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AN INVITATION TO BUDAPEST

The 17th Conference of the International Society for Fluoride Research will be held June 22-25, 1989, at the Sporthall in Budapest, Hungary. All interested scientists are welcome.

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HARMFUL EFFECTS OF WATER FLUORIDATION IN HOLLAND

A Review of

Fluoride: The Freedom Fight

by Dr. Hans Moolenburgh

Inhabitants of the ancient cathedral city of Haarlem, Holland, have had a long history of defending or regaining their freedom from conquerors. Perhaps because of this fact, Dr. Hans Moolenburgh, a highly regarded family physician consultant who resides there, was particularly sensitive to events that led to his writing this remarkable book. As a young lad he had lived through the dreadful Nazi occupation of his country. His father, who became a public prosecutor in 1933, dedicated himself to destroying the German spy network operating in Holland. Later, during the war, whenever possible, he assisted Jews in escaping from the Gestapo. Inevitably the Nazis scheduled him for extinction, but he miraculously escaped because he happened to be absent from Amsterdam on that fateful day. In his place someone else was shot.

Consequently, when the Haarlems Dagblad, the local newspaper, casually announced on 26 October, 1968, that fluoridation of the city's water supply would begin in June, 1970, Dr. Moolenburgh immediately wrote a letter to the paper which stated in part: "When I turn the tap on what I want to see is water, not a medicine I did not ask for." So much public support for his view followed that the Chief Inspector of Health for North Holland called Dr. Moolenburgh into his office where he told him: "You are not allowed to write things like this because you have a disquieting effect on the population I have to forbid you to write like that." But the letter had made its impact, and the Haarlem Municipal Council rescinded its decision to fluoridate. In retaliation, the dentists wrote to the newspaper suggesting that people use a "filter" on their water tap to remove the fluoride — a device the director of water supplies of Haarlem called an "unhygienic time bomb."

Later, when another community was about to start fluoridation, an appeal to the Crown brought the following response: "The Crown finds this licence [to fluoridate] in conflict with the principles of decent government, as these are based on the general awareness of what is right. This means that the caution which should always be observed by the government had been violated, preventing consumers with objections to fluoridation to obtain non-fluoridated water in a practical way." The matter, however, did not come to rest. Proponents in the Health Department then circulated a booklet entitled Advice No. 19 containing a chapter on "medical aspects" that "seemed," to Dr. Moolenburgh, "to have been written by someone with hardly any medical experience."

Fluoride: The Freedom Fight especially warrants the attention of readers of Fluoride because of a number of important scientific observations that are recorded in it. It was not written specifically to provide detailed evidence concerning the pros and cons of adding fluoride to water supplies. Originally, at the start of their investigations, the author and his associates anticipated that "good, solid scientific reports would have persuasive power with the authorities." They quickly learned, however, that such an idea was "ridiculously naive." Indeed, in areas of Holland that had recently been fluoridated, adverse

health effects were already occurring, manifesting symptoms which my late husband, Dr. George L. Waldbott, had been recording in the scientific literature as early as 1955. His first case, a 35-year-old female, living in fluoridated Highland Park, Michigan, had become bedridden after exhibiting a wide variety of progressively more debilitating symptoms which local physicians were unable to diagnose. From her history, Dr. Waldbott learned that in her early years she had lived in China where her drinking water contained sufficient natural fluoride to have caused her very noticeable dental fluorosis. Following her hospitalization for extensive diagnostic studies, eight of Detroit's most prominent specialists in the areas of her symptoms concurred that she was seriously ill, but they were at a loss to establish an overall diagnosis.

Neither in the hospital nor after her discharge was she given any medication. Instead, she was instructed strictly to avoid fluoridated water, not only for drinking but also for cooking. She was likewise told to avoid both tea and seafood because of their high fluoride content. Very soon her headaches, eye disturbances, and muscular weakness disappeared in a most dramatic manner. After about two weeks her mind began to clear, and she underwent a complete change in personality. For the first time in two years she was able to undertake her household tasks without repeatedly having to stop and rest. Within a four-week period she had gained five pounds and no longer suffered from her previous symptoms.

Clinical details of this case were published by Dr. Waldbott in Internat. Arch. Allergy Appl. Immunol., 7:70-74, 1955, and in his comprehensive book Fluoridation: The Great Dilemma (Coronado Press, 1978), written in collaboration with Professors A.W. Burgstahler and H.L. McKinney of the University of Kansas. During over 25 years which followed, Dr. Waldbott carried out extensive basic research on fluoride and chronic fluoride intoxication from air-borne as well as water-borne fluoride. His more than 80 clinical reports, articles, and reviews in this area appeared in highly respected U.S., European, and International journals. Founder of the International Society for Fluoride Research, a multi-disciplinary organization devoted especially to the investigation of the biological effects of fluoride, he served as its Secretary and Editor of its journal Fluoride from its inception in 1968 until his death in July, 1982.

To return to what was happening in Holland, adverse side effects, as already mentioned, were being encountered in fluoridated areas that were not seen elsewhere. Chronic fluoride poisoning, Dr. Moolenburgh found, presents itself by general complaints: "headache, inflated tummy, an itchy skin, nausea, tiredness, pains here and there in the joints." It is often very erratic, making a correct diagnosis "most difficult," especially for a physician who has not been alerted to the presence of a toxic substance in the environment which may be responsible for the illness.

In the months following publicity of his clinical observations, Dr. Moolenburgh "received hundreds of letters and most of them concerned real fluoride poisoning. The complaints went away when using non-fluoridated water, came back with fluoridated water and went away again with non-fluoridated water. This could be proved time and again." But "the general practitioners laughed" and called these results "pure imagination." After all, "Had not the authorities assured them that it [fluoridation] was safe for everyone? Oh, they had read those articles by mad Moolenburgh? Yes, what could you

expect? The man was apparently mad as a hatter, and people like him should not be allowed in medical practice.' So, while my warnings cured many people, they also earned me the scorn of many GPs."

On the other hand, when it came to public accountability for their unqualified claims of the absolute safety of water fluoridation, professional proponents were more cautious. Invited to sign a guarantee of financial responsibility in the event of scientifically-proven side effects, the dentist or health official making such claims would adamantly refuse to do so. For example, Dr. A, when asked to sign on the dotted line, "turned white as a sheet and then stammered: 'I will not even consider doing such a thing!'"

As part of his effort, Dr. Moolenburgh organized a research group of local GPs with no preconceived ideas about possible health effects of fluoridated water. If anything, they were more inclined to be skeptical than credulous. For orientation, they were asked to read key scientific studies. Their attitude soon changed, however, when one of them began to suffer a nagging gastrointestinal pain which, in spite of his incredulity, proved beyond question to have been caused by the fluoride in the drinking water. "It was as if a company of colour-blind people suddenly recovered their ability to see colours." Eventually others in the group also observed cases of fluoride intoxication from fluoridated drinking water.

To obtain unassailable proof that nothing but fluoride in the water was responsible and that the ill effects were not imaginary, Dr. Moolenburgh's group turned to a double blind procedure. A cooperating pharmacist prepared eight numbered bottles of drinking water, some with fluoride, some without. Only the pharmacist knew which ones contained fluoride. Their numbers, placed in a sealed envelope, were sent to the group's attorney. Patients who had recovered from side effects after changing to non-fluoridated water drank, daily, one liter of water from one of the bottles given to them. If symptoms occurred, the number of the bottle was sent to the attorney in a sealed envelope. Only after all the envelopes were received were they opened in the presence of witnesses to avoid any possibility of bias. The positive results of these double-blind tests were published in Fluoride (7:146-152, July, 1974) in an article by Dr. G.W. Grimbergen, one of the members of the group organized by Dr. Moolenburgh.

In addition to the original 12 physicians practicing in Haarlem and some of its surrounding fluoridated areas, various individuals with training in biology, chemistry, and neurology also participated in the above study. The main symptoms exhibited by the patients were gastro-intestinal, stomatitis, joint pains, polydipsia, headaches, visual disturbances, muscular weakness, and extreme tiredness. A definite relationship between the symptoms and fluoride in water was clearly established. Thus this research, carried out independently in the Netherlands, provides unequivocal confirmation of Dr. Waldbott's extensive earlier published clinical findings on fluoride of a similar nature.

Other interesting research findings are also recorded in Fluoride: The Freedom Fight. In the fluoridated town of Aalsmeer, a flower grower who was raising pheasants observed peculiar birth defects among chicks of mother pheasants drinking fluoridated water, namely, an extra toe or a deformed beak. Moreover, these fluoridated chicks were also unusually tame; they made no attempt to flutter away on being touched, in contrast to non-fluoridated chicks.

One of the scientists in the group, Dr. Mien, a neurologist, had done a dissertation on cholinesterase inhibition by fluoride and had prepared a presentation of it for the ISFR conference held in Holland in February, 1976. Dr. Mien's experiments had been conducted on blood outside the body. To finalize her research, however, it was imperative to determine the effect of fluoridated water on cholinesterase inside the body. With the discontinuation of fluoridation in Amsterdam following the Dutch High Court decision in 1973 to outlaw fluoridation, the constant stream of patients with side effects had dried up. To solve this problem, the majority of the doctors and their wives in the research group volunteered to become subjects for the study.

The results were dramatic. "A severe disruption of the cholinesterase function was demonstrated in each case. In a normal person cholinesterase shows a sort of slow, undulating pattern, but in the fluoridated individuals chaos prevailed. There was a definite inhibition of cholinesterase activity." Since, in addition to its role in nerve impulses, cholinesterase is important in maintaining stability in membrane permeability, its inhibition by fluoridation could make "the cell membrane more porous — one more reason why we should take care." In this way, fluoride could be behaving like "a gatekeeper who welcomed dangerous substances into the cells." Clearly, this research showed that "fluoridation has an effect on every cell of the body." Does it also explain why the fluoridated pheasant chicks made no attempt to flutter out of the hand as did the non-fluoridated ones?

Today in Holland, as Dr. Moolenburgh points out, after more than 10 years of freedom from fluoridation, tooth decay has not only not increased but has continued to decrease, and overall dental health has improved greatly. In various industrialized countries children's teeth in fluoridated areas are no better than in non-fluoridated areas. In fact, the trend toward better teeth appears to be independent of fluoridation, indicating that other factors are probably playing a greater role in the reduction in dental caries.

When fluoridation came to an end in Holland after eight years of relentless fighting, as Dr. Moolenburgh expressed it, "we had conquered an insidious spiritual dictatorship against overwhelming odds." Humbly, he gives major credit to the "Great Stage Manager" who "took pleasure in the victory of little people over powerful titans." Dr. Moolenburgh calls this victory "an example of how moral right can prevail over the thought pattern of a sick society." In other words: Members of parliament were never meant "to decide what we should eat or drink."

Highly readable and informative, and filled with sparkling wit and humor, Fluoride: The Freedom Fight is must reading for scientists who are unaware of the clinical realities of fluoridation as well as for those who are confronted with their fight for freedom in dealing with fluoridation.

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E.M.W.

EVALUATION OF FLUORIDE LEVELS AND EFFECTS ON HONEY BEES (Apis mellifera L.) (Hymenoptera: Apidae)

by

D.F. Mayer*, J.D. Lunden and L.H. Weinstein
Prosser, Washington, USA

SUMMARY: A three year study (1984-1986) conducted in the Puyallup Valley, WA, showed that fluoride was present in live honey bees, dead honey bees, teneral adults and stored pollen. Fluoride levels depended upon the location of the colonies in relation to a fluoride emitting source, — an aluminum smelter, and prevailing wind direction. Data on frames of adult bees, frames of brood, brood survival, brood population dynamics and honey production were collected during the study. Results indicate that the levels of fluoride found were not detrimental to the colonies over the length of the study.

KEY WORDS: Fluoride; Honey bees, Apis mellifera.

Introduction

Honey bees, Apis mellifera L. are subject to diseases, pests, pesticide poisoning and industrial pollutants which may result in bee mortality and loss of colony vigor. Fluoride gases and particulates are released as by-products from aluminum smelters and other industrial factories. Airborne gaseous fluorides can affect the growth, quality, and productivity of plants and animals (1).

Reports concerning fluoride levels in honey bees and their response to airborne fluoride emission are meager. This is especially true in North America where improvements in air cleaning equipment have removed most of the fluoride emitted from smelters (2). Dewey (3) found higher fluoride concentration in honey bees collected near an aluminum factory than in bees collected at least 80 km away from the plant. Bromenshenk et al. (4) found a higher fluoride concentration in honey bees collected near an industrial area than in bees collected some kilometers away. Atkins, Anderson and Greywood (5) demonstrated that continuous exposure to fluoride gases of 4-5 ppb (3.2-4.0 µg/cubic meter) shortens the life-span of caged worker bees.

This paper reports results of research on effects and biological responses of honey bee colonies to different levels of airborne fluoride emissions under field conditions.

Materials and Methods

In January 1984, four honey bee colonies were placed at each of three

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locations in and near the Puyallup Valley, Pierce County, WA selected to provide high, medium and low exposures of the bees to fluoride. Locations were separated by several kilometers at different distances and directions from the Kaiser Aluminum plant located at Fife, WA. The Fife Heights (FH) location was 0.8 km directly downwind and in the fume path from the smelter, adjacent to a station that measured among other variables, ambient air fluoride concentration. The Valley Road Farm (VRF) location was 6.4 km east of the plant in an area occasionally subjected to fumes from the smelter. The third location was 10 km south southeast of the plant at the Washington State University Research Station (REC), an area not directly subjected to fumes from the smelter. A control location was established in a non-industrial area near Prosser, WA, approximately 200 km east of the Puyallup Valley. In March 1984, all colonies were inspected and those not surviving the winter were replaced with viable colonies from outside the area. All colonies were removed from the locations in the fall of 1984. In April, 1985, four honey bee colonies established from package bees were again placed at each of the three locations and at Prosser.

Live and dead adult honey bees, teneral adults, stored pollen, and honey obtained from each colony at each location were collected and analyzed for fluoride content. Samples for fluoride analysis were collected from each colony as follows: (1) live adult bees at the hive entrance; (2) dead bees from Todd traps (6); (3) teneral adults from 50 capped cells; (4) stored pollen from 50 cells per colony by use of forceps; and (5) all the honey from one frame per colony at two different dates each year. Adult bees (200 to 400 per sample) and pollen were analyzed at Boyce Thompson Institute, Ithaca, NY by the semi-automated method for fluoride. Honey was analyzed for fluoride at the Irrigated Agriculture Research and Extension Center by the fluoride specific electrode method.

Each year, beginning in April 1984 and continuing until fall, each individual colony was inspected every 10 to 14 days. Amounts of brood were estimated in terms of number of square centimeters of brood. In 1986, only amounts of capped brood were estimated. Number of adult bees was estimated in terms of numbers of frames covered with bees. In August, honey production was evaluated in terms of number of Langstroth combs or tenths. At each inspection 100 cells containing eggs were tagged with stick pins to outline that section of the brood comb. At the next inspection, the number of cells with brood vs empty cells were counted to obtain an estimate of brood survival. Brood population dynamics were determined by removing the cell cap and recording the number of brood at different stages of growth. During July, August, and September, Todd traps were attached to the colonies for one or two weeks to obtain estimates of adult mortality. Duncan's Multiple Range Test was used to analyze data for statistical differences between locations in concentrations of fluoride and biological parameters (7).

Results and Discussion

Fluoride Content. The mean contents of fluoride in (or on) live bees, dead bees, stored pollen and honey from the four locations are presented in Tables 1, 2 and 3.

Mean fluoride concentrations of live adult bees was significantly different between all locations in 1984 (Table 1). Fluoride concentrations were highest

Table 1

Mean Fluoride Content of Live Honey Bees Collected at Different Locations.* Values are ppm Fluoride on a Dry Weight Basis.

Location	May		June		July			August		
	1985	1986	1985	1986	1984	1985	1986	1984	1985	1986
REC	58 ¹	28 ¹	67 ¹	48 ¹	97 ¹	35 ¹	66 ¹	138 ¹	112 ¹	99 ¹
VRF	73 ¹	64 ²	42 ¹	50 ¹	163 ²	123 ²	87 ¹	151 ¹	223 ²	181 ²
FH	244 ²	123 ³	149 ²	128 ²	129 ^{1,2}	181 ³	236 ²	251 ²	263 ²	186 ²
Prosser	9 ³	—	10 ³	7 ³	18 ³	12 ²	8 ³	15 ³	6 ³	6 ³

Location	September			October		Mean		
	1984	1985	1986	1984	1986	1984	1985	1986
REC	85 ¹	66 ^{1,2}	72 ¹	27 ¹	30 ¹	86 ¹	68 ¹	57 ¹
VRF	90 ¹	118 ²	211 ²	28 ¹	66 ²	108 ²	118 ¹	110 ²
FH	219 ²	257 ³	261 ²	82 ²	130 ³	170 ³	219 ²	177 ³
Prosser	5 ³	16 ⁴	—	—	5 ⁴	13 ⁴	11 ³	7 ⁴

* REC = Washington State University Research Station; VRF = Valley Road Farm; and FH = Fife Heights.

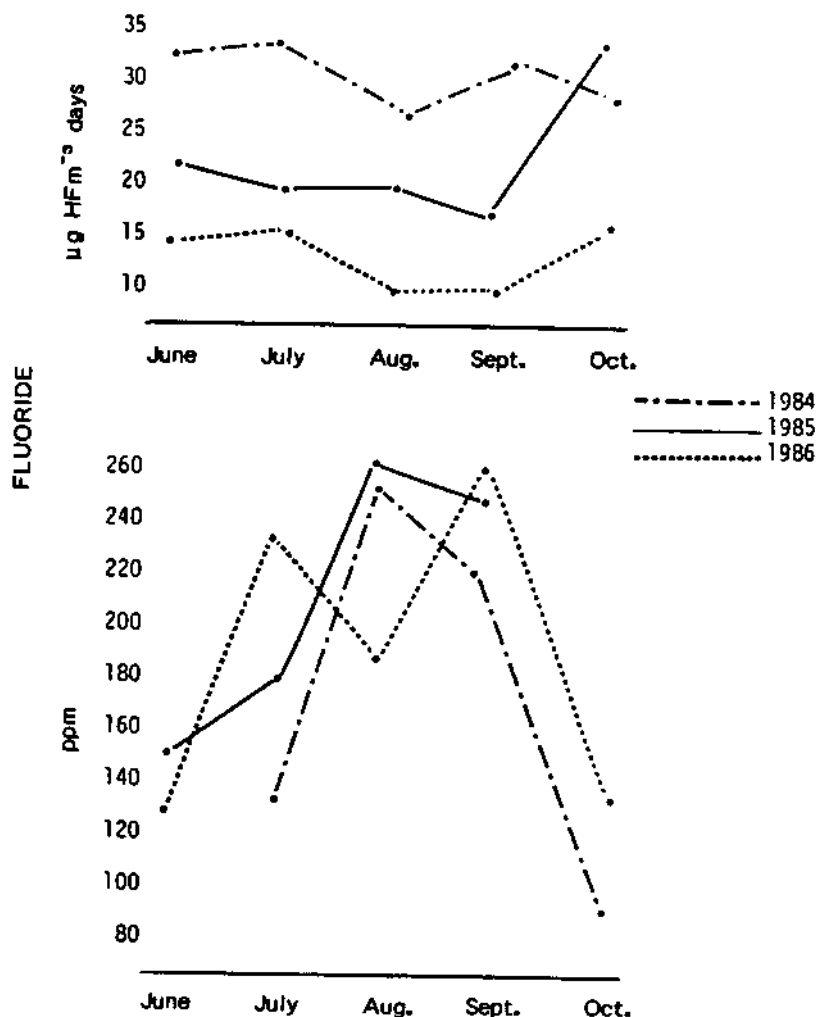
Means within a column referenced by the same number are not significantly different ($p \leq 0.5$; by Duncan's [1951] Multiple Range Test).

at the FH location and the lowest at Prosser, with the REC and VRF locations intermediate. Dewey (3) found 10.5 ppm fluoride in control honey bees and 221.0 ppm in live bees near a fluoride emission source in Montana. Bromenshenk et al. (4) found 4 ppm fluoride in control honey bees and 182 ppm in live bees near an industrial area in the same area of Puget Sound, Washington. In neither study, was there any indication that levels of fluoride found had contributed to honey bee mortality. In Europe, Dreher (8) determined that foraging bees exposed to heavy fluorine pollution contained 9.2 to 10.5 $\mu\text{g}/\text{bee}$. At FH, our area of highest fluoride concentration, we found a mean high of 263 ppm (10.5 μg) in August and a mean low of 82 ppm (3.3 μg) in October. There appears to be an increase in mean fluoride concentration obtained from live bees during July, August, and September and a reduction in October. Also, this fall reduction is not clearly related to the fluoride concentration in the air at the site (Figure 1). We speculate that decreased pollen foraging in October results in lower concentrations in the fluoride content of live bees.

Mean fluoride concentration of dead bees was significantly different between locations (Table 2). There were no significant differences in fluoride between live and dead bees at the four locations indicating that fluoride toxicity was not related to mortality. Dreher (8) has suggested that the LD_{50} for fluorine toxicity is 10 $\mu\text{g}/\text{bee}$. We found 358 ppm (14.3 μg) fluoride from live bees collected from one colony at FH suggesting 10 $\mu\text{g}/\text{bee}$ is not a toxic concentration. We suspect that much of the fluoride is on the surface of the body of the bee rather than inside it.

Figure 1

Monthly mean $\mu\text{g HFm}^{-3}$ at FH Monitoring Station and Monthly Mean ppm of Live Honey Bees at FH in 1984, 1985, and 1986.



Fluoride was significantly higher only from teneral adults collected at FH in both years. Mean values of fluoride levels in teneral adults were: REC 3 ppm, VRF 3 ppm, FH 15 ppm, and Prosser 1 ppm, for 1985 and REC 8 ppm, VRF 11 ppm, and FH 17 ppm for 1986.

Fluoride in stored pollen was lowest at Prosser and highest at FH. The VRF site was significantly higher than the REC site only in 1986 (Table 3).

Table 2

Mean Fluoride Content of Dead Honey Bees Collected in Todd Traps at Different Locations. Values are ppm Fluoride on a Dry Weight Basis.

Location	1984	1985
REC	102 ^a	74 ^a
VRF	144 ^b	130 ^b
FH	223 ^c	219 ^c
Prosser	15 ^d	—

Means within a column followed by the same letter are not significantly different ($p \leq 0.5$; by Duncan's [1951] Multiple Range Test).

Table 3

Mean Fluoride Content of Pollen and Honey at Different Locations. Values are ppm Fluoride on a Dry Weight Basis.

Location	1984		1985		1986	
	Pollen	Honey	Pollen	Honey	Pollen	Honey
REC	16 ^a	1.2 ^a	18 ^a	0.4 ^a	18 ^a	0.9 ^{ab}
VRF	19 ^a	1.2 ^a	21 ^a	0.7 ^b	32 ^b	0.8 ^b
FH	33 ^b	0.9 ^a	37 ^b	0.7 ^b	61 ^c	1.4 ^a
Prosser	—	—	8 ^c	0.3 ^a	2 ^d	0.4 ^c

Means within a column followed by the same letter are now significantly different ($p \leq 0.5$; by Duncan's [1951] Multiple Range Test).

There have been few analyses of the fluoride content in stored pollen, although Maurizio and Staub (9) reported from 9 to 18 ppm fluoride in various pollens collected in Switzerland.

Fluoride concentrations in honey were always low (Table 3). Tong et al. (10) who reported concentrations of 0.001 to 8.9 ppm fluorine in honey from New York, did not feel that the high amount was hazardous. We found a high of 1.4 ppm fluoride for honey in our study.

No consistent patterns related to fluoride emerged during the three years of the experiment (Table 4) i.e. fluoride had no effect on adult or brood populations. The existing brood, and colony build up during the summer and decline in fall were normal for honey bees.

Mean brood survival for the 3 year study was 91% at REC, 90.3% at VHF, 91% at FH and 91.6% at Prosser. In 1984 and 1986 significant differences were detected in brood survival between locations, but they were not related to the presence of airborne fluoride. No differences were detected in 1985. Harbo (11) found average egg mortality of 7% and concluded that mortality was influenced by both colony and queen. Sakagami and Fukuda (12) found 4-11%

Table 4
Bee Populations in Colonies at Different Locations in the Puyallup Valley

Location	Mean Number of Frames per Colony Covered by Adult Bees			Mean Square Centimeters of Brood per Colony		
	July			August		
	1984	1985	1986	1984	1985	1986*
REC	22 ^a	16 ^a	12 ^a	5321 ^a	10217 ^a	3483 ^a
VRF	22 ^a	17 ^a	22 ^b	8727 ^{ab}	10210 ^a	5572 ^{ab}
FH	11 ^a	18 ^a	24 ^b	7359 ^{ab}	10681 ^a	6095 ^a

* Mean square centimeters of capped brood per colony.

Means within a column followed by the same letter are not significantly different ($p \leq 0.5$; by Duncan's [1951] Multiple Range Test.

mortality to the capped cell stage in normal, healthy colonies, about the same as that we found.

Stage of brood development 14 days after eggs were marked is presented in Figure 2. No consistent differences were evident between locations or time of year of sampling in the percent of individuals at the different stages of development. Milum (13) showed that variations for complete development times of individual worker bees ranged from slightly less than 19 $\frac{7}{8}$ days to slightly more than 24 days and developmental time of a population approached a bell-shaped curve. Our data show a similar curve for an immature population of honey bees.

The mean number of dead bees per day ranged from 6 to 85, but there were no significant differences between locations. The number of dead bees were in the normal range of less than 100 per day at all locations (14).

There were no significant differences between locations in honey production in 1984 (Table 5); there were, however, in 1985 and 1986. Woyke (15) showed that honey production is correlated to a varying degree with worker population and average length of productive life of workers.

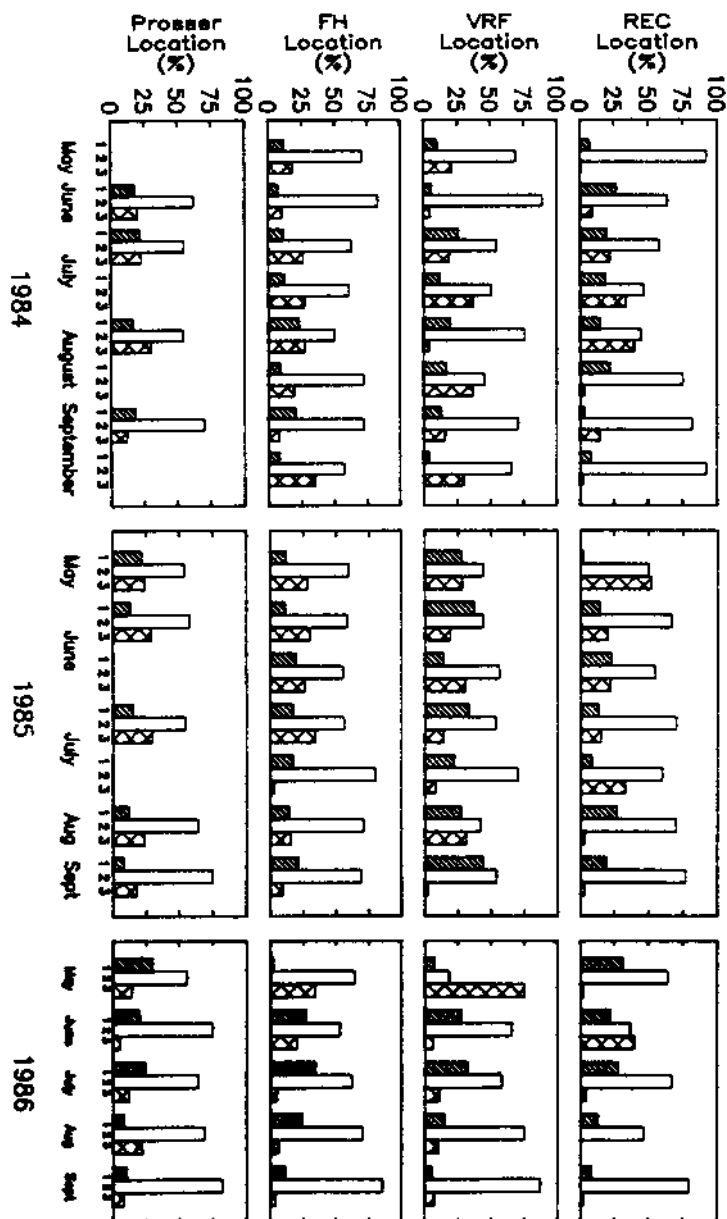
Table 5
Mean Frames of Honey in August at Different Locations

Location	1984	1985	1986
REC	7.5 ^a	2.8 ^a	2.3 ^a
VRF	10.6 ^a	1.5 ^a	2.2 ^a
FH	9.2 ^a	5.9 ^b	7.3 ^b
Prosser	—	9.3 ^c	11 ^c

Means within a column followed by the same letter are not significantly different ($p \leq 0.5$; by Duncan's [1951] Multiple Range Test.

Figure 2

Percent of honey bee immatures at different stages of development 10 to 14 days after eggs were laid at different locations (1 = prepupa, 2 = white-eyed pupa and 3 = pink-eyed pupa).



Conclusion

Since in our study honey production was equal to or higher at FH than at the other locations in the Puyallup Valley, the higher concentrations of fluoride appeared to have no effect on the size of worker populations or adult life span.

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DENTAL FLUOROSIS IN RELATION TO TEA DRINKING IN JORDAN

by

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SUMMARY: A total of 2,516 children aged 6 to 13, residing in a chronic dental fluorosis area of Jordan, were examined. Fluorosis and daily quantity of tea drinking were recorded for each child. Drinking waters from all visited cities were collected and analyzed. The daily mean of cups of tea drunk by each child was 4.13 ± 0.48 cups. Correlation between dental fluorosis and the daily mean of tea drinking was positive.

KEY WORDS: Dental fluorosis; Jordan; Tea.

Introduction

Epidemiological studies in areas with high and low concentrations of fluoride in drinking water and soil have been conducted around the world during recent decades (1-13). It is well known that waters high in fluoride are found at the foot of high mountains and in areas with geological deposits of marine origin. The geographical belt from Syria, through Jordan to Morocco, and the Rift Valley through Sudan and Kenya is typical (15). Fluorides, which originate from volcanic activity, are also distributed in the atmosphere from industrial emissions and from the dust of fluoride-containing soils (8,13,15-16).

Epidemiological studies conducted in Kenya (23,8,13,16) and in Morocco (16,8,13) showed an unexpectedly high prevalence and severity of dental fluorosis in populations living in areas with relatively low fluoride levels in drinking water. Reports to the Jordanian Ministry of Health (17,18) had previously indicated that fluoride concentrations in drinking waters range from 0.40 ppm F to 1 ppm F in most of the country except near the Al-Hassa phosphate mines where it was 2 ppm F.

The purpose of our study was to determine the prevalence of dental fluorosis and the source of high fluoride intake in Jordanian children.

Materials and Methods

2,516 children (1,180 girls and 1,336 boys), aged 6 to 13 years, were examined in 26 schools in 12 cities from north to south by only two examiners. Frequent inter- and intra-examiner rating controls were done during the survey.

Schools were chosen randomly by the examiners who did not announce in advance time or date of visiting towns or schools. The two examiners had official permission to visit schools whenever and wherever they desired.

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All children were examined in their schools, outdoors, seated facing the examiner, illuminated by natural daylight (direct sunlight was avoided). Name, sex, year and place of birth, profession of parents, number of persons in the family and the number of cups of tea taken daily by each child were noted prior to intra oral examination. Each examiner, who ignored the origin of children, examined 10 subjects while the other recorded clinical data. The whole dentition was examined. Data on dental fluorosis was recorded according to the modified Dean Index (19) from 0 to 5.

Drinking water samples were collected by the examiners from water taps in visited cities and analyzed by Orion R. by specific electrode for fluoride analysis using Tisab III reagent. Methods for data reduction and statistical analysis followed those recommended by Schwartz and Lazar (20).

Results

Drinking water analysis revealed concentrations ranging between 0.2 ppm F and 0.98 ppm F (Table 1). Only 43 boys and 21 girls were free from dental fluorosis; 2 boys and 4 girls were classified degree 1 while 1,291 boys and 1,155 girls presented 2nd, 3rd, 4th and 5th degree according to Dean (Figures 1,2).

Figure 1

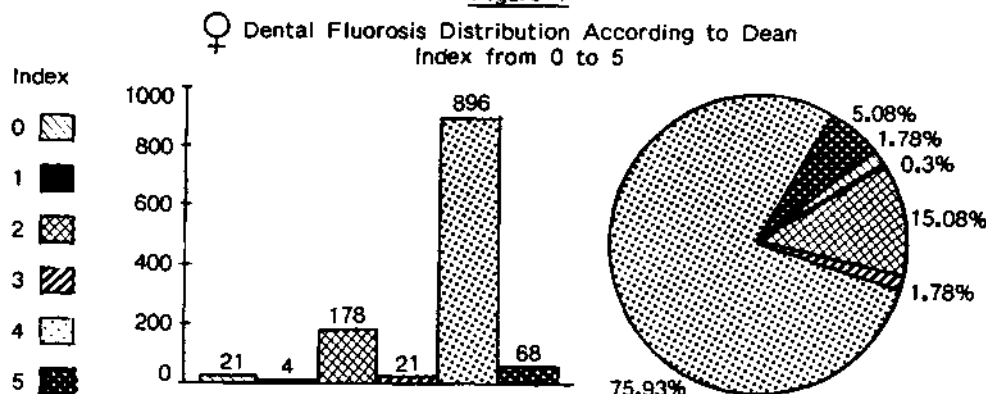


Figure 2

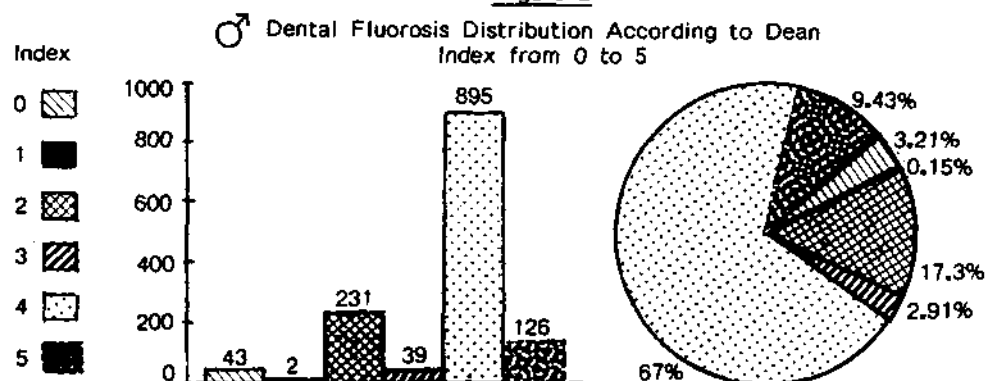


Table 1
Fluoride Concentration in Drinking Waters in Some Visited Towns
and Their Geographic Situation

Town	Fluoride Concentration in ppm	Geographic Situation
Irbid	0.21	North
Qweira	0.60	South
Amman	0.61	Center
Al Hassa	0.65	South
Wadi Musa	0.68	South
Ma'an	0.75	South
Akaba	0.98	Extreme South

The children who were compared, attended two schools located in the same residential area in the capital, Amman. However, they had a different social standard and way of living: the private school had a high standard of living, the public school a lower standard. In the public school, the mean fluorosis index was 3.31 ± 0.29 whereas in the private school the mean fluorosis index was 2.10 ± 0.21 . The public school in Amman when compared with another public school in the city of Ma'an (rural area) revealed that in Ma'an the mean fluorosis index was 4.07 ± 0.10 (Table 2).

Table 2
Comparison Between Three Schools, Two in the Capital Amman (Private and Public Schools) and the Third, a Public School in Ma'an (rural area).

City	School Category	Number of Children	F ₁ Mean*	Daily mean of tea cups taken	Degree of Significance
Amman	Private	108	2.10 ± 0.21	1.42 ± 0.32	H.S.**
Amman	Public	80	3.31 ± 0.29	2.57 ± 0.32	H.S.**
Ma'an	Public	97	4.07 ± 0.10	5.84 ± 0.69	H.S.**

* \pm Standard Deviation with $p < 0.05$

** H.S. = Highly Significant

Tea Drinking: In general the mean number of cups of tea taken daily by each child was 4.13 ± 0.48 cups. The comparison between boys and girls in all cities visited where children of both sexes were examined revealed a higher score in boys than in girls (Table 3).

In the two schools (private and public) in Amman, the capital, and in the third school in Ma'an (rural area) a daily mean cups of tea was 1.42 ± 0.32 cups for the first, 2.57 ± 0.32 cups for the second and 5.84 ± 0.69 cups for the third (Table 2).

Table 3

Daily Mean of Tea Drinking, comparison between Boys and Girls in Visited Cities where Both Sexes Were Examined

City	BOYS		GIRLS	
	Number of children	Mean of daily tea cups taken by each child*	Number of children	Mean of daily tea cups taken by each child*
Amman	188	1.91 \pm 0.23	79	1.56 \pm 0.23
Irbid	100	3.43 \pm 0.39	109	2.10 \pm 0.23
Rasaifa	83	4.13 \pm 0.33	64	3.12 \pm 0.45
Aguaba	316	4.47 \pm 0.20	233	3.22 \pm 0.21
Kerak	71	4.85 \pm 0.50	47	3.36 \pm 0.50
Shobak	47	5.12 \pm 0.46	56	3.73 \pm 0.41
Wadi Musa	263	5.65 \pm 0.24	214	4.52 \pm 0.27
Al Hassa	49	5.73 \pm 0.88	27	3.03 \pm 0.60
Ma'an	97	5.84 \pm 0.69	83	4.07 \pm 0.55
Madaba	91	6.93 \pm 0.61	157	3.74 \pm 0.35

* \pm Standard Deviation, $p < 0.05$

Discussion

The majority of our sample presented severe forms of dental fluorosis. Analysis of drinking water differed slightly according to Zohni (17) and Oweisa (18). The difference may be explained by the fact that we analyzed drinking water collected from water taps whereas Zohni and Oweiss who found higher fluoride concentrations, analyzed water in wells. Haikel et al. (13) also found lower fluoride concentrations in tap water than in wells.

These fluoride concentrations which range from 0.2 ppm F to 0.98 ppm F are similar to the schedules published by the American Academy of Pediatrics (21) reviewed in 1979 and the American Dental Association (22) which authorized fluoride supplementation in drinking water.

The high prevalence and severity of dental fluorosis in our results was unexpected. Similar results were reported by Manji et al. (23) in areas with similar fluoride concentrations. The temperature and altitude were the principal cause. This factor must be taken into account in our study since the mean annual temperature in Jordan ranges from 14°C to 24°C from north to south (Climatic Atlas of Jordan) (24).

On the other hand, Haikel et al. (13) who reported a high prevalence of fluorosis in areas with only 0.13 to 0.21 ppm F in drinking water explained this phenomenon by the presence of phosphate mines in these areas. On the contrary, in Jordan the highest prevalence and severity of dental fluorosis was found in areas located 150 kilometers from phosphate mines with only

0.75 ppm F in drinking water. Thus the source of high continuous fluoride intake in our sample must be found elsewhere.

The comparison of the three schools, two of them located in the capital, Amman, in the same residential area, the third in Ma'an, considered a rural area, rule out factors such as temperature or phosphate dust. In fact, both cities are outside the phosphate mines areas; their annual mean daily temperature is 15°C (20); fluoride concentrations in their waters, namely 0.6 ppm F in Amman and 0.7 ppm F in Ma'an are similar. The only difference between the two schools compared in Amman, the capital, is that the first one is a private school with a high standard of living whereas the second one is a public school where children drink tea instead of more expensive fruit juice. In the private school where mean daily tea drinking was 1.42 ± 0.32 cups per child, the mean dental fluorosis index was 2.10 ± 0.21 , whereas in the second school where the mean daily tea drinking was 2.5 ± 0.32 cups per child, mean dental fluorosis index was 3.31 ± 0.29 . Comparison of the public school in Amman with the public school in Ma'an confirmed the positive correlation between tea drinking and severity in dental fluorosis; in Ma'an the daily mean of cups of tea per child was 5.84 ± 0.69 , the mean fluorosis index was 4.07 ± 0.10 .

Conclusion

The severity of dental fluorosis in Jordanian children may be related to excessive daily tea drinking.

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BIOCHEMICAL EFFECTS OF FLUORIDE ON THYROID GLAND DURING EXPERIMENTAL FLUOROSIS

by

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SUMMARY: Fluorosis leads to fat accumulation in the thyroid gland, hyperlipidemia and hypertriglyceridemia. The administration of sodium fluoride in the concentrations of 5, 10, 20 and 50 mg/kg b.w./day to normal rabbits for 100 days increased the lipid components, total lipids and triglycerides in thyroid gland. The increase in content was highly significant compared to controls. The level of free fatty acids was significantly reduced in thyroid gland of fluoride-treated rabbits.

KEY WORDS: Free fatty acids; goitre; Sodium fluoride; Total lipids; Triglycerides.

Introduction

One of the most outstanding features of pre-skeletal fluorosis is the extraordinary general fatigue experienced by most sufferers. Such marked weakness is linked by physicians to a low activity of the thyroid gland. The role of thyroid gland in fluorosis became the subject of controversy when Maumené (1), who was studying the toxicity of fluoride, observed a tumor, presumably a goitre, on the neck of a dog to which he had administered 20 to 120 mg of sodium fluoride daily for four months. A high incidence of goitre has been observed in countries where skeletal fluorosis is endemic (2). In India, goitres have been connected directly to high concentrations of fluoride in drinking water in persons 14 to 17 years of age (3). Fluoride, a potent inhibitor of anaerobic glycolysis, is an important trace element of hard and soft tissue organs, whose effects on lipid synthesis by endocrine glands have not been observed. The following experiments were conducted to make observations on the thyroid gland.

Materials and Methods

Albino rabbits of both sexes in the weight range of 400-650 gm, procured from Kaila Scientific Corporation, Agra (India), were divided into five groups of 12 rabbits (6 males, 6 females). All the animals were fed standard pellet diet obtained from Hindustan Lever Ltd., Bombay, and water was supplied ad libitum. The animals were weighed prior to the start of the experiment. One group given 1 cc distilled water/kg/day served as control. The remaining four groups were administered subcutaneous injections of sodium fluoride solutions in the concentrations of 5, 10, 20 and 50 mg/kg/day respectively. After 100 days, all animals were weighed again and kept for overnight fasting. The following day, the animals were sacrificed under ether anesthesia. The thyroid gland was removed immediately for various biochemical estimations. The extraction of total lipids was done with the method of Folch et al. (4). Total lipids were gravimetrically estimated.

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Thin Layer Chromatography: The neutral lipids were separated by thin layer chromatography. The method of Freeman and West (5) was used with slight modification for the preparation of silica gel G thin layer plates (20 x 20 cms) for chromatography. The plates were activated at 110°C for 90 minutes and allowed to cool before use. The plates were developed in a solvent system, n-hexane:diethyl ether:acetic acid [galacial] 90:10:1 v/v. The developed plates were air dried and then placed in sealed chambers saturated with iodine vapors which provided yellow spots, which were marked and analyzed. After identifying and marking the different spots, they were scratched into centrifuge tubes containing the extracting solvent (n-hexane:diethyl ether, 1:1 v/v). The pooled extracts, evaporated to dryness under reduced pressure, were taken up in a known volume of chloroform:methanol (1:1 v/v) and used for the colorimetric estimation of various lipid fractions. Triglycerides were determined by the method of Vanhandle and Zilversmith (6). Free fatty acids were estimated according to the method of Chakrabarty et al. (7).

Results

The results are given in Table 1. The administration of fluoride to rabbits leads to abnormal accumulation of fat in the thyroid gland (Table 1). In males, total lipid levels were higher in all fluoride-treated groups of rabbits. The

Table 1
Effects of Fluoride on Total Lipids and its Fractions in Thyroid Gland of Rabbit (Values are mean \pm S.D.)

Parameter studied	Treatment F ⁻ mg/kg b.w.	Male	Percentage of control	Female	Percentage of control
Total Lipids	0 (control)	46 \pm 4		53 \pm 6	
	5	71 \pm 9 ^a	+56	67 \pm 8 ^b	+26
	10	87 \pm 4 ^{a,d}	+92	94 \pm 9 ^{a,d}	+76
	20	127 \pm 2 ^{a,c}	+177	146 \pm 13 ^{a,c}	+173
	50	167 \pm 21 ^{a,d}	+265	193 \pm 10 ^{a,c}	+262
Triglycerides	0 (control)	14 \pm 0.6		17 \pm 4	
	5	22 \pm 0.6 ^a	+51	26 \pm 0.3 ^a	+56
	10	31 \pm 0.3 ^{a,a}	+123	37 \pm 0.7 ^{a,a}	+126
	20	71 \pm 1.0 ^{a,a}	+412	67 \pm 0.7 ^{a,a}	+302
	50	104 \pm 0.6 ^{a,a}	+652	88 \pm 0.4 ^{a,a}	+431
Free fatty acid	0 (control)	14 \pm 0.1		23 \pm 0.1	
	5	13 \pm 2.2	-7	16 \pm 0.4 ^a	-33
	10	7 \pm 0.2 ^{a,a}	-48	12 \pm 0.1 ^{a,a}	-48
	20	7 \pm 0.2 ^{a,e}	-50	6 \pm 0.2 ^{a,a}	-76
	50	11 \pm 0.5 ^{a,a}	-21	20 \pm 0.3 ^{a,a}	-12

All values are expressed in mg/g w.w. of tissue, level of significance between the groups was calculated by student's t-test; significant values in treated groups compared to the control are: a p < 0.001, b p < 0.02; and significant values in 5 mg vs 10 mg F⁻ group, 10 mg vs. 20 mg F⁻ group and 20 mg vs. 50 mg F⁻ group are c p < 0.001, d p < 0.01, and e p < 0.05.

highest percentage increase (26%) was seen in animals administered 50 mg/kg of fluoride. In females, the total lipids showed rapid elevation in thyroid gland of all experimental groups. The concentration of triglyceride in thyroid gland registered a significant increase ($p < 0.001$) in fluoride-treated animals of both sexes compared to controls. The males showed a higher percent increase in triglyceride levels of thyroid gland in 20 and 50 mg F^- groups compared to females (412% vs. 302% in 20 mg F^- group and 652% vs 431% in 50 mg F^- group). The free fatty acid content in thyroid gland of males did not show any significant change in animals treated with 5 mg/ F^- /kg. The free fatty acids in the other F^- -treated groups showed a significant decline ($p < 0.001$) compared to controls. In both sexes, decrease in 5 mg vs. 10 mg F^- group ($p < 0.001$), 10 mg vs. 20 mg F^- group ($p < 0.05$ in male, $p < 0.001$ in female) and increase in 20 mg vs. 50 mg F^- group ($p < 0.001$) was observed.

Discussion

The thyroid gland is the most sensitive organ in its histopathological and functional responses to expressive amounts of fluoride. The lowest dose of fluoride that has been reported to influence thyroid function is 0.5 mg/kg/day in experimental animals (8). If thyroid concentrates fluoride as it does iodine, excessive amounts of fluoride in the thyroid gland might interfere with the normal activities of the thyroid, perhaps by poisoning enzymes. Triglycerides undergo hydrolysis by a hormone-sensitive lipase to form free fatty acids and glycerol. It has been pointed out that this enzyme is most susceptible to the inhibitory action of fluoride in amounts as low as 1 part in 5 million which may result in increased levels of triglycerides in thyroid (9). The increase in total lipids seems to be due to the deficiency of a specific hydrolytic enzyme necessary to break down the lipid.

Fluoride has been known to cause a decrease in the active ion transport at the cell membrane and an increase in the membrane permeability of cells because of its inhibition of pyrophosphatase activity (10). This inhibition also interferes with fatty acid oxidation (11). Fluoride also inhibits enzymes involved in fatty acid oxidation like acyl CO-A synthetase. During glycolysis, 2-phosphopyruvic acid undergoes degradation to form phosphoenol pyruvic acid and water. This reaction is catalyzed by enolase, an enzyme which requires magnesium and is thus susceptible to fluoride inhibition (12). The addition of fluoride to an actively glycolyzing system, results in the accumulation of phosphoglyceric acid and decreased production of ATP (13). As ATP is a requisite for lipid synthesis, the observed decrease in level of free fatty acids is not unexpected. Growth hormone accelerates the release of free fatty acids from adipose tissue and raises the plasma free fatty acid concentration by increasing the rate of lipolysis of the triacylglycerols stores. It also activates the hormone sensitive lipase and increases glucose utilization as well. The latter has been attributed to stimulation of esterification by the increased production of free fatty acid. These lipolytic processes require the presence of glyccorticoids and thyroid hormone (14). If fluoride competes with iodine, so that not enough thyroxine can be formed, there might be an increase in goitre. When the total iodine pool of the body is low, however, fluoride interferes with the function of the gland and thereby produces a fluoride-iodine antagonism. This interpretation is further supported by a survey of 648 people in 13 mountainous villages in Nepal where the iodine content

of water is low (0.001 ppm) and where goitre is prevalent. A close correlation between fluoride intake and the incidence of goitre (15) was noted.

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ALTERATIONS IN THE ACID AND ALKALINE PHOSPHATASE QUANTITIES IN FLUORIDE-EXPOSED ESTUARINE GOBY, Boleophthalmus Dussumieri

by

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SUMMARY: An edible goby Boleophthalmus dussumieri, which abounds in the estuaries of South Gujarat coast (21° 12'N and 72° 50'E) has been chosen for current experiments. The fish were subjected to sublethal concentrations of sodium fluoride, namely, 40 and 80 ppm, (LD₅₀ at 96h : 120 ppm). Acid and alkaline phosphatases, which are significantly associated with the structural and metabolic aspects of tissues, have been examined quantitatively to assess their involvement in the pollutant-induced derangement in the fish. Initial depletion in both these hydrolases paralleled low levels of total proteins in the two target tissues (liver and muscles). These observations in conjunction with the histopathological and metabolic alterations seen in the fluoride-exposed fish confirmed the extent of disturbance caused by this pollutant. However, later signs were evidenced of recovery by enzyme and metabolite concentrations close to those in the pre-exposure period.

KEY WORDS: Boleophthalmus dussumieri; Mudskipper; Phosphatases; Sodium fluoride.

Introduction

Phosphatases are significantly associated with many functions at the cellular level. The hydrolases are capable of degrading all major classes of cell materials. Acid phosphatase is involved in a number of activities such as phagocytosis (1), dissolution of tissue components (2), fat absorption in intestine (3), cellular differentiation (4) and keratinization (5-7). Alkaline phosphatase is associated with the formation of fibrous proteins (8) and phosphate transfer in DNA metabolism (9). Pollutants bring about derangement in an organism through their specific and generalized effects on cellular structure and metabolism. Consequently pollutant-induced changes in activities of these hydrolases may be expected.

A wide range of industrial chemicals with potential pollutant effects have been shown to cause adverse effects on acid and alkaline phosphatase activities. Progressive depletion of acid phosphatase activity in the rat liver following treatment with dimethylaminoazobenzene has been reported (10). Similarly, inhibition of acid phosphatase activity in the liver and gills of Clarius batrachus treated with malathion has also been shown (11). Alkaline phosphatase activity in kidney, liver and muscles of Mystus vitatus was inhibited when exposed to rogor (12). Cadmium has been reported to lower alkaline phosphatase activity in gills of C. batrachus (13).

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In view of the vital role played by the phosphatases in structural integration and in the metabolic activities of animals combined with a lack of information on fluoride-induced effects on them, especially on aquatic vertebrates like fish, the present study was carried out.

Materials and Methods

Healthy edible mudskippers, *Boleophthalmus dussumieri* collected from the Dumas coast of South Gujarat (21° 12'N and 72° 50'E) during ebb-tides were brought to the laboratory and acclimated in artificial sea water (14) for a week (Photoperiod: 12 h D/N, Temperature: 25 ± 2°C, salinity 24 ± 0.1%, pH 8.05 ± 0.1). During this period they were given commercial fish food twice a day. Feeding was discontinued 24 h prior to experimentation. Twenty-five fish (weight: 10-14 g, length: 9-12 cm) were exposed to different sublethal concentrations of fluoride (40.0 and 80.0 ppm F), prepared by dissolving the appropriate amount of commercial grade sodium fluoride (NaF) in artificial sea water (LD₅₀ for 96 h is 120 ppm F). Fish from the same size group maintained in media (artificial sea water) without fluoride served as controls. Fish exposed to fluoride media as well as controls were given fish-food twice a day and exposure media were changed daily. At the end of 24 h, 3 fish were removed from each container and were anesthetized by general hypothermia. Liver and muscles of the fish were isolated and acid and alkaline phosphatases were estimated as given in Sigma Technical Bulletin No. 104 (15), using p-nitrophenyl phosphate as substrate. Enzyme activities were obtained as Bessy-Lowry units (one unit of phosphatase activity liberates 1 μ m of p-nitrophenol [1 μ m = 0.1391 mg/h under specified conditions] and were expressed as μ g of p-nitrophenol released/100 mg protein/30 min. The fish were removed to determine the enzyme activities at intervals of 24 h to 96 h, after which the intervals were increased to 48 h. The experiment was terminated at the end of 288 h. Total proteins were estimated by Folin-phenol method of Lowry et al. (16).

Results

Acid Phosphatase

Muscles: Fish exposed to different concentrations of fluoride registered low activity of acid phosphatase in muscles. In fish exposed to 40.0 ppm F, acid phosphatase activity increased until 96 h; at 144 h it was at its lowest level. In fish exposed to 80.0 ppm F, higher activity was registered at 48 h, it decreased considerably from 96 to 288 h when it reached its lowest level (Figure 1). In muscles of fish exposed to 40.0 ppm F, the enzyme activity never reached the normal level.

Liver: Acid phosphatase activity was lower in the liver of fish exposed to different concentrations of fluoride. Fish exposed to 40.0 ppm F exhibited more enzyme activity at 24 h, but it decreased gradually; at 192 h it reached its lowest point. In fish exposed to 80.0 ppm F, lower activity was noted at 72 h; it increased and peaked at 192 h then decreased gradually (Figure 2).

Alkaline Phosphatase

Muscles: Fish exposed to different concentrations of fluoride in media exhibited fluctuations in alkaline phosphatase activity in the muscles which, on the whole, was considerably lower compared to controls. In fish exposed to 40.0 ppm F,

Figure 1

Acid phosphatase activity (μg of p-nitrophenol released/100 mg protein/30 min.) in muscles of *B. dussumieri* exposed to sublethal concentrations of fluoride.

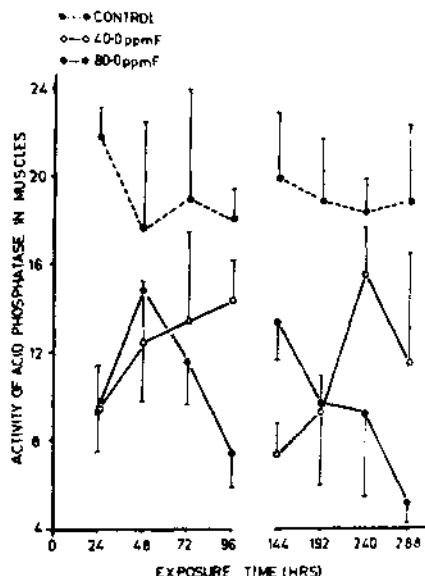
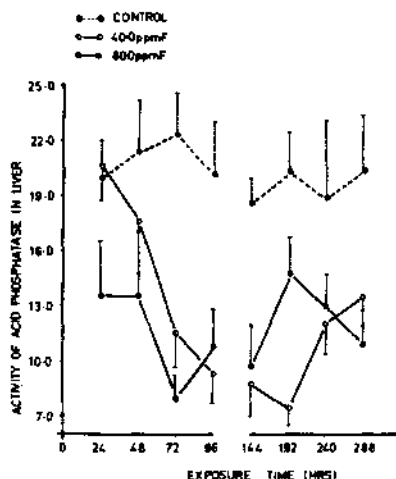


Figure 2

Acid phosphatase activity (μg of p-nitrophenol released/100 mg protein/30 min.) in liver of *B. dussumieri* exposed to sublethal concentrations of fluoride.



the enzyme activity declined from 24 h to 72 h. At 144 h, it was much lower; it gradually rose until 240 h, after which it suddenly fell to 288 h. In fish exposed to 80.0 ppm F, the enzyme activity declined after 24 h; at 96 h it reached its lowest point. Enzyme activity in the muscles of 80.0 ppm F exposed fish never reached the control level (Figure 3).

Liver: Alkaline phosphatase activity in the liver of fish exposed to different concentrations of fluoride for different durations of time was lower than in controls. In fish exposed to 40.0 ppm F, after an initial rise at 24 h, the enzyme activity declined and continued to fall till 72 h, after which an increase was noted. After 240 h, the enzyme activity declined and, at 288 h, it reached its lowest level. In fish exposed to 80.0 ppm F, enzyme activity was at its lowest level at 48 h, it increased only to decline again at 96 h. At 192 h, it reached near control level and then decreased gradually (Figure 4).

Discussion

Abnormal levels of pollutants such as fluoride present in the environment tend to accumulate in the living organism. When the edible mudskipper, *B. dussumieri*, was exposed to various concentrations of fluoride-containing effluent, soft tissues such as liver, muscles and intestine absorbed and stored fairly large amounts of this element (17). Such accumulation would be expected to cause derangement in the cellular metabolic machinery. The decrease in

Figure 3

Alkaline phosphatase activity (μg of p-nitrophenol released/100 mg protein/30 min.) in muscles of *B. dussumieri* exposed to sublethal concentrations of fluoride.

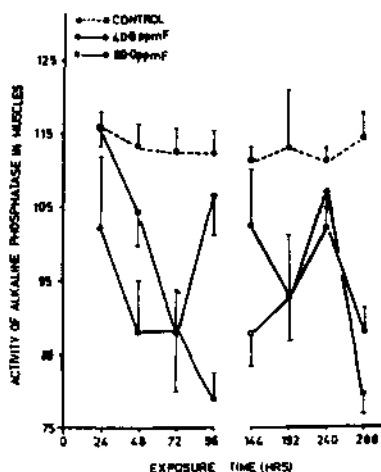
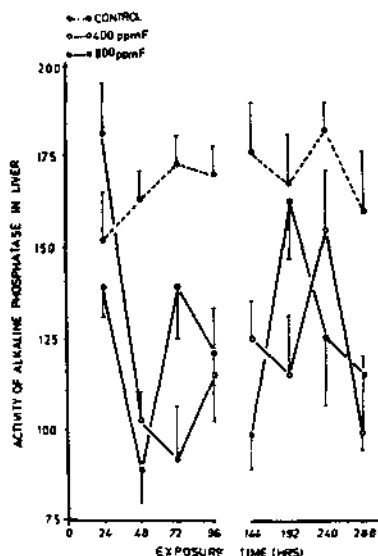


Figure 4

Alkaline phosphatase activity (μg of p-nitrophenol released/100 mg protein/30 min.) in liver of *B. dussumieri* exposed to sublethal concentrations of fluoride.



acid and alkaline phosphatase activities in liver and muscles of the mudskipper exposed to sublethal concentrations of sodium fluoride exhibit one such facet of metabolic disturbance.

Several studies involving pollutant effects on tissue acid and alkaline phosphatase activities have been reported. When *Notopterus notopterus* was exposed to phenol compounds, there was a significant decrease in the acid phosphatase activity in the liver (18). The activity of alkaline phosphatase has been reported to decrease in *Tilapia mossambica* exposed to sublethal concentration of monocrotophos (19). Sharma and Sastry (20) showed that the acid and alkaline phosphatase in the liver of *Channa punctatus* were inhibited when the fish were exposed to sublethal concentrations of endrin for 30 days.

A decrease in Mg-dependent enzymes such as alkaline phosphatase and certain esterases in the serum of rats given water containing 100 ppm F for 50 days has been reported (21). Inhibition of acid phosphatase by fluoride in human saliva and prostate gland was observed by Smith *et al.* (22). Decrease in plasma acid phosphatase was reported in rats when they were given 50 mg fluoride/kg body weight (single oral dose) (23). Acid phosphatase is particularly fluoride sensitive as shown by Belfanti *et al.* (24) and Kustscher (25). Recently Takagi and Shiraki (26) showed that acid phosphatase is among the highly impaired enzymes in the early stages of acute toxic sodium fluoride nephropathy. Fluoride can partly bring about enzyme inhibition by being absorbed on (and thus blocking) the active sites of the enzyme required for formation of enzyme-

substrate complex. The inhibition of the enzyme activity in liver indicates that the transphosphorylation reaction is adversely affected in this organ. Similar inhibition of acid and alkaline phosphatase was reported in kidney of rats which were administered subcutaneously 12 mg fluoride/kg body weight, daily for 12 weeks (27).

On histological examination, the liver of fluoride-treated mudskipper revealed ruptured cell membranes, indistinct cellular content and damaged hepatocyte cords. Large vacuoles appeared and fatty degeneration became extensive; no identifiable remnants of hepatic architecture could be seen (28). The damage of hepatic cells is also a reason for inhibition of normal liver functions, reflected in the low activity level of phosphatases.

Acknowledgements

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CORRECTION: Editorial Book Review: "Fluoridation: The Australian Scene" (Fluoride, 21:51-53, 1988). The following footnote referring to publication of Fluoride in Australia - A Case to Answer, by Wendy Varney, was accidentally omitted in the printing:

- Published by Hale & Iremonger Pty, Ltd., GPO Box 2552, Sydney 2001, NSW, Australia, Nov., 1986, (\$12.95 p/b; \$25.95 h/b, Australian dollars).

EPIDEMIOLOGICAL STUDY OF DENTAL FLUOROSIS IN TRIBALS RESIDING NEAR FLUORSPAR MINES

by

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SUMMARY: A total of 4544 tribals from 24 villages living in the vicinity of mines were examined in a house to house survey. 2637 tribals from downstream villages (along the river into which fluorspar plant effluent is discharged) and 1907 tribals from surrounding villages (within 8 km radius from mines) were studied. Water fluoride level of villages varied from 0.4-5.6 ppm.

The prevalence of dental fluorosis was 35.3% and dental caries was 3.0%. Dental fluorosis showed significant positive association and dental caries showed significant negative association with water fluoride level. River water fluoride level was 17.0 ppm at the point of effluent discharge which gradually declined with increasing distance from that point. Dental fluorosis also showed gradual decline with increasing distance from the point of effluent discharge in downstream villages. The pattern of dental fluorosis, urinary fluoride excretion and radiological changes suggestive of skeletal fluorosis was similar in tribals.

KEY WORDS: Dental caries; Dental fluorosis; Fluorspar mines; Tribals; Urinary fluoride.

Introduction

The study of human fluorosis is more than fifty years old in this country. At present, it has been identified in some 10 states with more than one million people afflicted with skeletal fluorosis; in addition, several millions are exposed to the risk of developing fluorosis at any time. Fluorotic dental changes provide important diagnostic criteria of human fluorosis; it can be easily recognized and studied under even extreme field conditions.

Between 1938 and 1942 Dean observed that the water fluoride level is positively related to dental fluorosis and negatively to dental caries (1) which was further confirmed by several studies in different countries (2).

The symptomatology, clinical manifestations, dental fluorosis, skeletal fluorosis and urinary fluoride excretion are parameters used in diagnosing endemic fluorosis. Although dental fluorosis is easily recognizable and studied it is necessary to determine to what extent it represents a pattern of other manifestations; it can help in identifying regions at risk of developing fluorosis.

Keeping this situation in mind, analysis of information is aimed at studying

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dental fluorosis and caries in tribals in relation to their drinking water fluoride level, and the pattern of dental fluorosis, skeletal fluorosis and urinary fluoride excretion in relation to the water fluoride level.

Materials and Methods

In a house to house survey during 1981-83, 4544 tribals from 24 villages were interrogated and examined. Water samples were collected from all sources from each village. 341 first morning urine samples and 309 x-rays of right forearm were taken from randomly selected individuals. Water and urine fluoride estimation was done with the help of Orion ion specific electrode.

Results and Discussion

2637 tribals from 12 downstream villages (located along the course of the river in which fluorspar plant effluent is discharged) and 1907 tribals from 12 surrounding villages (within 8 km radius from mines) were examined. As shown in Table 1, in surrounding villages, 56.1% of tribals and in downstream villages, 41.3% of tribals had <1 ppm drinking water F level.

Table 1

Distribution of Tribals According to Their Drinking Water Fluoride Level

Location of Village	Drinking Water Fluoride Level (ppm)				Total
	<1	1.1-2.0	2.1-3.0	3.1-4.0	
Surrounding	1070 (56.1)	564 (29.6)	164 (8.6)	109 (5.7)	1907 (100.0)
Down Stream	1090 (41.3)	1207 (45.8)	77 (12.9)	263 (10.0)	2637 (100.0)
Total	2160 (47.5)	1771 (39.0)	241 (5.3)	372 (8.2)	4544 (100.0)

Table 2

Dental Fluorosis in Tribals

Dental Changes	No.	Prevalence
Chalky White (C)	425	9.7
Pitting (P)	34	0.8
Mottling (M)	322	7.4
C + P	182	4.2
C + M	333	7.6
P + M	55	1.3
C + P + M	189	4.3
Total	1540	35.3

Table 3

Dental Changes in Tribals

Dental Changes	No.	Prevalence (%)
Dental Fluorosis	1540	35.3
Dental Caries	144	3.3
Dental Fluorosis & Caries together	52	1.2
Normal Teeth	2652	60.2
Total	4361	100.0

Overall prevalence of dental fluorosis was 35.3%. Chalky white changes were commonest of all followed by mottling and pitting. 49.2% of individuals with fluorotic dental changes had various combinations of changes (Table 2).

Dental changes in tribals were analyzed after excluding individuals without teeth. Results revealed that 60.2% of individuals had normal teeth, 35.3% had dental fluorosis, 3.3% had dental caries while 1.2% had dental fluorosis as well as dental caries (Table 3). Prevalence of both dental fluorosis and caries was significantly higher in the group 6-15 years of age whereas in children below five years of age dental fluorosis was 13.6% and caries was 0.5%. Dental fluorosis was significantly higher and caries was significantly lower in tribal males than in females (Tables 4-5).

Table 4
Dental Changes in Tribals in Relation to Age

Dental Changes	Age Groups in Years			Total
	< 5	6-15	> 16	
Dental Fluorosis	119 (13.6)	679 (51.3)	742 (34.3)	1540 (35.3)
Dental Caries	4 (0.5)	69 (5.2)	71 (3.3)	144 (3.3)
Normal Teeth	751 (85.9)	575 (43.5)	1351 (62.4)	2677 (61.4)
Total	874	1323	2164	4361

Table 5
Dental Changes
in Males and Females

Dental Changes	Male	Female
Dental Fluorosis	801 (37.6)	739 (33.1)
Dental Caries	62 (2.9)	82 (3.7)
Normal	1266 (59.5)	1411 (63.2)
Total	2129	2232

The relation between dental fluorosis and F level in drinking water was significantly positive; that between caries and F level in drinking water significantly negative (Table 6). At less than 1 ppm F in water, 26.7% of tribals had dental fluorosis and 4.9% had dental caries. At more than 2 ppm F in water, 46.6% of tribals had dental fluorosis and 1.4% of tribals had dental caries.

The pattern of dental fluorosis, radiological changes suggestive of fluorosis and mean urinary fluoride level were related to F level in drinking water; the lowest symptom level was at less than 1 ppm; the

highest was at 2.1-3 ppm F. At more than 3 ppm F symptoms were slightly less than the mid-range group overall, but the difference was not statistically significant (Table 7).

Table 6
Dental Changes in Tribals According to Drinking Water F

Dental Changes	Drinking Water F (ppm)			
	< 1	1.1-2.0	2.1-3.0	> 3
Dental Fluorosis	376	679	129	157
	2160	1771	241	372
	(26.7)	(38.3)	(53.5)	(42.2)
Dental Caries	105	30	4	5
	2160	1771	241	372
	(4.9)	(1.7)	(1.7)	(1.3)

Table 7
Observation in Relation to Drinking Water F Level

Drinking Water F (ppm)	Dental Fluorosis (%)	+ VE X-rays (%)	\bar{X} Urinary F Level
< 1	26.7	7.1	3.5
1.1-2	38.3	5.3	3.6
2.1-3	53.5	12.5	5.7
> 3.1	42.2	15.4	4.7

Observations in relation to distance from effluent discharge also showed a similar pattern with the highest level at 2-6 km distance with declining trend up to 14 km distance (Table 8). Some of the disparities observed in relation to the distance from the effluent discharge, when analyzed in detail for dental fluorosis, showed that, although at the closest site river water F level was highest (17.0 ppm), it was not used for drinking purposes. In areas at 20-24 km distant river water was likewise not used. This may be the reason that these two extremes failed to show the same trend.

Table 8
Observations in Relation to Distance from Effluent Discharge

Distance (in km)	Dental Fluorosis	Symptoms (%)	+ VE X-Rays (%)	\bar{X} Urinary F Level
< 2	38.8	28.5	-	3.8
2-6	69.9	76.4	27.9	4.5
6-8	54.8	59.3	15.4	3.8
10-14	13.7	12.5	4.3	2.0
14-20	17.9	16.3	6.9	3.4
20-24	34.7	22.3	10.0	3.3

The results clearly showed that dental fluorosis increased with increasing water F level. Dental fluorosis showed a trend similar to radiological changes and urinary fluoride levels in the study area. Dental fluorosis also followed a pattern of water pollution due to effluent discharge.

Water intake in tribals under study was almost double that of others due to hard work and hot climate. This explains why dental fluorosis as high as 26.7% was recorded at less than 1 ppm water F level.

In the absence of crippling deformity in the present study how far dental fluorosis and radiological changes reveal a public health problem at F levels from 0.4-5.6 ppm is a question. However symptoms of backache and joint pain in as high as 17.0% of tribals indicate suffering due to high fluoride intake.

In this study prevalence of dental fluorosis without grading also showed a trend in relation to water F level; radiological changes and urinary fluoride excretion was similar.

This indicates that a prevalence study without grading will help in identification of a high fluorosis area under similar conditions; even trained para-medical workers can help in identifying an at risk area which can be further investigated in detail. This is important when new regions are still coming up with the problem of endemic fluorosis in this country.

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DENTAL FLUOROSIS IN SCHOOL CHILDREN IN THE VICINITY OF AN ALUMINUM FACTORY IN INDIA

by

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SUMMARY: Dental fluorosis is endemic for the school age children aged 5-16 residing in the vicinity of an aluminum factory at Hirakud, Orissa. Of school children examined, 60.9% of boys and 47.8% of girls showed mild to severe symptoms of dental fluorosis in the form of variously stained and worn-out teeth with wearing enamel. Severe grade of mottling due to ingestion of fluoride-contaminated water (1.0 to 52.5 ppm) and to the fluoride-polluted atmosphere in this industrial area.

KEY WORDS: Aluminum factory; Dental and skeletal fluorosis; Fluoride air and water pollution; India.

Introduction

Ingestion of fluoride-contaminated water gives rise to dental fluorosis as reported from many parts of India (1-4). In the Hirakud area of Orissa, school children are markedly affected by this lesion due to the high fluoride level of their water sources (5). On prolonged drinking of fluoride-contaminated water, they develop toxicity symptoms such as staining, mottling and abrasion of their developing teeth. The degrees of these symptoms are related to the concentration of fluoride in the source of water.

Indian Aluminium Company Limited (INDAL) was established at Hirakud in the western part of Orissa in 1957. It has four lines containing 172 pots and started production in 1959. Although fluoride pollution is endemic in this industrial area, to this day no work has been done to tackle this problem. Necrosis in plants and fluorosis in domestic animals were recorded in 1985 (5). The same year the present study was started with financial assistance from the Department of Environment, Government of India, New Delhi to assess the degrees of dental fluorosis for school children residing in the aluminum factory area at Hirakud, Orissa.

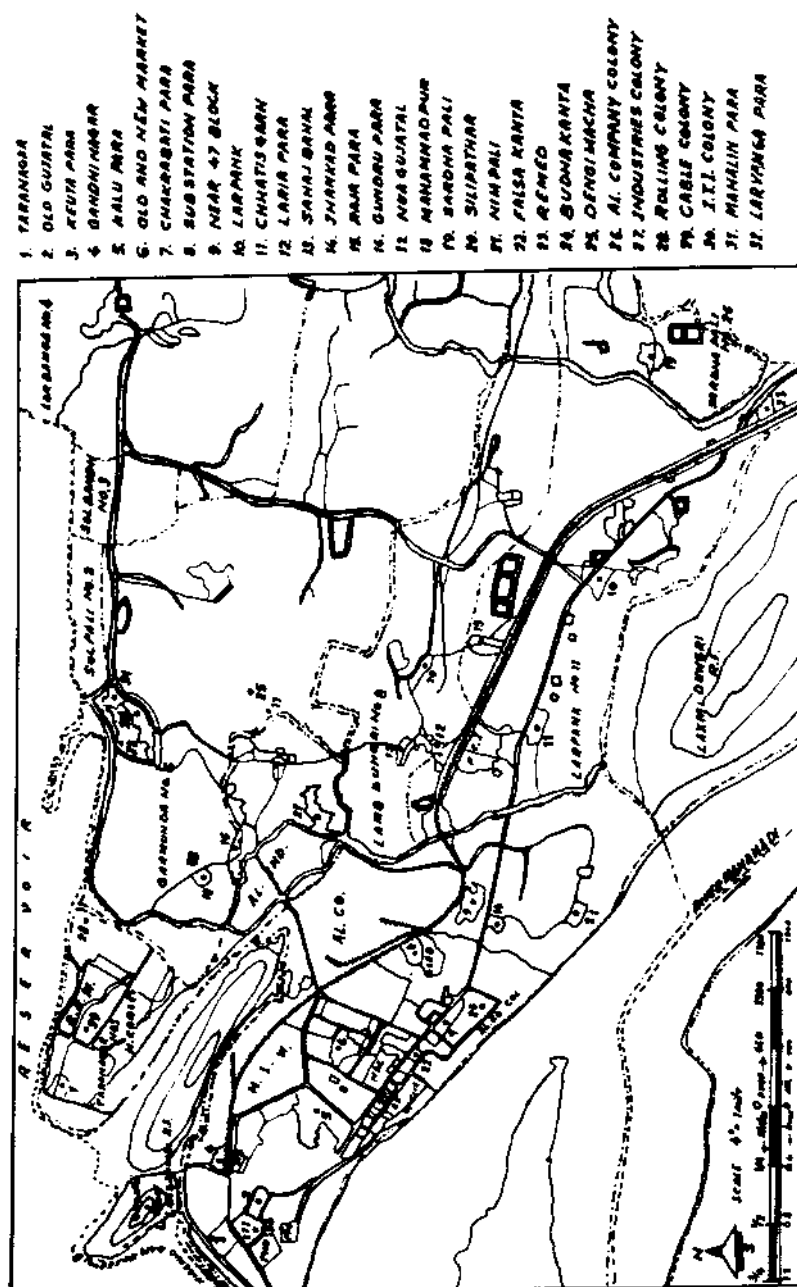
Materials and Methods

The study area, Hirakud, is geographically situated at about 21°31'N latitude, 83°54'E longitude and at about 522 ft. above the mean sea level. The climate is hot and the temperature reaches as high as 45°C in summer. The schools such as Jamada U.P. and M.E. School, Mahammadpur U.G., M.E. and High School, Govt. Girls High School, First Gap U.P. and M.E. School, Hirakud, U.G.M.E. School, Hirakhand U.P. School, Hirakud High School, Nadikhand U.P. School and Govt. M.E. School of Hirakud in Sambalpur district, Orissa were selected for investigation. The children studying in these schools come from surrounding villages in the vicinity of the aluminum factory (Figure 1).

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Figure 1

Showing the Villages of Hirakud Area, Orissa, India



Fluoride

Dental Examination: The children of the schools were carefully examined during class hours for lesions in their teeth. A total of 2044 children of different age groups was studied and extent of their lesion was expressed on a dental score 0-3 as follows: 0. Normal: Teeth appearing normal in shape and size with smooth and glossy white enamel; 1. Mild Effect: Teeth with horizontal yellow lines/yellow glistening patches on the enamel; 2. Moderate Effect: Chalky white teeth mottling with distinct yellowish brown streaks/areas on the surface/at the base; 3. Severe Effect: Cream colored teeth mottling with brown to black areas, wearing enamel and worn-out/chipped-off edges.

Results

The results of dental examination of school children are shown in Table 1 and Figures 3, 4, 5 and 6. Of 1149 boys and 895 girls examined, 60.9% of boys and 47.8% of girls had characteristic markings on their teeth. Various degrees of mottling, staining, wearing and chipped-off edges were involved, sometimes molars and pre-molars appearing cream colored with pitting enamel. Most of the boys and girls of age group 5-7 yrs were suffering from mild to moderate types in the form of horizontal yellow lines or yellow glistening patches and yellowing brown areas on the enamel; in groups 8-10 yrs., 11-13

Table 1

Occurrence of Dental Fluorosis in School Children in the Vicinity of the Aluminum Factory at Hirakud, Orissa (India).

Child sex	Age groups (Yrs.)	No. of children examined	Dental Score				Children with normal teeth		Children with fluorotic teeth	
			0	1	2	3	No.	%	No.	%
BOYS	5-7	137	53	45	22	17	53	38.7	84	61.3
	8-10	416	142	149	44	81	142	34.1	274	65.9
	11-13	420	170	118	57	75	170	40.5	250	59.5
	14-16	176	84	48	26	18	84	47.7	92	52.3
	Total	1149	449	360	149	191	449	39.1	700	60.9
GIRLS	5-7	142	70	35	23	14	70	49.3	72	50.7
	8-10	352	165	110	32	45	165	46.9	187	53.1
	11-13	311	170	86	19	36	170	54.7	141	45.3
	14-16	90	62	14	5	9	62	68.9	28	31.1
	Total	895	467	245	79	104	467	52.2	428	47.8
Grand Total		2044	916	605	228	295	916	44.8	1128	55.2

N.B. Dental Score 0-3

0. Normal: Teeth appearing normal shape and size with smooth and glossy white enamel.
1. Mild Effect: Teeth with horizontal yellow lines/yellow glistening patches on the enamel.
2. Moderate Effect: Chalky-white mottling with distinct yellowish brown streaks/areas on the surface/at the base.
3. Severe Effect: Cream colored mottling with brown to black areas, wearing enamel and worn-out/chipped off edges.

Figure 2

Teeth of school children showing various degrees of dental fluorosis. A. Teeth with normal appearance. B. Teeth with yellow glistening patches on the surface. C. Chalky-white teeth mottling with distinct yellowish brown streaks. D. Cream colored teeth mottling with black areas and chipped-off edges.



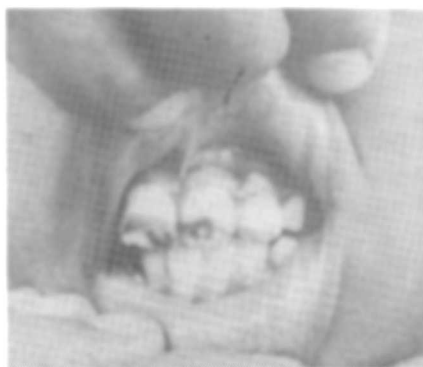
A.



B.



C.



D.

yrs., and 14-16 yrs. teeth were damaged by mottling with brownish streaks, worn-out edges, reduced thickness and length and wearing enamel. The concentration of fluoride in different water sources of the area, i.e., ponds, canals, wells and rivers varied from 1.0 to 52.5 ppm.

Discussion

Dental fluorosis was largely confined to the permanent teeth although in a few cases the deciduous teeth were affected. It is prevalent in children who are born and brought up in an endemic fluorosis area. However, if an individual above 16 years is brought from a non-endemic area, no lesion in the teeth will be observed. He/she may become afflicted with skeletal fluorosis and may also suffer from non-skeletal manifestations (6).

Figure 3

Occurrence of Dental Fluorosis in School Children in the Vicinity of Aluminum Factory at Hirakud, Orissa, India.

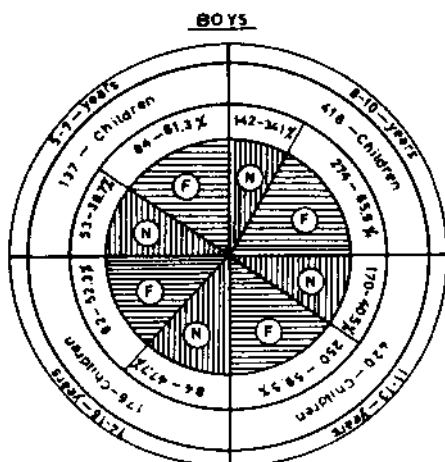


Figure 4

Occurrence of Dental Fluorosis in School Children in the Vicinity of Aluminum Factory at Hirakud, Orissa, India.

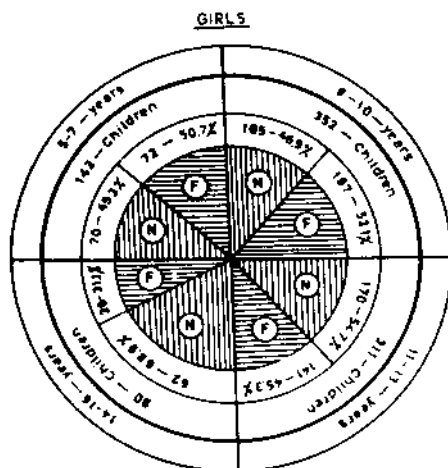


Figure 5

Occurrence of Dental Fluorosis in School Children in the Vicinity of Aluminum Factory at Hirakud, Orissa, India.

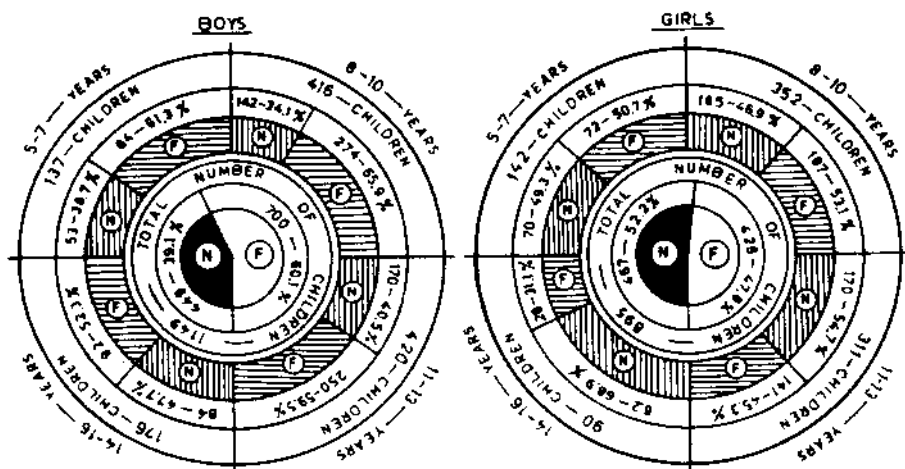
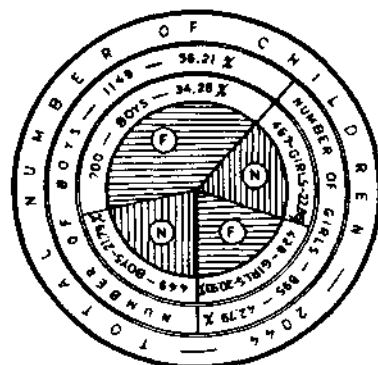


Figure 6

Occurrence of Dental Fluorosis in School Children in the Vicinity of Aluminum Factory at Hirakud, Orissa, India.



If above-normal fluoride ingestion takes place during the formative stage of the teeth, both enamel and dentine are adversely affected. Specific ameloblastic and odontoblastic damage will change with intake of varying levels of fluoride. The matrix laid down by damaged ameloblasts and odontoblasts can not accept minerals as does a normal matrix (7). Probably this damage is due to the effect of fluoride on the major inorganic constituent, the calcium hydroxylapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ of growing teeth. This aspect needs further in depth study. These teeth when fully formed and erupted, have varying degrees of mottling and staining.

Venkateswarlu et al. (8) and Siddiqui (9) found that 0.9 to 1 ppm in drinking water is associated with mottled enamel. In the Hirakud area, children of the villages drink and bathe in canals, ponds and rivers, where the water fluoride content is high (5).

Investigation has shown that the children of factory workers and the children using water from a captive pond in the vicinity of factory and canal flowing to the river, had teeth with cream colored appearance, mottling with brown streaks, wearing enamel, reduced thickness and length and chipped-off edges. Mottling, clearly evident in the age group 8-10, was more pronounced in the age group 10-16.

Conclusion

The authors conclude that since food habits are more or less the same, prolonged ingestion of fluoride-contaminated water is the main cause of occurrence of dental fluorosis in school children in this area.

Acknowledgements

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THE EFFECT OF SODIUM FLUORIDE ON THE GROWTH AND DIFFERENTIATION OF HUMAN FETAL OSTEOBLASTS - AN IN VITRO STUDY

by

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SUMMARY: Human fetal osteoblast (OB) culture has been established as a model of osteofluorosis. Fluoride affects not only the morphology of osteoblasts, but also their metabolism, as shown by ^{45}Ca uptake rate, activity of alkaline phosphatase (ALP) and cell protein. In addition, we also observed that alkaline phosphatase activity showed a biphasic pattern at different cytotoxic doses of fluoride. The preliminary explanation for these findings is discussed.

KEY WORDS: Cell culture; Cell morphology; Fluorosis; Human osteoblasts; Metabolism.

Introduction

Fluoride may be toxic for various organs including bone. In fluorosis, various kinds of lesions, such as osteomalacia, osteopetrosis and osteoporosis, may occur in the bone. Recently reports on the major metabolic changes in fluorosis involving the effect of fluoride on the synthesis and catabolism of protein, nucleic acids and enzymes and on calcium-phosphorous metabolism have appeared (1-3). But the real mechanism of the toxic effect of fluoride on bone metabolism is unclear. In this experiment, human OB culture was used as a model of fluorosis. Based on several indices of bone cell morphology and metabolism, the effect of cytotoxic doses of fluoride on human OB growth and differentiation was observed. The results are discussed in relation to the mechanism of osteofluorosis.

Materials and Methods

Characterization of Osteoblasts in Culture: Bone cells from a five month old male fetus from a therapeutic abortion were prepared from the long bone by a modification of the method reported by Luben (4). The isolated OB were cultured in 10% fetal calf serum (FCS) in 199 medium at 37°C in a humidified atmosphere of 95% air and 5% CO_2 . Pure fibroblast (FB) culture from the lungs of the same fetus was used for comparison (5).

Culture Medium and Reagents: 199 powder culture medium (DIFCO), Crude collagenase type II (SIGMA), Trypsin (DIFCO), Coomassie brilliant blue G-250 (FLUKA), β -glycerophosphate sodium (E. MERCK), α -naphthol phosphate (FLUKA), Fast blue RR (FLUKA), $^{45}\text{CaCl}_2$ (120 mCi/mL Ca^{++} , Beijing Institute of Atomic Energy). The amount of fluoride in fetal calf serum was 0.002 mM and that in 199 medium 0.001 mM.

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Design of Experiment: Both morphologic observation of OB and Biochemical determination on bone cell metabolism were done on each fetal bone cell culture. Adopting a self-contrast experimental design, the culture flasks containing OB were randomly arranged into three groups: 1. Blank control 2. 0.17 mM NaF and 3. 1 mM NaF groups. On day 2, 5, 10, 15 after culture, the three flasks in every group were measured respectively.

Morphologic Observation: 1. Living cells: Cells in culture were viewed under an inverted microscope with phase contrast. 2. Electron microscopic observation: OB culture at tenth day was fixed by 2.5% glutaraldehyde in 0.2 M phosphate buffer after the cell was sedimented by centrifugation at 200 g at room temperature. The OB specimen was embedded in Epon 812 and stained with lead citrate and ultra thin sections were made by LKB ultramicrotome. The specimens were observed with a JEM-100CX electron microscope.

Biochemical Index: 1. After extraction of cells with 0.2 M NaOH at 60°C for 2 hours, the protein content was assayed by the method of Bradford (6). 2. The cellular layers were washed three times with D-Hank's buffer and lysed by addition of 0.5 mL distilled water, followed by three freeze-thaw cycles. ALP activity on the lysates were determined by method of β -glycerophosphate sodium (7). 3. Ten day old OB cultures were stained on the slide according to the ALP couple method as described by Kiernan (8). After which, the OD values were determined on 50 ALP positive OB randomly in each slide, with a scanning microphotometer system (Microscope Photometer OPTON SMP-03, LEITZ). The spectral maximal absorbance was at 568 nm and amplifying was with a 16 objective (9). Data were calculated by HP 9825 micro-computer. The mean OD value of a single OB in each group was compared with the other. 4. Liquid scintillation counting (Model 1210, LKB) was used to determine the treated/control ration of ^{45}Ca incorporated in OB in each culture flask by the modification of Binderman method (10).

Results

Morphologic Change: 1. In the early stage of culture, the shape of OB in each group was similar. In the 5 day culture, in the medium supplemented

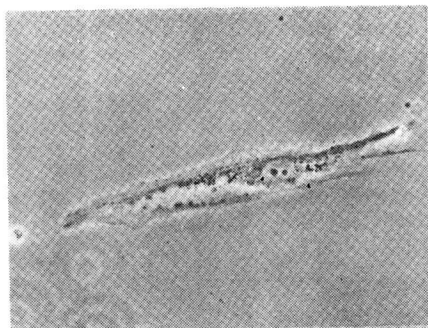
Figure 1

Phase-contrast micrographs of OB in control group. Cell cultured by day 10. x200.



Figure 2

Phase-contrast micrographs of OB in 0.17 mM fluoride group. Cell cultured by day 10. x200.



with fluoride at a concentration of 0.17 mM or 1 mM, the cell size was smaller than that in the control. On day 10, cell size in the control group was increased and cytoplasm seemed to be more abundant and transparent (Figure 1). The cells in medium supplemented with fluoride showed cytotoxic phenomenon. The cells in 0.17 mM fluoride group were a little smaller than OB in controls (Figure 2). In the 1 mM fluoride group, in which cells were fewer, cells were spindle-shaped with dark cytoplasm (Figure 3). In addition, several cells were swollen with foamy cytoplasm and disintegration of the cell body.

2. After 10 days in culture, the OB had abundant cytoplasm, containing a well developed rough endoplasmic reticulum, mitochondria, numerous scattered polyribosomes and secretory vacuoles (Figure 4). Those cultured in a medium with 0.17 mM fluoride also contained numerous well developed endoplasmic reticulum and mitochondria (Figure 5) and a high level of metabolism. Those with 1 mM fluoride exhibited serious changes of intoxication, hydropic degeneration of mitochondria and a break-down of the rough endoplasmic reticulum (Figure 6). Compared with FB, the OB cultures showed the following characteristics: 1. Histochemical staining of ALP in OB cytoplasm was strongly positive,

Figure 3

Phase-contrast micrographs of OB in 1 mM fluoride group. Cell cultured by day 10. x200.

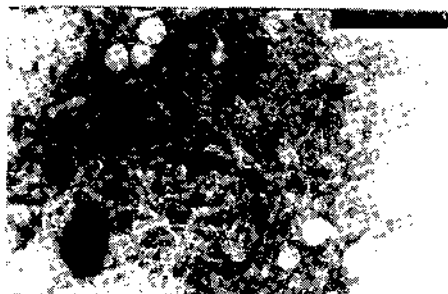


Figure 4

Electron micrographs of OB in control group. x6600.

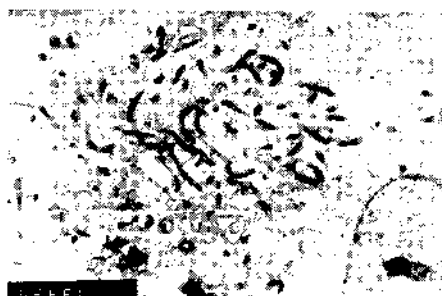


Figure 5

Electron micrographs of OB in 0.17 mM fluoride group. x6600.

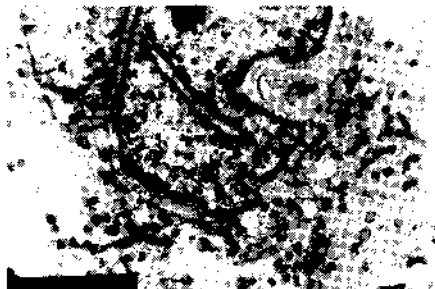


Figure 6

Electron micrographs of OB in 1 mM fluoride group. x6600.



whereas FB showed negative staining. 2. The ^{45}Ca -incorporation rate of OB was 10 times higher than that of FB. 3. The activity of ALP in OB was 10 times higher than that of FB. 4. When cultured for up to 20 days and stained by a modification of Von Kossa method, OB showed positive whereas FB a negative result.

Assay of Biochemical Parameters: 1. Two days after the addition of fluoride to culture, a small peak of increase in protein content was observed in the 0.17 mM fluoride group (Table 1). After 5, an inhibiting effect of fluoride on cell growth was seen in the two groups with fluoride supplement. The cells with 1 mM fluoride concentration were inhibited more strongly than those supplemented with 0.17 mM fluoride, statistically is very significant ($p < 0.01$). On the other hand, the inhibiting effect of fluoride on cell protein content in these two groups differed, the higher the concentration of fluoride, the stronger the inhibition (Figure 7).

2. On day 2 of culture, all cell groups had the same ALP activity. On day 5 ALP increased in all cultures. 0.17 mM fluoride caused a small increase in ALP activity, but a decrease with 1 mM fluoride (Table 2). By day 10,

Table 1
The Effect of Fluoride on OB Protein Content
($\bar{X} \pm \text{SD}$ $\mu\text{g}/\text{per flask}$ $n = 9$)

Group	2	5	Day 10	15
Control	40 \pm 3	61 \pm 4	93 \pm 5	158 \pm 20
0.17 mM NaF	50 \pm 2*	54 \pm 2**	75 \pm 5**	138 \pm 25**
1 mM NaF	39.5 \pm 3 ^a	50 \pm 2** ^b	67 \pm 5** ^a	109 \pm 15** ^b

* $p < 0.005$, ** $p < 0.01$: Comparison between control and experimental groups

^a $p < 0.05$, ^b $p < 0.01$: Comparison between the two experimental groups.

Table 2
The Effect of Fluoride on Alkaline Phosphatase Activity
($\bar{X} \pm \text{SD}$ $\mu/\text{mg pro} \times 10^{-3}$ $n = 9$)

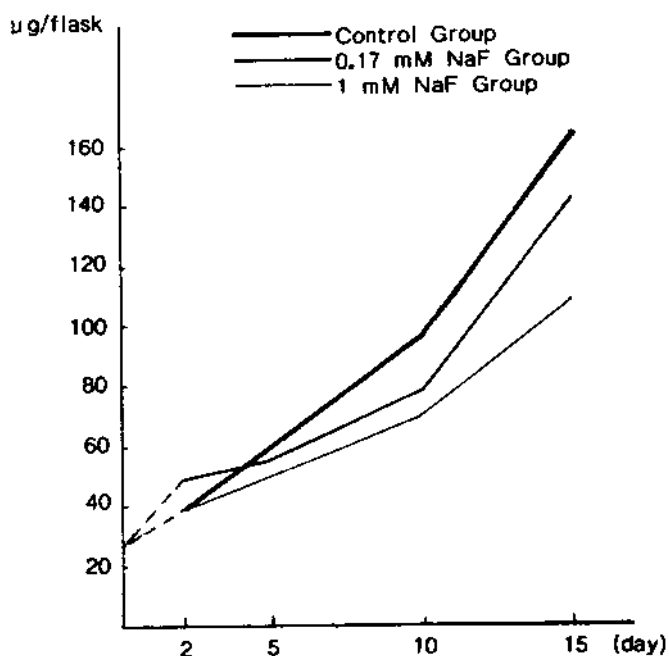
Group	2	5	Day 10	15
Control	111 \pm 10	230 \pm 30	345 \pm 40	258 \pm 30
0.17 mM NaF	115 \pm 8	283 \pm 20**	497 \pm 70**	286 \pm 30*
1 mM NaF	110 \pm 10	210 \pm 20* ^b	265 \pm 30** ^b	221 \pm 50* ^b

* $p < 0.005$, ** $p < 0.01$: Comparison between control and experimental groups

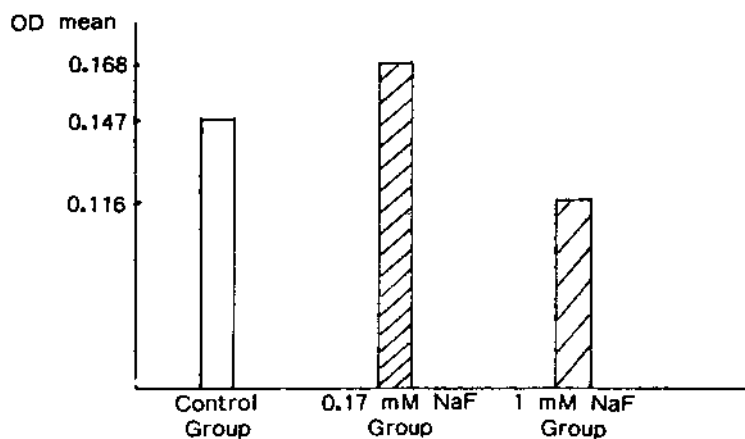
^a $p < 0.05$, ^b $p < 0.01$: Comparison between the two experimental groups.

Figure 7

The Effect of Fluoride on OB Protein Content per Day

Figure 8

ALP Activity of Single OB on the Tenth Day in Each Group.



ALP activity was maximum, a large increase for the culture with 0.17 mM NaF, a large decrease for the 1 mM NaF culture ($p < 0.01$).

3. The same results were observed in single cell histochemical measure at 10 days (Figure 8). The index of cell differentiation as expressed by the percentage of matured cell was increased in the 0.17 mM fluoride (76%). In the control group, it was 53% and in the 1 mM fluoride group, it was only 20% (Table 3).

4. Both fluoride concentrations showed inhibitory effect on the incorporation of ^{45}Ca into OB (Table 4); the higher the concentration of fluoride, the stronger is the inhibitory effect.

Table 3
The Effect of Fluoride on Index of Cellular Differentiation

Item	Groups		
	Control	0.17 mM NaF	1 mM NaF
Total number of cells scanned	150	100	141
Cell numbers above threshold*	80	76	25
Differentiation Index (%)	53	76	20

1. ALP mean value in control group as the threshold, counting the cell number that contains the amount above the threshold.

2. Differentiation index = $\frac{\text{the cell numbers above threshold}}{\text{total number of cells scanned in each group}}$

Table 4
Changes of ^{45}Ca Uptake Rate of OB Culture on Day 10

Groups	^{45}Ca count cpm/flask	Protein content $\mu\text{g}/\text{flask}$	^{45}Ca incorporation cpm/mg protein	Percentage (%)
Control	3280 \pm 400	93 \pm 5	35.5	100
0.17 mM NaF	2220 \pm 150	75 \pm 5	30*	85
1 mM NaF	1665 \pm 400	67 \pm 5	24.2** ^a	68

* $p < 0.005$, ** $p < 0.01$: Comparison between control and experimental groups

^a $p < 0.05$, ^b $p < 0.01$: Comparison between the two experimental groups.

Discussion

The average physiological concentration of fluoride in serum is 7.5 μM to 25 μM . In our experiment, the fluoride concentration in both groups was 7-40 times that of the maximum physiological state. Thus it is considered to be a very toxic substance for the body or for the cell culture.

1. OB in culture showed inhibition of growth at the fluoride concentration of 0.17 mM, but the subcellular organs seemed well-developed and the function of OB which contact 0.17 mM NaF seemed increased. Lough (11) observed active osteoid production of OB in a bone sample taken from a patient with renal failure and fluorosis, he observed no subcellular change. Further experiments should be performed to determine whether this difference is due to the high dosage of fluoride or the conditions of the experiment. Morphologic change of OB affected by 1 mM fluoride concentration was consistent with that reported by Lavrushenko (12) on rats with fluorosis. Fluoride at this concentration had become a cytotoxic factor and showed a suppressed effect on cell metabolism and function.

2. The protein content of a cell may directly reflect its growth state. Taves (13) reported that fluoride concentration at 2 to 50 μ M may promote bone formation in vivo. But some have reported that drinking water containing fluoride above 4 mM for a long time may result in increase in number of OB and amount of bone collagen formation in animal model of chronic fluorosis (14). However, the in vitro study produced different results. Chow (15) proved that the concentration of 0.1 mM fluoride may significantly inhibit protein synthesis in rabbit OB culture. In the present study, we demonstrated the same result in human fetal OB culture and the result correlated well with the morphologic observations. The mechanism of fluoride toxicity is still to be elucidated, the following possibilities may be considered:

1. Fluoride may inhibit amino acid uptake by cells and reduce protein synthesis (16).
2. Fluoride may affect the action of Na-K-ATPase in the cell membrane, which may influence the transport of amino acid into the cell (17).
3. Fluoride may directly impair the peptide chain initiation (18).
4. Fluoride may inhibit the key enzyme of glycolytic pathway and thus reduce energy metabolism and protein synthesis (19).

3. ALP mainly exists in bone tissue and is produced by OB. The amount of ALP in bones is 5-40 times higher than that in non-bone tissue (20). ALP is a good marker for the function state and differentiation of osteoblasts (21). Farley et al. (22) observed the increase in skeletal specific ALP activity in a patient with osteoporosis as they investigated the effect of a therapeutic dose of NaF. A similar result was observed on the cultured OB at the non-toxic concentration of NaF (26).

On the other hand, Miyazaki (23) observed marked inhibitory effect of a toxic concentration of fluoride (0.5-5 mM) on OB culture from a chicken embryo. In our experiment, we observed that both toxic concentrations of fluoride showed different effects on OB function as assessed by ALP activity. The results corresponded with that from the ultrastructural study. These findings may explain the varied manifestation of bone lesions in fluorosis. But the above exact mechanism of fluoride on enzyme is still obscure. The decline of ALP activity in the 15 day culture of each group may be related to the contact inhibition of cells in the pre-calcification state. Using a scanning microspectrophotometer, we were able to measure the ALP activity in a single cell which permitted us to count the differentiated OB. This result suggests that fluoride may directly affect the maturation and differentiation of OB in vitro.

4. Matthews (24) observed that radiocalcium may accumulate in the position where the bone cell growth and calcification would occur and suggested that the osteocyte might be the mineralizing system by combining or collecting calcium and also the earliest location of formation of the calcium crystallized nucleus. Rolle (25) presented evidence that the accumulation of calcium in the osteocytes, osteoblasts and chondrocytes was directly related to the state of extracellular calcification. The above finding was also supported by the in vitro experiment, according to which, the OB showed a very high calcium uptake rate and a very good ability for calcification (10).

Farley (26) demonstrated that a physiological concentration of fluoride at 5-25 μM may enhance calcium uptake by OB culture and accelerate deposition of calcium in bone. Taves (27) demonstrated that fluoride at 7.5 μM may promote calcification in vitro. It is known that fluoride at high concentration is a non-biological factor inhibiting mineralization in the body and administration of a large dose of fluoride for a long time may retard or inhibit the biophysiological process of calcification. Moreover, both Eisenman (28) and Miyazaki (23) observed similar results in in vivo and in vitro experiments respectively.

In the present experiment, it was demonstrated that a high concentration of fluoride may inhibit uptake of calcium by human fetal OB culture; this effect was increased in direct proportion to the fluoride concentration. It has been suggested that intracellular fluoride can irreversibly block most of the calcium pathways in the cell membrane and result in a production of calcium influx (29).

5. Both fluoride concentrations had a direct inhibitory effect on cell growth, but the effect of fluoride on cell function showed a biphasic character. These findings suggest that fluoride may affect OB growth and enzymatic activities in different ways. There may be no synchronous correlation between the growth and ALP activity of osteoblast. To estimate the cytotoxic effect of fluoride on mouse fibroblast culture, Holland (30) suggested that at the same concentration of fluoride, the synthesis of structural protein was first inhibited then stopped cell growth. The NaF concentration necessary for inhibiting functional expression was much higher than that for inhibiting protein synthesis. A similar result was observed in human fetal OB culture in our experiment.

Conclusion

Cultured human fetal osteoblasts were used as a model of osteofluorosis. Different concentrations of fluoride were tested for their effect on growth and function of OB to explore the mechanism of osteofluorosis. The conclusions were as follows:

1. The fluoride ion may affect OB in multiple ways, including the structure, growth, functional state and the ^{45}Ca uptake rate.
2. Inhibition of growth and function of OB by fluoride may occur at the same level; 0.17 mM NaF may inhibit OB growth while 1 mM NaF was necessary to inhibit the ALP activity of OB.
3. The cytotoxic dose of fluoride may express biphasic action on ALP activity; 0.17 mM of NaF promotes ALP activity of OB while 1 mM NaF may inhibit it.

4. Cytotoxic dose of fluoride directly affects the differentiation of OB; a small dose (0.17 mM) may promote the maturation of OB whereas a large dose of NaF (1 mM) usually inhibits it.
5. Both toxic doses of NaF inhibit the ^{45}Ca uptake rate and the degree of inhibition is directly proportional to the dosage of NaF.

Acknowledgement

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PATTERNS OF DENTAL FLUOROSIS IN A EUROPEAN COUNTRY
IN RELATION TO THE FLUORIDE CONCENTRATION
OF DRINKING WATER

by

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(Abstracted from J. Dent. Res. 66:10-12, 1987)

Children (456) 14-16 yrs. old residing continuously since birth in the same areas were divided into four groups, according to the fluoride level in their drinking water: 1) less than or equal to 0.1 ppm; 2) 0.3-0.5 ppm; 3) 0.5-1.25 ppm; and 4) 1.26-2.0 ppm. Fluoride concentration in drinking water influenced the occurrence of dental fluorosis by resulting in a steeper profile of the prevalence from lower incisors to second molars rather than increasing prevalence in all teeth.

Fluorosis increased in relation to the increase in fluoride content of water for all tooth types except lower incisors. Fluorosis was greatest in those teeth that were mineralized later in childhood. Recent research, using fluoride tablets, indicates that levels of fluorosis are related to fluoride intake during the time the individual tooth is being formed. The later in childhood the tooth is formed, the greater are the actual prevalence and degree of severity of dental fluorosis.

There can be little doubt that exposure to sources of fluoride, such as fluoride toothpaste and other prophylactic fluoride measures, namely those operated by the school dental service, increases greatly from birth to age of 10 years and at a rate that increases the daily intake of fluoride per kg body weight.

Another possible explanation relates to findings showing that the effects of fluoride on the mineralized tissues are closely related to calcium metabolism. Ample supplies of calcium and phosphate and the rapidly growing skeleton in baby rats may result in increased protection against ingestion of elevated amounts of fluoride. A similar mechanism may operate during tooth formation in human infancy.

The fluoride concentration in plasma increases with age and with the fluoride concentration in drinking water. Therefore, even disregarding the effects of changes in calcium phosphate metabolism, exposure of the forming enamel to fluoride may increase over the years.

Accordingly, the hypothesis is advanced that the amount of fluoride ingested daily in relation to the mineralization of the individual tooth controls the occurrence of dental fluorosis, presumably modified by the general calcium-phosphate status.

At present, the use of preventive fluoride measures is increasing, particularly in early childhood, and the fluoride concentration in dentifrices is under reconsideration. Possible biological effects of such preventive measures cannot be completely evaluated until after 10-15 years, when incisors, pre-

molars, and molars formed under this regime have erupted.

KEY WORDS: Dental fluorosis; Fluoride dentifrices; Waterborne fluoride.

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**DENTAL CARIES AND STAINING AFTER 28 MONTHS OF RINSING
WITH STANNOUS FLUORIDE OR SODIUM FLUORIDE**

by

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(Abstracted from J. Dent. Res. 65:424-427, 1986)

The aim of this study was to compare the effects of daily mouth-rinsing with aqueous solutions of 0.05% NaF or 0.1% SnF₂ on dental caries and tooth staining. A total of 437 children, aged from 12 to 15 years at baseline, were residing in a non-fluoridated community. One group rinsed daily under supervision for 28 months with the NaF solution, the other with the SnF₂ solution. The SnF₂ group exhibited four to five times as much extrinsic stain as did the NaF group. There were no statistically significant differences between the two groups in terms of total DMFS. However, the increment of pit and fissure caries was 0.9 surfaces fewer for the SnF₂ group ($p = 0.04$), whereas the increment of smooth surface caries was 0.6 surfaces fewer for the NaF group ($p = 0.04$). These data suggest that there may be a difference in mechanism of action between SnF₂ and NaF rinses.

Over the 28-month period of the study, 169 out of 437 (38.7%) subjects were lost. Of the 268 subjects remaining at the end of the study, about two-thirds of each group were present and actually rinsed at 75% or more of the opportunities. The qualitative stain scores for the SnF₂ group increased by four to five times over the baseline scores.

The SnF₂ group had a significantly lower DMFS after 28 months. The same phenomenon was demonstrated for occlusal surfaces, with the difference again favoring the SnF₂ group. There was also a statistically significant difference for approximal surfaces, although in this case the DMFS was lower in the NaF group. SnF₂ mouthrinse inhibited caries on pit and fissure surfaces significantly better than NaF mouthrinse, whereas the latter inhibited caries on smooth surfaces significantly better.

KEY WORDS: Dental caries; Mouth rinses; Sodium fluoride; Stannous fluoride; Enamel staining.

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GENOTOXIC EFFECTS OF FLUORIDE: A CONTROVERSIAL ISSUE

by

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(Abstracted from Mutation Research, 195:127-136, 1988)

Increasing amounts of fluoride in the water-food-chain of the American population have raised further questions about the assumed safety of such exposure levels (1 ppm in fluoridated drinking water and up to 12,300 ppm in certain dental products). In particular, there is special concern about potential genotoxic effects of fluoride. Numerous investigations have been directed at this problem, but the results to date have been conflicting and contradictory. This article reviews most of these studies and notes differences in the forms and concentrations of fluoride used, exposure periods, types of systems studied, and nature of the effects observed. Although there is strong evidence that fluoride can exert genotoxic effects, the threshold doses and the conditions under which such effects occur need to be confirmed and ultimately related to human exposure levels.

KEY WORDS: Chromosome damage; Fluoride; Genotoxic effects.

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IS FLUOROSIS AN ETIOLOGICAL FACTOR IN OVERUSE INJURIES (RSI)?

by

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(Abstracted from Medical Hypotheses 21:369-371, 1986)

Painful and crippling conditions, mainly of the fingers and arms, associated with overuse in performing repetitive movements, are termed repetitive strain injury (RSI) in Australia and New Zealand. They are usually thought to be caused by ergonomic factors namely incorrect working methods and postures.

However, actions similar to those now associated with RSI have been performed for many years with similar faulty postures but with few complaints, which suggests that a new factor has arisen during the last few years which has made some people much more susceptible to the development of RSI. One such factor is the recent marked increase in the fluoride content of the environment. Habitually drinking artificially fluoridated water greatly increases the amount of fluoride deposited in the bones ($p < 0.001$) causing

Fluoride

"fluorosis" which is due to a high level of bone fluoride. Deposition occurs especially in much-exercised bones with a high metabolic rate.

Fluoride is deposited in bone mainly around the canaliculae and the osteocyte lacunae; when the osteocytes resorb this high-fluoride bone, sufficient fluoride is released to poison the osteocytes.

Symptoms of fluorosis: aches and stiffness in muscles/bones (in arms, shoulders, neck, legs, jaws and lower back), sometimes accompanied by muscular weakness, muscle spasms or tingling sensations in fingers and feet are similar to those of RSI. The recent increase in the fluoride content of the environment suggests that RSI might be due partly to excessive fluoride absorption.

An abnormally high fluoride level in bone affects the resorbing osteocytes, disrupting the remodelling process; it leads to reduced functional efficiency, discomfort and pain, which are features of fluorosis.

A 1985 pilot study in New Zealand to test this hypothesis dealt with young Melbourne women who had drunk fluoridated water for seven years - all were employed in office work. In 12 subjects with RSI (mean age 27 years) the mean fluoride concentration in alveolar bone was 2737 ppm - significantly ($p < 0.001$) greater than the mean of 1687 ppm in the alveolar bone of 12 similar women (mean age 26 years) without RSI. The annual increase in fluoride concentration was 103 ppm in those with RSI but only 50 ppm in controls.

KEY WORDS: Alveolar bone; Fluoride accumulation; Repetitive strain injury (RSI).

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Editor's Note: See also editorial by Dr. Sutton, 20:151-153, 1987, entitled "Toxic Effects of Fluoride Released by Bone Resorption."

INSTRUCTIONS TO AUTHORS

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

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