of the authors (MVN) is gratefully acknowledged.

References

1 Schultz JA, Lamb AR. The effect of fluorine as sodium fluoride on growth and reproduction of albino rats. *Science 61* 93-94 1925.
2 Udall DH, Kellers KP. A report on fluorosis in cattle in Columbia River Valley. *Cornell Veterinarian 42* 159-184 1952.
3 Vogel E. Strong antimutagenic effects of fluoride on mutation induction by trenimon and 1-phenyl-3-dimethyltriazene in *Drosophila melanogaster*. *Mutation Research 20* 339-352 1973.
4 Tokar VI, Savchenko ON. The influence of inorganic fluorine compounds on functional condition of the hypophysis-testis system. *Problems of Endocrinology 23* 104-107 1977.
5 Li Y, Dunipace AJ, Stookey GK. Effects of fluoride on the mouse sperm morphology test. *Journal of Dental Research 66* 1509-1511 1987.
6 Chinoy NJ, Sequeira E. Effects of fluoride on the histoarchitecture of reproductive organs of male mouse. *Reproductive Toxicology 3* (4) 261-267 1989.
7 Chinoy NJ, Rao MV, Narayana MV, Neelakanta E. Microdose vasal injection of sodium fluoride in the rat. *Reproductive Toxicology 5* (6) 505-512 1991.
8 Chinoy NJ, Sequeira E. Fluoride induced biochemical changes in reproductive organs of male mice. *Fluoride 22* (2) 78-85 1989.
9 Chinoy NJ, Sequeira E, Narayana MV. Effects of vitamin C and calcium on the reversibility of fluoride induced alterations in spermatozoa of rabbit. *Fluoride 24* (1) 29-39 1991.
10 Davis KR, Ohio C. Fluoride: A critical review. *Journal of Occupational Medicine* 593-601 1961.
11 Pearson S, Stern S, McGavack TH. A rapid accurate method for the determination of total cholesterol in serum. *Analytical Chemistry 25* 813-814 1953.
12 Talalay P. Hydroxysteroid dehydrogenases. *Enzymology 6* 512-516 1962.
13 Peterson M, Swerdloff RS. Separation of bound from free hormone in radioimmunoassay of leutinizing hormone and follicle stimulating hormone, separation with double antibody PEG combination. *Clinical Chemistry 25* 1239 1979.
14 Neelam K, Suhasini RV, Sudhakar RY. Incidence on prevalence of infertility among married male members of endemic fluorosis district of Andhra Pradesh. *Proceedings of Conference of International Society for Fluoride Research* (Abstract). ISFR, Nyon, Switzerland 1987.

15 Saralakumari D, Varadacharyulu NCh, Ramakrishna Rao P. Effect of fluoride toxicity on growth and lipids in liver, kidney and serum in rats. *Arogya Journal of Health Sciences 15* 24-29 1988.
16 Chinoy NJ, Narayana MV, Sequeira E *et al.* Studies on effects of fluoride in 36 villages of Mehsana District, North Gujarat. *Fluoride 25* (3) 101-110 1992.
17 Shashi A. Biochemical effects of fluoride on lipid metabolism in the reproductive organs of male rabbits. *Fluoride 25* (3) 149-154 1992.
18 Susheela AK, Kharb P. Aortic calcification in chronic fluoride poisoning: biochemical and electronmicroscopic evidence. *Experimental and Molecular Pathology 53* 72-80 1990.
19 Zahvoronkov AA, Strochkova LS. Fluorosis: Geographical pathology and some experimental findings. *Fluoride 14* (4) 183-191 1981.
20 Fain JN. Involvement of phosphatidyl inositol breakdown in elevation of cytosolic calcium by hormones and relationship to prostaglandin formation. In: Kohn LD (Ed). *Hormone Receptors*. John Wiley and Sons, 1982 pp 237-276.

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

IS THERE A DENTAL BENEFIT FROM WATER FLUORIDE?

John Colquhoun
Auckland, New Zealand

SUMMARY: Dental data collected for virtually all New Zealand children, as well as comprehensive data from other countries, indicate no dental benefit from water fluoridation. Claims for a benefit depend on small-scale studies of selected samples of children. The classic fluoridation research is critically re-examined.

Key words: Dental caries; Fluoridation research; New Zealand; Water fluoride.

A. RECENT EVIDENCE

Data for all children

Almost all (98% of) New Zealand children are enrolled with the School Dental Service. Dental data, including the fluoridation status of each child's place of residence, has been collected since 1980 for all the 12- and 13-year-old children as they leave the care of the Service, and since 1988 for all the 5-year-old children. The Table below contains the most recent data for such children in the six main population centres (1).

TABLE. Caries-free percentages and mean number of decayed, missing or filled teeth in 1989, for all 12- and 13-year-old children and for all 5-year-old children, from all school dental clinics in non-fluoridated Christchurch and in other, fluoridated, main population areas of New Zealand.

Centre	12-13 year olds (No. of children)	caries-free %	mean DMFT	5 year olds (No. of children)	caries-free %	mean dmft
NON-FLUORIDATED						
Christchurch	(5822)	37%	1.9	(3849)	55%	1.8
FLUORIDATED						
Auckland	(11464)	33%	2.0	(9611)	53%	1.8
Hamilton	(2689)	30%	2.3	(2266)	47%	2.3
Palmerston Nth.	(1025)	31%	2.3	(950)	55%	1.8
Wellington	(4237)	36%	1.8	(3344)	58%	1.6
Dunedin	(1168)	29%	2.2	(994)	56%	1.5

The New Zealand Department of Health, a long-time advocate of water fluoridation, presented the 12-13-year-old data in its annual reports by comparing the combined fluoridated with the combined nonfluoridated areas of New Zealand (2). The differences were very small (only 1% for the caries-free percentage in each kind

Honorary Research Fellow, Education Department, University of Auckland, Private Bag, Auckland, New Zealand.

Part A was presented at the XIXth Conference of the International Society for Fluoride Research, Kyoto, Japan, September 1992. Part B is condensed from "Flawed foundation: A re-examination of the scientific basis for a dental benefit from fluoridation", first published in *Community Health Studies 14* 288-296 1990.

of area, and less than half a tooth for the mean number of decayed, missing or filled teeth) but suggested a small benefit from fluoridation. However, the areas being compared were dissimilar, one being mostly urban with higher average incomes, and the other mostly small-town-rural with lower average incomes. When similar kinds of communities were compared, the teeth were actually slightly better in the nonfluoridated areas (3,4).

There was a correlation between the dental health of children and the income level of their parents. In poorer areas, where there is poorer nutrition, there are poorer teeth. Christchurch, the only nonfluoridated large city, was the poorest in income levels (4), so should have had the poorest teeth for that reason alone. Instead, Christchurch 12-13-year-olds' teeth compared favourably with those in most of the fluoridated centres. The most recent data (Table) show that situation still applies, to both the older and younger children.

Comprehensive data from other countries (5-10), including data from the WHO Oral Health Data Bank (9) and the Teotias' 30-year surveys of over 400,000 Indian children (10), support the New Zealand School Dental Service data - *i.e.*, show no benefit from ingestion of water fluoride.

Data from small samples

Other New Zealand studies, of small samples of 5-year-olds (11), 7-year-olds (12) and 9-year-olds (13) claimed that there was a small but significant benefit resulting from fluoridation. These studies, which were contradicted by the data collected for all 12- and 13-year-olds, were discussed in my earlier study (4) which concluded: "If the above studies did demonstrate a fluoridation benefit to younger children, any lasting benefit must be small." Since then, the authors of the 9-year-olds study (13) and its follow-up (14) have admitted that their low-fluoride sample used for comparison "probably was biassed towards children of dentally unaware and low socioeconomic parents - a factor that would tend to increase their caries prevalence" (15).

My earlier study (4) had also investigated the socioeconomic status of three pairs of New Zealand communities which were being presented as exemplifying the benefit of fluoridation. In each case the fluoridated community was of higher income level than the nonfluoridated community.

Recently another small-sample non-blind study has been published, claiming to demonstrate the benefit of fluoridation for the whole of New Zealand (16). Small samples of 5-year-old children were examined, from selected fluoridated and non-fluoridated communities in Otago and Canterbury provinces. The results (left side of Figure 1) claimed up to 60% less tooth decay in the fluoridated communities, with a nonfluoridated community which had recently defluoridated (Timaru) occupying an "in-between" position. But, when I obtained the School Dental Service data for *all* 5-year-olds in the fluoridated and nonfluoridated areas of these two provinces, and for *all* 5-year-olds in the recently defluoridated community, the claimed differences did not exist (right side of Figure 1). School Dental Service data also revealed that the nonfluoridated Otago community selected for the study (Oamaru) had poorer child dental health than other nonfluoridated communities in the province, and that there had been no deterioration in child dental health following defluoridation in Timaru.

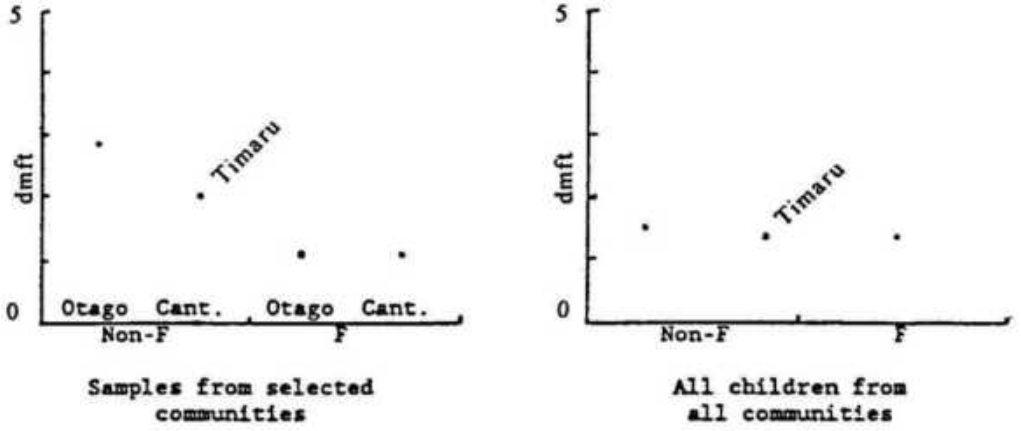


FIGURE 1. Left: Results of Canterbury-Otago dental survey of 5-year-olds (15). Right: Data for all 5-year-olds from all Canterbury and Otago fluoridated and nonfluoridated communities and from Timaru.

Conclusions from recent evidence

One can conclude, from recent evidence on tooth decay prevalences, that: 1) the claimed benefits of fluoridation are, at best, greatly exaggerated; 2) they are probably non-existent; and 3) they are possibly the reverse of what is claimed. That is, by weakening tooth structure, fluoridation actually makes teeth, in the long term, more prone to decay.

B. RE-EXAMINATION OF FLUORIDATION RESEARCH

Why do the faculties of our dental schools continue to produce studies of selected small samples which still claim a benefit from fluoridation? Why do prestigious review panels continue to produce one-sided reviews of evidence which support the claim? Why the abiding faith of dental and many medical professionals in the fluoridation theory? The answer may lie in the nature of professional education. Thomas Kuhn and others have described the hold which theories, once accepted, can exert on the minds of the professionals involved. Subsequent research becomes "a strenuous and devoted attempt to force nature into the conceptual boxes supplied by professional education" (17). I once shared that devotion and faith, but when the comprehensive School Dental Service data conflicted with the theory, I undertook a thorough re-examination of the classic research on which that theory is based. My findings were published in the peer-reviewed journal of the Australian and New Zealand Public Health Association (18), and are here summarized.

The "classic" research of fluoridation led to its endorsement in the 1950s. The research was in two stages: that establishing acceptance of an inverse fluoride-caries relationship (towns with higher natural water fluoride levels were supposed to have lower average levels of tooth decay); and the North American fluoridation trials.

The Fluoride-Caries Relationship

Researchers in the United States Public Health Service claimed to establish an inverse relationship between natural water fluoride levels and dental caries. This inverse relationship was in addition to an earlier established direct relationship between water fluoride levels and prevalence of dental fluorosis. Dean reported the fluorosis relationship based on studies in the 1930s of hundreds of United States communities (19,20). In contrast, his famous 1942 report of an inverse fluoride-caries relationship (21,22), which became the basis of widespread endorsement and practice of water fluoridation, presented data from only 21 communities in four States (Figure 2). Yet data from earlier caries surveys in hundreds of communities were available to Dean (23-26). The question arises: why did Dean later present only 21 of them?

The early fluorosis studies, mainly by others, were reported on by Dean who included on his survey forms a place for each child's caries status. Although not as uniformly collected as his later personal surveys, caries data were included - seen clearly from the facsimile of the survey form (see page 18) - in the array of fluorosis surveys (19,24,25) as well as in a survey made in 1933-34 in 26 States, which did not include data on fluorosis (26). Only two cities from Dean's 1938 paper on caries data (23) were included in his 1942 paper (22). Some data from the 1933-34 survey (26) were included (particularly for Indiana and Ohio), but not data from South Dakota and Wisconsin. Fluoridation proponents argue that Dean could not have had enough data on fluoride levels from the latter communities. That could be only partly true, because he had in fact a great deal of data for South Dakota (27). In 1939 he and McKay reported fluorosis data from 375 areas in 26 States which included also caries data (28). These caries data could have been compared with later data from low-fluoride areas where fluorosis was not endemic. Instead, caries scores from only a few of the communities were ever published.

Dean argued that he had to limit his analysis to the smaller number of cities because of the need to have reliable information like water histories. Proponents today echo the argument by claiming that the earlier studies showed less rigour, but Dean later acknowledged (29) that even the 21 cities' water histories were not accurate using his own criteria, namely continuous exposure of the observed group during childhood and an unchanged water source (19,20). Critics argue that the earlier data from the other communities, which were collected with less pro-fluoridation bias, should also have been reported. Also, factors other than fluoride (*e.g.* socio-economic and dietary differences, or other drinking water elements), not considered by Dean and other authors, could explain the variations in caries incidence. After Dean's studies reporting an inverse fluoride-caries relationship, about 23 others followed (30). They all presented data from selected communities. It was claimed that as water fluoride levels rose above 0.15 parts per million there was a corresponding reduction in tooth decay rates.

Critics claim that belief in the fluoride-caries relationship at low natural water-fluoride levels, on which the whole fluoridation theory rests, arose from presentation of highly selected data (31,32). When all the available published data were put together for the greater than 0.15 ppm areas (32), the claimed correlation disappeared (Figure 3). We cannot know what a plotting of randomly selected

communities would have shown. But the lack of correlation when the published studies are combined suggests that they were of communities selected to demonstrate the alleged association.

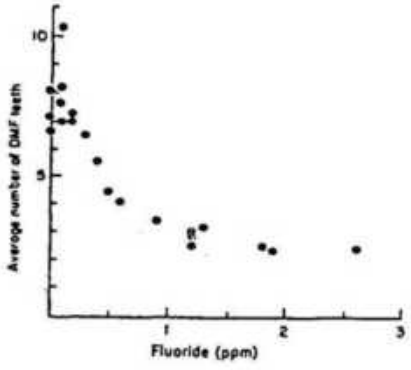


FIGURE 2. From Dean's classic 21 City Study, of "white children aged 12 to 14 years of 13 cities in 4 States" (22). The graph was compiled by adding data from 9 Chicago suburbs reported in an earlier study (21).

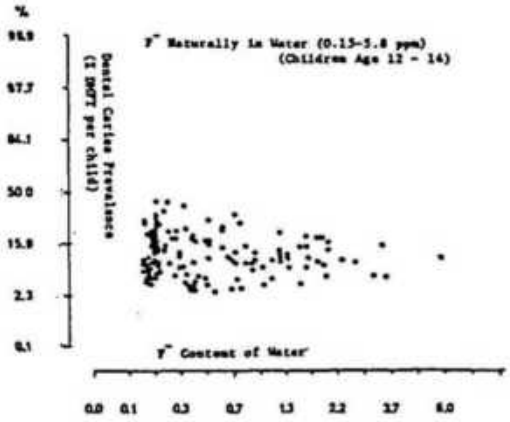


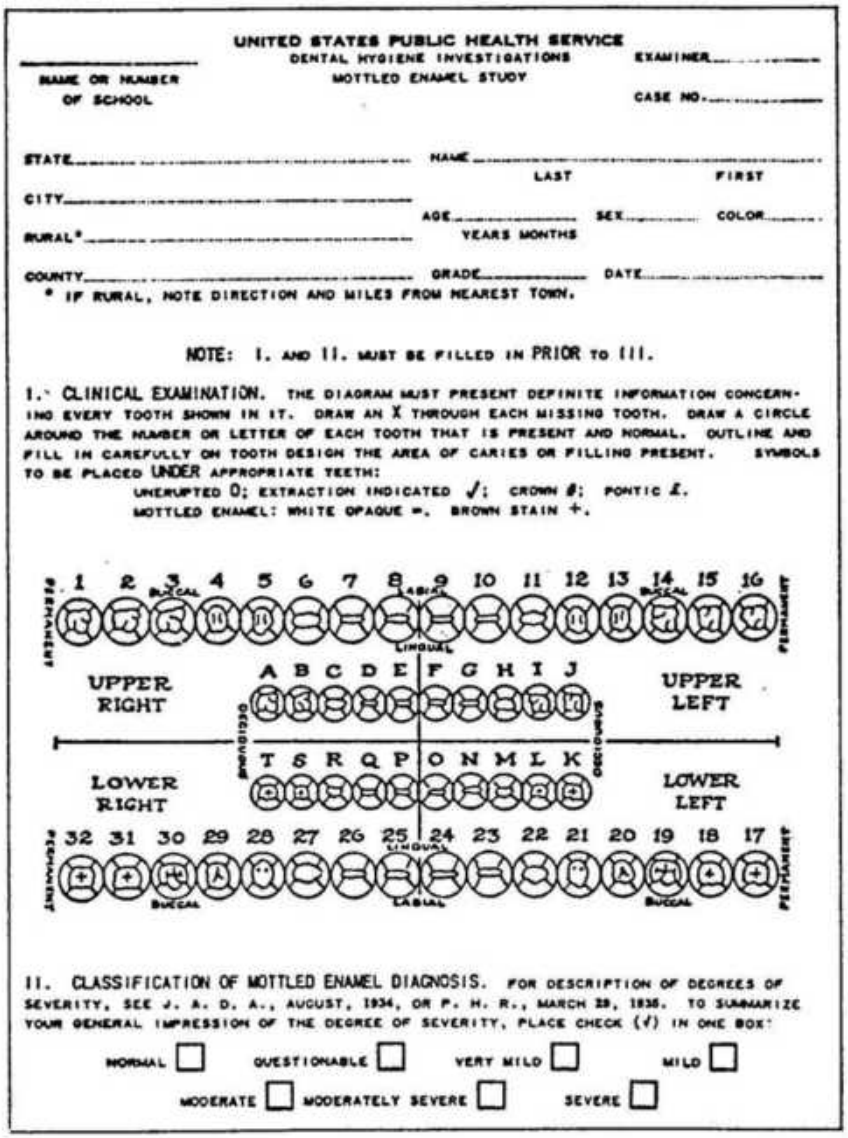
FIGURE 3. Natural water fluoride and tooth decay. Data for areas over 0.15 ppm water fluoride, from all published studies in North America and Europe, showing no correlation. From Ziegelbecker (32)

Fluoridation proponents have argued that the studies providing the caries data set in Figure 3 had been carried out by different examiners using different standards of diagnosis (33). That does not explain why Dean reported on only 21 communities in his classic study, out of the hundreds for which data were available.

Busse and associates have claimed that a re-analysis of Figure 3 data, involving manipulation to allow for regional variations and other multifactorial effects, confirm a fluoride-caries relationship (34). One would expect appropriate manipulation to show some fluoride-caries relationship because Figure 3 aggregates data mostly selected to support such a relationship. Brown has similarly argued that aggregation of data as in Figure 3 is invalid because "each region or community has its own caries gradient" (35). Yet Dean had also aggregated data from different regions to reach his result. Diesendorf has observed (personal communication) that any such analysis, whether by defenders or critics, is constrained by its limitation to cross-sectional, instead of time-dependent, studies.

It seems clear that the claimed inverse relationship between caries and water fluoride was, at best, exaggerated. It is true that caries is affected by many factors. But, given the magnitude of the claimed fluoride benefit, one could reasonably expect a fluoride-caries correlation to be more evident in Figure 3. Some earlier data (36-39) as well as the recent data already mentioned (5-10) do not support such a relationship.

Facsimile of the clinical examination section of one of Dean's survey forms. Similar forms were used in other surveys.



UNITED STATES PUBLIC HEALTH SERVICE
DENTAL HYGIENE INVESTIGATIONS
MOTTLED ENAMEL STUDY

NAME OR NUMBER OF SCHOOL _____ EXAMINER _____
CASE NO. _____

STATE _____ NAME _____ (LAST) (FIRST)
CITY _____ AGE _____ (YEARS MONTHS) SEX _____ COLOR _____
RURAL* _____ GRADE _____ DATE _____
COUNTY _____
* IF RURAL, NOTE DIRECTION AND MILES FROM NEAREST TOWN.

NOTE: I. AND II. MUST BE FILLED IN PRIOR TO III.

I. CLINICAL EXAMINATION. THE DIAGRAM MUST PRESENT DEFINITE INFORMATION CONCERNING EVERY TOOTH SHOWN IN IT. DRAW AN X THROUGH EACH MISSING TOOTH. DRAW A CIRCLE AROUND THE NUMBER OR LETTER OF EACH TOOTH THAT IS PRESENT AND NORMAL. OUTLINE AND FILL IN CAREFULLY ON TOOTH DESIGN THE AREA OF CARIES OR FILLING PRESENT. SYMBOLS TO BE PLACED UNDER APPROPRIATE TEETH:
UNERUPTED O; EXTRACTION INDICATED √; CROWN #; PONTIC Σ.
MOTTLED ENAMEL: WHITE OPAQUE =, BROWN STAIN +.

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
BUCCAL LABIAL BUCCAL
PERMANENT LINGUAL PERMANENT
UPPER RIGHT A B C D E F G H I J UPPER LEFT
DISTO-BUCCAL MESIO-BUCCAL
LOWER RIGHT T S R Q P O N M L K LOWER LEFT
32 31 30 29 28 27 26 25 24 23 22 21 20 19 18 17
PERMANENT LINGUAL PERMANENT
BUCCAL LABIAL BUCCAL

II. CLASSIFICATION OF MOTTLED ENAMEL DIAGNOSIS. FOR DESCRIPTION OF DEGREES OF SEVERITY, SEE J. A. D. A., AUGUST, 1934, OR P. H. R., MARCH 29, 1935. TO SUMMARIZE YOUR GENERAL IMPRESSION OF THE DEGREE OF SEVERITY, PLACE CHECK (√) IN ONE BOX:

NORMAL ☐ QUESTIONABLE ☐ VERY MILD ☐ MILD ☐
MODERATE ☐ MODERATELY SEVERE ☐ SEVERE ☐

The North American Fluoridation Trials

The preliminary results from the first (Grand Rapids-Muskegon) North American fluoridation trial (40), which commenced in 1945, led to the official endorsement of fluoridation in 1950, by the United States Public Health Service, soon followed by the Dental and Medical Associations and various other professional organizations and authorities. Other critics (31,41,42) drew my attention to the flaws in this trial, which are here described.

The preliminary results had claimed a 51.3% reduction in decay, after 4½ years, for children aged 6 years in Grand Rapids. But when we look at the scores for

6-year-olds which were published three years later (43) we find that an impossible 70.5% reduction was recorded in the first year of the trial and that there was then an increase but no overall reduction in the following years (Figure 4). The explanation is not hard to find. All children from the 79 schools in Grand Rapids ware examined at the commencement of the trial, but in succeeding years only a selected sample, claimed to be representative, being the children from 25 of the 79 schools. In the control city of Muskegon all children were examined throughout the period.

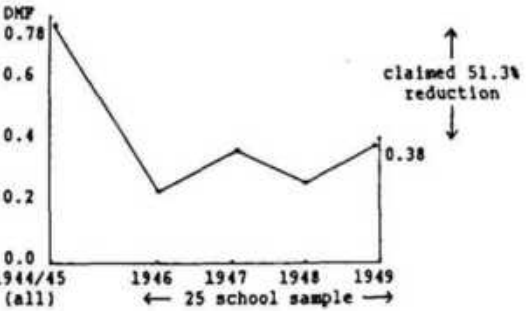


FIGURE 4. Published results of first fluoridation trial. Average DMF teeth of 6-year-olds in Grand Rapids during 4½ years from start of fluoridation (43)

Examination of the published data for other age groups also confirms that the sample of 25 schools was not representative of the population being studied. The reported DMFT of some age groups, approximately one year after the initial examinations, was lower than that of the same children when they were a year younger. For example. the 10-year-olds had DMFT 4.9 in 1945. In 1946, when they had become 11-year-olds, they had DMFT 4.2. Fluoridated water cannot turn decayed, missing and filled teeth into sound ones. It follows that the large recorded tooth decay reductions, which were mostly in the first year, resulted from selecting an unrepresentative sample.

Muskegon was fluoridated 6½ years after the trial commenced, and so was lost as an experimental control. An official report at the time has revealed that decay had also declined in the control city (44).

A critical examination of other early fluoridation trials revealed similar flaws, also detected by others. The basic limitations of the classic fluoridation trials were described over 30 years ago: poor research design including inadequate experimental controls, poor adjustment of sample sizes, lack of "blind" examinations to safeguard against examiner bias and variability, inadequate baseline measures and negligible statistical analyses (41). Diesendorf has subjected the Australian fluoridation trials to similar critical analysis (45). Textbook accounts, both of the caries-fluoride relationship and of the fluoridation trials, do not reveal the flaws which characterize most of the early research.

The Anglesey Trial

Critiques (46,47) of the more recent British "Anglesey" study have explained why its claimed "strictly blind conditions" were worthless. A fluoridated semi-rural island was compared with a nonfluoridated mainland urban "control", with no pre-fluoridation information on it, where one might expect a higher caries rate.

Defluoridation Studies

Studies claiming to show an increase in tooth decay following defluoridation - for example, in Wick (48) and Stranraer (49) in Scotland - have similar flaws. In Stranraer the DMF of 10-year-olds was reported to rise, after defluoridation, by only 4% in 6 years, the increase consisting wholly of the "missing and filled" component. In Wick, the children examined were 5- to 6-year-olds who have few if any permanent teeth. The "rise" consisted almost entirely of a 61% increase in the number of temporary teeth extracted. The "decayed and filled" component increased by only 0.4%. Such an increase reflects a change in the treatment pattern of Wick dentists, following the decision to defluoridate. No doubt the dentists believed they were responding to real increases in decay. But statistics obtained from much larger numbers of children (50,51) show no increase in tooth decay following stopping of fluoridation.

New Zealand's Fluoridation Trial

Most New Zealand water supplies were fluoridated following reports of "spectacular reductions" in dental decay during the nation's first fluoridation trial in the city of Hastings (52). Health Department files, which became available for public scrutiny after application under New Zealand's Official Information Act, revealed that the reductions in dental decay in Hastings were mainly the result of instructions to change diagnostic procedures in school dental clinics, affecting the definition of "decay" and the number of fillings inserted (53,54). The instructions were given after the study commenced and in the area of the experiment only, but were not mentioned in published reports of the study. The experimental control city was abandoned when its decay rates were found to be lower than in fluoridated Hastings.

The revelations about the Hastings fluoridation trial have not been refuted. In fact, it has been admitted that a diagnostic change occurred, but claimed that it affected only occlusal (biting) surfaces of back teeth which are now thought to be least affected by fluoridation (35,55). The authors of the Hastings exposure have pointed out (56,57): first, that the initial claims of the "dramatic reductions" in tooth decay, made after the Hastings and most other fluoridation trials, were for such biting surfaces of 6- and 7-year-olds' teeth; and second, the released archives showed that instructions, to change diagnosis affecting all tooth surfaces, were given later than and in addition to earlier instructions affecting only occlusal surfaces.

Of the Hastings exposure the authors stated: "there is no doubt about the good intentions and sincere commitment of the professionals who conducted the experiment. They had faith in their theory that fluoridation would provide an immense benefit, based on their acceptance of evidence from the United States" (53).

References

1 Data collected by Area Health Boards and sent to Department of Health.
2 Annual Reports. Department of Health, from 1981. Government printer, Wellington.
3 Colquhoun J. Influence of social class and fluoridation on child dental health. *Community Dentistry and Oral Epidemiology 13* 37-41 1985.
4 Colquhoun J. Child dental health differences in New Zealand. *Community Health Studies 11* 85-90 1987.
5 Diesendorf M. The mystery of declining tooth decay. *Nature 322* 125-129 1986.
6 Yiamouyiannis JA. Water fluoridation and tooth decay: Results from the 1986-1987 national survey of US schoolchildren. *Fluoride 23* 55-67 1990.

7 Steelink C, Fowler M, Osborn M *et al*. Findings and recommendations of subcommittee on fluoridation. City of Tucson AZ 1992 (PO Box 27210).
8 Hildebolt CF, Elvin-Lewis M, Molnar S *et al*. Caries prevalences among geochemical regions of Missouri. *American Journal of Physical Anthropology 78* 79-92 1989.
9 Ziegelbecker RC, Ziegelbecker R. WHO data on dental caries and natural water fluoride levels. *Fluoride 26* 263-266 1993.
10 Teotia SPS, Teotia M. Dental caries: a disorder of high fluoride and low dietary calcium interactions (30 years of personal research) *Fluoride 27* 1994 (in press).
11 Evans RW, Beck DJ, Brown RH, Silva PA. Relationship between fluoridation and socioeconomic status on dental caries experience in 5-year-old New Zealand children. *Community Dentistry and Oral Epidemiology 12* 5-9 1984.
12 Fergusson DM, Harwood IJ. Exposure to additional fluoride, social background and dental health in 7-year-old children. *Community Dentistry and Oral Epidemiology 14* 48-52 1986.
13 de Liefde B, Herbison GP. Prevalence of developmental defects of enamel and dental caries in New Zealand children receiving differing fluoride supplementation. *Community Dentistry and Oral Epidemiology 13* 164-167 1985.
14 de Liefde B, Herbison GP. The prevalence of developmental defects and dental caries in New Zealand children receiving differing fluoride supplementation, in 1982 and 1985. *New Zealand Dental Journal 85* 2-8 1989.
15 de Liefde B, Herbison GP. Reply to letter. *New Zealand Dental Journal 86* 72 1990.
16 Treasure ET, Dever JG. The prevalence of caries in 5-year-old children living in fluoridated and non-fluoridated communities in New Zealand. *New Zealand Dental Journal 88* 9-13 1992.
17 Kuhn TS. *The Structure of Scientific Revolutions*. University of Chicago Press, Chicago 1970.
18 Colquhoun J. Flawed foundation: A re-examinationof the scientific basis for a dental benefit from fluoridation. *Community Health Studies 14* 288-296 1990.
19 Dean HT. Distribution of mottled enamel in the United States. *Public Health Reports 48* 703-734 1933.
20 Dean HT, Elvove E. Some epidemiological aspects of chronic endemic dental fluorosis. *American Journal of Public Health 26* 267-275 1936.
21 Dean HT, Jay P, Arnold FA, Elvove E. Domestic water and dental caries. II. A study of 2,832 white children aged 12-14 years, of eight suburban Chicago communities, including *L. acidophilus* studies of 1,761 children. *Public Health Reports 56* 761-792 1941.
22 Dean HT, Arnold FA, Elvove E. Domestic water and dental caries. V. 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.
23 Dean HT. Endemic fluorosis and its relation to dental caries. *Public Health Reports 53* 1443-1452 1938.
24 Dean HT, Elvove E. Studies on the minimal threshold of the dental sign of chronic endemic fluorosis (mottled enamel). *Public Health Reports 50* 1719-1729 1935.
25 Dean HT, McKay FS, Elvove E. Mottled enamel survey of Bauxite, Ark, ten years after a change in the common water supply. *Public Health Reports 53* 1736-1748 1938.
26 Messner CT, Gafafer WM, Cady FC, Dean HT. Dental survey of school children, ages 6-14 years in 1933-34 in 26 States. *Public Health Bulletin no. 226*. May 1936.
27 Dean HT, Elvove E, Poston RF. Mottled enamel in South Dakota. *Public Health Reports 54* 212-228 1939.
28 Dean HT, McKay FS. Production of mottled enamel halted by a change in common water supply. *American Journal of Public Health 29* 590-596 1939.
29 Proceedings: City of Oroville vs Public Utilities Commission, State of California, October 20-21 1955. Proceedings: Injunction Suit Chicago Citizens vs City of Chicago, May 13 1960.
30 The studies are listed in references 32 and 34.

31 Exner FB, Waldbott GL. *The American Fluoridation Experiment*. Devin-Adair, New York 1957.
32 Ziegelbecker R. Fluoridated water and teeth. *Fluoride 14* 123-128 1981.
33 Murray JJ, Rugg-Gunn A. *Fluoridation and Declining Decay. A Reply to Diesendorf.* British Fluoridation Society, London 1987.
34 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.
35 Brown RH. Fluoride and the prevention of dental caries. Part I: The role of fluoride in the decline of caries. *New Zealand Dental Journal 84* 103-108 1988.
36 Hewat RET, Eastcott DF. *Dental Caries in New Zealand*. Medical Research Council of New Zealand, Christchurch 1954.
37 Imai Y. Study of the relationship between fluoride ions in drinking water and dental caries in Japan. *Japanese Journal of Dental Health 22* 144-196 1972.
38 Forsman B. Dental fluorosis and caries in high-fluoride districts in Sweden. *Community Dentistry and Oral Epidemiology 2* 132-148 1974.
39 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.
40 Dean HT, Arnold FA, Jay P *et al.* Studies on mass control of dental caries through fluoridation of the public water supply. *Public Health Reports 65* 1403-1408 1950.
41 Sutton PRN. *Fluoridation: Errors and Omissions in Experimental Trials.* Melbourne University Press, Melbourne 1960.
42 Ziegelbecker R. Open letter to Lord Jauncey (summary). *National Fluoridation News 29* 3-4 1983.
43 Arnold FA, Dean HT, Knutson JW. Effect of fluoridated public water supplies on dental caries prevalence. Results of the seventh year of the study at Grand Rapids and Muskegon. *Michigan Public Health Reports 68* 141-148 1953.
44 Report of the ad hoc committee on fluoridation of water supplies. National Research Council publication no. 214. National Academy of Sciences, 1952. *Journal of the American Water Works Association 44* 1-9 1956.
45 Diesendorf M. A re-examination of Australian fluoridation trials. *Search 17* 256-261 1986.
46 Diesendorf M. Anglesey fluoridation trials re-examined. *Fluoride 22* 53-58 1989.
47 Colquhoun J. Letter. *Nature 324* 298 1986.
48 Stephen KW, McCall DR, Tallis JI. Caries prevalence in Northern Scotland before, and 5 years after, water defluoridation. *British Dental Journal 163* 324-326 1987.
49 Attwood D, Blinkhorn AS. Trends in dental health of ten-year-old children in South-West Scotland after cessation of fluoridation. *Lancet 2* 266-267 1988.
50 Kalsbeek H. Evidence of decrease in prevalence of dental caries in the Netherlands: An evaluation of epidemiological caries surveys on 4-6- and 11-15-year-old children, performed between 1965 and 1980. *Journal of Dental Research 61* (Special Issue) 1321-1326 1982.
51 New Zealand Department of Health dental statistics for all 12- and 13-year-old School Dental Service patients in communities which stopped fluoridation.
52 Ludwig TG. The Hastings fluoridation project. *New Zealand Dental Journal 54* 165-172 1958, *55* 176-179 1959, *58* 22-24 1962 (co-author EIF Pearce), *59* 298-301 1963, *61* 175-179 1965, *67* 155-160 1971.
53 Colquhoun J, Mann R. The Hastings fluoridation experiment: Science or swindle? *The Ecologist 16* (6) 243-248 1986, *17* (2) 125-126 1987.
54 Department of Health fluoridation files. New Zealand National Archives, Wellington.
55 Hunter PB, Storey E. The Hastings experiment confirmed. Unpublished article circulated by New Zealand Department of Health, 1987.
56 Colquhoun J, Mann R. *Hastings Fluoridation: Reply to Health Department* (contains reprint of reference 55). Published by Colquhoun and Mann, Auckland 1988.
57 Colquhoun J, Mann R. Letter. *New Zealand Medical Journal 102* 143 1989.

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

TERATOGENIC EFFECTS OF FLUORIDE ON CHICK EMBRYO

S Krupanidhi and T M Dhanarajan
Prasanthinilayam, India

SUMMARY:

1) The teratogenic effects of sodium fluoride on the ankle joint and toe bones of white leghorn chick embryos are reported.

2) Due to endochondrial osteofluorosis, the excessive growth of trabeculae in the epiphyses of distal tibiotarsus and proximal tarsometatarsus obliterated the ankle synovial joint space.

3) An increase occurred in the volume of bones, such as tibiotarsus, tarsometatarsus and phalanx.

4) Toe bones, namely phalanx and their articular surfaces with fibrocartilage containing spherical chondrocytes, were observed in control embryos. In fluoride-treated embryos, the distal and proximal regions of the phalanx grew enormously and irregularly, leaving little space for articulation.

5) Blackening and falling of feathers occurred in the fluoride-treated chick embryos.

Key words: Ankle joint; Chick embryo; Fluoride; Phalanx; Teratogenic effect.

Introduction

Animals normally ingest, in their routine life through their food and water, small amounts of fluoride which most uncommonly cause deleterious effects. The histopathological responses in animals that ingest excessive fluoride are governed by a number of factors (1). Fluoride commonly substitutes for hydroxyl ions present in bone, and a series of bone seeking cations, namely radium, strontium, and lead substitute for calcium. It has been observed that the gradual accumulation of fluoride over an appreciable length of time results in changes in structure of bone, *i.e.* osteofluorotic lesions of ribs, mandible, and metaphyseal areas of metatarsal and metacarpal bones (2). The morphometric measurements of bones of lower leg and forearm have been considered primarly in diagnosis of fluorotic changes in bones, and significantly higher values of mataphyseal and diaphyseal cortical indices of tibia and radioulna have been reported in workers of an aluminium factory in the age group of 10 to 27 years (3). Despite the availability of voluminous information on the deleterious effect of fluoride toxicity, comparatively little is known about its teratogenic effects.

The avian embryo, particularly that of chick, has been used as a convenient material to investigate the direct effects of drugs and chemicals on developing morphogenetic systems since maternal influences are excluded (4,5,6). The chick embryo possesses its own intact metabolic system, and comparable studies have claimed a predictive value of the chick embryo system comparable to other *in vitro* systems, as well as to whole animal systems (7).

Department of Biosciences, Sri Sathya Sai Institute of Higher Learning, Prasanthinilayam 515 134, Andhra Pradesh, India.

Therefore, an attempt has been made in the present investigation to study teratogenic effects of sodium fluoride on the synovial ankle joint and toe bones of developing chick embryos of white leghorn breed.

Materials and Methods

Fertilized eggs of white leghorn breed obtained from Bangalore Agricultural University, were wiped clean with 70% alcohol and incubated in a BOD incubator at 37±1°C and 60% RH for 96 hours. They were then divided into four batches, each comprising 20 eggs in the following order: The first batch, treated as control, was injected with 0.5 ml of distilled water; the second batch was injected with 1 ml sodium fluoride solution containing 50 mg; the third batch was injected with 0.5 ml sodium fluoride solution containing 25 mg; and the fourth batch with 0.1 ml of sodium fluoride solution containing 5 mg. The quantity of sodium fluoride solution as indicated above was injected into eggs deep into the yolk sac through its broader surface and later the bore was sealed with transparent tape and kept for incubation. Candling was done daily to observe development in eggs. The eggs incubated for 19 days (stage, 45) (8) were chosen for the present investigation.

Alizarin Red S Staining: Embryos cleaned in Locke's chick ringer solution were processed for fixation, clearing, staining and destaining as described by Richmond and Bennett (9). The photographs were taken using a Carl Zeiss stereomicroscope with a magnification of 1.6 x 0.5 times.

Results

To investigate teratogenic effects of fluoride on skeletal abnormalities, the phalanx of ankle joints and toe bones were selected for study in developing chick embryos. Control embryos incubated for 19 days showed a clean synovial ankle joint with articular surface containing fibrocartilage between tibiotarsus and tarsometatarsus (Figure 1). The embryos injected with 25 mg of NaF revealed conspicuous effects compared to treatment with 5 mg of NaF. The embryos injected with 50 mg of NaF were dead at an early stage. The experimental embryos showed an overgrowth in epiphyses of the distal tibiotarsus and the proximal tarsometatarsus, obliterating complete synovial joint and leaving little space for articulation (Figure 2). This reduces the fibrous connective tissue capsule which would lead to the impairment of mobility. There is also an increase in bone volume as is evident in Figures 2 and 5 due to endochondrial osteofluorosis. In response to fluoride toxicity metaphyses of tibiotarsus and tarsometatarsus in the region of the ankle joint could have differentiated into a layer of osteoprotenitor cells which upon differentiating into osteoblasts and later osteocytes possibly facilitated an increase in growth of bone at the articular surface.

The interphalangeal joints in toes are hinge joints facilitating uni-axial articulation, and their articular surface is provided with fibrocartilage (Figure 3). In control chick embryos, in between two phalanges there is the development of the articular surface with fibrous cartilage filled with transparent ground substance enclosing spherical chondrocytes (Figure 4). Whereas, in a comparable stage of treated embryos, there is an excessive and irregular growth of osteoid tissue of phalanx (Figures 5 and 6).

Figure 1 (above). Control chick embryo. The synovial ankle joint between tibiotarsus and tarsometatarsus with articular surface of fibrocartilage.
Figure 2 (below). Fluoride treated (25 mg). Obliteration of ankle joint space due to excessive growth of tibiotarsus and tarsometatarsus.
Note increase in volume of bones *cf.* Figure 1.

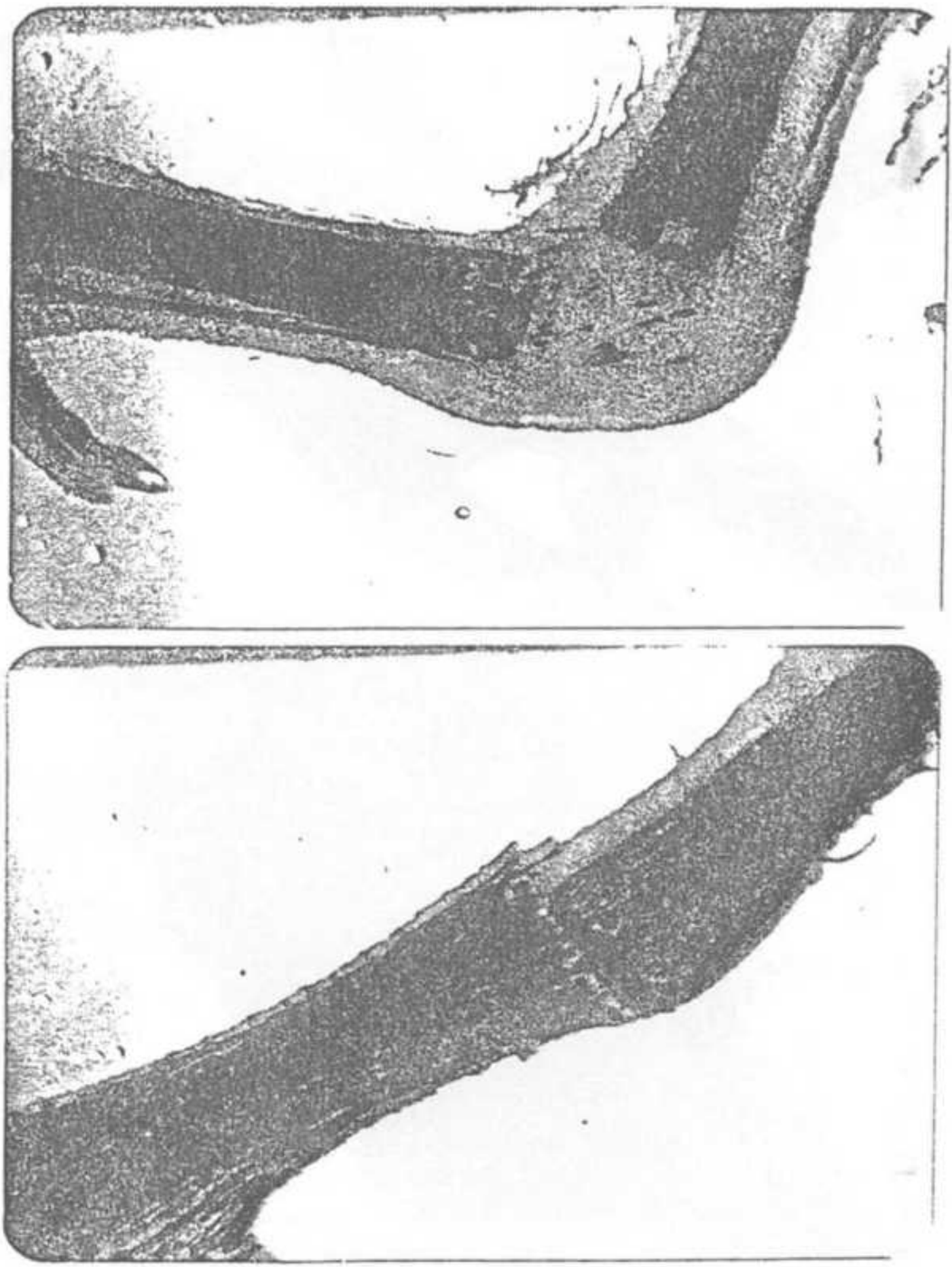
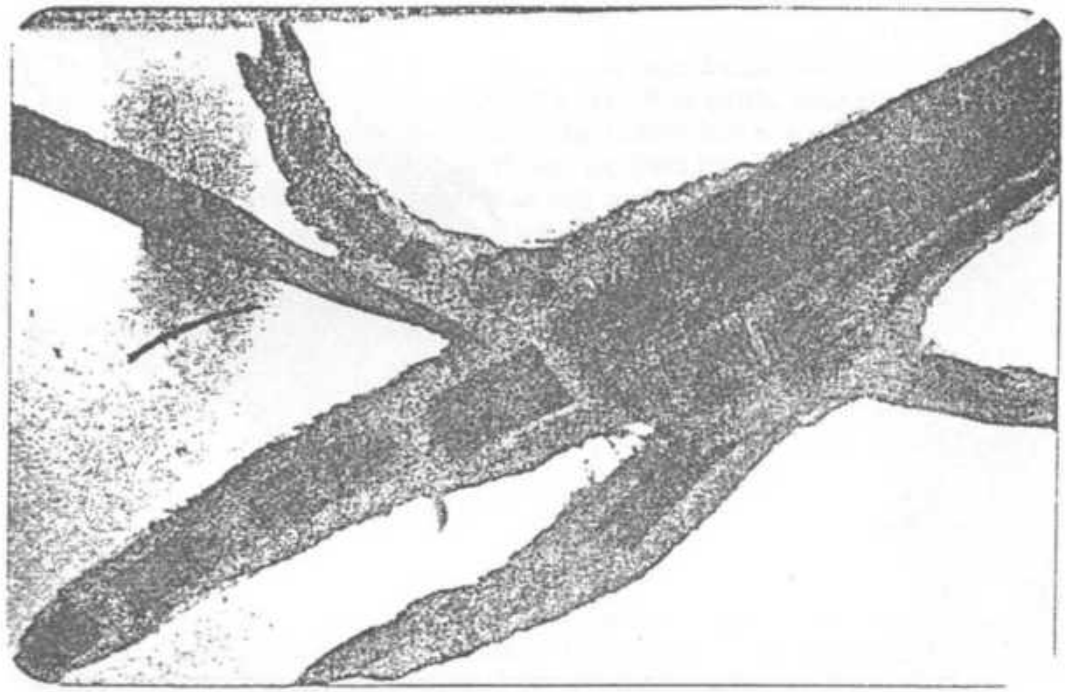


Figure 3 (above). Control embryo. Hind limb showing four digits with phalanx and uniform articular surface between them.

Figure 4 (below). Control embryo. Fibrocartilage between phalanx of toes with spherical chondrocytes in a ground transparent substance.



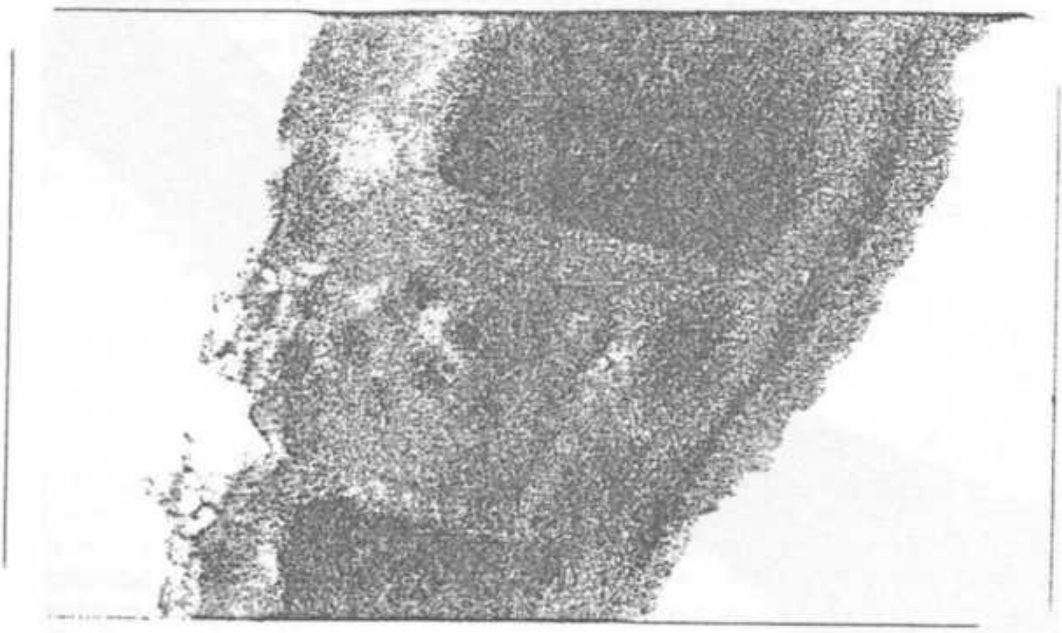
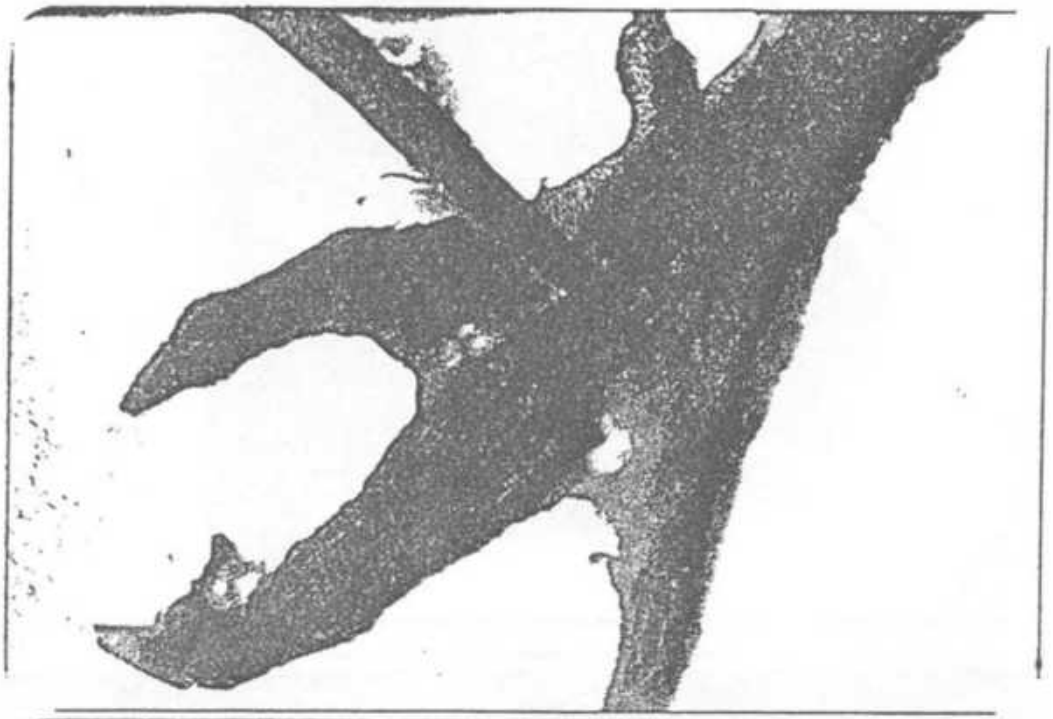


Figure 5 (above). Fluoride-treated. Irregular and abnormal growth of phalanges displacing fibrocartilage between them and impairing uni-axial articulation.
Figure 6 (below). Fluoride-treated. Proximal and distal terminals of phalanx with abnormally prominent trabeculae due to excessive ossification and corresponding reduction in fibrocartilage in the hinge-joint.



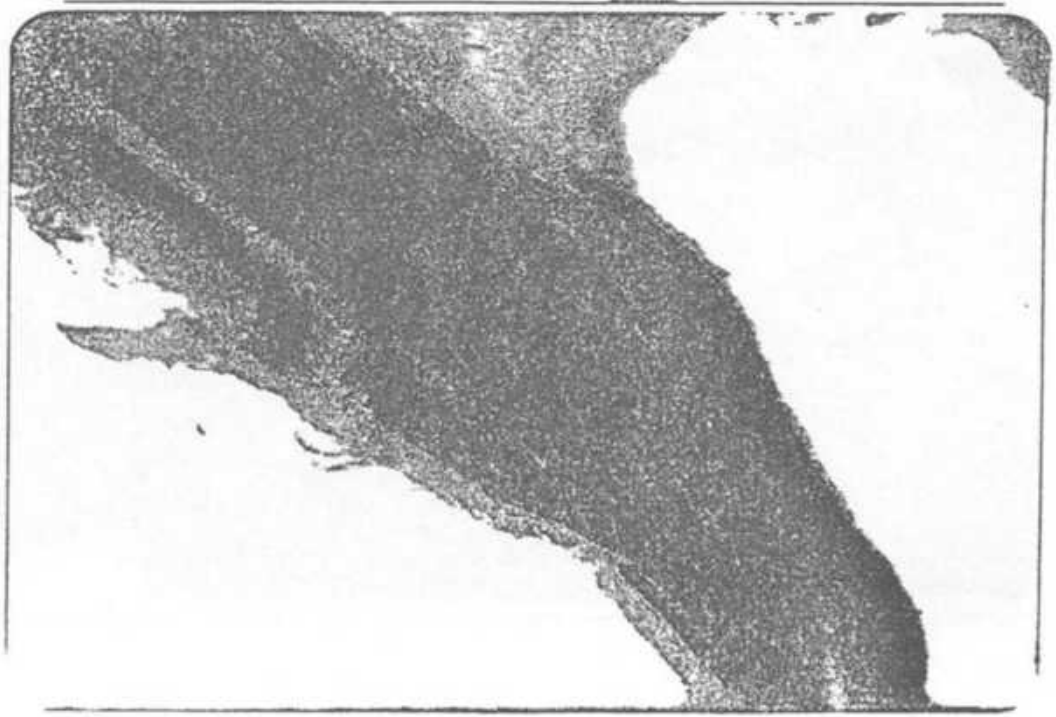


Figure 7 (above). Fluoride-treated. Falling of neossoptiles and teleoptiles and their blackening.
Figure 8 (below). Control chick embryo with white feathers.

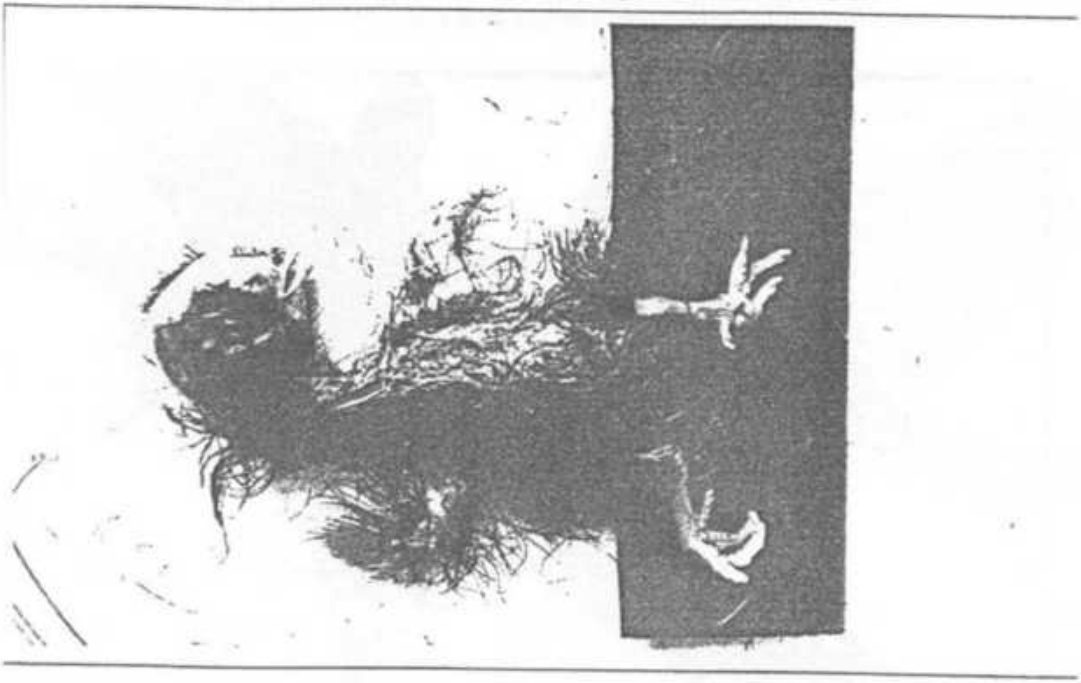
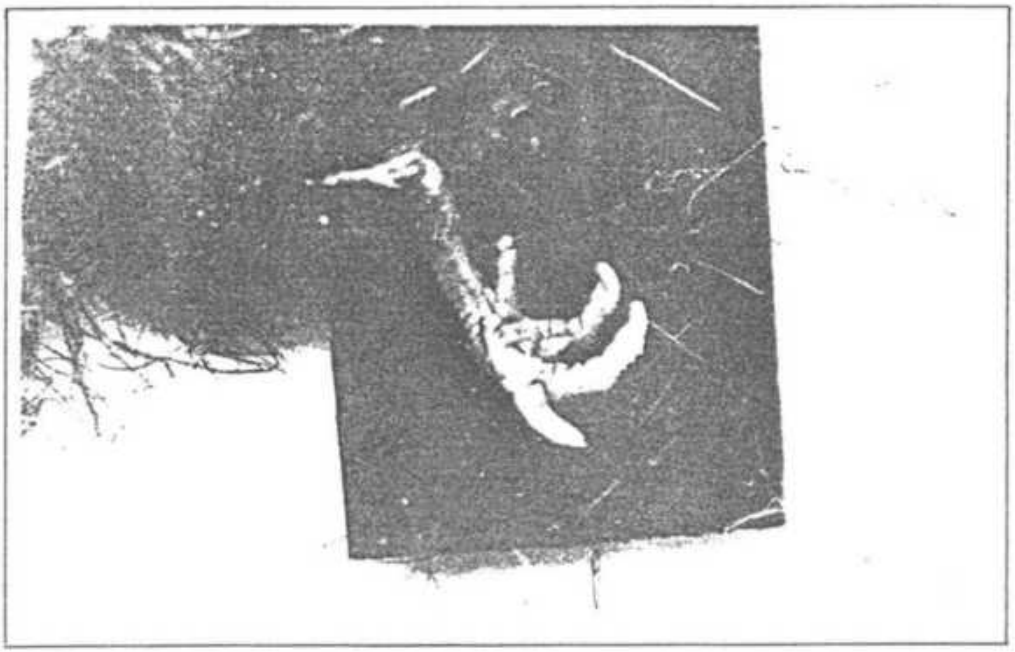
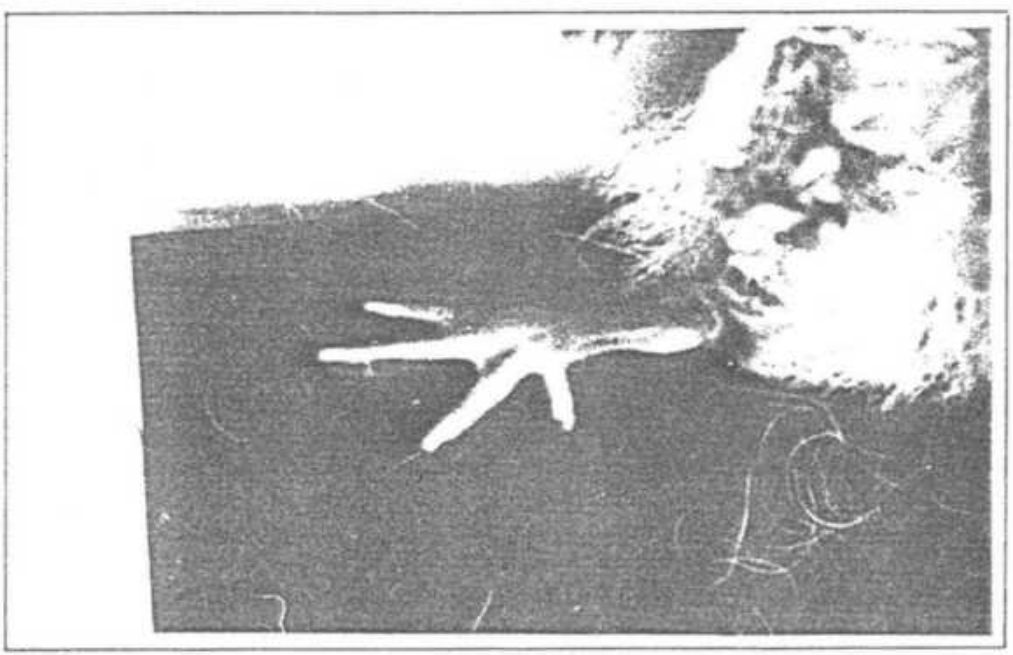


Figure 9 (above). Fluoride-treated. The toes showing stiffness and loss of flexibility.

Figure 10 (below). Control chick embryos with normal digits spreading.





Apart from endoskeletal malformations, the exoskeletal derivatives (feathers) are also severely affected due to fluoride toxicity. The loss of neossoptiles and teleoptiles and their blackening are noticed in the fluoride treated (25 mg) embryos incubated for 19 days (Figure 7). However, in control embryos, the feathers remained the usual silvery white in colour (Figure 8). In general, the embryos exposed to fluoride became weak, joints lost flexibility, and bones became fragile (Figures 7 and 9) compared to control embryos (Figure 10).

Discussion

The results discussed here pertained to the embryos treated with 25 mg of sodium fluoride, which is equivalent to 115 ppm of fluoride after considering the egg volume approximately as 100 ml. The fluoride tolerance levels in feed and water for growing chickens were 150 ppm and 11 ppm, respectively, without any appreciable clinical signs and lesions (2). As the developing embryonic system is more sensitive, fluoride imparts teratogenic effects at a concentration well below the tolerance limits prescribed for growing chickens. In the present discussion the comparison with the embryos treated with 5 mg of NaF was not made, as the results obtained were not consistent and conspicuous enough to visualize through photographs.

The excessive osteoid growth in synovial ankle joint displacing fibrous cartilage and ligaments, resulting from fluoride treatment, causes a severe impairment in bearing the total weight of the bird during perching. Therefore, the chick after hatching will be handicapped for normal movements. The sites of muscular and tendinous insertions could have been abnormally prominent in experimental embryos due to the development of multiple exostoses, namely the increase in density/volume of bones (Figures 2 and 5). A similar observation was made in the bony biopsy obtained from the tibia and iliac crest in 21 cases of affected human beings, revealing the growth of osteoid tissue in spongy bone among well formed trabeculae containing considerably high amounts of calcium (10). The irregular development of distal and proximal articular surfaces of phalanx causes the stiffness of toes, impairing uni-axial articulation of hinge joints, which in turn could affect the perching mechanism, locomotion, defence, and predation.

Virgintino *et al* (11) have reported teratogenic effects of methyl carbamate on the avian skeletal apparatus, namely Meckel's cartilage, and found that the cartilages undergo deformities with the death of chondroblasts in the extracellular matrix. In the present findings the fibrous cartilage in the hinge joints of control chick embryos shows spherical chondrocytes providing articular surface (Figure 4). However, irregular growth of trabeculae is conspicuous in the fluoride treatment (Figure 6), occupying the space in the hinge joints and thereby depriving the articular surface for free uni-axial movements. Furthermore, as suggested by Virgintino *et al* (11), the fibrous cartilage between hinge joints of phalanx of experimental embryos possibly has undergone deformation with the death of chondroblasts. The space provided could have been invaded by the osteoid growth of phalanx due to endochondrial osteofluorosis. These findings reveal that fluoride, like organophosphorus and methyl carbamate compounds (11), brings about abnormalities in the skeletal

apparatus during embryogenesis. This could possibly be carried further with greater intensity in the adult bird, impairing its normal life, as has been observed in fluoride toxicosis in animals and humans (2), thus suggesting fluoride induced teratogenicity.

References

1 Kintner RR. Dietary fluoride intake in the USA revisited (Part 2). *Fluoride 24* 51-61 1991.

2 Shupe JL, Olson AE. Clinical and pathological aspects of fluoride toxicants in animals. In: *Proceedings of International Fluoride Symposium.* Utah State University, Logan UT 1982 pp 319-337.

3 Czerwinski K. Morphometric measurements in the diagnosis of fluorotic changes in the long bones. *Fluoride 11* (2) 46-54 1978.

4 Wilson JG. Survey of *in vitro* systems, their potential use in teratogenicity screening. In: Wilson JG, Fraser FR (Eds). *Handbook of Teratology.* Plenum Press, New York 1978 pp 135-153.

5 Clemedson C, Schmid B, Walum E. Effects of carbon tetrachloride on embryonic development studies in the post-implantation rat embryo culture system and in chick embryos *in ovo. Toxicology In-Vitro 3* (4) 271-275 1989.

6 Hemminki K. Toxicity of rubber chemicals in the chicken embryos. *Scandinavian Journal of Workplace and Environmental Health 9* (12) 73-77 1983.

7 Jelinek R. Use of chick embryo in screening for embryo toxicity. *Teratogen Carcinogen Mutagen 2* (3/4) 225-263 1982.

8 Hamburger V, Hamilton H. A series of normal stages in the development of the chick embryo. *Journal of Morphology 88* 49-92 1951.

9 Richmond GW, Bennett L. Modified Dawson's method for staining fœtal skeletons. *Stain Technology 13* 77 1938.

10 Jolly SS. An epidemological, clinical and biochemical study of endemic, dental and skeletal fluorosis in Punjab. *Fluoride 1* (2) 65-75 1968.

11 Virgintino DNL, Abbate D, Ribatti M *et al.* Methylcarbamate effects on Meckel's cartilages in the chick embryo. *Biological Structure and Morphology 8* (3) 85-88 1989.

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

CASE REPORT: MASS FLUORIDE POISONING, HOOPER BAY, ALASKA.

A REVIEW OF THE FINAL REPORT OF THE ALASKA DEPARTMENT OF HEALTH AND HUMAN SERVICES, APRIL 12, 1993.

Richard G Foulkes
Abbotsford, Canada

SUMMARY: The death of a 41-year-old male and the illness of approximately 296 others on May 21-23, 1992, in Hooper Bay Alaska has been shown to be due to acute fluoride intoxication caused by malfunction of the fluoridation equipment of system 1 of the village's two-system (well) water supply. Fluoride levels were reported to be as high as 150 ppm.

The "Final Report" prepared by the Section of Epidemiology, Alaska Department of Environmental Conservation (DEC) and the US Public Health Service (USPHS) is dated April 12, 1993. This document shows that gastro-intestinal symptoms were predominant. The investigators "suggest" that the minimum lethal dose for fluoride, when consumed by humans over 24-32 hours, is 20 mg/kg. This is higher than the "probably toxic dose" of 5 mg/kg calculated for a single ingestion (Whitford 1989). Serum half life of 3.5 hours of the hospitalized patient was within the range previously reported (Ekstrand *et al* 1980). However, the recovery time of 19 days for plasma fluoride and systemic toxicity was longer than previously reported (Heifetz and Horowitz 1986). The level of dose causing illness, 0.3 mg/kg, was 27 times lower than the dose previously reported; for example, the "maximum safely tolerated dose" of 8.0 mg/kg (Heifetz and Horowitz 1986).

The final report cites a number of reasons for the system failure. These include human error, mechanical failure, lack of safety features and failure to comply with regulations.

The report recommends re-affirmation of fluoridation by the Alaska Division of Public Health; the determination by USPHS and DEC that operational safety features are in place; and, that DEC should ensure compliance with regulations.

Key words: Fluoride poisoning; Hooper Bay, Alaska.

Location

Hooper Bay is a Yup'ik Eskimo settlement of approximately 950 people on the Bering Sea coast of Alaska. In Hooper Bay, as in 150 other rural Alaskan communities, fluoride has been added to drinking water for many years for the purpose of preventing dental caries. A press report states that a survey carried out by the Indian Health service examiners in 1990 on the North Slope Borough of Alaska showed that the dental health of these natives was "the worst in the US" (*Ketchikan Daily News*).

The enthusiasm for fluoridation of native communities in Alaska, as well as in many areas of the "lower 48 states", includes adjusting some school supplies to 5 ppm, a value in excess of the EPA's Maximum Contaminant Level of 4 ppm (USPHS/CDC 1985).

Richard G Foulkes BA MD, PO Box 278, Abbotsford BC, Canada V2S 4N9.

In Hooper Bay, both system 1 and system 2 (well) water supplies were fluoridated at 1.2 ppm. On May 21-23, 1992, system 1, owing to a malfunction, delivered fluoride at a level of 150 ppm. This resulted in the death of a 41-year-old male and the illness of approximately 296 others, including one adult female who was admitted to hospital in serious condition.

Symptoms

Among 81 people, interviewed during a household survey, who were served by system 1, 51 (63%) had illness meeting the case definition. This suggested that among all 470 residents of system 1 63%, or 296 of them, developed fluoride poisoning.

All were Alaskan Native of whom 50% were female. Their ages ranged from 6 months to 73 years (median 21 years).

Symptoms. were related primarily to the gastro-intestinal system, *i.e.*, nausea, vomiting, diarrhea, and abdominal pain. Other symptoms included headache, itching, weakness, numbness or tingling of an extremity, shortness of breath, and fatigue. The median latency period (38 cases) ranged from less than 1 to 150 minutes (median 7 minutes) and the median duration of illness (53 cases) ranged from 1 to 132 hours (median 24 hours).

The case of the individual who died was characterized by repeated vomiting and drinking multiple glasses of water. A summary in the Final Report stated that post mortem examination revealed aspiration of gastric contents with pulmonary necrosis and inflammation. Serum calcium was 4.9 mg/dL (norm = 8.4-10.2); magnesium 3.4 mg/dL (norm = 1.7-2.2); and alcohol 0.028%. The urine fluoride level, not corrected for creatinine, was 55 mg/dL. The death certificate listed.the final cause of death as "Acute Overdose, Sodium Fluoride".

Investigation of survivors

The persons from whom information was collected during the household surveys were asked to give urine and blood samples. 41 and 26 consented to have urine and blood, specimens collected, respectively. Urine samples were analyzed for fluoride; serum was analyzed for lactic acid dehydrogenases (LDH), creatinine kinase (CK), aspartate serum transferase (AST), calcium, phosphorus, and magnesium. Both plasma and serum were analyzed for fluoride.

On June 5, 5 of the 7 people with highest urine fluoride levels agreed to have repeat specimens collected. On June 9, 9 of 11 ill people with elevated initial urine fluoride levels agreed to have repeat urine specimens collected and 12 of 20 ill people with abnormal serum chemistry or elevated serum fluoride (greater than 0.03 mgF/L) agreed to have repeat blood specimens.

Results

The mean fluoride level of urine collected May 27-28 (6-7 days after ingestion) was 6.5 mgF/L (n = 26) (range 28.7-1.1 mgF/L). In non-cases (n = 15), the mean was 2.0 mgF/L (range 0.4-3.4 mgF/L).

Serum fluoride levels on samples collected May 27-28 from case-patients (n = 23) were a mean of 0.100 mgF/L and a median of 0.071 mgF/L. Non-cases (n = 3) had a mean of 0.036 mgF/L.

Serum chemistry analysis on May 27-28 (n = 15 case-patients) showed that all had elevated LDH and normal calcium and magnesium. Five had elevated phosphorus; 10 had elevated AST; and 1 had elevated CK. The Report states that on retesting on June 9, continued serum chemistry abnormalities were noted compared to normal values: 8 had low magnesium; 4 had high phosphorus; 2 had high LDH; and 1 had a high AST. A detailed table for these results is not provided, as is the case with the May results.

Dosage Estimates

The level of fluoride in system 1 on May 21-23 was, according to the Report, 150 mgF/L. Estimated intakes of water by 62 persons interviewed ranged from 2 to 140 cc/kg with a mean of 36 cc/kg. From these, the investigators estimated that fluoride doses ranged from 0.3 to 21.0 mg/kg. The man who died consumed an estimated 17.9 mg/kg. Among case-patients, doses and percentages were: 16% had less than 1.0 mg/kg; 34%, less than 2.0 mg/kg; and 21% had greater than 8.0 mg/kg.

The authors note that the estimates did not include fluoride consumed in water used to prepare cooked foods such as soup or vegetables. They state that their studies "suggest" that the minimum lethal dose of fluoride for humans, when consumed over 24 to 32 hours is in the range of 20 mg/kg. This is higher than the "probably toxic dose" of 5 mg/kg calculated for a single acute ingestion (Whitford 1989). The initial serum fluoride level of 9.1 mg/L from the most seriously ill survivor (hospitalized) "provides a value that may be close to the lethal serum level".

Other Findings

Fluoride is cleared from serum exponentially. The serum fluoride half-life for the hospitalized survivor was 3.5 hours. This was within the range (2.4-4.3 hours) previously reported (Ekstrand *et al* 1980).

Serum fluoride levels in all patients who had at least three fluoride determinations did not return to normal levels within 24 hours as previously reported (Heifetz and Horowitz 1986). The mean fluoride level in case-patients approximately 19 days after ingestion was 0.092 mg/L, *nearly three times* the mean among the three non-cases on May 27-28. In addition, evidence of systemic toxicity, including abnormalities of magnesium, phosphorus, LDH, and AST, also persisted 19 days. The authors are of the opinion that this has not been previously reported.

The minimum estimated dose that caused illness (assuming a fluoride concentration of 150 mg/L) was 0.3 mg/kg or "approximately, 28 mg of total fluoride". This level is *lower* than other reports referred to by the authors (Augenstein *et al* 1991, Duxbury *et al* 1982, Spoerke *et al* 1980), and *27 times less* than the 8.0 mg/kg recommended as a maximum safely tolerated dose in another report (Heifetz and Horowitz 1986). Further, for the case-patients for whom fluoride dose was estimated, 16% consumed less than 1.0 mg/kg and 34% consumed less than 2.0 mg/kg. The authors write that this "implies that *both acute gastro-intestinal symptoms and systemic toxicity may result from doses lower than previously believed.*"

Sequel

Owing to the adverse publicity surrounding this episode of hyperfluoridation in Hooper Bay and in light of the many problems that led to it, 11 of 36 villages

(36%) in the Yukon-Kuskokwin Delta voted to discontinue fluoridating their water supplies.

Following the outbreak, the Department of Environmental Conservation (DEC) and the US Public Health Service (USPHS) reviewed other village water systems in Alaska. Of 123 with systems that were evaluated, 12 (10%) were "missing at least one safety feature". None had a twist-lock plug for the fluoride pump electrical connection that is, evidently, a requirement.

The Workers' Compensation Board of British Columbia now includes in its publication, *Water Fluoridation, A Manual of Standard Practice* (1993), a list of problems identified by those investigating the Hooper Bay incident. These are: operator has minimal training; no fluoride testing by operator; slow response to high fluoride values identified by testing; inadequate labelling of pumps and piping; electrical wiring that signals water pump was corroded (water pump did not turn on); high water level indicator in holding tank that signals on/off operation of water pump and fluoride pump was not activated (fluoride pump was in continuous operation); defective ball valve in fluoride pump allowed pump to operate at 7 times the normal rate; no daily or continuous monitoring of treated water; and possible cross-connection between treated water in the holding tank and fluoride solution in tank (siphon action possible upon incorrect use of long supply hose).

Recommendations

The following recommendations were made by the investigators:

"1. The Alaska Division of Public Health strongly reaffirms its commitment to fluoridation of public water supplies. Water fluoridation is one of the most important methods to prevent dental caries in Alaskan children.

"2. USPHS and DEC should determine that communities with fluoridated water systems have operational standard safety features in place....

"3. DEC should ensure that all community water systems have complied with existing regulations regarding installation of standard safety equipment, training of water system operators, and routine, systematic monitoring and follow-up of fluoride levels and inspection of fluoridation units."

Commentary

The mass poisoning incident in Hooper Bay, euphemistically called "hyperfluoridation", has not shaken the strongly held belief of Public Health personnel in the safety of fluoridation and its effectiveness in the reduction of dental caries. If the results of the dental survey of the North Slope are applicable to the people of the Yukon-Kuskokwin Delta, the effectiveness of fluoridation is obviously questionable.

So strongly held is the belief in the safety of fluoridation that Public Health personnel in British Columbia could not accept the finding that the death in Hooper Bay was due to acute fluoride intoxication until they were presented with the official Report of the incident.

Although conclusions reached by the investigators regarding the dosage required to produce symptoms are based on assumptions and do not include the ingestion of fluoride from food, they should provide a warning that symptoms, especially of the gastro-intestinal system, may be frequent and unrecognized, particularly among

children consuming water in schools fluoridated at 5 mgF/L (up to 5.4 mgF/L) in addition to exposure at home. It is interesting to note that the minimum estimated dose causing illness was interpreted from 0.3 mg/kg to be 28 mg fluoride. Usually, for example, in the USPHS publication *Review of Fluoride Benefits and Risks* (1991), calculations are based on a 50-kg adult and a 20-kg child dose. These figures indicate total fluoride of 15 mg for adults and 6 mg for children. Is this a deliberate understatement or do the Native people studied weigh more than the "standard" used by USPHS?

It is also interesting to speculate on whether "mandatory" fluoridation of Alaskan Natives plays a role in the "climbing death rates from cancer" reported in the Alaskan press as a result of their review of a report by the US Department of Health and Human Services on death rates for 1984-1988 (*Anchorage Daily News* 1992). The Alaska Native total death rates from cancer are higher at 211/100,000 than the Alaska non-native total, 166.9/100,000, and the US total (all races) of 171.3/100,000.

Overall, the discrepancy found by the investigators when compared to "conventional" assumptions and the revelation of the obvious hazards of fluoridation systems, especially in rural areas, should call for an extensive review by unbiased observers.

References

Alaska Department of Health and Social Services. *Hooper Bay Waterborne Outbreak - Fluoride, Final Report*. April 12 1993.

Anchorage Daily News. Cancer Plagues Natives. p 1 May 28 1992.

Augenstein WL, Spoerke DG, Kulig KW *et al*. Fluoride ingestion in children: A review of 87 cases. *Pediatrics 88* 907-912 1991.

Duxbury AJ, Leach FN, Duxbury JT. Acute fluoride toxicity. *British Dental Journal 153* 64-66 1982.

Ekstrand J., Ehrnebo M, Whitford GM, Jamberg PO. Fluoride pharmacokinetics during acid-base changes in man. *European Journal of Clinical Pharmacology 18* 189-194 1980.

Heifetz SB, Horowitz HS. Amounts of fluoride in self administered dental products: Safety considerations for children. *Pediatrics 77* 876-882 1986.

Ketchikan Daily News. North Slope Dental Health Called Worst in US. Dec.17 1990.

Spoerke DG, Bennett DL, Gullekson DJ. Toxicity related to acute low dose sodium fluoride ingestions. *Journal of Family Practice 10* 139-140 1980.

US Department of Health and Human Services, PHS. *Review of Fluoride Benefits and Risks*. Feb 1991

USPHS/CDC. *Fluoridation Census 1985*. Department of Health and Human Services, Bethesda MD 1985 pp 28-32.

Whitford GM. *The Metabolism and Toxicity of Fluoride*. Monographs in Oral Science. Karger, Basel 1989.

Workers' Compensation Board of British Columbia. *Water Fluoridation, A Manual of Standard Practice 1993*. p 50.

A CRITIQUE OF RECENT PAPERS BY DR JOHN COLQUHOUN

G Neil Jenkins *
Newcastle upon Tyne, England

The Editor of Fluoride has generously invited me to write an article in support of fluoridation and I have gladly accepted paying special attention to the critical papers of Dr Colquhoun.

Criticism of the "21 City Survey"

The first major publication showing the fluoride-caries relationship was by Trendley Dean (1) and is usually referred to as the "Survey of the 21 Cities". Colquhoun (2) raised doubts about this work and, by implication, the whole basis of fluoridation and asks how were the 21 cities chosen out of the "hundreds" of communities in which the fluoride of the water had been related to enamel mottling (later called fluorosis after its ætiology was discovered). While Dean (3) did identify, by sending questionnaires to the officers of dental societies, 335 communities in which mottled teeth occurred, the fluoride of the water and the caries scores, were collected only in 8 of them (3). The quantitative relation of fluoride to mottling seems to have been originally established by data from fewer than 40 communities (4). Dean clearly stated his criteria for choosing the 21 cities, namely, a population of sufficient size and a water supply with the desired fluoride concentration from a stable source without "interfering relevant variables" (5). Colquhoun (2) is fully aware of these criteria and quotes Dean's admission (6) that he did not always adhere to them. Colquhoun also complains that socio-economic and dietary differences were not considered. This is true but it would be an inconceivable coincidence if these factors differed between the F and non-F communities to produce a reduction in caries as large and as clearly related to fluoride levels as has been found worldwide.

Twelve other studies have been published from USA and Europe which show the quantitative relationship between caries scores and the fluoride of the drinking water over the range 0 to 2 ppm (7). Colquhoun (2) mentions 23 such studies but his list is not accessible to me.

Ziegelbecker and Ziegelbecker (8) quote figures from the WHO data bank for five countries which do not show lower caries scores with higher concentrations of fluoride in water but rather a weak positive correlation. Without a knowledge of conditions in these countries it is impossible to make any firm comments. However, in countries with large variations in temperature and rainfall, there are often wide variations in the fluoride level of water (higher in hot dry seasons) which makes estimation of intake difficult. The fluoride/caries relationship is only quantitative over a range of intake equivalent to 0 to 2 ppm in water: if a population receives from its diet (*e.g.* from fish or tea) fluoride equal to intake from water containing more than 2 ppm, the maximal effect would be exerted throughout the population and would not be related to dose from water. The low DMFT rates in Sri Lanka and Malta suggests this possibility or that some other factor makes the caries situation

* Emeritus Professor of Oral Physiology, University of Newcastle upon Tyne. Address for correspondence: 4 Jesmond Dene Terrace, Newcastle upon Tyne, NE2 2ET England.

different from that elsewhere. In Hungary, salt is fluoridated which may eclipse an effect from fluoride in water. Data from some countries failing to show that fluoride in water reduces caries in no way refutes the reality of the 113 positive effects reported from 23 countries (see later).

The roundabout way in which the effect of fluoride on caries was discovered is evidence for the validity of the relationship. McKay (9) was the first to relate mottling to water supplies but stated with an unfortunate inversion of emphasis, that in spite of the "obviously and unmistakably defective" nature of mottled enamel it shows "no greater susceptibility to the onset of caries than does (normal) enamel". It was the British dentist Ainsworth (10) who, in the 1920's, found that mottled teeth showed a marked *resistance* to caries (7.9% permanent mottled teeth with caries, compared with a national average of 13.1%). The discovery that fluoride was the cause of mottling naturally suggested that it also reduced caries as confirmed by Dean's survey.

The effect of fluoride was, in effect, rediscovered independently in Britain in the 1940's, during World War II. Children from industrial towns in Britain were evacuated to the country as a precaution against bombing. A dentist examining the evacuees alongside the rural children noted that evacuees from South Shields had "remarkably good teeth - much better than those of the local children" (11). This led to a systematic survey in South Shields and neighbouring North Shields which confirmed the low caries rate in the former and analyses of their water supplies for F showed them to be 1.4 ppm and less than 0.25 ppm F, respectively (12). In this case, as with Ainsworth's data the caries difference was discovered first, followed by its association with fluoride. A full account of the early history of the fluoride-caries relationship has recently been published (11).

The DMF Scoring of Caries

Caries is usually scored by counting the number of decayed, missing or filled teeth (DMFT) or surfaces (DMFS): for deciduous teeth the acronyms are put in small letters (dmft; dmfs or sometimes deft or defs, the "e" referring to extracted teeth as distinct from teeth shed naturally). Colquhoun (13) is critical of this method of scoring emphasising that the "M" and "F" depend on subjective diagnostic criteria and that in collecting data in a fluoridation scheme (possibly subconscious?) bias may occur, as dentists have been led to expect a lower score in F areas or even, Colquhoun hints, encouraged to produce one (13).

As evidence of the subjective nature of caries diagnosis, Colquhoun reported (13) that the average DMF recorded by 73 dentists, who were paid for each filling, varied from 0.5 to 8.5 and the average was three times higher than that of the School Dental Officers who were paid a fixed salary! Most of the data on which the case for fluoridation rests were from children treated by school dentists who would have no incentive to raise the DMF. The criteria for making these decisions changed markedly about 20 years ago, following the realisation that very early carious lesions may, under favourable circumstances (including exposure to fluoride), undergo reversal, *i.e.* a remineralisation (14,15). If a lesion is judged to be capable of this change it is undesirable to fill it and in 1977 the New Zealand School Dental Service changed their diagnostic policy with the effect of reducing the number of

fillings (2,13). This means that DMF or dmf scores collected before 1977 will tend to be higher than after 1977 - a possible source of spurious "reductions in caries", including, according to Colquhoun, the results of the Hastings fluoridation study (2).

Incidentally, Colquhoun ignores this point in his remark that "fluoridated water cannot turn decayed, missing or filled teeth into sound ones" (16). In fact, fluoridated water, or fluoride in other forms, can turn a very early lesion (without cavitation) into an area of remineralised enamel higher in fluoride and more resistant to acid than neighbouring enamel free from caries (15,17). This reversal of caries has been found in fluoridated areas (18) and in experimental caries *in vivo* (19,20).

The criticism of the DMF method of scoring is particularly relevant to fluoridation surveys because the caries scoring was rarely done "blind". The subjects were examined in their own towns whose fluoride status was known. When clinical examinations were accompanied by radiography (as in Culemberg-Tiel) examination of the radiographs was done "blind" (18).

The *possibility* that some optimistic bias in recording and reporting DMF may have occurred in some fluoridation studies cannot be denied but it would be absurd to think that such defects are major factors among the 113 fluoridation schemes worldwide that have been traced and tabulated (11). Colquhoun admits that his criticism of the results of fluoridation schemes are based on the study of DMF scores which he justifies because "he has no option but to use what data are available" (13).

It is now widely agreed that the DMFT score is less than satisfactory and it is unfortunate that the more sensitive DMFS (or defs) scores have not been used more widely in fluoridation studies. I agree with Colquhoun that percentages based on comparisons of small fractions of a DMF are misleading (*e.g.* F and non-F groups with rises of 0.2 and 0.4 respectively being described as a 50% reduction). I think Colquhoun is overstating his case by stating that differences in mean DMF of less than *one* tooth or surface, although statistically significant, can be of *no* clinical significance, but would agree if this read "much less than one"(13).

Colquhoun's criticism of the survey that was not blind

The one survey that did attempt, with great care, to collect data blindly (21) was dismissed by Colquhoun (2) as "worthless", on the grounds that the fluoride group lived in a semi-rural island (Anglesey) whereas the control group lived in two mainland towns which "one would expect to have a higher caries rate".No reference was given to support the statement that rural and urban populations differ in caries rates but Ziegelbecker and Ziegelbecker (8) quote data that do not support this "expectation". They compare caries rates in ten urban and rural areas: in three, the urban rate is higher, in four the rural and in three there is no difference. I have traced one reference (22) that showed that in Northeast England deft and defs did not differ in non-F rural or urban areas but there was a small difference in F areas (0.6 of a deft) but much smaller than the differences produced by fluoride (3.5 in urban and 4.1 in rural areas) (Table 1) (22). In any case, the semi-rural area of Anglesey included Holyhead (population 10,620) and the towns referred to were of similar size (populations 9,260 and 14,558) so the two areas did not differ greatly.

TABLE 1
Comparison of caries in urban and rural populations in N.E. England

	deft		defs	
	F	NoF	F	NoF
Urban	2.6	6.1	4.5	11.6
Rural	2.0	6.1	3.1	11.7

Note that the difference in caries between urban and rural is much smaller than the difference between the F and non F

The Effect of Social Status

Colquhoun has stated that some of the differences between the DMF in F and non-F areas were more related to social status than to fluoride intake (23). Other workers in New Zealand (24,25) and elsewhere (26,27) have found that fluoride reduces caries at all levels of social status but the groups compared were not always similar in age or exposure to fluoride and the DMF was not always scored by the same examiners. In the most careful survey on this topic, the groups were of uniform age and of known fluoride exposure, caries was scored by the same individuals throughout under standardised conditions and the social class was assessed for each child (27). (Colquhoun related it merely to the social rank of the community served by a clinic). The results showed a reduction at all levels of social status but was largest (71%) in the lowest income group.

The Recent Decline in Caries

The fall in caries scores that has occurred in developed countries in recent decades (28,29) has made the measurement of the effect of fluoride more difficult because by lowering the DMF in the control, non-F areas, it has reduced the difference between them and the fluoridated areas. Colquhoun states that caries began to decline in New Zealand long before fluoride was introduced either in the water or in toothpastes and quotes data showing that fluoridation had little effect on an already descending curve (30). He also states that the reductions in DMF in F and non-F areas are about the same or even slightly greater in some non-F areas in New Zealand (30). This conclusion does not agree with other figures in New Zealand (24,25), or elsewhere (26,31) which show that the effects of fluoridation are still detectable (though smaller) against a background of the general decline (Table 2). An important piece of evidence for the validity of the effect of fluoridation (which Colquhoun and Diesendorf doubt or think has been exaggerated) is that positive effects have been reported in areas where the trend has been for caries to rise, *e.g.* in the Culemborg-Tiel (32) and Karl Marx Stadt-Plauen (33) schemes (Table 3). In Japan, although there was a rise in the only fluoridated area in that country it was smaller by 38% than the rise in the control(34).

The cause of the fall probably differs from country to country but a factor common to all is the widespread use of fluoride toothpastes, supplemented in some countries with fluoride in the water, in rinses or tablets. Other causes have been suggested such as the effect of the more cautious diagnosis of caries, changes in diet, the use of antibiotics which might inadvertently change the oral bacteria and the possibility that caries shows a cyclic incidence. These may contribute to the effect although there is little evidence for them (28,29). There have been changes in the

diet in New Zealand quoted by Colquhoun (30) but I doubt whether they could produce a decline in caries as large as that which has occurred. The fall in sugar consumption in New Zealand is quite small (and a much larger drop in Britain was neutralised from the dental point of view by a sharp rise in sugar-containing soft drink consumption (35)). More fruit and vegetables were eaten but their role in caries is doubtful(36). Cheese consumption has risen markedly; although laboratory experiments (37,38) and intra-oral caries tests (39) have shown that cheese has anti-caries properties, evidence for an effect in the normal diet is slight and circumstantial (40).

TABLE 2. Effect of fluoridation on caries in populations in which caries was falling

	1976	1981	difference (%)
Newcastle (F added 1968)	2.2	1.4	0.8 (36%)
Northumberland (No F)	5.4	3.4	2.0 (37%)
Difference (%)	3.2 (59%)	2.0 (58%)	

dmft of 5-year-old children

Note that the percentage fall with F is the same in both years in spite of a 37% fall in the control

TABLE 3. Effect of fluoridation on populations with a background of increasing caries

	1953	1960	1969
Tiel (F added 1953)	6.2	5.7	2.5
Culemborg (No F)	5.7	8.5	9.8

Average number of proximal cavities in 15-year-old children

	1959	1967	1971
Karl Marx Stadt (F added 1959)	3.6	2.4	2.0
Plauen (No F)	3.5	4.5	4.8

Average DMF in 15-year-old children

The Effects of Defluoridation on Caries

In attempting to show that defluoridation had not led to a significant rise in caries, Colquhoun states (correctly (2)) that the DMF of 10-year-old children had risen in Stranraer after defluoridation by "only" 4% in 6 years but (with a bias in reporting of which he accuses others) omitted to point out (or did not notice) that the DMF in the control town had *fallen* by 16% (41). A more realistic rise would therefore be 20% (4+16). Although he points out that the "rise" (his quote marks) was in the missing and filled components of DMF this is a misleading emphasis. The same information would be conveyed by stating that the D of the DMF did not rise but fell 33% in the control town (41). In other words, the effect of defluoridation was to neutralise the uncertain factors which reduced caries in the control town, as in the developed world generally.

Five years after defluoridation in Wick, the number of caries-free 6-year-old children fell by 10% (from 27.4 to 24.6) and the DMF rose by 33% (from 2.63 to 3.92, $p < 0.001$) (41) consisting, as Colquhoun points out (2), of a massive rise in

extractions. I am assured by Professor K Stephen (personal communication) that there was no change in the criteria for extraction. Colquhoun states that results obtained from "much larger" groups show no increase in caries after defluoridation. One of the two sources is official statistics in New Zealand to which I have no access and I cannot comment. The other data is from the 6-year-old children in the Culemborg-Tiel study in the Netherlands (32), the numbers being 93 and 158, hardly "much larger" than the 106 and 126 children in Wick! While there was no overall rise in caries in Tiel after defluoridation in this age group (but a slight fall), the percentage with a dmfs greater than 15 fell massively in Culemborg (from more than 50 to less than 20), it remained unchanged in Tiel and the percentage of caries-free rose more than in Tiel. The DMFS of the 15-year-old subjects in this trial did rise for about 10 years after defluoridation (from 10.8 in 1968 to 12.7 in 1979-80) then it began to fall as the effect of the general decline became apparent but did not fall below the 1969 figure until 1985-6 (43). Thus, the removal of F had delayed the fall that had occurred elsewhere by about 10 years.

The Alleged Uniformity of the Results of Fluoridation

Colquhoun (2) quotes Diesendorf in his statement that the results of fluoridation studies are nearly always a drop of 50 to 60% DMF in permanent teeth. Colquhoun suggests that papers reporting the "magic 50 to 60%" were more likely to be approved by referees and editors and implies that results with a lower reduction were either not submitted for publication or were rejected. In fact, perusal of the results of the 113 schemes in 23 countries (11) show that 50% is simply the mode of the results, 28 % of all the results are over 60% reduction and 18% are below 30% reduction. The "magic figure" is 40 to 49% reduction for deciduous teeth (30 % of the results) and 50 to 59% reduction for permanent teeth (38% of the figures not "mostly" as Diesendorf stated). Clearly the results show a very typical normal distribution.

Conclusion

The effect of fluoride can only be judged by looking at the evidence as a whole. Colquhoun's criticism is based on a selection of limited or anomalous evidence and in no way refutes the conclusion that fluoride, whether natural or used artificially, is the most powerful known weapon against caries.

References

1 Dean HT, Arnold FA, Elvove E. Domestic water and dental caries. V. 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.

2 Colquhoun J. Flawed foundation: A re-examinationof the scientific basis for a dental benefit from fluoridation. *Community Health Studies 14* 288-296 1990.

3 Dean HT. Distribution of mottled enamel in the United States. *Public Health Reports 48* 703-734 1933.

4 Dean HT, Dixon RM, Cohen C. Mottled enamel in Texas. *Public Health Reports 50* 424-442 1935.

5 Dean HT, Jay P, Arnold AA Jr, Elvove E. Donestic water and dental caries. *Public Health Reports 56* 761-792 1941.

6 Proceedings: City of Oroville vs Public Utilities Commission, State of California, October 20-21 1955. Proceedings: Injunction Suit Chicago Citizens vs City of Chicago, May 13 1960.
7 Murray JJ. Rugg-Gunn A. *Fluoridation and Declining Decay. A Reply to Diesendorf.* British Fluoridation Society, London 1987.
8 Ziegelbecker R, Ziegelbecker RC. WHO data on dental caries and natural water fluoride levels. *Fluoride 26* 263-266 1993.
9 McKay FS. The relation of mottled enamel to caries. *Journal of the American Dental Association 15* 1429-1437 1928.
10 Ainsworth's findings were published in: *The Incidence of Dental Disease in Children.* MRC Spec Rep Series (London) No. 91 1925.
11 Murray JJ, Rugg-Gunn A, Jenkins GN. *Fluorides in Caries Prevention.* Butterworth Heinemann, London 1991.
12 Weaver R. Fluoride and dental caries on Tyneside. *British Dental Journal 76* 29-40 1944.
13 Colquhoun J. Some investigations into the "DMF" measure of fluoride dental benefit. *Fluoride 23* 111-118 1990.
14 Koulourides T, Keller SE, Mansson-Hing L, Lilley V. Enhancement of fluoride effectiveness by experimental cariogenic priming of human enamel. *Caries Research 14* 32-39 1980.
15 Silverstone LM. Remineralization phenomena. *Caries Research 11* Supplement 1 59-79 1977.
16 Colquhoun J. Fluoridation. *Australian Journal of Public Health 15* 308-309 1991.
17 Dowse CM, Jenkins GN. Fluoride uptake *in vivo* in enamel defects and its significance. *Journal of Dental Research 36* 816 1957.
18 Backer-Dirks O. Posteruptive changes in dental enamel. *Journal of Dental Research 45* 503-511 1966.
19 von de Fehr FR, Loe H, Theilade E. Experimental caries in man. *Caries Research 4* 131-148 1970.
20 Edgar WM, Geddes DAM, Jenkins GN *et al.* Experimental caries in man. *Archives of Oral Biology 23* 655-661 1978.
21 Jackson D, James PMC, Thomas FD. Fluoridation in Anglesey 1983: a clinical study of dental caries. *British Dental Journal 158* 45-49 1985.
22 Rugg-Gunn AJ, Carmichael CL, French AD *et al.* Fluoridation in Newcastle and Northumberland. *British Dental Journal 142* 395-402 1977.
23 Colquhoun J. Influence of social class and fluoridation on child dental health. *Community Dentistry and Oral Epidemiology 13* 37-41 1985.
24 Evans RW, Beck DJ, Brown RH *et al.* Relationship between fluoridation and socio-economic status on dental caries experience in 5-year-old New Zealand children. *Community Dentistry and Oral Epidemiology 12* 5-9 1984.
25 Fergusson DM, Horwood LJ. Relationships between exposure to additional fluoride, social background and dental health in 7 year-old children. *Community Dentistry and Oral Epidemiology 14* 48-52 1986.
26 Hausen H, Milen E, Heinomen 0 *et al.* Caries in primary dentition and social class in high and low fluoride areas. *Community Dentistry and Oral Epidemiology 10* 33-36 1982.
27 Carmichael CL, Rugg-Gunn AJ, French AD *et al.* The effect ot fluondanon upon the relationship between caries experience and social class in 5 year-old children in Newcastle and Northumberland. *British Dental Journal 149* 163-167 1980.
28 Glass RL (Ed). Symposium on the decline in dental caries. *Journal of Dental Research 61* (Special Issue) 1301-1383 1982.

29 Jenkins GN. Recent changes in dental caries. *British Medical Journal 291* 1197-1198 1985.
30 Colquhoun J. Fluorides and the decline in tooth decay in New Zealand. *Fluoride 26* 125-134 1993.
31 Rugg-Gunn AJ, Carmichael CL, Ferrell RS. Effect of fluoridation and secular trend on caries in 5 year old children living in Newcastle and Northumberland. *British Dental Journal 165* 359-364 1988.
32 Kalsbeek H. Evidence of decrease in prevalence of dental caries in The Netherlands. *Journal of Dental Research 61* (Special Issue) 1321-1326 1988.
33 Kunzel W. Trinkwasserfluoridierung Karl MarxStadt XIII. *Stomatologie DDR 26* 458-465 1976.
34 Minoguchi G. *Bulletin in Stomatology Kyoto University 4* 45-124 1964.
35 Rugg-Gunn AJ. Sugar consumption in the United Kingdom. *British Dental Journal 161* 359-364 1986.
36 Rugg-Gunn AJ. *Nutrition and Dental Health.* Oxford Medical Publications, Oxford 1993.
37 Rugg-Gunn AJ, Edgar WM, Geddes DAM *et al.* The effect af different meal patterns upon plaque pH in human subjecs. *British Dental Journal 139* 351-356 1975.
38 Jenkins GN, Hargreaves JA. Effect of eating cheese on Ca and P concentrations of whole mouth saliva. *Caries Research 23* 159-164 1989.
39 Silva MFA, Jenkins GN, Burgess RC. Effects of cheese on experimental caries in man. *Caries Research 20* 263-269 1986.
40 Rugg-Gunn AJ, Hackett AF, Appleton D *et al.* Relationship between dietary habits and caries increments assessed over the years in 403 English adolescent schoolchildren. *Archives of Oral Biology 29* 983-992 1984.
41 Attwood D, Blinkhorn AS. Trends in dental health of ten-year-old children in South-West Scotland after cessation of fluoridation. *Lancet 2* 266-267 1988.
42 Stephen KW, McCall DR, Tallis JI. Caries prevalence in Northern Scotland before, and 5 years after, water defluoridation. *British Dental Journal 163* 324-326 1987.
43 Kalsbeek H, Kwant GW, Groeneveld A *et al.* Caries experience of 15-year-old children in the Netherlands after discontinuation of water fluoridation. *Caries Research 27* 201-205 1993.

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

REPLY TO PROFESSOR JENKINS' CRITIQUE

John Colquhoun
Auckland, New Zealand

21 City Survey

Jenkins simply restates Dean's claim that insufficient data were available from the earlier studies. The survey forms used by Dean in the fluorosis studies (1-5) show clearly that clinical information on decayed, missing and filled teeth was obtained then, as well as from a 1933-34 survey in 26 States (6). So Dean did have caries data on more than the 21 cities in 4 States (7) that he later compared. Jenkins' "only 8" refers to only one study. The evidence was set out in the paper (8) which Jenkins criticizes. Readers can judge which interpretation is more convincing.*

The criticized paper (8) noted that the 23 other studies reporting a caries/natural fluoride relationship were listed in the paper by Busse *et al* (9). Jenkins suggests reasons for the lack of a fluoride/caries relationship in recent WHO data, but does not apply the same caution to the early studies which claimed the relationship.

Many will be unconvinced by Jenkins' suggestion that the way the relationship was "discovered" is evidence for its validity. Many of the early reported fluorosed teeth were from areas with very high water fluoride levels, where a topical caries-delaying effect could have operated. I have not disputed the possibility of such an effect. It is the later claimed 1-part-per-million "optimal" water fluoride effect which is now in doubt.

Concerning the Shields reports from England: Jenkins fails to mention that the author he cites had first examined very young Shields children, and found a fluoride effect (10) - but later reported (11) little if any difference in tooth decay, among older children and adults, between the fluoridated and nonfluoridated parts of Shields (though infant mortality was significantly higher in the fluoridated part).

DMF scoring of caries

The change in diagnostic procedures of dentists was not disputed in my papers. In fact I drew attention to it. Jenkins' claim - that the now recognized remineralization of early carious lesions could account for the absurd claimed reduction in the number of carious cavities, in the same children, in the Grand Rapids fluoridation trial - does not bear examination. Only a cavity, not "a very early lesion (without cavitation)", was counted as D (decayed) in the DMF scores of the fluoridation trials.

Jenkins admits the possibility of bias in "some" fluoridation studies, but suggests it is "absurd" to think such bias could be a major factor in "the 113 fluoridation schemes that have been traced and tabulated." Dr Philip Sutton (12) devastatingly analyzed the "tabulation" by the authors whom Jenkins cites. Few "studies" remained in existence after allowing for the tabulation's duplications and dubious inclusions. Apart from the four original trials, long ago shown to be seriously defective (13), only 19 could today be found. Not one of them meets present-day standards of research design.

It was pointed out in the other study which Jenkins criticizes: "In the history of science many examples of unconscious bias of professionals have resulted in

* The case is restated, with facsimile of a survey form, in the article on pages 13-22.

malleability of apparently objective quantitative data. The problem was greater when the professionals were firmly committed to acceptance of a theory, as well as firmly convinced of their own objectivity" (14).

The worthless "blind" survey

Jenkins has not answered at all my main criticism of the Anglesey "blind" study: the fact that pre-fluoridation data were not presented for the non-fluoridated control area. Without that information we can never know whether the decay differences reported (and claimed to be due to fluoridation) were not present before fluoridation started. Comparisons of communities, without knowledge of such pre-fluoridation dental status, *are* worthless.

In the Anglesey study, children on a fluoridated off-shore island had less tooth decay than children in a nonfluoridated mainland urban area. In Auckland, New Zealand, children on nonfluoridated off-shore islands have less tooth decay than children in the fluoridated mainland urban area. Neither situation, in itself, proves anything about the effect of fluoridation..

An early, very comprehensive, New Zealand survey reported differences in dental decay rates between urban and rural communities (15). There was less tooth decay in the countryside, probably reflecting dietary differences at that time. Today there tends to be more tooth decay in rural, lower-income, areas. The fact that such differences were not found in a selected area of England does not rule out the need to choose similar kinds of communities for comparisons.

Effect of social status

Jenkins cites other New Zealand authors who compared data on small numbers of young children from a fluoridated and from a nonfluoridated area. The English study by his Newcastle colleagues (16), which Jenkins describes as "the most careful study on this topic", similarly compared 5-year-olds from two areas. The data which Jenkins criticizes (17,18) were for *all* children aged 12-13 years in New Zealand's most populous regions, and showed no difference in dental health between the fluoridated and nonfluoridated areas. In fact, when similar socio-economic areas of differing fluoridation status were compared, dental health was better in the non-fluoridated areas.

The recent decline in caries

Again, Jenkins rebuts my data collected for *all* children by citing studies based on samples of children from selected areas. Whether the communities he compares were suitable for comparison has been questioned. The results do not accord with more recent data from more comprehensive surveys (19-21).

I do not dispute (though I have doubts) that the widespread use of fluoride toothpastes and other fluoride products may have contributed to the decline in tooth decay in some countries. The fact remains that recent large-scale surveys caste doubt on the effect of water fluoridation.

For example, the recent comprehensive study of all geochemically defined regions in Missouri (19) found: "there were no significant differences between those children drinking optimally fluoridated water and those drinking suboptimally fluoridated water." This finding contradicts the early pro-fluoridation claim, which compared "nine selected Missouri cities" (22).

The effects of defluoridation on caries

Again Jenkins relies on small-scale studies of a few (usually only two) communities. Were Tiel and Culemborg, for example, suitable for comparison? Critics have shown (23) that the two cities were dissimilar in many respects, while the small samples of children compared were probably not representative. Even so, the reported trends indicated a delay in the appearance of lesions rather than an actual reduction in decay.

Again, many of the studies on which Jenkins relies were of very young children. Larger surveys suggest that any delaying effect of fluoridation on decay in these children's teeth (possibly reflecting a delay in eruption of their teeth) has disappeared by the time the children are 12 or 13 years old.

Jenkins is persuaded by an assurance that there was no change in criteria for extraction of deciduous teeth in Wick, following defluoridation. With long experience in the practice of childrens' dentistry, I find the claimed need for a massive (60%) increase in such extractions, five years after defluoridation, less than credible.

Jenkins incorrectly states that I cited 6-year-olds data from Tiel and Culemborg as "much larger" than the Wick and Stranraer small samples. The study I had cited was of numerous surveys throughout the Netherlands of 11,659 children, which revealed no increase in tooth decay following defluoridation in that country (24).

Uniformity of results of early fluoridation trials

The results of the early studies can hardly be described as "a normal distribution" when they all showed marked reductions in decay associated with fluoridation, while recent, more comprehensive, data show virtually no differences in decay rates when fluoridation is compared with nonfluoridation.

Conclusion

Comprehensive surveys from several countries, including information on whole populations of New Zealand children, indicate little if any dental benefit from water fluoride. Thus "the evidence as a whole" does not support Professor Jenkins' conclusion.

References

1 Dean HT. Distribution of mottled enamel in the United States. *Public Health Reports 48* 703-734 1933.

2 Dean HT, Dixon RM, Cohen C. Mottled enamel in Texas. *Public Health Reports 50* 424-442 1935.

3 Dean HT, Elvove E. Studies on the minimal threshold of the dental sign of chronic endemic fluorosis (mottled enamel). *Public Health Reports 50* 1719-1729 1935.

4 Dean HT, Elvove E. Further studies on the minimal threshold of chronic endemic dental fluorosis. *Public Health Reports 52* 1249-1264 1937.

5 Dean HT, McKay FS, Elvove E. Mottled enamel survey of Bauxite, Ark. ten years after a change in the common water supply. *Public Health Reports 53* 1736-1748 1938.

6 Messner CT, Gafafer WM, Cady FC, Dean HT. Dental survey of school children, ages 6-14 years in 1933-34 in 26 States. *Public Health Bulletin no. 226*. May 1936.

7 Dean HT, Arnold FA, Elvove E. Domestic water and dental caries. V. 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.

8 Colquhoun J. Flawed foundation: A re-examination of the scientific basis for a dental benefit from fluoridation. *Community Health Studies 14* 288-296 1990.
9 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.
10 Weaver R. Fluoride and dental caries on Tyneside. *British Dental Journal 76* 29-40 1944.
11 Weaver R. Fluorine and dental caries: Further investigation on Tyneside and in Sunderland. *British Dental Journal 77* 185-193 1944.
12 Sutton PRN. The failure of fluoridation. *Fluoride 23* 1-4 1990.
13 Sutton PRN. *Fluoridation: Errors and Omissions in Experimental Trials.* Melbourne University Press, Melbourne 1959 (2nd edition 1960).
14 Colquhoun J. Some investigations into the "DMF" measure of fluoride dental benefit. *Fluoride 23* 111-118 1990.
15 Hewat RET, Eastcott DF. *Dental Caries in New Zealand.* Medical Research Council of New Zealand, Christchurch 1954.
16 Carmichael CL, Rugg-Gunn AJ, French AD, Cranage JD. The effect of fluoridation upon the relationship between caries experience and social class in 5-year-old children in Newcastle and Northumberland. *British Dental Journal 149* 163-167 1980.
17 Colquhoun J. Influence of social class and fluoridation on child dental health. *Community Dentistry and Oral Epidemiology 13* 37-41 1985.
18 Colquhoun J. Child dental health differences in New Zealand. *Community Health Studies 11* 85-90 1987.
19 Hildebolt CF, Elvin-Lewis M, Molnar S *et al.* Caries prevalences among geochemical regions of Missouri. *American Journal of Physical Anthropology 78* 79-92 1989.
20 Yiamouyiannis JA. Water fluoridation and tooth decay: Results from the 1986-1987 national survey of US schoolchildren. *Fluoride 23* 55-67 1990.
21 Diesendorf M. The mystery of declining tooth decay. *Nature 322* 125-129 1986.
22 McClure FJ. *Water Fluoridation: The Search and the Victory.* US Department of Health, Education and Welfare (National Institutes of Health), Bethesda MD 1970 p 99.
23 Ziegelbecker R, Thomson HM. Sixteen years of water fluoridation in the Netherlands and its influence on dental decay. *Fluoride 6* 57-63 1973.
24 Kalsbeek H. Evidence of decrease in prevalence of dental caries in the Netherlands: An evaluation of epidemiological caries surveys on 4-6- and 11-15-year-old children, performed between 1965 and 1980. *Journal of Dental Research 61* (Special Issue) 1321-1326 1982.

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

Abstracts of two papers presented to the XIXth Conference of the International Society for Fluoride research, Kyoto, Japan, September 1992.

DENTAL EFFECTS OF DISCONTINUED WATER FLUORIDATION IN GUANGZHOU, CHINA

A Tsutsui,[1] O Sakai,[1] S Kobayashi,[2] M Yagi,[2] K Horii [2] and Y Shen [3]

In China, water fluoridation began in Guangdong province in Guangzhou in 1965 and in Dongguan in 1974. However, fluoridation ceased in these places in 1983 and 1986, respectively. There is at present no fluoridation in China. Both cities are located to the north of Hong Kong and belong to the subtropical zone (mean temperature = 71.1°F). On the basis of Minoguchi's formula, their optimal fluoride concentration is 0.6 ppm. In December 1990, seven years after fluoridation was discontinued, a dental survey of schoolchildren aged 15 was made in Guangzhou, with Fushan (0.1 ppm) as control area. Two dentists examined dental caries, and one dentist examined dental fluorosis with Dean's Index. Other enamel defects were also recorded. Subjects were 62 students in Guangzhou and 71 students in Fushan. The mean DMFT was 0.44 (SE = 0.10) in Guangzhou and 2.20 (0.33) in Fushan. The per cent difference was 80% ($p < 0.001$). The prevalence rate of dental fluorosis was 21% in Guangzhou and 2.8% in Fushan. The community fluorosis index was 0.42 and 0.08, respectively. Many dark-brown teeth, not caused by fluoride, were found. They may have been caused by tetracycline. The fluoride concentration was unstable for the first 10 years, ranging from 0.7 ppm to 0.9 ppm, higher than the optimal fluoride concentration. Afterwards it ranged from 0.3 ppm to 0.7 ppm. We conclude that the fluoride concentration of drinking water in Guangzhou was sometimes higher than optimal.

1 Department of Preventive Dentistry, Fukuoka Dental College, Fukuoka, JAPAN.
2 Department of Preventive Dentistry, School of Dentistry, Niigata, JAPAN.
3 Department of Stomatology, First Affiliated Hospital, Sun Yat-Sen University of Medical Science, Guangzhou, CHINA.

The following DISCUSSION took place after presentation of the above paper.

Dr Tohru Murakami (President, Japanese Society for Fluoride Research):
My question is in six parts.

1) Why was fluoridation stopped in Guangdong and Dongguan?
2) Was it because it did more harm than good?
3) Why do you believe that Minoguchi's formula is applicable to China?
4) Was the dental survey a blind test? Why were the samples so small and were the compared communities similar?
5) Because 80% is a bigger difference than ever claimed before, does that not show that the compared communities were dissimilar?
6) Why did you make the comment on tetracycline, which has nothing to do with fluoride?

Dr A Tsutsui: The reason for the discontinuation of fluoridation was that the fluoride concentration used was unsafe and too high, so much dental fluorosis

occurred. But from 1977 the fluoride concentration was stable and there was no problem. However, the high level of dental fluorosis led to fluoridation being discontinued. In some other parts of China dental fluorosis is caused by fluoride content in foods. Tetracycline used to be prescribed for children but is now prohibited for children up to 8 years of age. So many of the dark patches on children's teeth were caused by tetracycline, or some kind of fever. Minoguchi's formula is reasonable to use in China, as in Japan. The formula is in the textbook on fluorides and children's health published by the WHO and is a useful formula.

THE PREVALENCE OF DENTAL FLUOROSIS AND DENTAL CARIES IN THE NATURAL FLUORIDE AREA OF CENTRAL TAIWAN

M Yagi,[1] S Kobayashi,[1] K Horii,[1] A Tsutsui,[2]
Y Gunjishima,[2] O Sakai [2] and M Chou [3]

An epidemiological survey was carried out in 1991 to study the prevalence of dental fluorosis and dental caries in the natural fluoride area of Central Taiwan. Primary school children residing in the fluoride districts brought samples of water from their private wells. The fluoride concentration in the drinking water samples ranged from 0.0 ppm to 5.6 ppm. Children aged 10 - 11 (N = 128 in total) were selected for analysis and were classified into four categories according to their place of residence and the relative level of fluoride in the place of residence: 1) Deficient (N = 41), 2) Low (N = 21), 3) Medium (N = 50), and 4) High (N = 16). Dental fluorosis was not a public health problem in the Deficient and Low areas (CFI < 0.21). Fluorosis was borderline in the Medium areas (CFI = 0.56), and epidemic in the High areas (CFI = 1.21). The mean DMFT score was 2.51 in the Deficient, 0.90 in the Low, 0.58 in the Medium, and 0.25 in the High areas. Although dental fluorosis was increasing and dental caries was declining from Deficient to High, we could not decide the optimal fluoride concentration in Central Taiwan. In deciding the optimal fluoride concentration in the same area, it is also necessary to refer to the data from different studies on the artificial water fluoridation which was implemented in a city of Central Taiwan from 1972 to 1986.

1 Department of Preventive Dentistry, School of Dentistry, Niigata University, Niigata, JAPAN.
2 Department of Preventive Dentistry, Fukuoka Dental College, Fukuoka, JAPAN.
3 Department of Oral Pathology, School of Dentistry, National Yang-Ming Medical College, Taipei, TAIWAN.

The following DISCUSSION took place after presentation of the above paper

Dr Tohru Murakami: Fluoridation was stopped in Taiwan many years ago, because it caused many harmful effects - for example dental fluorosis even as severe as the "moderate" type. Do you still think it would be better to start fluoridation again in Taiwan?

Dr Yagi: Yes. We did not find any moderate or severe fluorosis.

Professor A W Burgstahler: As Dr Colquhoun pointed out, the large-scale study just completed in Tucson, Arizona, showed that the higher the fluoride content of the drinking water the greater the tooth decay. It was then discovered that the higher rate of tooth decay was associated with poorer nutrition in the lower socio-economic classes. Now in your study: did the higher fluoride area have better diets and nutrition? If you have not looked at that aspect you have a very incomplete picture. The other point I would note is that the number of children in your high fluoride area was only 16. The study in geochemical areas of Missouri which I described earlier included every single child of known water history of lifetime ingestion, in every one of those communities. It was a very thorough study - a complete total population survey. It seems to me that this sampling-type technique is very risky.

Dr Yagi: I have a question about your presentation. Why don't you speak about the other uses of fluoride than water fluoridation in the USA?

Professor Burgstahler: You are talking about toothpastes and other topical uses like mouthrinses and so on?

Dr Yagi: Yes. And how effective are the drinks consumed which come from fluoridated areas in USA?

Professor Burgstahler: Well, the Missouri study is interesting in another respect. They also examined the teeth of prehistoric indigenous inhabitants of those areas - the native American Indians - and the same pattern was observed: lower caries rates among children in those areas were not related to water fluoride. The study was published in the *American Journal of Physical Anthropology* and gave a prehistorical picture as well as a contemporary one. I agree that the widespread use of other forms of fluoride does confound the picture. But the Tucson study showed that, even when you have fluoride toothpastes and so on, you still have that direct correlation between high fluoride levels and high tooth decay.

Dr John R Lee: You had 128 total in the group, 16 in the high fluoride group and small numbers in each of the other groups. Did you have a statistician calculate the significance of the difference relative to the number of subjects? I have gone through many papers like this one where the N value is low. You need much higher numbers. You cannot make any conclusions from a group as small as 16. One child with 8 cavities will totally distort the result if it happens to be one of the 16.

Chair: Do you wish to comment on the statistical analysis?

Dr Yagi: No.

REVERSIBLE IMPAIRMENT OF GLUCOSE TOLERANCE IN PATIENTS WITH ENDEMIC FLUOROSIS

N Trivedi, A Mithal, S K Gupta and M M Godbole
Lucknow, India

Abstract from *Diabetologia 36* 826-828 1993

Endemic fluorosis is a condition resulting from prolonged ingestion of drinking water which contains excess fluoride. Studies on rats have suggested that fluoride toxicity may produce glucose intolerance and abnormalities in insulin secretion. We studied glucose and insulin profiles following an oral glucose load in patients with endemic fluorosis. Twenty-five young adults (age range, 15-30 years) with endemic fluorosis, and an equal number of matched healthy control subjects with normal fluoride intake were studied. Impaired glucose tolerance was demonstrated in 10 of 25 (40%) patients with endemic fluorosis. Patients with impaired glucose tolerance had significantly higher fasting serum immunoreactive insulin ($p < 0.05$), higher fasting serum fluoride ($p < 0.001$), and a significantly lower fasting glucose to insulin ratio than that in patients with normal glucose tolerance ($p < 0.001$) or control subjects ($p < 0.05$). The fasting serum fluoride levels correlated positively with the area under the glucose curve ($r = 0.80$, $p < 0.01$) in patients with impaired glucose tolerance. Interestingly these abnormalities could be reversed when the village was provided drinking water with fluoride levels within acceptable limits. The present study shows that chronic fluoride toxicity in humans could result in significant abnormalities in glucose tolerance which are reversible upon removal of the excess fluoride.

Key words: Fluoride; Glucose; Insulin; Insulin resistance.
Reprints: A Mithal, Department of Medical Endocrinology, Sanjay Gandhi Post-graduate Institute of Medical Sciences, POBox 375, Lucknow 226 001, India.

URINE, SERUM AND HAIR MONITORING OF HYDROFLUORIC ACID WORKERS

K Kono, Y Yoshida, M Watanabe, Y Orita, T Dote and Y Bessho
Osaka, Japan

Abstract from *International Archives of Occupational and Environmental Health 65* (1 Suppl) S95-S98 1993

To define the relationship between fluoride (F) concentration in the serum, urine and hair of workers and the concentration of hydrofluoric acid (HF) in the work environment, pre- and postshift serum and urine samples of 142 HF exposed workers and 237 unexposed workers were examined. Hair specimens were also collected for the determination of F. To determine whether external contamination influences hair analysis, the control hair samples were kept in the work environment for one week. The pre-exposure levels in serum and urinary F in HF workers were higher ($P < 0.01$) than the control values. This suggests that F excretion from the body continues for at least 12 hours. The postshift serum and urinary F concentra-

tions of these workers were significantly higher ($P < 0.01$) than the preshift concentrations. The levels of F in the hair of HF workers were also higher than in the control subjects. The concentrations of F in postshift serum and urine, and hair were in good correlation to each other. There was a linear relationship between mean serum and urinary F concentrations and HF concentration in the workplace. A mean F concentration of 82.3 μg/L in serum and 4 mg/L in urine with a lower fiducial limit (95%, $P = 0.05$) of 57.9 μg/L in serum and 2 mg in urine were estimated to correspond to an atmospheric HF concentration of 3 ppm, which is the maximum allowable concentration recommended by Japan Association of Industrial Health and also the threshold limit value suggested by American Conference of Governmental Industrial Hygienists. F concentration in the hair increased after the hair samples were retained, however, it decreased to the reference value after washing treatment. The results support the speculation that F is excreted into the hair after long-term exposure to HF. From the results obtained, it was suggested that exposure to HF can be monitored by determining the serum, urinary and hair F concentrations.

Key words: Hair; Hydrofluoric acid worker; Monitoring; Serum; Urine.

Reprints: K Kono, Osaka Medical College, Department of Hygiene and Public Health, 2-7 Daigakumachi, Takatsuki, Osaka 569, Japan.

EFFECT OF CONTROLLED LOCAL RELEASE OF SODIUM FLUORIDE ON BONE FORMATION - FILLING A DEFECT IN THE PROXIMAL FEMORAL CORTEX

A P McCormack, P A Anderson and A F Tencer
Seattle, Washington, USA

Abstract from *Journal of Orthopaedic Research 11* (4) 548-555 1993

To assess the effect of sodium fluoride (NaF) in the healing of a defect in cortical bone, an experimental model was created by the drilling of 5.0 mm holes in the proximal ends of both femora of 12 adult male New Zealand White rabbits. An interlocking intramedullary implant constructed of poly(d,l-lactic acid) containing NaF was placed in the right femur and an identical implant without NaF (sham), in the left. The implant in the right femur was designed to release NaF in a controlled manner over the duration of the experiment. Ten weeks after implantation, the specimens were removed and were tested in torsion. The mechanical properties were not significantly different between the groups. The femora exposed to NaF had an 18.6% increase in intact cortex near the defect ($p = 0.023$), however, the deposition of mineralized bone within the defect was not significantly greater. In fact, healing appeared to be impaired by the presence of NaF. There was complete closure of the defect in all but one of the femora with a sham implant, but the tissue had not yet calcified. In contrast, only one femoral defect exposed to NaF had closed. Examination of the material filling the defects of the femora exposed to NaF showed that it was predominantly uncalcified osteogenic mesenchymal tissue.

Key words: Bone formation; Femur; Sodium fluoride

Reprints: A F Tencer, University of Washington, Harborview Medical Center Department of Orthopedics, 325 9th Ave, Seattle, WA 98104, USA.

PROTECTIVE EFFECT OF TOPICALLY APPLIED FLUORIDE IN RELATION TO FLUORIDE SENSITIVITY OF MUTANS STREPTOCOCCI

C Vanloveren, J F Buijs and J M Tencate
Amsterdam, Netherlands

Abstract from *Journal of Dental Research* 72 1184-1190 1993

The aim of the present *in vitro* experiments was to determine whether the protection of enamel by topically applied fluoride against demineralization by bacterial acids would depend on the fluoride sensitivity of the bacteria. Glucose-agarose gel suspensions of fluoride-sensitive and fluoride-resistant mutans streptococci were placed on bovine enamel specimens with different amounts of fluoride. One group of specimens was untreated, a second group had been pretreated with a F-lacquer, and a third group had been pretreated with the F-lacquer and rinsed subsequently with a KOH-solution, to remove deposited CaF_2. After 22-hour incubations at 37°C, the amounts of calcium and lactate and the pH of the agarose gels were determined. This procedure was repeated on three consecutive days. Two parent *S. mutans* strains, one parent *S. sobrinus* strain, and five fluoride-resistant derivatives were tested. Both pretreatments gave a significant protection to the enamel specimens. For the *S. mutans* strains, the degree of protection did not depend on the fluoride sensitivity of the strains. For the *S. sobrinus* strains, the results suggested a reduced protection against demineralization by the fluoride-resistant derivatives. Only from the second group of enamel specimens was enough fluoride released for inhibition of bacterial metabolism. Presumably, it was released by the dissolution of CaF_2. It is concluded that a possible adaptation of mutans streptococci in dental plaque to frequent exposures to fluoride will not necessarily decrease the caries-preventive effects caused by topically applied fluoride agents.

Key words: Fluoride sensitivity; Mutans streptococci; Topical fluoride.
Reprints: C Vanloveren, Academic Centre for Dentistry Amsterdam, Department of Cariology and Endodontology, Louwesweg 1, 1066 EA Amsterdam, The Netherlands.

PERCEPTIONS OF DENTAL FLUOROSIS

P J Riordan
Como WA, Australia

Abstract from *Journal of Dental Research* 72 (9) 1268-1274 1993

Mild dental fluorosis has long been accepted as a side-effect of water fluoridation and, more recently, has been recognized as a consequence of the use of other fluoride-based caries-preventive strategies. Traditionally, dental health professionals have not seen this as being of public health importance, but members of the public have not been asked their opinion. The purpose of the present study was to gather the opinions of lay groups concerning the appearance of the teeth of children with various degrees of fluorosis. Twenty-eight children, born in 1978, who had earlier participated in a study of fluorosis in Perth (Western Australia), allowed 10

observers to look at their upper central incisors under good viewing conditions. Fluorosis in these teeth ranged from TF score 0 (no fluorosis) to TF score 3. The observers were university students, parents, public servants, or dentists. They responded to statement items about the appearance of the teeth. The results, based on just over 3000 responses, showed that lay and dental observers could distinguish between different fluorosis levels. In response to a statement that the teeth appeared pleasing, a large majority agreed when the TF score was 0, but agreement declined as the TF score increased; when the TF score was 3, most people disagreed. Similarly, observers felt that the appearance would increasingly embarrass the child as the TF score increased. Observers, except the dentists, tended to feel that higher TF scores indicated neglect on the part of the child. Most observer groups felt that fluorosis would be no greater an esthetic problem for girls than for boys, but for more severe fluorosis, the dentists saw the appearance as being a greater problem for girls. The dentists responded that most fluorosis did not require any treatment, but when the TF score was 3, a majority of them felt that esthetic treatment would be warranted. The results suggest that, for these observers, fluorosis score TF = 2 or greater was easily noticed, and when the TF score was 3, fluorosis aroused concern in most observers. Recent epidemiological studies reported TF scores of 2 or more in 11.3% of 12-year-olds and 17.5% of 7-year-olds in Perth. Strategies to reduce the prevalence of fluorosis with TF score 2 and avoid fluorosis with TF score 3 - including reduction of toothpaste ingestion, removal of fluoride from infant formulae, and avoidance of inappropriate supplement use-should be devised and implemented.

Key words: Dental fluorosis; Fluoridation; Fluoride supplements; Lay opinion.
Reprints: P J Riordan, Health Department of Western Australia, Dental Service, P O Box 50, Como, WA 6152, Australia.

ENAMEL DEFECTS IN 4-YEAR-OLD TO 5-YEAR-OLD CHILDREN IN FLUORIDATED AND NON-FLUORIDATED PARTS OF CHESHIRE, UK

K J Weeks, K M Milsom and M A Lennon
Liverpool, England

Abstract from *Caries Research* 27 4 317-320 1993

The aim of this study was to compare the prevalence of developmental defects of enamel in the deciduous dentition of 4- to 5-year-old children residing in fluoridated (1 ppm F) and non-fluoridated (less than 0.2 ppm F) communities in Cheshire, UK. The significant difference in the prevalence of developmental defects of enamel between the two communities was accounted for by the higher prevalence of diffuse opacities in fluoridated Nantwich (29%), than in non-fluoridated Northwich (14%). The results also showed that when controlling for the age at which parents claimed toothbrushing commenced, the children in fluoridated Nantwich still had significantly more diffuse defects than the children in Northwich.

Key words: Deciduous dentition; Dental fluorosis; Enamel defects; Fluoride.
Reprints: M A Lennon, University of Liverpool Department of Clinical Dental Science, P O Box 147, Liverpool L69 3BX, England.

SEALANTS REVISITED - AN UPDATE OF THE EFFECTIVENESS OF PIT-AND-FISSURE SEALANTS

L W Ripa
Stonybrook NY, USA

Abstract from *Caries Research 27* (Suppl 1) 77-82 1993

The first sealant clinical trials used cyanoacrylate-based materials. These were replaced by dimethacrylate-based products which were marketed. A major difference between marketed sealants is their method of polymerization. First-generation sealants were initiated by ultraviolet light, second-generation sealants are autopolymerized, and third-generation sealants use visible light. Over time, clinical retention was found to be greater for second generation as compared with first-generation sealants. Five to 7 years after initial application the pits and fissures of approximately one third of teeth treated with first-generation sealants were fully protected as compared with two thirds of the teeth treated with second-generation sealants. First-generation, ultraviolet light initiated, sealants are no longer marketed. Clinical reports indicate that retention is similar for second- and third-generation systems, but longer clinical evaluations are necessary. A recent innovation is the addition of fluoride to sealants. Fluoride release to the saliva from a fluoride sealant system is rapid, but clinical studies are needed to determine if the fluoride addition improves caries inhibition.

Key words: Caries prevention; Fluoride; Pit-and-fissure sealants.
Reprints: L W Ripa, State University of New York School of Dentistry, Department of Childrens Dentistry, Stony Brook, NY 11794, USA.

INTEGRATED CARIES PREVENTION EFFECT OF A NEEDS-RELATED PREVENTIVE PROGRAM ON DENTAL CARIES IN CHILDREN COUNTY OF VARMLAND, SWEDEN - RESULTS AFTER 12 YEARS

P Axelsson, J Paulander, G Svardstrom, G Tollskog and S Nordensten
Karlstad, Sweden

Abstract from *Caries Research 27* (Suppl 1) 83-94 1993

Twenty years ago the caries prevalence in Swedish children was among the highest in the world; within Sweden, it was highest in the County of Varmland. Ongoing clinical research projects were initiated to evaluate the separate and combined effects of preventive measures, and in 1978 a preventive program, based on caries risk assessment, was introduced for 3- to 19-year-olds in the county. The effects were monitored by a computerized epidemiological system. From 1979 to 1991, caries prevalence and incidence decreased by 75-90 and 75-85%, respectively. The percentage of caries-free 3-year-old children increased from 51 to 94%, and in

12-year-old children, the caries prevalence decreased from 6.5 to 1.0 DFS, the lowest in Sweden. The program is very cost-effective, and in 1990 the mean annual treatment time per child was the lowest in Sweden. Currently, new methods of caries risk prediction and integrated caries prevention are being developed, with special reference to cost-effectiveness.

Key words: Children; Dental caries, epidemiology; Dietary habits; Educational level; Fissure sealants; Fluorides; Plaque control; Preventive program, cost-effectiveness.
Reprints: P Axelsson, Public Dental Health Service, Department of Preventive Dentistry, Alvgatan 47, S-65230 Karlstad, Sweden.

AN EPIDEMIOLOGICAL ASSESSMENT OF THE CHRONOLOGICAL DISTRIBUTION OF DENTAL FLUOROSIS IN HUMAN MAXILLARY CENTRAL INCISORS

R W Evans
Melbourne, Australia

Abstract from *Journal of Dental Research* 72 (5) 883-890 1993

The objectives of this study were 1) to develop and test a new index, the Chronological Fluorosis Assessment (CFA) Index, for measuring variation in the chronological distribution and intensity of dental fluorosis, and 2) to determine whether the new index was sufficiently sensitive for detection of a decline in dental fluorosis corresponding to the reduction in the waterborne fluoride level in Hong Kong. According to the CFA Index, the cervical, middle, and incisal third divisions of the labial surface of a maxillary central incisor crown are each classified into one of six categories of an ordinal scale of fluorosis. Data from 1295 life-long resident Hong Kong Chinese children exposed to known water fluoride concentrations were analyzed. Fluorosis declined from incisal to cervical in 41% of cases, and a reverse gradient was apparent in 29%. Overall, fluorosis intensity was higher on incisal thirds than on both middle and cervical thirds. However, when tooth thirds were regrouped according to common 16-month developmental periods, in order to control for time-related variation in fluoride concentration, the incisal-middle difference was of reduced statistical significance, and both the incisal-cervical and the middle-cervical differences became insignificant. A regression analysis of CFA Index on cohort indicated a significant cohort-related fluorosis decrease, in relation to cervical, middle, and incisal tooth thirds. It was concluded 1) that fluorosed enamel which forms in chronological sequence tends toward a uniform intensity, and 2) that the new index was sufficiently sensitive for a decline to be measured in dental fluorosis in Hong Kong.

Key words: Chronological Fluorosis Assessment; Dental fluorosis; Fluoridation; Hong Kong.
Reprints: R W Evans, University of Melbourne School of Dental Science, 711 Elizabeth St, Melbourne, Vic 3000, Australia.

ROLE OF GTP-BINDING PROTEINS IN REGULATION OF Na^+/H^+-EXCHANGE AND $Na^+,K^+,2Cl^-$-CO-TRANSPORT - EFFECT OF FLUORIDE ION

I A Kolosova, J Bernhardt, S N Orlov and F R Buhler
Moscow, Russia.

Abstract from *Biochemistry - Russia 58* (3) 295-298 1993

The effects of NaF and $AlCl_3$ on basal and hyperosmotic shrinking-stimulated Na^+/H^+-exchange and $Na^+,K^+,2Cl^-$-co-transport activities in rat smooth muscles and erythrocytes have been studied. Preincubation of smooth muscle cells with 10 mM NaF inhibits both basal (by 45%) and hyperosmotic shrinking-stimulated (by 190%) $Na^+,K^+,2Cl^-$-co-transport. Sodium fluoride causes 80% activation of basal $Na^+,K^+,2Cl^-$-co-transport in erythrocytes with no effect on the activation of transport by hyperosmotic shrinking. The effect of NaF on $Na^+,K^+,2Cl^-$-co-transport in erythrocytes does not depend on Al^{3+}. Basal Na^+/H^+-exchange is stimulated by fluoride in smooth muscle cells by 780%, but is insensitive to fluoride in erythrocytes. Under hyperosmotic conditions, NaF activates Na^+/H^+-exchange by 100% in smooth muscle cells and by 130% in erythrocytes. In the latter case the effect is potentiated by Al^{3+}. The data suggest that GTP-binding proteins are involved in activation of Na^+/H^+-exchange by hyperosmotic shrinking.

Keywords: Smooth muscle cells; Erythrocytes; Hyperosmotic shrinking; Na^+/H^+-exchange; $Na^+,K^+,2Cl^-$-co-transport; GTP-binding proteins.
Reprints: I A Kolosova, M V Lomonosov State University School of Biology, Moscow, Russia.

STOICHIOMETRY OF TIGHT BINDING OF MAGNESIUM AND FLUORIDE TO PHOSPHORYLATION AND HIGH-AFFINITY BINDING OF ATP, VANADATE, AND CALCIUM IN THE SARCOPLASMIC RETICULUM Ca^{2+}-ATPase

T Daiho, T Kubota and T Kanazawa
Asahikawa, Japan.

Abstract from *Biochemistry 32* (38) 10021-10026 1993

We previously showed that, when the purified Ca^{2+}-ATPase from sarcoplasmic reticulum (SR) is treated with fluoride (F-) in the presence of Mg^{2+}, a complete inactivation of the enzyme is induced by tight binding of approximately 2 mol of Mg^{2+} and 4 mol of F^- to the catalytic site per mole of phosphorylation site (Kubota T, Daiho T and Kanazawa T. *Biochimica Biophysica Acta 1163* 131-143 1993). Contradictorily, on the basis of the postulated content of the Ca^{2+}-ATPase in F^--treated SR vesicles, Coll and Murphy (*Journal of Biological Chemistry 267* 21584-21587 1992) suggested that each inactivated enzyme contains one tightly-bound Mg^{2+} and two tightly-bound F^-. The present study has been made to resolve this conflict. The contents of phosphorylation site, high-affinity ATP-binding site, high-affinity vanadate-binding site, and high-affinity Ca^{2+}-binding site in the SR vesicles used were 3.33 ± 0.06, 3.54 ± 0.12, 3.34 ± 0.04, and 6.98 ± 0.16 nmol/mg, respectively. When the vesicles were incubated with F^- in the presence of Mg^{2+}, the Ca^{2+}-ATPase was inactivated progressively. After removal of unbound Mg^{2+} and F^- by gel filtration, tightly-bound Mg^{2+} and F^- were determined by use of an atomic absorption spectrophotometer and a F^--selective electrode. A linear relationship existed between the extent of the enzyme inactivation and the contents of the tightly-bound ligands. The contents of tightly-bound Mg^{2+} and F^- in the fully inactivated vesicles were 6.65 and 12.6 nmol/mg, respectively. The same stoichiometry was obtained with another preparation of SR vesicles. These results demonstrate that tight binding of 2 mol of Mg^{2+} and 4 mol of F^- per mole of phosphorylation site, per mole of high-affinity ATP- or vanadate-binding site, and per 2 mol of high-affinity Ca^{2+}-binding site leads to a complete inactivation of the Ca^{2+}-ATPase in SR vesicles.

Key words: ATPase; Ca^{2+} binding; Mg^{2+} binding; Phosphorylation; Sarcoplasmic reticulum; Vanadium binding.
Reprints: T Kanazawa, Asahikawa Medical College, Dept. of Biochemistry, Asahikawa 078, Japan.

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.