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TABLE OF CONTENTS

EDITORIAL

- Urinary Fluoride: Measure of Toxicity? 207-209

ORIGINAL ARTICLES

- Stimulation of Adenylate Cyclase Activity by $\text{Na}_2\text{PO}_3\text{F}$ and NaF in Intact Rat Hepatocytes and Plasma Membrane Fractions—by A.R. Shahed and D.W. Allmann, Indianapolis, Indiana 210-217
- Effect of F^- on Major Salivary Glands, the Amylase Activity, Stimulated Salivary Flow Response and cAMP Levels in Parotid Gland by Rats Consuming F^- Via Drinking Water — by I. Boros, Gy. Mozsik, and P. Keszler, Budapest, Hungary 217-223
- Effect of F^- Effluent on Some Metabolites and Minerals in Fry of *Catla catla* (Hamilton) — by K. S. Pillai, and U. H. Mane, Aurangabad City, India 224-233
- Disfiguring Dental Fluorosis in Auckland, New Zealand — by John Colquhoun, Auckland, New Zealand 234-242
- Urinary Fluoride Excretion in Skeletal Fluorosis — by D. Raja Reddy, N.V. Rammohan Rao, J.M.K. Murthy, and N. Geetanjali, Hyderabad, India 243-246
- Studies of the Relative Fluoride Content of Normal and Pathologically Mineralized Human Tissues — by S.M. Mohamedally, London, England 246-251
- Impact of Fluoride Emissions on Chlorophyll Content of Shrubs in Vicinity of a Surat Fluorine Industry — by K.S. Pillai, Surat, India 252-258
- Report on the Second Fluorine Symposium in Szczecin — by Z. Machoy, Szczecin, Poland 259-260

ABSTRACTS

- Sodium Fluoride-Induced Morphological and Neoplastic Transformation, Chromosome Aberrations, Sister Chromatid Exchanges and Unscheduled DNA Synthesis in Cultured Syrian Hamster Embryo Cells — by Takeki Tsutsui, Nobuko Suzuki, and Manabu Ohmori, Tokyo, Japan 261-262

Acid Reactivity of Carbonated Apatites with Strontium and Fluoride Substitutions — by J.D.B. Featherstone, C.P. Shields, K. Khademazad, and M.D. Oldershaw, Rochester, N.Y.	263-264
Pharmacokinetics of Fluoride Gels in Children and Adults — by J. Ekstrand, G. Koch, L.E. Lindgren, and L.G. Peterson, Stockholm, Sweden	264-265
Fluoride Pharmacokinetics During Acid-Base Balance Changes in Man — by J. Ekstrand, M. Ehrengren, G.M. Whitford and P.O. Jarnberg, Stockholm, Sweden	266
Fluoride in Waters, Soils and Vegetation in Western Buenos Aires Province — by R.S. Lavado, N.B. Reinaudi, and J.A. Vaquero, LaPlata, Argentina	266-267
Mud-bath Therapy of a Patient with Osteoarthritis Deformans Exposed to Fluorine and Its Compounds — by L.A. Kozlava and L.G. Zhukova, Sverdlovsk, USSR	267
AUTHOR INDEX	268-269
SUBJECT INDEX	270-274

The Fourteenth Conference of the International Society for Fluoride Research will be held in Morioka, Japan, June 12-15, 1985, at the Iwate Medical Association Hall. The program Committee is now soliciting abstracts (up to 300 words) of papers dealing with any aspect of fluoride research for presentation at the conference. Abstracts should be written in English in the format shown below and sent by the end of February, 1985 to: Professor Humio Tsunoda, Dept of Hygiene and Public Health, School of Medicine, Iwate Medical University, Morioka 020 Japan.

Note: Morioka, a city of 250,000 and the historical capital of Iwate Prefecture, is located about 500 km (300 mi) north of Tokyo on Honshu, the main island of Japan. Bullet train service from Tokyo to Morioka is scheduled to begin in March, 1985. Domestic airline service from Tokyo is also available.

ABSTRACT — Please be brief — 300 words maximum if possible. Title of paper should be ALL CAPS; author(s) listed by first name, middle initial, last name; indicate full address w/zip code. Single space, black carbon ribbon.

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

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URINARY FLUORIDE: MEASURE OF TOXICITY?

The question whether urinary fluoride is correlated with fluoride intake and whether the level of urinary fluoride can be equated with fluoride-induced illness is a matter of importance not only to scientists engaged in fluoride research, but also to government agencies responsible for establishing standards, as well as to attorneys involved in fluoride connected litigation, and most especially to clinicians confronted with the diagnosis of fluoride intoxication. Some authors (1) have proposed that the degree of intoxication can be judged on the basis of the level of urinary fluoride. However, more and more evidence is accumulating which points to the fallacy of such a theory.

In general it is true that urinary fluoride rises in relation to fluoride intake. Whether the level of urinary fluoride parallels adverse effects of fluoride has been examined extensively by Waldbott (this Journal Oct. 81, p. 148; July 82, p. 109) who pinpointed the fallacies in extensive surveys on fertilizer and aluminum factory workers (2,3) which purport to "prove" that low urinary fluoride signifies lack of ill effect.

Many factors account for wide individual differences among workers exposed to atmospheric fluoride, some of which follow:

1. Growing children retain more fluoride in their bones than adults and therefore excrete less.
2. Age, the extent of kidney and liver function, and gastric acidity alter the pattern of fluoride excretion, i.e. patient's whose kidneys malfunction retain more fluoride (4).
3. During pregnancy, urinary fluoride is reduced (5).
4. Simultaneous intake of substances which bind fluoride, such as calcium, aluminum, and other metals, causes increased fluoride elimination through the gastrointestinal tract and therefore decreases urinary excretion (6).
5. Persons previously exposed to high fluoride intake, on a low fluoride regime prior to sampling, eliminate more fluoride than they take into their bodies (7,8).
6. From day to day and from hour to hour the variability of consumption of fluoride in food and water and the extent of activity of a fluoride-emitting factory at the time of sampling, make spot sampling less reliable than 24-hour urine. The specific gravity (9) and acidity (pH) (10) of urine and possible contact of urine with metal, glass, or enamel will have to be considered.

In statistical studies, individuals who are suffering ill effects may fail to be identified. To establish the diagnosis careful clinical observations over prolonged periods of individuals both at the top and at the bottom of the statistical scale are necessary.

In the July 1984 issue of this Journal, Chandra and Thergaonkar reported new observations on the relationship of fluoride in drinking water to

fluoride in urine and the incidence of mottled teeth. They found a significantly positive relationship between the fluoride level in drinking water and urinary fluoride whereas that between the calcium content of drinking water and urinary fluoride was significantly negative. Comparison of urinary fluoride with drinking water fluoride and severity of dental fluorosis revealed that up to 4 ppm F⁻ in drinking water, urinary F⁻ excretion was higher than drinking water F⁻. As the level of F⁻ in drinking water increased, urinary F⁻ values decreased. The lowest urinary F⁻ value, 0.8 ppm, was detected in a child with very mild dental fluorosis whose drinking water contained 1.42 ppm F⁻. On the other hand, a child manifesting severe dental fluorosis excreted 30.4 ppm F⁻ in urine while consuming 4 ppm F⁻ in water. From 9 years of age upwards, mean F⁻ excretion through urine was always higher than mean drinking water F⁻. Increasing severity of dental fluorosis was directly related to increase in urinary F⁻ excretion (117% to 150% among fluorotics).

Reddy et al., in this issue (pages 224-227), measured urinary fluoride levels in 24-hour samples over a period of 7-38 days from 16 cases of established skeletal fluorosis. The level of fluoride in the drinking water ranged between 3.8 and 11.4 ppm. Minimum urinary fluoride ranged between 0.5 and 4.48 ppm, maximum between 1.5 and 13 ppm. No correlation was observed between urinary fluoride, clinical stage of fluorosis, or neurological sequelae. Reddy stresses the importance of 24-hour sampling over a prolonged period because urinary fluoride varies widely from day to day in the same individual. He concludes that high urinary fluoride is consistent with fluorosis. Low urinary excretion, however, does not necessarily exclude fluoride-induced illness.

The foregoing studies amplify and confirm Waldbott's repeated findings, namely that fluoride-induced injury cannot be reliably determined on the basis of the level of urinary fluoride and that spot samples or single 24-hour samples of urine can be very misleading. In a survey in his own practice of a selected group of allergic patients, 24-hr. urinary fluoride output was highly inconsistent, both in pre-1967 low-fluoride (0.1 ppm) Detroit and in fluoridated (1 ppm) cities. Twenty-four hour fluoride excretion ranged up to 5.6 mg. The majority were between 0.0 and 1.0 mg. Following a test dose (6.8 mg NaF), given orally, one patient eliminated only 3.6% of it in the 24-hour urine, another 99.5% (11). Some of his patients with severe symptoms eliminated little fluoride in urine whereas others, with high urinary excretion, were symptom-free (12). He explains: "Low urinary F⁻ levels signify that either little F⁻ is taken into the system, that F⁻ is being retained in the system, or that larger than usual amounts are being excreted through the bowels, sweat or saliva" (12).

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STIMULATION OF ADENYLATE CYCLASE ACTIVITY BY $\text{Na}_2\text{PO}_3\text{F}$ AND NaF
IN INTACT RAT HEPATOCYTES AND PLASMA MEMBRANE FRACTIONS

by

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SUMMARY: The effect of $\text{Na}_2\text{PO}_3\text{F}$ (MFP) on cAMP production and adenylate cyclase activity in rat hepatocytes was investigated. MFP (1-10 mM) increased cAMP accumulation in hepatocytes isolated from both fed and fasted rats. MFP increased adenylate cyclase activity (a) in plasma membrane fractions and (b) in intact hepatocytes under conditions where hydrolysis of MFP was negligible. It is proposed that MFP, as a complex, stimulates adenylate cyclase activity and thus increases cAMP accumulation in isolated hepatocytes.

KEY WORDS: NaF ; $\text{Na}_2\text{PO}_3\text{F}$; cAMP; Adenylate cyclase; Hepatocytes

Introduction

In our metabolic studies with liver cells, both NaF and MFP enhanced glycogenolysis, inhibited glycolysis and altered the activity of several enzymes involved in these pathways (1-3). Furthermore, both NaF and MFP increased gluconeogenesis from lactate and dihydroxyacetone (1). Since NaF has been shown to increase cAMP accumulation in intact hepatocytes (4,5), it was proposed that NaF increased glycogenolysis and decreased glycolysis in a cAMP-dependent manner.

MFP is a compound in which F is covalently bound to phosphorus and is not readily hydrolyzed (see the Discussion section for a description of MFP hydrolysis). MFP, like other fluorine containing compounds, e.g. Na_2SiF_6 , SnF_2 , and NaF etc., is present in concentrations ranging from 0.05 to 650 mM in many products commonly used in dental care. Recent work (6-8) has shown that up to 50% of the topically applied fluoride can be ingested. Due to these facts and our continued interest in metabolic effects of fluorine-containing compounds and our above-mentioned parallel observations with NaF and MFP on carbohydrate metabolism, the present investigation was undertaken.

The current report describes the effect of MFP on cAMP accumulation, adenylate cyclase activity, and phosphodiesterase activity in isolated rat hepatocytes.

Materials and Methods

Animals and diet. Male Wistar rats (150-174 g) were maintained on a fluoride deficient diet [IU 500 (10)] and distilled water for at least

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a week before being used for the isolation of hepatocytes or plasma membrane preparations.

Isolation of plasma membrane fractions. Plasma membrane fractions were obtained either from whole liver or from isolated rat hepatocytes that had been pretreated with NaF or MFP. When whole liver was the starting material, the tissue was homogenized in 1 mM NaHCO₃ containing 0.5 mM CaCl₂ at pH 7.5 and the plasma membrane fraction was isolated according to Neville (11). When plasma membrane preparations were obtained from pretreated rat hepatocytes, the hepatocytes were isolated using a standard collagenase method and preincubated with NaF or MFP for 10 min as described previously (4). The incubated cell suspension was centrifuged for 1 min at 100 x g and the cell pellet was homogenized in 1 mM NaHCO₃ containing 0.5 mM CaCl₂ at pH 7.5 followed by centrifugation for 10 min at 1500 x g. The pellet was washed twice in the same buffer by resuspending. The plasma membrane fractions were stored in liquid N₂ until used for assaying adenylate cyclase activity. Plasma membrane fractions obtained from hepatocytes preincubated with NaF or MFP contained negligible amounts of the fluoride which was added during the preincubation.

Assay of adenylate cyclase activity. Adenylate cyclase activity was determined by measuring the amount of cAMP produced as described by Johnson et al. (12). The reaction was initiated by the addition of a plasma membrane fraction (0.2-0.4 mg protein) and terminated 10 min later by the addition of 0.2 ml of 0.3 M HClO₄ containing ³H-cAMP (5000 - 10,000 cpm). The mixture was boiled for 5 min. The samples were centrifuged and cAMP was determined using the protein kinase binding assay as described previously (4).

Phosphodiesterase activity. Phosphodiesterase activity was assayed in a 30,000 x g (30 min) supernatant of a rat hepatocyte homogenate in a two step reaction according to Thompson and Appleman (13).

Hydrolysis of MFP. Hepatocyte (50-100 mg) suspensions were incubated in the presence of various concentrations of MFP for 10 min in 2 ml of Krebs-Henseliet buffer pH 7.4 gassed with 95% O₂-5% CO₂, containing 2.5% bovine serum albumin and 1 mM theophylline in a shaking water bath at 37° C, centrifuged (1 min at 100 x g) and analyzed for free fluoride in the supernatant using a fluoride specific electrode. The purified sample of MFP (Ozark-Mahonning Co., Tulsa, OK) used in these experiments contained less than 4.5% free fluoride.

Results

Hydrolysis of MFP. Data shown in Table 1 clearly indicate that only 2-6% of the added MFP was hydrolyzed to free fluoride by hepatocytes in 10 min under the experimental conditions employed. MFP hydrolysis was slow and time dependent (data not shown). The percent hydrolysis of MFP by plasma membrane fractions was insignificant (data not shown).

Effect of MFP on cAMP accumulation in rat hepatocytes. The data presented in Table 2 illustrate that MFP significantly increased cAMP levels

Table 1
Hydrolysis of MFP in Isolated Hepatocytes

MFP Added (μ M)	Conc. of F^- in the medium (M) (- Hepatocytes)	Conc. of F^- in the medium (M) (+ Hepatocytes)	Net Hydrolysis %
0	0.5	0.5	-
100	4.0	10.1	5.6
500	17.5	50.5	6.5
1,000	37.0	83.5	4.6
5,000	200.0	33.5	2.7
10,000	430.0	610.0	1.8
Conc. of free F^- Conc. of free F^- free (F)			
Net Free(F) = in medium plus - in medium minus - in the			
hepatocytes hepatocytes buffer			

Results are representative of several such experiments. MFP samples used for this experiment contained less than 4.5% free F^- . Hepatocytes were incubated for 10 min with indicated MFP concentrations in Krebs-Henseleit buffer, pH 7.4, 2.5% bovine serum albumin and 1 mM theophylline previously gassed with 5% CO_2 -95% O_2 .

Table 2
Effect of MFP on cAMP Formation in Hepatocytes
From Fed and Fasted Rats

MFP (mM)	pmoles cAMP/g wet wt./10 min.	
	Fed	24-Hr. Fasted
0.0	390 \pm 30	1200 \pm 180
1.0	1140 \pm 170*	1890 \pm 350
2.5	1340 \pm 225*	2510 \pm 200*
5.0	1110 \pm 140*	2940 \pm 210*
10.2	2140 \pm 680*	3250 \pm 280*

*p < 0.05

Results expressed as means \pm S.E.M. of 5-6 different hepatocyte preparations. Hepatocytes were incubated for 10 min. with MFP in Krebs-Henseleit buffer, pH 7.4, 2.5% bovine serum albumin, and 1 mM theophylline.

in hepatocytes isolated from either fed or fasted rats. Approximately a 3-fold increase in cAMP was observed when 1 mM MFP was present. Based on the data in Table 1, when 1 mM MFP was present in the incubation system the free fluoride concentration was about 83 μ M. As previously shown (4) a concentration of 5 mM NaF was required to induce a similar 3-fold increase in hepatocyte cAMP accumulation. Thus the data in Table 2 cannot be easily explained by the hydrolysis of MFP to free fluoride ions. Therefore it appears that (a) cAMP accumulation is not the result of hydrolysis of MFP to free fluoride, and (b) that MFP appears to be more effective than NaF in increasing hepatocyte cAMP levels.

Effect of NaF and MFP on adenylate cyclase activity in a crude plasma membrane preparation. In view of our observations described above, it was of interest to explore whether MFP, like NaF, would also stimulate adenylate cyclase activity in a plasma membrane preparation. The results presented in Table 3 show that MFP like NaF significantly stimulated adenylate cyclase activity in a crude plasma membrane fraction. The increase in adenylate cyclase activity was in a concentration-dependent manner. A partially purified plasma membrane fraction from whole liver gave a similar stimulation of adenylate cyclase activity in the presence of NaF, MFP and glucagon (data not shown). Since both phosphate and pyrophosphate could be present as impurities in MFP, their effect on adenylate cyclase activity was also studied. Pyrophosphate inhibited adenylate cyclase activity as has been shown by others (14,15), whereas phosphate had no effect (data not shown). Thus the increase in cAMP levels seen with MFP could not be due to pyrophosphate or phosphate contamination. All experiments with hepatocytes contained theophylline to inhibit phosphodiesterase activity. Therefore it is apparent that the MFP dependent increase in cAMP accumulation is most likely the result of a direct stimulation of adenylate cyclase activity.

Table 3

MFP and NaF Adenylate Cyclase Activity in Crude Plasma Membrane Fractions from Rat Hepatocytes

Concentration	Adenylate Cyclase Activity*		
	MFP	NaF	Glucagon
0.0	20 ± 4	20 ± 4	
1.0 mM	62 ± 16**	54 ± 5**	
2.5 mM	73 ± 20**	108 ± 29**	
5.0 mM	81 ± 18**	161 ± 23**	
10.0 mM	94 ± 13**	148 ± 32**	
100.0 nM	-	-	168 ± 28**

*pmoles cAMP/mg protein 10 min., ** p < 0.005

Results expressed as means ± S.E.M. of five different plasma membrane preparations. Hepatocytes from fed rats used to prepare crude membrane fractions as described under Methods. MFP, NaF or glucagon present during assay of adenylate cyclase.

Effect of MFP on cAMP phosphodiesterase activity. Another mechanism that could lead to an increase in the hepatocyte cAMP level could be the inhibition of phosphodiesterase activity by MFP or NaF. Phosphodiesterase activity was measured in a high speed supernatant of a rat hepatocyte homogenate. NaF and MFP did not have any significant effect on phosphodiesterase activity (Table 4). As expected, theophylline significantly inhibited phosphodiesterase activity. Thus the stimulation of cAMP levels seen with intact hepatocytes above was not due to an effect of MFP or NaF on the phosphodiesterase activity.

Table 4
F⁻ Cytoplasmic Phosphodiesterase Activity

Concentration	pmoles adenosine released/mg protein/min		
	NaF	MFP	Theophylline
0	170 ± 20	170 ± 20	170 ± 20
1 mM	220 ± 40	160 ± 10	100 ± 10*
5 mM	340 ± 90	170 ± 10	7 ± 0.1*

* $p < 0.05$

Results expressed as mean ± S.E.M. of four different preparations. Phosphodiesterase activity measured in the supernatant of a rat hepatocyte homogenate as described in Methods.

Effect of NaF and MFP on hepatocyte adenylate cyclase activity. This experiment was designed to examine whether MFP and NaF would interact with adenylate cyclase in intact hepatocytes in some manner as to render the enzyme in an active form. It has been shown that plasma membrane preparations pretreated with NaF and the subsequent removal of NaF resulted in an irreversible activation of adenylate cyclase activity (14). To examine whether NaF and MFP would also activate adenylate cyclase in an irreversible manner, in intact cells, hepatocytes were preincubated with NaF and MFP for 10 min, centrifuged, and the cell pellet was then washed with buffer and subsequently used to prepare the plasma membrane fraction (see Methods). During this procedure more than 99% of the added fluoride was removed and its concentration in the adenylate cyclase assay was negligible. Adenylate cyclase activity in crude plasma membrane fractions prepared from hepatocytes pretreated with NaF and MFP was elevated by 5-fold and 16-fold respectively (Table 5). As seen with cAMP accumulation in intact hepatocytes (Table 2) and reference 4 for NaF data and the activation of intact hepatocyte adenylate cyclase (Table 5), it appears that MFP is more effective in stimulating adenylate cyclase activity and cAMP accumulation in hepatocytes than NaF.

Discussion

The results of this investigation, for the first time to our knowledge, clearly demonstrate that MFP, a compound in which F is covalently bound to phosphorus, stimulates cAMP accumulation in intact hepatocytes from fed and starved rats (Table 2). It also shows that MFP induced increase in cAMP formation is dependent on the stimulation of adenylate cyclase activity (Tables 3,5) and not on an inhibition of cAMP phosphodiesterase, since the latter is not affected by either NaF or MFP (Table 4). This is the first report to show that MFP stimulated adenylate cyclase activity in plasma membrane fractions prepared from isolated hepatocytes (Table 3). The data presented in Table 5 show that MFP and NaF increased adenylate cyclase of rat hepatocytes irreversibly. In this experiment, intact hepatocytes were preincubated with either NaF or MFP, and then cells

Table 5
NaF and MFP Adenylate Cyclase Activity
in Rat Hepatocytes

Preincubation concentration of NaF or MFP	pmoles cAMP/mg protein/10 min	
	NaF	MFP
0 mM	3.3 ± 0.4	3.3 ± 0.4
0.5 mM	8.4 ± 0.3*	N.D.
2.5 mM	11.8 ± 2.1*	15.9 ± 3.1*
5.0 mM	15.6 ± 2.1*	29.2 ± 11.1*
10.0 mM	20.6 ± 5.1*	50.7 ± 15.1*

* $p < 0.05$

Results expressed as means ± S.E.M. of 6-8 different hepatocyte preparations. Hepatocytes incubated for 10 min. with either NaF or MFP. Plasma membrane fractions prepared and adenylate cyclase assayed as described in Methods in absence of added NaF or MFP. Under these conditions free F⁻ concentration in assay negligible.

were washed to remove NaF and MFP (more than 99% being removed), and a plasma membrane fraction prepared and assayed for adenylate cyclase activity. Under these conditions adenylate cyclase activity was stimulated 5 and 16-fold following pretreatment with NaF and MFP respectively (Table 5). The stimulation of adenylate cyclase activity by MFP appears to be the result of the whole complex rather than due to the release of fluoride as a result of hydrolysis of MFP, since under these conditions the concentration of free F⁻ during the adenylate cyclase assay was negligible. The data in Table 1 show only 2-6% hydrolysis of MFP, and the concentration of free fluoride was estimated to be only 600 μ M when 10 mM MFP was used under the present experimental conditions. Therefore it appears that free fluoride generated from the hydrolysis of MFP cannot account for adenylate cyclase activation observed since the K_m for fluoride is 3-5 mM (15). MFP can be hydrolyzed under acidic conditions (16) e.g. pH less than 3, or when low concentrations (<0.05 mM) of MFP are incubated with different enzyme preparations (17). However it has been shown in this paper (Table 1) and by Ericsson and Patterson (17) that the percent hydrolysis of MFP is inversely related to the substrate concentration. That is when the concentration of MFP is greater than 0.05 mM the percent hydrolysis is reduced. Thus it is possible that under the conditions employed in these experiments with MFP concentrations of 1-10 mM and a low protein content during preincubation very little hydrolysis of MFP was observed.

It is concluded that MFP, acting as an intact compound, can stimulate adenylate cyclase activity in an irreversible manner in intact hepatocytes as well as plasma membrane fractions and thus increase cAMP accumulation in isolated rat hepatocytes.

Acknowledgement

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EFFECT OF F^- ON MAJOR SALIVARY GLANDS. THE AMYLASE ACTIVITY, STIMULATED SALIVARY FLOW RESPONSE AND cAMP LEVELS IN PAROTID GLAND OF RATS CONSUMING F^- VIA DRINKING WATER

by

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SUMMARY: This study examined the effect of intakes of 25 or 50 ppm fluoride via drinking water for four weeks on the amylase activity of the parotid gland, isoproterenol-stimulated (1 mg/100 g b.w., i.p.) salivary flow and on the amylase activity in saliva fractions. A significant elevation of the tissue amylase activity was seen in the F₂₅ and F₅₀ groups compared to control. The volume of saliva collected for 30 min after isoproterenol injection was higher in the fluoride-treated groups; the amylase activity was also increased. In the glandular tissue cAMP level was augmented. It appears that fluoride may affect the parotid function; it may also influence the salivary amylase activity, presumably by acting on the adenyl cyclase activity.

KEY WORDS: Fluoride consumption via drinking water; Parotid amylase activity; Salivary flow; Amylase activity in saliva; cAMP level parotid gland.

Introduction

Although fluoride is a well known inhibitor of many enzyme systems in vitro (1) and fluoride stimulates cAMP production in broken cell systems (2) only little is known about its in vivo effects. The skeletal and dental changes due to fluoride administration have been extensively studied (3-9). However little attention has been paid to metabolic changes occurring in tissues of intact animals consuming fluoride in

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drinking water. Several studies have been performed on the salivary fluoride concentrations (10-14), but only few studies are concerned with the effects of fluoride on the salivary glands which are known to have high metabolic activity (15-18). Few data are available on the influence of fluoride on the salivary enzymes (19-21).

The purpose of the present investigation was to study the alterations in the parotid amylase, isoproterenol-stimulated salivary flow and in the amylase activity of the saliva in rats consuming fluoride in drinking water for four weeks. The authors also wished to find out whether or not the intake of fluoride (25 to 50 ppm) in drinking water could affect parotid cAMP concentrations.

Materials and Methods

Female Wistar rats were used with an average body weight of 75grams. Animals were fed on standard laboratory chow (LATI, Hungary) with a mean fluoride concentration of 48 ± 5.2 ppm. They were also given fluoride in drinking water with concentrations of 0, 25 or 50 ppm. Both food and water were provided ad libitum. On the 28th day, after 16-hour starvation period (free access to water) rats were anesthetized with sodium pentobarbital. Eight rats from each group were bled through the femoral vein. The parotid glands were promptly removed, weighed and homogenized in distilled water for 2 min. at 0°C , using Ultra-Turrax blender system. Samples were centrifuged (3000 rpm, 0°C , 10 min). The amylase activity of the supernatants were measured by the starch splitting capacity of the enzyme and expressed in mg starch split per min per total glandular tissue. To investigate the effect of isoproterenol (1 mg/100 g b.w., i.p.) on parotid saliva secretion, a direct quantitative method was employed. Rats were placed onto a thermostated (37°C) table and they were intubated. The parotid duct was prepared and exposed to cannulation. The cut end of the duct was placed in the end of a calibrated polyethylene capillary tube for saliva collection. Saliva was collected over a period of 30 min in fractions ten minutes each. The activity of amylase was expressed in mg starch split per min per μl of ten-minute fraction or mg starch split per min per μl of saliva secreted during 30 min.

For measuring the cAMP content, parotid tissues from 13 rats in each group were removed, frozen in liquid N_2 and the cAMP content was assayed using a cAMP Kit (^{125}I , Becton Dickinson, N.Y.). Results were expressed in pmole per mg glandular protein. The protein content of samples were determined by the method of Lowry et al. (23).

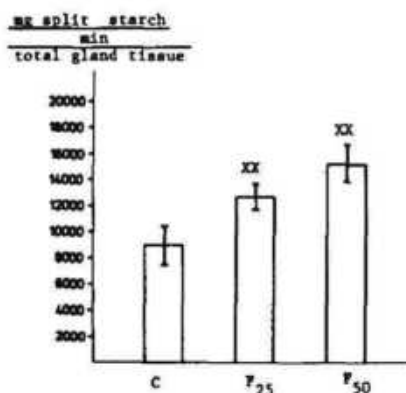
Results

1. Effect of F^- treatment on the parotid amylase activity is illustrated in Figure 1. F^- -treated rats exhibited substantially higher amylase activities than controls (C: 8600 ± 1460 ; F₂₅: $12,800 \pm 110$; F₅₀: 15400 ± 1370 mg starch split/min/total glandular tissue).

2. Isoproterenol-stimulated salivary flow response and amylase secretion of the parotid glands: Using isoproterenol as secretory stimulant

Figure 1

Effect of F^- Treatment on the Amylase Activity
of the Parotid Gland



F^- consumed via drinking water. C = control (consumed deionized water); F₂₅ = 25 ppm NaF in drinking water; F₅₀ = 50 ppm F^- . Data are means ± S.D. Significance, XX: $p < .01$.

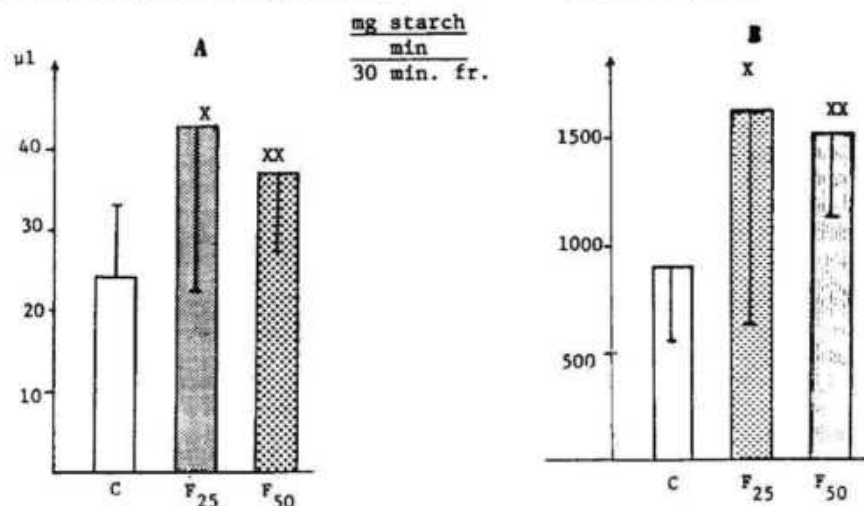
salivary flow, in groups F₂₅ and F₅₀, was significantly elevated compared to control (Fig. 2/A, C: 24.1 ± 9.3 ; F₂₅: 43.1 ± 20.7 ; F₅₀: 36.8 ± 9.5 μ l of saliva secreted during 30 min.). This was associated with concomitant increase of the amylase activity in saliva collected as well (Fig. 2/B, C: 900 ± 351 ; F₂₅: 1620 ± 1020 ; F₅₀: 1510 ± 380 mg starch split/min μ l of saliva secreted during 30 min).

3. cAMP levels in parotid gland: Parotid gland tissue cAMP levels are shown in Table 1. In tissues of the F^- -treated groups, cAMP levels were significantly elevated.

Discussion

Some biochemical alterations in the parotid glands of rats treated with fluoride via drinking water for four weeks were examined. Investigations were performed on an animal model that had been shown to be suitable for the study of the in vivo effects of fluoride treatment (22). The concentrations of fluoride found in the sera of rats receiving F^- in water in a concentration of 25 or 50 ppm were in that range, where fluoride concentrations are generally less than capable of influencing enzymic activities in vitro but it was also reported that cells with high metabolic rate are very sensitive to fluoride and a 2-3-fold elevation of cytoplasmic fluoride concentration may be toxic (24,25). This especially holds true for the fluoride sensitive cells, which are not able to lower intracellular concentration of fluoride (26). Singh (27) published data about the effect of long-term fluoride intake (10 and 25 ppm F^- via drink-

Figure 2

Salivary Flow Response of the
Parotid Gland After IsoproterenolAmylase Activity in Stimulated
Saliva Fraction

1 mg/100 g b.w., i.p. Stimulation in rats consuming F⁻ in drinking water for four weeks. :C (control group deionized water); :F₂₅ (25 ppm F⁻); :F₅₀ (50 ppm F⁻).

30 min. :C; :F₂₅; :F₅₀
Significance, X: $p < 0.05$; XX: $p < 0.01$

Table 1

Changes in Tissue Levels of cAMP in Parotid Glands of Rats Consuming Fluoridated Drinking Water for Four Weeks

Treatment	cAMP
Control (13) (consumed deionized water)	3.91 ± 0.63
F ₂₅ (13) (consumed 25 ppm F ⁻ as NaF in drinking water)	6.84 ± 0.75
F ₅₀ (13) (consumed 50 ppm F ⁻ as NaF in drinking water)	11.16 ± 1.56

$p < 0.01$
 $p < 0.01$
 $p < 0.001$

ing water) on the enzyme activities of liver and kidney of rats and he found that the activities of alkaline phosphatase, acid phosphatase and succinic dehydrogenase were decreased in the F^- -treated groups. At the same time Ferguson (28) was not able to show any significant changes in the activities of acid phosphatase, alkaline phosphatase, GOT, GPT and LAP in the liver and intestine after a six-week fluoride dosage (10 ppm). According to the enzyme studies of McClure (21) fluoride in drinking water was found to have no effect on the activity of salivary amylase. However, investigations failed to examine the possibility of physiological effect of fluorides absorbed from drinking water and food. In view of these findings and those of Whitford (29) and Wallace-Durbin (30), which indicated that salivary glands may concentrate fluoride, we decided to study the effect of fluoride treatment on the amylase activity of the rat parotid gland. The observation that the activity of amylase was significantly increased in both of the F^- groups prompted us to make further investigations to ascertain whether any relationship could be found between the fluoride effect and the salivary gland function and glandular enzyme activities.

It is well known that the glandular function, secretion and composition of the saliva are interrelated, but no such studies have been performed on fluoride-treated rats. This was the reason why we examined the effect of a single intraperitoneal injection of isoproterenol on the salivary flow response of the parotid gland. It was found that the saliva secretion in groups F_{25} and F_{50} was significantly elevated as compared to control suggesting that the four-week fluoride treatment could affect the secretory function of the gland. Similar results have been published for the submandibular gland by Boros (31).

Previous studies have indicated that the concentration and secretion of specific salivary components in the parotid gland are dependent on the stimulation of specific receptors (32) and amylase is the predominating protein secreted after isoproterenol stimulation. In our study, the saliva secreted by control and fluoride-treated glands differed not only in flow rate but also in the activity of salivary amylase. Fluoride administration produced an increase in the amylase activity of parotid saliva collected for 30 min after isoproterenol stimulation.

Dietary fluoride intake (20 ppm F^-) to rats results in an increase of tissue cAMP concentrations (liver, kidneys and submaxillary gland) by the end of a four-week period as compared to those of controls kept on a low fluoride diet (33). These results correlated with those published by Allmann (34) in which rats receiving fluoridated water (1 ppm F^-) had a significant elevation in cAMP in liver, tibia, femur, heart, kidneys and submaxillary gland, especially owing to an increased adenyl cyclase activity. In a study on rats receiving fluoride, an increased excretion of urinary cAMP has also been reported (35), although determination of urinary cyclic AMP may not be directly related to intracellular cAMP levels, because increased amounts of cAMP detected in urine after fluoride administration may be of renal origin (36).

Current evidence suggests that after treatment with NaF (10 mg NaF/kg) in rabbits, cAMP concentrations significantly increased in blood

plasma, liver and kidneys (37) and it was also shown that increased cAMP levels were due to the direct stimulatory effect of fluoride on adenyl cyclase.

In the present study the cAMP level of the parotid gland significantly increased in groups F₂₅ and F₅₀ as compared to control. We suppose that the alterations observed were also due to the specific effect of fluoride exerted on the adenyl cyclase even more because evidence has been provided that the enzyme adenyl cyclase, prepared from the rat parotid gland, can also be activated by fluoride (38).

The enhancement of the amylase activity in unstimulated, fluoride-treated rat parotid gland and the increased flow rate and amylase activity of the saliva after isoproterenol stimulation in the F⁻ groups suggest that fluoride causes an elevation of intracellular cAMP level presumably by activating adenyl cyclase, which may in turn phosphorylate the glandular proteins via cAMP-dependent protein kinase (39), and may also be involved in the enzyme (amylase) secretion from the exocrine glands (40,41).

Further studies are required on the effects of fluoride in the salivary glands in order to elucidate the mechanism by which fluoride regulates the salivary amylase activity and secretion process.

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FLUORIDE

EFFECT OF F⁻ EFFLUENT ON SOME METABOLITES AND
MINERALS IN FRY OF CATLA CATLA (HAMILTON)

by

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SUMMARY: Fry of Catla catla were exposed to fluoride effluent dilutions of 1.2, 2.5, 4.3, 7.2, and 13.2ppm from a fluorine emitting industry situated at Surat City in Gujarat State for 96 hours. Accumulation of fluoride increased with increase in exposure time and fluoride content in effluent dilutions. Fluoride concentrations from 1.2 ppm upwards affected protein, from 4.3 ppm upwards affected glycogen and iron, and 7.2 ppm upwards affected lipid content in fry at all durations of exposure. The sodium content was unaffected after exposure to the above effluent dilutions. Thus, protein content was most affected followed by glycogen and lipid, and iron content in fry.

KEY WORDS: Fluoride; Metabolites; Catla catla; Effluent; Lipids; Glycogen; Iron; Protein

Introduction

Though studies have been made on the toxicity of various metallic pollutants, little attention is being paid to toxicity of trace elements to members of aquatic ecosystems. Among trace elements, fluoride plays a major role in deteriorating aquatic ecosystems. Studies on the ecological significance of exposure of aquatic animals to fluoride are limited (1). Reports on fluoride toxicity to aquatic invertebrates, namely crabs (2,3), bivalve molluscs (4-6), are available but, on aquatic vertebrates, namely fishes, reports are sporadic. In 1974, Woodwiss and Fretwell estimated LC₅₀ of 125 ppm F⁻ for brown trout yearlings (7). Wright (8) estimated 100 ppm F⁻ (wet weight basis) in the whole body of brown trout exposed to 5 ppm fluoride for 200 hrs. Channa punctatus exposed to 10 ppm F⁻ exhibited hyperglycemia, increase in cholesterol and sodium, decrease in blood glucose, hematocrit values and oxygen consumption (9-11).

Considering the effects of fluoride in various ways on fish life, the present study aims to find out the biochemical changes associated with treatment of fluoride effluent discharged from the fluorine industry at City Surat in Gujarat State on the fresh water fish, Catla catla (Hamilton). Changes in total protein, glycogen, lipid, sodium and iron content along with the fluoride accumulation in the whole body due to treatment of the effluent were studied.

From the Department of Biosciences, University of South Gujarat, City Surat and Department of Zoology, University of Marathwada, City Aurangabad, India. Presented at the 13th conference of the International Society for Fluoride Research, Nov. 14-17, 1983, New Delhi, India.

Materials and Methods

Fry of Catla catla (2.5 to 4.0 cm in length and 900 to 1200 g in weight) obtained from Inland Fisheries Station, Ukai (Gujarat State) were acclimated in 5 aquaria (10 L capacity) for 8 days. Supply of air was constant, the water was changed every alternate day.

The effluent collected from the industry was diluted from 100% (the original concentration) to 10% (with interval treatment of 10%) and transferred into polypropylene containers (4 L capacity). Ten fry were maintained in each container. Since more than 75% mortality in 20% effluent dilution occurred within 24 hrs, the fry were next exposed to 10, 5.6, 3.2, 1.8 and 1% effluent dilutions (containing 13.2, 7.2, 4.3, 2.5 and 1.2 ppm fluoride, in respective effluent dilution). Twelve polypropylene containers, containing 1% effluent dilution with 10 fry in each, were maintained for 96 hrs. Four containers, with fresh tap water and 10 fry in each, served as control. After every 24 hrs, living fry exposed to the 1% effluent dilution were removed from 3 containers, and from 1 control container. The experiment was repeated with 1.8, 3.2, 5.6 and 10% effluent dilutions. Five samples of controls exposed for a different number of hours to different effluent dilutions were separately analyzed, the mean became the control value for 24, 48, 72 and 96 hrs. The fry, belonging to the effluent dilutions and control, were placed in an oven at 80°C and dried for a constant weight.

Temperature, pH, dissolved oxygen (12) and fluoride content in effluent were measured using an Orion fluoride electrode. Protein (13), glycogen (14) and lipid (15) concentrations were estimated in oven-dried fry. The fry were ashed at 600°C in a muffle furnace and fluoride content estimated (16). For estimation of sodium (using a flame photometer) and iron (17) the samples were digested in concentrated H₂SO₄.

Results are expressed on a dry weight basis. Significance in changes in metabolites or minerals was compared with the control using the student's 't' test. Analysis of variance (two-way with replicates) was used to find out the significance in changes in the content of fry exposed to different effluent dilutions. Percent deviation of mineral/metabolite in fluoride-exposed fry, from control is expressed as:

$$\frac{\text{mineral/metabolite in fluoride exposed fry} - \text{mineral/metabolite in fry from control group}}{\text{mineral/metabolite in fry from control group}} \times 100$$

Results and Discussion

Temperature, pH and dissolved oxygen in effluent media fluctuated between 35.8 and 36.8°C, 7.03 and 7.27, and 5.20 and 5.80 ppm, respectively. Dissolved pH and oxygen of control were 7.21 and 5.67 ± 0.51 ppm, respectively. Protein content in fry exposed to various concentrations of effluent was lower compared to controls. When exposed to 1.8% effluent dilutions, at 24, 48, 72 and 96 hrs., protein content in fry was higher than in those exposed to 1%. On the other hand, protein content declined at all periods of exposure in fry exposed to 3.2% effluent dilution, in

fry exposed to 5.6% and 10% it decreased considerably at 96 hrs. In fry exposed to 10% effluent dilution, protein content was higher up to 72 hrs. than in those exposed to 5.6% dilution. Thus, there was a significant change in protein content ($P < 0.0005$) in the fry exposed to different effluent dilutions during different intervals of exposure (Table 1).

Table 1
Protein Content (mg/g) in *C. catla* Fry

Exposure Time (hrs)	Effluent Dilution (%) (ppm F^-)					
	C	1.0	1.8	3.2	5.6	10.0
	0 ppm	1.2 ppm	2.5 ppm	4.3 ppm	9.2 ppm	13.2 ppm
24	261.64 ± 10.81	247.12 ± 5.24	247.45 ± 11.51	238.93 ± 10.07	242.98 ± 12.07	270.83 ± 8.33
48	269.72 ± 4.72	251.88 ± 9.82	259.37 ± 11.02	237.24 ± 6.70	248.81 ± 12.11	269.24 ± 7.80
72	278.98 ± 12.53	250.88 ± 6.50	258.67 ± 11.61	233.78 ± 4.89	259.71 ± 11.78	272.31 ± 19.50
96	263.84 ± 7.72	239.62 ± 13.90	254.60 ± 26.18	233.82 ± 20.61	224.98 ± 16.44	220.55 ± 3.12

Mean values ± Standard Deviation (n=3); C - Control

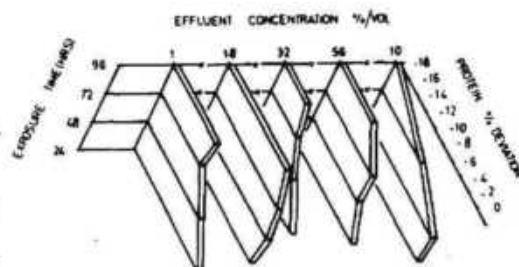
one more variable, the exposure hours (X_1) was added, the equations could be modified. The amount of fluoride in the exposure media ($\beta X_2 = 22.353$), and the exposure hours ($\beta X_1 = -0.8094$) might have played minor roles in decreasing the protein content in fry compared to the amount of fluoride accumulated in the fry ($\beta Z = -20.1918$). The percentage deviation of protein from the control was low in fry exposed to 10% effluent dilution at 96 hrs (Fig. 1).

The decline in protein was greater in higher concentrations, but it was not directly related to the fluoride in the effluent. Fluoride has the capacity to bind plasma protein and intracellular proteins (18). Several *in vitro* studies have confirmed that fluoride inhibits protein synthesis in Landshutz ascites (19). Reduction in protein content due to inhibition of fluoride on protein synthesis, has been reported in tissue pieces (*in vitro*), namely dental pulp tissues (20), islets of Langerhans from rats (21) and bone (22). Inhibition of protein synthesis

revealed that protein estimated in the fry exposed to various effluent dilutions (vertical) at different exposure hours (horizontal) are significantly different at 1% probability level. The interaction (concentration of fluoride in the effluent X exposure duration) is significant at 5% probability level ($F_{4,9} = 2.25$). A multiple regression equation is established; where Y is the protein content and X_2 is the fluoride accumulation in fry. However, the multiple correlation coefficient was not so high ($R = 0.52$). When

Figure 1

% Deviation of Protein in Fry of *C. catla* Exposed to Effluent from Control



by fluoride has been concluded to result from the inhibition on initiation of new peptide chains and the dissociation of 80S ribosome (23).

The fry exposed to 1.8% effluent dilution showed a higher elevation of glycogen content than those exposed to 1% at 24, 48, and 96 hrs. Glycogen content declined in fry exposed to 3.2% effluent dilution compared to those exposed to 1.8% dilution. It decreased more at 96 hrs. The glycogen content was lower in fry exposed to 10% effluent dilutions than in those exposed to 4.6% at 48, 72 and 96 hrs. Thus, although the glycogen content in controls of fry showed fluctuations, in fry exposed to different effluent dilutions it decreased significantly ($P < 0.01$) and the content at different hours decreased significantly ($P < 0.025$) (Table 2). The

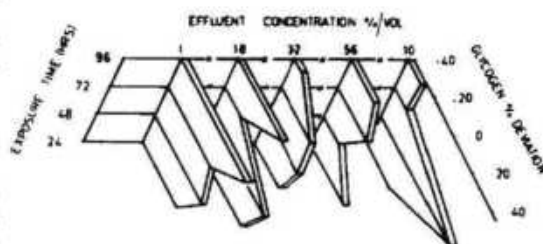
Table 2
Glycogen Content (mg/g) in C. catla Fry

Exposure Time (hrs)	Effluent Dilution (I) (ppm F ⁻)					
	C	1.0	1.8	3.2	5.6	10.0
	0 ppm	1.2 ppm	2.5 ppm	4.3 ppm	9.2 ppm	13.2 ppm
24	0.62 ± 0.05	0.56 ± 0.04	0.72 ± 0.05	0.51 ± 0.09	0.55 ± 0.07	0.57 ± 0.04
48	0.61 ± 0.05	0.59 ± 0.06	0.73 ± 0.08	0.50 ± 0.04	0.51 ± 0.03	0.48 ± 0.07
72	0.60 ± 0.04	0.61 ± 0.05	0.50 ± 0.21	0.53 ± 0.09	0.51 ± 0.11	0.41 ± 0.04
96	0.60 ± 0.05	0.68 ± 0.07	0.71 ± 0.05	0.40 ± 0.01	0.48 ± 0.01	0.47 ± 0.04

Mean values ± Standard Deviation (n=3); C - Control

lowest percentage deviation of glycogen content was observed in fry exposed to 10% effluent dilution. In other dilutions (1 and 1.8%), the percentage deviation of glycogen from the control fluctuated in a wide range. The fluoride level in these two dilutions of effluent might not have been high enough to induce a change in glycogen metabolism in the fry (Fig. 2). 3.2% effluent dilution (containing 4.3 ppm fluoride) or above has shown inhibition on glycogen synthesis in the fry. A lesser amount of fluoride accumulated in the fry exposed to a series of lower dilutions (1.8 and 1%) might have inhibited ATPase in fry. Similarly, elevation of glycogen content in fry exposed to 1.8 and 1% effluent dilution at the following exposure times (24 hrs. in 1.8%,

Figure 2
% Deviation of Glycogen in Fry of C. catla
Exposed to Effluent from Control



FLUORIDE

48 hrs in 1 and 1.8%, 72 hrs in 1%, and 96 hrs in 1 and 1.8%) might be due to inhibition of ATPase in fry. Fluoride inhibition on ATPase has been reported (24-26). If fluoride is given rapidly in the growing cell (*in vitro*), the ATP level could be lowered (27,28). The reason for reduction in glycogen in fry exposed to higher dilutions of effluent could be attributed to fluoride inhibition of enolase (29,30).

Lipid content in fry, exposed to the effluent dilutions, decreased predominantly in higher concentrations when compared to controls. When exposed to 5.6% effluent dilution, lipid content in fry increased compared to controls. Lipid content was lowest in fry exposed to 10% at 72 hrs. At 24 and 72 hrs the content decreased as the concentration of effluent increased. At 48 and 96 hrs, the lipid content in fry exposed to 1.8% effluent dilution was higher than in those exposed to 1%. The lipid content in fry exposed to 3.2% effluent dilution declined at 48 and 96 hrs. In controls, it showed comparatively little change (Table 3). A significant decline in lipid was observed in fry exposed to various exposure hours compared to controls ($P < 0.05$). The percent decrease in lipid was low in fry exposed to 10% effluent dilution at 96 hrs (Fig. 3). According to Phillips and Hart (31), fluoride has the capacity to alter lipid metabolism. In recent studies, Goodstein (32) reported a reduction in phospholipids in pups given 50 ppm fluoride in water.

Table 3
Lipid Content (ug/g) in *C. catla* Fry

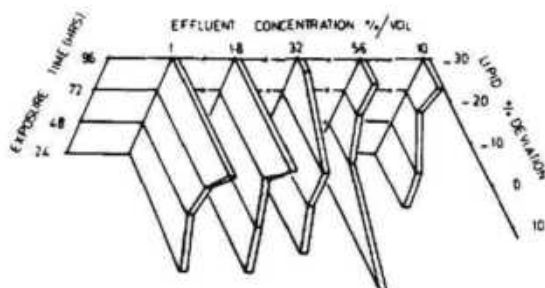
Exposure Time (hrs)	Effluent Dilution (%) (ppm F ⁻)					
	C	1.0	1.8	3.2	5.6	10.0
	0 ppm	1.2 ppm	2.5 ppm	4.3 ppm	9.2 ppm	13.2 ppm
24	282.67	302.21	285.83	277.03	251.69	224.90
	±	±	±	±	±	±
	24.54	23.10	18.38	17.07	10.46	30.56
48	276.94	250.43	265.03	238.80	240.44	218.44
	±	±	±	±	±	±
	14.78	9.56	29.74	48.57	9.75	26.59
72	291.42	284.98	265.69	262.03	226.63	205.13
	±	±	±	±	±	±
	11.99	30.30	24.21	37.59	21.73	29.60
96	282.43	270.13	284.95	213.97	210.01	210.45
	±	±	±	±	±	±
	9.99	43.30	21.94	15.26	11.30	26.19

Mean values ± Standard Deviation (n=3); C - Control

Whereas the fluoride content in fry exposed to effluent dilutions increased more than controls at all exposure hours, the increase was less in fry exposed to 1% effluent dilution. It gradually increased with the increase in effluent concentration and period of exposure (Table 4). In controls, the fluoride content in fry ranged between 84.35 ± 6.98 and 89.52 ± 13.25 ug/g which was less than in the treated fry. Those exposed

Figure 3

% Deviation of Lipid in Fry of C. catla
Exposed to Effluent from Control



duration of exposure increased fluoride in fry accumulated (Fig. 4).

Fish and other aquatic vertebrates accumulate fluoride from the environment primarily in the skeleton. The mechanism of incorporation of fluoride ion in the fish skeleton is the same as in other vertebrates (3). Fluoride's strong affinity for the hydroxyl lattice sites of apatite largely accounts for its mineral seeking property. Thus, it is likely that the greater amount of fluoride ingested in fry C. catla goes to the hydroxyl apatite, the major component in bone

to effluent dilutions accumulated significant amounts of fluoride ($P < 0.05$). Thus fluoride accumulation in fry differed in effluent dilutions at various exposure hours. ANOVA also stated that both exposure duration and exposure media significantly affect accumulation of fluoride in fry. The percentage deviation of fluoride in fry from the control group was maximum at 96 hrs. The lowest percentage deviation of fluoride was observed in fry exposed to 1% effluent dilution. Thus, as the concentration of fluoride in the exposure media rose and the

Table 4

F⁻ Content (mg/g in C. catla Fry

Effluent Dilution (%) (ppm F⁻)

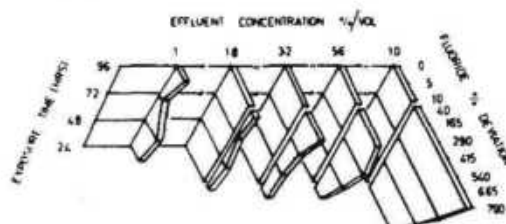
Exposure Time (hrs)	C	1.0	1.8	3.2	5.6	10.0
	0 ppm	1.2 ppm	2.5 ppm	4.3 ppm	9.2 ppm	13.2 ppm
24	85.33	95.32	127.94	159.55	196.66	382.14
	± 8.61	± 14.85	± 13.20	± 12.00	± 13.20	± 18.83
48	85.09	97.77	133.64	165.16	254.54	496.36
	± 6.82	± 3.65	± 24.05	± 9.15	± 15.68	± 17.15
72	84.35	93.31	136.41	199.10	317.00	629.96
	± 6.98	± 6.36	± 19.17	± 9.68	± 14.79	± 23.99
96	89.52	99.57	162.77	226.99	389.22	766.67
	± 13.25	± 4.12	± 8.87	± 9.94	± 25.44	± 55.08

Mean values ± Standard Deviation (n=3); C - Control

(33). Bone tissue undergoing active mineralization accumulates fluoride at a faster rate (34).

Figure 4

% Deviation of F⁻ in Fry of C. catla
Exposed to Effluent from Control



At all effluent dilutions, sodium content in fry decreased at 24, 48 and 72 hrs compared to controls. In fry, exposed 1.8% effluent dilution, the sodium content was higher at 24 hrs than in fry exposed to the remaining effluent dilutions as well as in controls. At 96 hrs

with 1 to 5.6% effluent dilutions, sodium content in fry was high compared to controls (Table 5) (Fig. 5). Similarly Quissell and Sutti (35) reported that intracellular (*in vitro*) sodium content is less sensitive to fluoride.

Table 5
Na Content (mg/g) in *C. catla* Fry

Exposure Time (hrs)	Effluent Dilution (%) (ppm F ⁻)					
	C	1.0	1.8	3.2	5.6	10.0
	0 ppm	1.2 ppm	2.5 ppm	4.3 ppm	9.2 ppm	13.2 ppm
24	69.50	69.20	73.40	68.80	69.20	69.50
	± 3.40	± 2.46	± 7.69	± 1.73	± 4.01	± 2.56
48	71.10	70.33	70.70	70.30	69.77	65.40
	± 2.35	± 2.05	± 2.69	± 18.20	± 3.00	± 4.92
72	72.40	67.00	70.30	71.00	67.80	70.07
	± 4.57	± 1.10	± 3.02	± 4.02	± 3.79	± 1.55
96	68.00	68.43	68.17	69.36	70.80	57.80
	± 4.80	± 4.03	± 16.11	± 4.18	± 3.57	± 2.08

Mean values ± Standard Deviation (n=3); C - Control

Iron content decreased in fry exposed to all effluent dilutions (except 1.8%) at all exposure hours (Table 6); the decrease was greater in fry belonging to 5.6% effluent dilution. The decrease in iron content in fry exposed to various effluent dilutions at different exposure hours was significant at 0.05% probability level. The lowest percentage deviation of iron content from the control was reached in fry exposed to 10% effluent dilution.

The percentage deviation of iron was comparatively low in fry exposed to 3.2 and 5.6% effluent dilution. Fry exposed to lower dilutions (1 and 1.8%) showed considerable fluctuation in iron content (Fig. 6). Adverse effects of fluoride on iron metabolism have been reported in rabbits. Fluorotic rabbits exhibited hypochromic anemia (36). Concomitant presence of iron enhanced fluoride absorption in rats (37).

Figure 5

% Deviation in Sodium in Fry of *C. catla*
Exposed to Effluent from Control

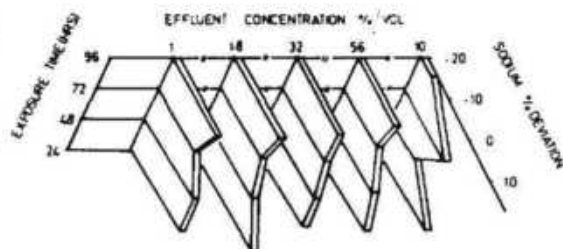


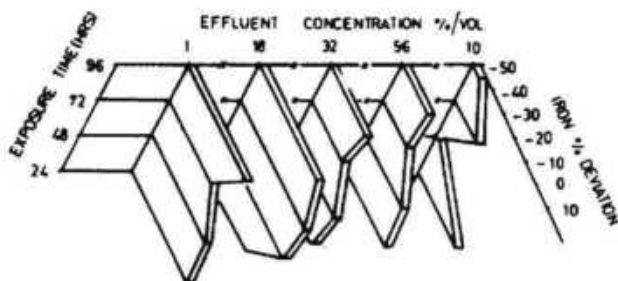
Table 6
Fe Content (mg/g) in C. catla Fry

Exposure Time (hrs)	Effluent Dilution (%) (ppm F ⁻)					
	C	1.0	1.8	3.2	5.6	10.0
	0 ppm	1.2 ppm	2.5 ppm	4.3 ppm	9.2 ppm	13.2 ppm
24	4.80	4.61	3.91	3.75	3.73	3.90
	± 0.69	± 0.54	± 0.57	± 0.66	± 0.83	± 0.94
48	5.50	5.17	5.60	4.56	4.20	2.82
	± 0.71	± 0.39	± 1.05	± 0.60	± 0.55	± 1.02
72	5.50	4.57	5.84	3.96	3.62	3.73
	± 0.80	± 0.61	± 0.96	± 1.07	± 0.56	± 0.98
96	4.91	4.87	5.50	3.89	3.43	2.72
	± 0.97	± 0.82	± 0.56	± 0.98	± 0.50	± 0.84

Mean values ± Standard Deviation (N-3); C - Control

Figure 6

% Deviation of Iron in Fry of C. catla
Exposed to Effluent from Control



Summary

Protein metabolism was more affected, followed by glycogen and lipid, and iron, when the fry of C. catla were exposed to effluent dilutions from the fluorine industry situated at Surat in Gujarat State (India).

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DISFIGURING DENTAL FLUOROSIS IN AUCKLAND, NEW ZEALAND

by

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SUMMARY: A survey of dental fluorosis in Auckland, New Zealand is reported. In the fluoridated (one part per million) area, 24.9% of children aged 7 to 12 years had dental fluorosis, and 3.6% had the advanced form of the condition (discolored or pitted enamel). In the nonfluoridated urban area (0.2 ppm groundwater) only 4.9% of children the same age had the very mild form. The incidence was lowest—2.9%—on an off-shore island, where the residents drink rainwater. Only cases which unquestionably answered the description of dental fluorosis—symmetrically arranged diffuse white opacities, clearly visible with the teeth undried—were recorded. In the larger fluoridated area, which has a greater range of income levels than the low-income nonfluoridated area, there was an inverse relationship between the advanced (discolored or pitted) condition and the socio-economic status of the suburbs where the children lived. In the Auckland district, the incidence of both dental fluorosis and dental decay indicates that child dental health is now better in the non-fluoridated area.

KEY WORDS: Dental fluorosis; Auckland, New Zealand

Introduction

Early in 1983, the author's attention was called to the existence of disfiguring dental defects, of the type described as dental fluorosis (1,2), in several children in school dental clinics in the Auckland Health District. The district's water supply had been fluoridated since 1966 to a level of 1 part per million.

The author personally verified the observations made by two dental operators serving in the district, who had examined 458 children aged 7 to 12 years. He found that over 25% of them exhibited variable degrees of diffuse white opaqueness (or "mottling"). The opaque areas followed the surface growth lines of the enamel (or "perikymata"), being more or less linear in arrangement when extensively present, and transverse to the long axis of a tooth. Symmetry (bilateral) was also present. That is, occurrence in a tooth on one side of a dental arch was similar to its occurrence in the corresponding tooth on the other side. The more severe cases exhibited discoloration, or even pitting, of the opaque enamel.

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He then examined the 390 school children of the same age in the non-fluoridated part of urban Auckland, which uses groundwater with a natural fluoride content of 0.2 ppm (3). He found that only 5% of them had the mildest form of the same condition.

His suggestion that a more extensive survey be made was met with disfavor. The Head Office of the Department of Health pointed out that surveys had already been carried out in Hastings (fluoridated since 1953) and elsewhere. These had not been published, but public statements had been made that there was no evidence of an increase in enamel opacities. Due to his imminent retirement, the author had insufficient time to complete a thoroughly detailed study in the way he would have wished. However, he proceeded with this fuller survey, with the help of some school dental clinic staff.

Material and Methods

To rule out the possibility that the population of 458 school children initially investigated was not representative of the fluoridated part of Auckland, the author made further observations of school children of the same age in six additional dental clinics chosen at random. A total of 1955 such children were examined in the suburban fluoridated area that included varying socio-economic levels (4).

A survey of an additional 342 children of the same age living on an off-shore island, which depends on nonfluoridated rainwater, brought the total number seen in the nonfluoridated area to 732. All such mainland and most island children, in the nonfluoridated area, were personally examined by the author.

Only cases of bilateral diffuse enamel opacities, clearly visible with the teeth undried, were recorded. Children with such mottling who were known to have grown up in areas different in fluoridation status from those where they were examined were excluded from the survey. The proportion of children at each clinic who were not life-long residents of the suburb was not ascertained. There is no reason to suppose, however, that proportions differed between areas. Age and equal sex distributions were the same in each area. Ethnic composition of the areas was similar, except for the usual higher proportion of Maori and Pacific Island people in the lower socio-economic areas. As no sex, race or ethnic relationship was apparent, these categories were not separated. Only erupted permanent teeth were considered, and all these were examined. Initial screenings were by the author or specially instructed dental clinic staff. Cases found were classified by the author.

Classification of Bilateral Diffuse Opacities: The level of severity assigned to each case was the one which corresponded to the two most severely affected teeth. Each pair of homologous teeth was classified into one of three such categories, as follows:

Table 1
Distribution of Dental Fluorosis in Auckland School Children (Examined October-November 1983)

Area	Fluoridation status	Age group (yrs)	No. of children	No. according to category of dental fluorosis:				No. with involvement of classes of teeth:				No. according to no. of fluorosed teeth:						
				ren	1	2	3	total	incisal	canine	pre-molar	1st molar	2nd molar	2	4	6	8	10
Fluoridated (1 ppm) High:\$798-9974 (20-47%)		7	152	26	1	2	29	16	-	-	28	-	4	10	8	4	3	-
		8	121	22	5	5	32	17	1	-	30	-	3	13	6	4	2	4
		9	155	35	9	4	48	31	9	10	44	-	6	12	9	10	5	6
		10	149	28	2	2	32	14	10	18	27	5	3	9	8	2	1	9
		11+	82	17	3	-	20	6	9	14	16	7	-	5	3	3	4	5
Totals			659	128	20	13	161	84	29	42	145	12	16	49	34	23	15	24
Percentages				5.0%	24.4%	12.7%												
Fluoridated (1 ppm) Middle:\$7067-7255 (27-33%)		7	148	28	1	2	31	12	-	-	31	-	7	15	4	5	-	-
		8	140	27	1	3	31	11	-	-	30	-	6	15	3	6	-	1
		9	151	28	2	5	35	12	7	7	35	1	2	17	5	3	2	6
		10	157	33	3	3	39	12	10	13	36	4	2	17	4	5	2	9
		11+	80	11	4	2	17	8	7	9	16	4	-	6	2	2	-	7
Totals			676	127	11	15	153	55	24	29	148	9	17	70	18	21	4	23
Percentages				3.8%	22.6%	8.1%												
Fluoridated (1 ppm) Low:\$5279-7003 (7-15%)		7	108	32	-	-	32	4	-	1	30	-	2	27	1	1	1	-
		8	121	31	1	2	34	8	2	1	33	-	5	19	5	2	1	2
		9	140	31	1	1	33	11	5	4	32	-	4	15	7	2	1	4
		10	111	26	2	1	29	14	8	16	24	7	2	5	2	8	6	6
		11+	140	41	2	1	44	12	9	32	27	20	2	10	9	6	4	13
Totals			620	161	6	5	172	49	24	54	146	27	15	76	24	19	13	25
Percentages				1.8%	27.7%	7.9%												
Fluoridated area:			Totals	416	37	33	486	188	77	125	439	48	48	195	76	63	32	72
Percentages				3.6%	24.9%	9.6%												
Nonfluoridated groundwater 0.2ppm Low:\$5105-6131 (9-16%)		7	100	4	-	-	4	1	-	-	4	-	3	1	-	-	-	-
		8	78	7	-	-	7	-	-	-	7	-	4	3	-	-	-	-
		9	71	3	-	-	3	1	-	-	3	-	2	-	-	1	-	-
		10	89	2	-	-	2	-	-	-	3	-	2	1	-	-	-	-
		11+	52	3	-	-	3	-	-	-	2	1	1	1	-	-	-	-
Totals			390	19	-	-	19	2	-	-	17	2	12	6	-	1	-	-
Percentages				-	-	-	4.9%	0.5%										
Nonfluoridated (rainwater) Low:\$3291 (16%)		7	61	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		8	69	4	-	-	4	2	-	-	4	-	1	1	-	-	2	-
		9	58	2	-	-	2	1	-	-	2	-	-	2	-	-	-	-
		10	49	-	-	-	1	1	1	1	-	1	-	-	-	-	-	1
		11+	105	2	-	1	3	1	-	1	1	1	2	1	1	-	-	1
Totals			342	8	1	1	10	5	1	2	7	3	2	4	-	-	-	2
Percentages				0.6%	2.9%	1.5%												
Nonfluoridated area:			Totals	27	1	1	29	7	1	2	24	5	14	10	-	1	2	2
Percentages				0.3%	4.0%	1.0%												

Table 2
Summary of the Prevalence of Dental Fluorosis
in Auckland School Children in 1983

Fluoridation status and socio-economic level of area	No. of child- ren	Percent of children with den- tal fluorosis:			Percent of fluorosis cases involving:		
		All cate- gories	Categories 2 and 3	Fluorosed incisors	2-4 teeth	6-10 teeth	Over 10 teeth
Fluoridated (1 ppm)							
High	659	24.4	5.0	12.7	40.4	44.7	14.9
Middle	676	22.6	3.8	8.1	56.9	28.1	15.0
Low	620	27.7	1.8	7.9	52.9	32.6	14.5
Total	1955	24.9	3.6	9.6	50.0	35.2	14.8
Nonfluoridated							
(ground) Low	390	4.9	0.0	0.5	94.7	5.3	0.0
(rain) Low	342	2.9	0.6	1.5	50.0	20.0	20.0
Total	732	4.0	0.3	1.0	82.8	10.3	6.9

Category 1. Any of the following, without discoloration or pitting:

- (a) small patches, up to 2 mm in diameter, mostly on cusp tips or incisal edges (Fig. 1, and lower teeth, Fig. 2)
- (b) larger patches, in the same position, or extending along marginal ridges and crown surfaces (Fig. 3,4)
- (c) continuous or broken horizontal lines on crown surfaces (Fig. 5,6) (these usually occur with some discoloration or pitting)

Table 3

F⁻ Toothpaste Consumption
in New Zealand*

Year	F ⁻ Toothpaste % Total Sales
1970-71	6
1972-73	15-17
1974-75	37-43
1976-77	51-56
1978-79	69-73
1980	76

Category 2. Any of the above, with some yellow or brown discolored patches also present (Figs. 7,8)

Category 3. Any of the above, with some pitting (Figs. 9-12)

The results are presented in Table 1 and summarized in Table 2.

Fluoride Toothpaste Use: Information on sales of fluoride toothpaste in New Zealand from 1970 through 1980 is given in Table 3.

Results

*Source: Dentifrice Prod. Mgr.
Lever Bros. Personal Communi-
cation to F.A.C. Wright.

There is a highly significant difference ($P < 0.001$ by Chi square test) between the incidence of dental fluorosis in the fluori-

dated and nonfluoridated areas: fluoridated 24.9%; nonfluoridated ground-water 4.9%; nonfluoridated rainwater 2.9% (Tables 1,2).

In all areas, the most commonly affected teeth were the first permanent molars (90% of all cases in the fluoridated area and 83% in the nonfluoridated) followed by incisors (39% and 24%), premolars (26% and 7%), canines (16% and 3%) and second permanent molars (10% and 17%). In the younger age groups, of course, only the first permanent molars and incisors were erupted.

In the fluoridated area, 50% of all cases involved only 2 or 4 teeth, and 15% involved more than 10 teeth. In the nonfluoridated area 83% of the cases involved only 2 or 4 teeth, and 7% (two fluoride tablet takers) involved more than 10 teeth.

There were no significant differences between incidences of dental fluorosis in the various age groups.

Within the fluoridated area, the over-all incidence of dental fluorosis was not significantly correlated with socio-economic level, though it was somewhat higher ($0.10 > P > 0.05$) in the low socio-economic area. However, the incidence of advanced (categories 2 and 3) dental fluorosis was inversely correlated with socio-economic level, and the differences are significant ($P < 0.05$).

Fluoride Toothpaste Use: Table 3 shows that a marked increase in the use of fluoride toothpaste has occurred in New Zealand since 1970. However, there is no trend toward a greater severity of dental fluorosis among younger children, thereby indicating that fluoride in the water supply is the controlling factor.

Disfigurement: Two objective measures are: (a) presence of advanced (category 2 or 3) dental fluorosis, and (b) involvement of front (incisor) teeth. By either criterion there is a very significant difference in the incidence of disfiguring cases between the high and low income suburbs of the fluoridated area ($P < 0.01$) and between the fluoridated and nonfluoridated areas ($P < 0.001$).

Special Observations: The two children with advanced dental fluorosis in the nonfluoridated area, and the only ones in that area with more than 10 teeth involved, had been given fluoride tablets, at the recommended dose regimen, since birth (one intermittently and the other regularly).

In the nonfluoridated, 0.2 ppm, natural fluoride area, the most advanced case, a 10-year-old Maori boy, displayed faint horizontal white lines on his upper central incisors. From an early age, he had drunk tea regularly, and his family was fond of fishing and seafoods.

In the high income fluoridated area, at least two of the children with advanced cases had been ingesting fluoride tablets, on the recommendation of the family dentist, despite living in a fluoridated area.

Some of the children with fluorosis reported the use and swallowing of fluoride toothpaste since an early age.

Discussion

Classification and Terminology: The three-category system of classification used in this study is similar to that of Smith et al., which has been described as most suitable for screening purposes (5). The author did not use the Federation Dentaire Internationale (FDI) index which has been used in other recent New Zealand studies, primarily because it would have been too time-consuming and complicated for the time available. It is based on descriptive rather than etiological criteria, and is designed for recording all kinds of enamel defects, not just dental fluorosis (6).

Other classification systems (7-9) do not adequately distinguish opacities associated with fluorosis from those due to other causes. Judging from preliminary reports of its use in New Zealand (10-12), the new FDI index is also not discriminatory enough.

There are many kinds of enamel defects. One involves mottling or opacities, which in turn may be of two kinds: (a) the nonfluoride "idiopathic" type (variously described as "demarcated", "discrete", "circumscribed" or "localized") and (b) the fluoride or "fluorosis" type (described as "diffuse", "lustreless" or "generalized"). The nonfluoride kind is distributed randomly over tooth surfaces, whereas dental fluorosis tends to be bilateral in distribution, following the perikymata. It is thus obviously developmental in origin (1,2).

There has been considerable confusion over terminology, which has obscured the problem of dental fluorosis. For example, the term "fluorotic-type defect" has been used for cases subjectively assessed by a single examiner as "cosmetically poor" (11). In reality, the term is meaningless, since it has been claimed that "fluorotic-type defects can be produced by factors other than fluoride" (11). The term "dental fluorosis" is apparently being avoided (9-12).

Much euphemistic and misleading phraseology — for example "very mild", "mild" and "moderate" to describe obviously disfiguring mottling (13) — seems to have facilitated the erroneous claim that dental fluorosis is "detectable only by close expert examination" (14). The fact that all categories of dental fluorosis reported in the present study were, on some occasions, pointed out to the school dental operators by the children's parents themselves, firmly disputes this claim.

Other New Zealand Studies: The 1957 Commission of Inquiry, which originally established acceptance of water fluoridation in New Zealand, predicted that barely detectable mottling "may be expected in 6 to 10% of children when the fluoride content of water is 1 ppm" (14). Reports of other recent New Zealand studies (10-12) confirm incidences well in excess of those predicted, and significantly higher in fluoridated areas than in nonfluoridated ones. Little information is available from these reports about objectively measured levels of severity. Auckland's cli-

CATEGORIES OF DENTAL FLUOROSIS

(Reproductions of color photographs by Jan Caris)



Fig. 1: boy, age 7 (perm. molar I)



Fig. 2: boy, age 10

Above: Category 1a (small patches on tooth tips or edges)

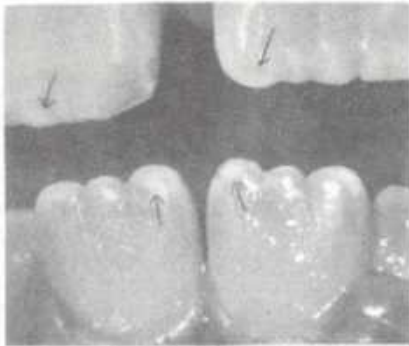


Fig. 3: boy, age 7



Fig. 4: boy, age 7

Above: Category 1b (larger patches on tooth tips or edges)

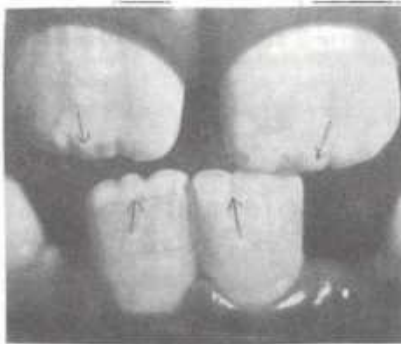


Fig. 5: boy, age 7



Fig. 6: girl, age 9

Above: Category 1c (continuous or broken horizontal lines on teeth)

(Arrows in Figs. 1-6 above indicate areas of representative fluorotic opacities.)



Fig. 7: girl, age 9

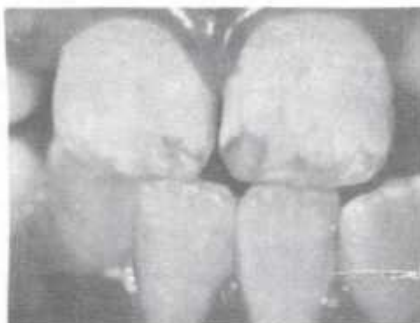


Fig. 8: boy, age 8

Above: Category 2 (yellow or brown coloration present)



Fig. 9: boy, age 9

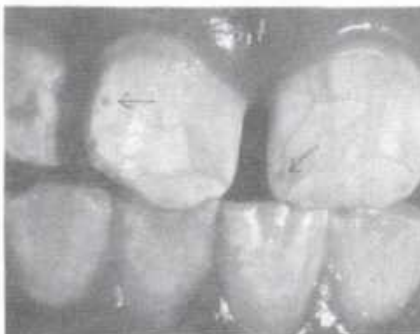


Fig. 10: boy, age 11

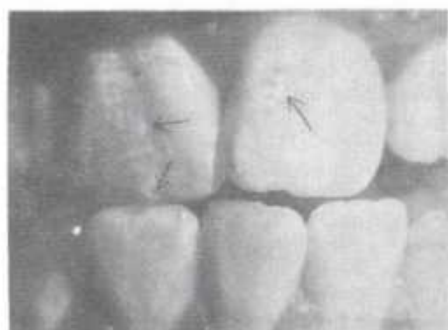


Fig. 11: girl, age 9

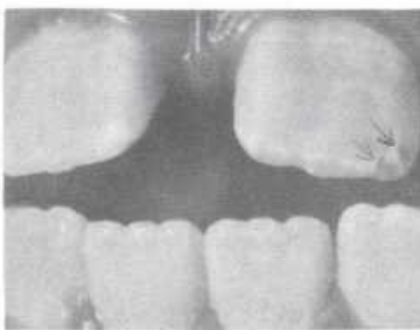


Fig. 12: boy, age 8

Above: Category 3 (as in Category 1 or 2 but with pitting)

(Arrows in Figs. 9-12 indicate regions of noticeable pitting.)

mate is subtropical, warmer than centers farther south, and this could affect fluorosis incidence and severity.

In 1970 it was reported that, on the basis of Dean's classification, 7.6% of children in Hastings, after 16 years of fluoridation, showed evidence of "questionable or very mild" dental fluorosis (15). But in 1982 according to the FDI index, diffuse mottling was 37% among nine-year-olds (10). The methods of measurement can hardly alone explain the great difference in incidence of such opacities in the same fluoridated community.

Other Countries: Increased levels of dental fluorosis have been reported from other countries (16-18). In an "optimal" fluoride (1.06ppm) community, Kewanee, Illinois, 2.4% of the children had definitely unsightly fluorosis, for which no cause, other than the fluoride in the water, could be found (13). This town had been surveyed by Dean more than three decades earlier. The authors state: "Unlike the present study, Dean did not diagnose any children in Kewanee as having either moderate or severe fluorosis" (13). These American researchers apparently did not experience the difficulty which those in New Zealand seem to have in diagnosing dental fluorosis, by its characteristic appearance.

Other Possible Causes: Attempts to link diffuse opacities in teeth with factors other than fluoride have not been particularly successful. Childhood fever, nutritional deficiencies, local injury, local infection, long-term illness, and genetic characteristics have been mentioned (10, (19-21). Critics who claim that the disfiguring tooth defects are uncommon (19) have failed to explain why school dental clinic staff and the author found so many "uncommon" cases in the fluoridated area, but not in the nonfluoridated one. Wilson and Cleaton-Jones (1978) were not able to show any correlation between enamel mottling and infectious exanthemata (22).

It has been claimed that "over 90 different factors" may be responsible for enamel defects (20). The original text referred to all kinds of enamel defects, including "enamel hypoplasia and intrinsic discolouration of the teeth", but in the report of the Victorian Committee of Inquiry, it is inaccurately stated that these factors are implicated in "similar enamel mottling" to dental fluorosis (21). Danish researchers have commented: "Although these factors should be taken into consideration when ascertaining the etiology of enamel defects in individuals, only 'excess fluoride' has been shown to be capable of inducing generalized enamel changes on a population scale as demonstrated by numerous studies" (16).

Balance of Risk versus Benefit: In the present study the caries experience of the Auckland children with dental fluorosis was not recorded. It did not, however, seem to be different from the average for the clinical-patient group to which they belonged.

In other studies, the author has shown that the dental decay treatment experience of Auckland children is related to the socio-economic

levels of the suburbs where they live (23,24). The prevalence of decayed, missing and filled teeth of 12 to 13-year-old children is now lower in the nonfluoridated part of the Auckland Health District than in the fluoridated part of the same economic level (4,25). The percentage of caries-free children is higher in the nonfluoridated areas, when the socio-economic variable is taken into account (24). This important variable has been overlooked in earlier research claiming to show the effectiveness of fluoridation.

The greater prevalence of advanced forms of dental fluorosis found here in association with higher socio-economic levels in the fluoridated area contrasts with a relatively lower incidence of dental caries in these groups (24). Moreover, current dental health statistics indicate that declines in caries rates are now occurring in nonfluoridated communities to about the same extent as in fluoridated ones (17, 24-30). Thus earlier predictions of the dental benefits of fluoridation appear to be considerably exaggerated, and the possible damage, especially from dental fluorosis, greatly underestimated.

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URINARY FLUORIDE EXCRETION IN SKELETAL FLUOROSIS

by

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SUMMARY: Urinary fluoride levels were estimated by 24-hour sampling over a period ranging between 7 and 38 days in 16 subjects of established skeletal fluorosis. The fluoride content of the well or village water, which the subjects were consuming, ranged between 3.8 and 11.4 ppm. Fluoride levels in urine which fluctuated widely from day to day, ranged from 0.5 to 4.48 ppm, minimum, and from 1.5 to 13 ppm, maximum.

No definite correlation was observed between urinary fluoride levels, clinical stage and neurological sequelae. The significance of these findings is discussed.

KEY WORDS: Urinary fluoride; Daily F^- excretion; Skeletal fluorosis

Introduction

Urinary fluoride excretion varies widely from one individual to another. It is reduced in growing children and in pregnant women (1) as well as by simultaneous intake of substances which bind fluoride, such as calcium, aluminum and other metals (2). In persons previously exposed to high fluoride intake, whose fluoride consumption is low prior to sampling, more fluoride is eliminated than taken into their bodies (3,4). Other factors that play a role in urinary fluoride excretion are specific gravity (5) and pH of the urine (6), as well as the fluoride content of food and water. These factors render spot sampling and 24-hr. sampling unreliable.

All previous studies of urinary fluoride excretion in skeletal and nonskeletal fluorosis have been carried out using either spot or 24-hour urinary sampling. For this reason, the present study was designed to evaluate serial excretion of urinary fluoride, of established cases of skeletal fluorosis, in an endemic area over a prolonged period of time.

Materials and Methods

Sixteen subjects of established skeletal fluorosis with or without neurological complications, randomly selected from endemic areas in Andhra Pradesh, India were subjected to a thorough clinical check-up. All

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subjects were evaluated radiologically and were classified into three grades (7). Blood urea nitrogen and serum creatinine clearance were determined in all subjects. The fluoride content of well or village water which the subjects were consuming was also measured. Each subject was advised to collect the 24-hour urine in the plastic container provided. A special buffered preservative* was added to the urine to prevent bacterial growth which might affect fluoride estimation. None of the subjects was taking drugs which could alter urinary pH or bind fluoride. Other variables were unchanged during the period of study. Urine and well water samples were analyzed for fluoride content by the fluoride electrode method (8).

Results

The study group comprised two females and fourteen males, whose ages ranged between 25 and 65 years. All had resided most of their lives in endemic villages and had consumed water containing high levels of fluoride. Radiologically, all cases were classified as having grade III skeletal fluorosis. Twelve of the sixteen subjects had neurological sequelae, eight had radiculomyelopathy, and four had only radiculopathy. Creatinine clearance was normal in all the subjects.

Table 1
24-Hour Urinary Fluoride Excretion by Sixteen
Subjects with Grade III Skeletal Fluorosis

Case No.	Sex	Age	Neurological Condition*	Days studied	F ⁻ in Drinking water		F ⁻ in 24-Hr Urine (ppm)			
					Min.	Max.	Mean	SD	Var-	SD
1	M	50	N	12	5.4	0.5	3.1	1.54	0.55	0.74
2	M	48	N	22	11.4	0.8	3.5	2.22	0.62	0.79
3	M	55	R	38	11.4	1.3	6.0	3.54	1.17	1.00
4	M	53	N	24	9.8	1.0	1.6	1.32	0.04	0.21
5	M	38	R+M	12	3.8	1.3	2.1	1.62	0.08	0.28
6	F	40	R	26	5.7	0.6	1.5	1.10	0.05	0.23
7	M	38	R	22	6.2	1.1	4.5	2.42	0.36	0.60
8	M	45	N	7	7.5	2.5	4.1	3.4	0.24	0.49
9	M	55	R+M	28	7.0	0.9	13.0	5.42	2.00	3.16
10	M	45	M	12	7.5	1.8	3.6	2.76	0.33	0.58
11	M	25	R	12	4.7	1.2	10.0	4.26	4.96	2.22
12	M	38	R+M	10	3.8	1.4	6.5	3.05	2.81	1.68
13	M	50	R+M	7	5.2	1.0	5.0	1.7	2.17	1.47
14	M	35	R	8	8.5	1.8	5.6	4.15	2.02	1.42
15	M	55	R+M	31	4.8	3.2	8.3	4.69	1.83	1.35
16	F	45	R+M	44	8.0	4.5	10.0	6.10	2.49	1.58

* N = Normal; M = Myelopathy; R = Radiculopathy

* Composed of 95% ethyl alcohol (960 ml), 37% formaldehyde (680 ml) and 1472 ml distilled water; citric acid (0.5 N) solution to reach 5.15 pH and a few crystals of thymol.

The fluoride content of the well or village water ranged between 3.8 and 11.4 ppm. The duration of the study of urinary samples ranged between 7 and 38 days, values of which are given in the table. Urinary fluoride levels, which fluctuated widely from day to day, ranged from a minimum of 0.5 - 4.48 ppm to a maximum of 1.5 - 13 ppm. No definite correlation was found between urinary fluoride levels, clinical stage, and neurological sequelae.

Discussion

This study clearly shows that neither spot nor 24-hour urinary sampling for fluoride excretion is a reliable index and that the variation between samples is explained by individual differences. Because urinary fluoride varies widely from day to day in the same individual, any study of urinary fluoride excretion in fluorosis using single spot or single 24-hour sampling is fallacious (9).

Similarly, in view of wide fluctuations in urinary fluoride from one individual to another, statistical analysis utilizing individual values is unreliable. This conclusion is further supported by the fact that the minimum and maximum urinary fluoride values were 0.5 to 4.5 ppm and 1.5 to 13.0 ppm, respectively. When the drinking water fluoride ranged between 3.8 to 11.4 ppm, the lowest minimum and maximum urinary fluoride levels were within the normal range of urinary fluoride in normal individuals consuming water containing 0.4 ppm fluoride (10). Thus, there is some, but no consistent, correlation of urinary fluoride excretion with fluoride intake. High urinary fluoride is consistent with fluorosis but low urinary excretion does not necessarily exclude fluoride-induced illness.

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STUDIES OF THE RELATIVE FLUORIDE CONTENT OF NORMAL AND PATHOLOGICALLY MINERALIZED HUMAN TISSUES

by

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SUMMARY: On the basis of specimens from 298 post-mortem cadavers, fluoride levels in normal and pathologically mineralized tissues were correlated statistically.

In normal arterial specimens, taken from aorta, renal and basilar artery, although fluoride levels were slightly increased in relation to age, the total fluoride concentration did not exceed 40 ppm in the aorta, 2.5 ppm in the renal and 0.8 ppm in the basilar artery. By contrast, in pathologically calcified arteries, the fluoride content was up to 700 ppm in the aorta, 12.5 ppm in the renal artery and 5.8 ppm in pathological arteries, which shows a similar pattern to that in normal bone, although later in onset.

These results suggest that fluoride accumulation in pathologically calcified tissues occurs by the same non-specific process as that in bone, as demonstrated by the linear characteristics of the correlation between values.

KEY WORDS: F^- levels in aorta; F^- in renal arteries; F^- in basilar arteries

Presented at the 13th conference of the International Society for Fluoride Research, Nov. 14-17, 1983, New Delhi, India. Dr. S.M. Mohamedally, 27 Woodside Gardens, Tottenham, London N17 6UY, England.

Introduction

Previously, Mohamedally and Wix (1) have shown a connection between bone mineralization and the rate of osteoid turnover in human subjects and that bone fluoride levels increase steadily with advancing age.

Haythorn and Taylor (2) reported that excess calcium is related to increasing age in mineralized tissues. Since calcium is almost always associated with fluoride in the mineralization process, the present study was undertaken to correlate, statistically, the fluoride levels in normal and pathologically mineralized tissues.

Methods

Post-mortem bone, aorta, renal and basilar artery material from 298 cadavers was obtained from London and Watford Medical Institutions. Fluoride was estimated in ash samples by means of the ion-specific electrode method MAFF (3). An extract of the ashed sample in perchloric acid 0.2M is added to Citrate Buffer pH 8.3. The final pH is adjusted to 5.4 ± 0.2 in order to release fluoride ion from metal complexes. Fluoride activity is measured in millivolt membrane potential and is related to fluoride concentration by a standard curve on 3-cycle log graph paper. The apparatus used was a Corning EEL electrode pH meter, model 12 fitted with a fluoride ion-selective electrode. The results were plotted as ppm versus age of deceased donor in years and examined statistically.

Results

The distribution of fluoride in human post-mortem materials from cases of accidental death in young healthy adults is shown in Table 1. Whereas fluoride accumulation in normal soft tissue is minimal, uptake by

Table 1
F⁻ Levels in P.P.M. (Ash Weight)

Tissues	Average F ⁻ Levels	Tissues	Average F ⁻ Levels
Prostate	0.3	Heart	3.0
Testis	0.3	Kidney	3.4
Pancreas	0.4	Spleen	3.5
Thyroid	0.6	Lung	4.2
Tongue	0.7	Aorta	30.0
Brain	0.7	Finger bone (small)	200.0
Stomach	2.4	Femur	2160.5
Liver	2.9	Skull bone (parietal)	3054.5

Figure 1

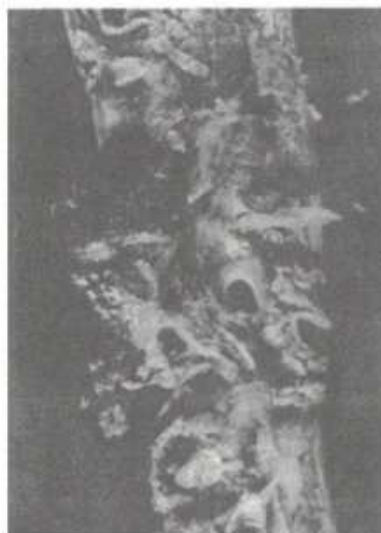
Normal Appearance of Descending
Aorta at Autopsy



Slight intimal lipid accumulation but no obvious calcification evident. F⁻ level 30 ppm.

Figure 2

Pathological Appearance of
Calcified Aorta

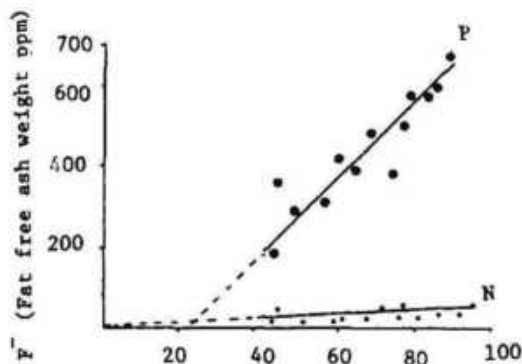


Thrombus near the origin of the mesenteric. F⁻ level in calcified region of aortic wall 600 ppm. Both cases above 50 years old.

pathologically calcified tissues appears to be significant as illustrated in the case of the aortic samples (Fig. 1 and 2). A fluoride level in the pathological artery of 600 ppm is 20 times greater than that in corresponding normal tissue. Fluoride levels of autopsy materials from normal and diseased aortas, as well as from renal and basilar arteries, as a function of age, are presented in Figs. 3, 4, and 5. In addition, the age-dependent increase in fluoride was related to the presence of pathological lesions. In the pathological aorta, levels of fluoride increase rapidly. Levels ranging between 200-700 ppm have been found in test material. The slope is 20 times the slope found in normal aortas and extrapolation to the abscissa shows that (if the process is linear), changes begin to take place at about 22 years of age. In renal and basilar artery, the fluoride content illustrates the same phenomenon but at reduced levels of fluoride. However, in the case of the renal artery, extrapolation of the data for pathological tissues gives the age of onset approximately 11 years earlier than the basilar artery or aortic mineralization.

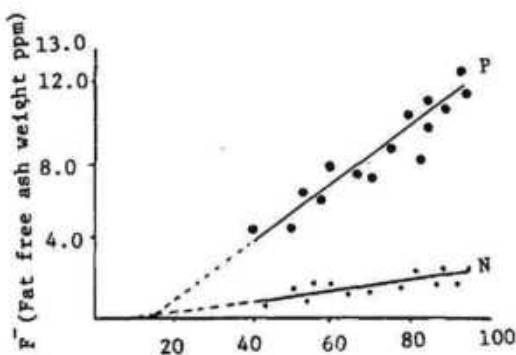
In the present study, 150 iliac bone samples were analyzed for fluoride levels (Fig. 6). Statistical assessments were made, the correlation

Figure 3

F⁻ Levels in Samples of Aorta
as Function of Age

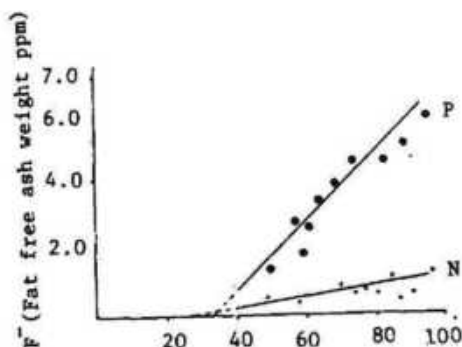
F⁻ levels in normal samples (marked N): Correlation coefficient (R) = 0.774; Slope = 0.401. Compared with pathological material (marked P): Correlation Coefficient (R) = 0.923; Slope = 8.671.

Figure 4

F⁻ Levels in Renal Artery Samples
as Function of Age

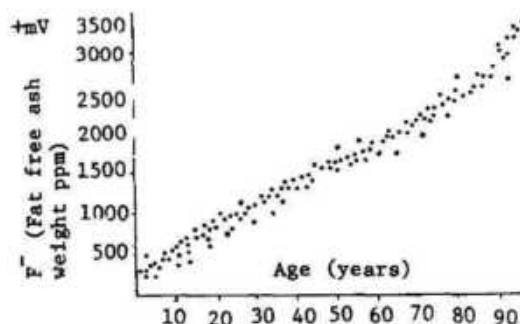
F⁻ levels in normal samples (N) give values of the correlation coefficient R = 0.787; Slope = 0.22, compared with pathological material (P): correlation coefficient (R) = 0.972; Slope = 0.149.

Figure 5

F⁻ Levels in Basilar Artery Samples
as Function of Age

Normal tissues (N) gives a correlation coefficient of 0.773 with a slope of 0.080. Pathological tissue (P) gives correlation coefficient (R) = 0.962 and slope = 0.095.

Figure 6

Bone F⁻ Levels as
Function of Age

The above data show a quasi-linear increase of F⁻ content. Some of the data are the same.

coefficient (R) between the fluoride levels and different age groups was found to be 0.935 and a slope (S) of 28.15. It can be argued that the fluoride content is either a measure of mineralization which is secondary to the pathological changes in the arteries, or that these pathological changes are a consequence of fluoride intake.

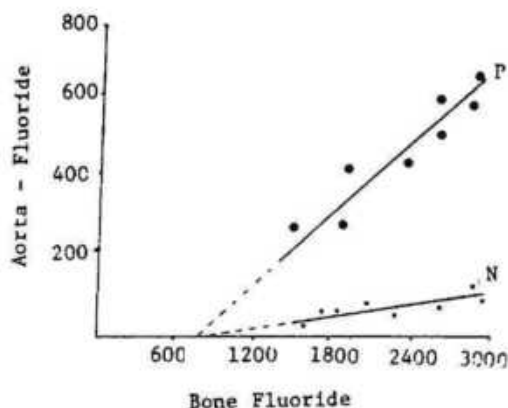
The latter possibility is excluded by the demonstration of separate categories of aortic fluoride levels in relation to bone fluoride levels in the same individual depending on whether arterial disease was present or absent (Fig. 7).

Conclusion

Our study agrees with the conclusion of Haythorn and Taylor (2) that the calcium content of aortic tissue rises linearly with age, since calcium and fluoride are combined in the apatite molecule.

The data are consistent with a view that fluoride uptake is a feature of calcified tissue and is not a special property of teeth and bone. Flu-

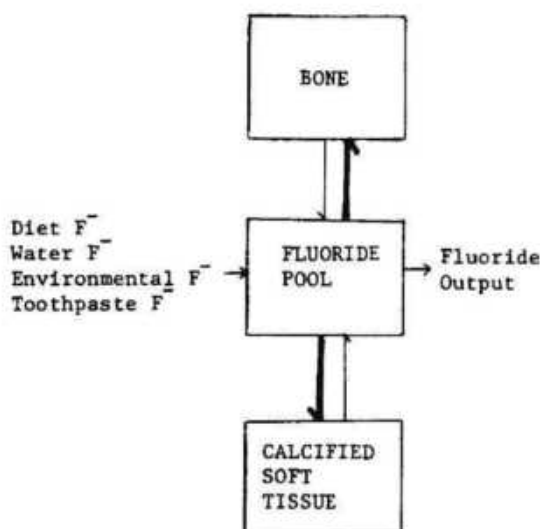
Figure 7
Correlation Between Aortic F^- and
Bone F^- Levels



oride uptake probably occurs by an exchange process summarized in Fig. 8. The age-dependent increase in bone fluoride levels indicates that fluoride uptake by calcified tissue is more rapid than its release which may suggest that fluoride-containing zones of calcification are less susceptible to demineralizing processes. A similar argument may be applied to the accumulation of fluoride in calcified soft tissue such as the aorta, renal and basilar artery which could be a factor leading to the irreversibility of the lesions affecting the arteries; they may be made resistant to demineralization. Therefore, patients with arterial disease may benefit from a restricted intake of fluoride or from treatment with a specific fluoride chelator if such a compound is available.

Figure 8

Diagrammatic Summary of F⁻ Exchange
with Bone and Soft Tissue



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IMPACT OF FLUORIDE EMISSIONS ON CHLOROPHYLL CONTENT OF
SHRUBS IN VICINITY OF A SURAT FLUORINE INDUSTRY

by

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SUMMARY: The impact of fluoride emissions from a fluorine industry situated at Surat, Gujarat State, on chlorophyll content of 4 species of shrubs, namely Prosopis juliflora, Acacia nilotica, Calotropis procera and Zizyphus nummularia was studied. Chlorophyll-b was more affected than chlorophyll-a in polluted plants. In C. procera the percent decrease in chlorophylls was higher than that in the other species under study. The results are discussed in the light of fluoride effect on leaves.

KEY WORDS: Fluoride, air pollution; Chlorophyll-a; Chlorophyll-b; Prosopis juliflora; Acacia nilotica; Calotropis procera; Zizyphus nummularia; Surat, India

Introduction

Since plants are much more sensitive to fluoride pollution than are man, animals or materials, extensive loss to agriculture and deterioration in natural ecosystems results. Therefore, studies of the effect of pollution on vegetation provide an important basis for measures in pollution control (1). The effect of fluoride on germination of seeds (2), growth of plants (3) and yield from plants (4,5), as well as the fact that plants can accumulate substantial amounts of fluoride causing necrosis in leaves (6-9) has been recorded.

Four species of shrubs, namely Prosopis juliflora, Acacia nilotica, Calotropis procera and Zizyphus nummularia growing in the vicinity of the fluoride industry, city of Surat, Gujarat State, India, were selected in the present report to determine the impact of fluoride emissions on chlorophyll-a and chlorophyll-b.

Materials and Methods

During 1978-1979, young, healthy leaves from 6 plants of P. juliflora, A. nilotica, C. procera and Z. nummularia, growing in the vicinity of the fluoride industry and on the nonpolluted South Gujarat University Campus about 12 kms distant from the industry (collected monthly October 1978 - September 1979) were brought to the South Gujarat University Laboratory. Polluted and nonpolluted leaves were separated and leaves were pooled species-wise. Three samples for each group were processed and analyzed for chlorophyll-a and chlorophyll-b content according to Holden (10) as described by Gordon et al. (11). The amount of chlorophylls is expressed as mg/g fresh weight with total chlorophyll

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being expressed as chlorophyll-a + chlorophyll-b. Percentage deviation in chlorophyll in polluted plants from nonpolluted plants is expressed as

$$\frac{\text{Chlorophyll in polluted plants} - \text{Chlorophyll in nonpolluted plants}}{\text{Chlorophyll in nonpolluted plants}} \times 100$$

The student's 't' test was employed to find the significance of the decrease in chlorophyll in the polluted plants.

Results

In *P. juliflora*, fluctuation in chlorophyll-a and -b was not considerable during October-April. On the other hand, chlorophyll-a and -b from both polluted and nonpolluted plants increased up to July then declined in September (Table 1). Chlorophyll-a was significantly reduced in August and chlorophyll-b in June, July and August, in polluted plants ($P < 0.05$). The lowest percent deviation of chlorophyll-a in polluted plants from nonpolluted ones occurred in August (-40.23%). They did not deviate in December, whereas chlorophyll-b of polluted plants deviated -57.36% in August and -2.53% in May.

Table 1

Monthly Fluctuation of Chlorophyll-a, Chlorophyll-b and Total Chlorophyll (mg/g) in *P. juliflora*

Species		1978					1979						
		Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.
Chlorophyll-a	NP	0.62	0.57	0.48	0.56	0.53	0.59	0.49	0.64	0.85	1.13	0.87	0.54
		± 0.04	± 0.05	± 0.03	± 0.04	± 0.07	± 0.04	± 0.07	± 0.08	± 0.17	± 0.05	± 0.05	± 0.07
	P	0.56	0.49	0.48	0.51	0.46	0.53	0.43	0.61	0.66	1.05	0.52	0.43
		± 0.03	± 0.06	± 0.02	± 0.03	± 0.03	± 0.04	± 0.04	± 0.04	± 0.04	± 0.03	± 0.07	± 0.01
	NP	0.71	0.65	0.72	0.64	0.69	0.69	0.65	0.79	0.92	1.54	1.29	0.61
		± 0.07	± 0.06	± 0.02	± 0.07	± 0.01	± 0.09	± 0.02	± 0.08	± 0.01	± 0.03	± 0.25	± 0.09
Chlorophyll-b	P	0.62	0.51	0.60	0.55	0.58	0.57	0.50	0.77	0.77	1.43	0.55	0.46
		± 0.05	± 0.07	± 0.19	± 0.04	± 0.19	± 0.04	± 0.14	± 0.25	± 0.03	± 0.03	± 0.07	± 0.01
	NP	1.33	1.22	1.28	1.20	1.23	1.28	1.25	1.45	1.77	2.68	2.16	1.14
		± 0.11	± 0.11	± 0.08	± 0.11	± 0.08	± 0.13	± 0.23	± 0.08	± 0.77	± 0.06	± 0.26	± 0.17
	P	1.18	1.00	1.09	1.05	1.04	1.11	0.71	1.38	1.43	2.48	1.04	0.90
		± 0.08	± 0.13	± 0.18	± 0.07	± 0.17	± 0.08	± 0.09	± 0.23	± 0.06	± 0.06	± 0.14	± 0.02

NP - Nonpolluted; P - Polluted

The two chlorophylls of *A. nilotica* did not vary considerably until May. Chlorophyll-a was highest in polluted plants in October and in nonpolluted in July. On the other hand, it was lowest in nonpolluted plants in October and in polluted plants in September. Chlorophyll-b was highest in polluted plants in October and in nonpolluted in

July. It was lowest in September, whereas in nonpolluted plants it was lowest in January (Table 2). Chlorophyll-a was significantly reduced in August and September, chlorophyll-b in September in polluted plants ($P < 0.05$). The percent deviations of chlorophyll-a and chlorophyll-b were low in September (-54.26 and -56.80%, respectively). The highest deviation of chlorophyll-b was in February (-10.32%).

Table 2
Monthly Fluctuation of Chlorophyll-a, Chlorophyll-b and
Total Chlorophyll (mg/g) in *A. nilotica*

Species		1978					1979						
		Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.
Chlorophyll-a	NP	0.81	0.96	1.00	0.95	0.91	0.98	0.89	0.96	1.20	1.23	1.02	0.94
		±	±	±	±	±	±	±	±	±	±	±	±
		0.03	0.03	0.03	0.06	0.03	0.05	0.04	0.03	0.03	0.21	0.04	0.01
	P	0.93	0.73	0.77	0.77	0.87	0.79	0.84	0.67	0.77	0.83	0.89	0.43
		±	±	±	±	±	±	±	±	±	±	±	±
		0.16	0.20	0.28	0.14	0.02	0.14	0.01	0.24	0.28	0.16	0.02	0.009
Chlorophyll-b	NP	1.53	1.6	1.22	1.01	1.26	1.05	1.24	1.11	1.45	2.12	1.40	1.25
		±	±	±	±	±	±	±	±	±	±	±	±
		0.10	0.15	0.16	0.27	0.03	0.28	0.03	0.21	0.19	0.70	0.03	0.11
	P	1.33	0.89	0.98	0.86	1.13	0.91	1.11	0.82	0.96	1.21	1.17	0.54
		±	±	±	±	±	±	±	±	±	±	±	±
		0.38	0.46	0.49	0.05	0.29	0.02	0.29	0.40	0.50	0.38	0.30	0.16
Total chlorophyll	NP	2.34	2.11	2.22	1.87	2.18	2.03	2.11	2.07	2.64	3.35	2.42	2.19
		±	±	±	±	±	±	±	±	±	±	±	±
		0.08	0.13	0.14	0.07	0.04	0.24	0.04	0.19	0.16	0.82	0.05	0.04
	P	2.26	1.62	1.74	1.63	2.00	1.71	1.95	1.49	1.72	2.04	2.06	0.97
		±	±	±	±	±	±	±	±	±	±	±	±
		0.53	0.71	0.76	0.15	0.31	0.16	0.31	0.63	0.75	0.50	0.31	0.17

NP - Nonpolluted; P - Polluted

In *C. procera*, chlorophyll-a and -b varied little during May and June (% deviations -4.08 and -5.36 in May, -10.34 and -8.11 in June respectively). Chlorophyll-a, in nonpolluted plants, was highest in July and, in polluted plants, in May. Chlorophyll-b was highest in August in both polluted and nonpolluted plants (Table 3). Chlorophyll-a's decrease in polluted plants was significant in all months, except May and June. The decrease in chlorophyll-b was significant except in May, June and August ($P < 0.05$). The percent deviation of chlorophyll-a was low in August (-50.9%); of chlorophyll-b, in January (-61.9%).

In *Z. nummularia*, chlorophyll-a was decreased in September in polluted plants, chlorophyll-b in August. Chlorophyll-a, in nonpolluted plants, was highest in April, chlorophyll-b in August (Table 4). The decrease in chlorophyll-a, observed in polluted plants, was not significant ($P > 0.05$). In October and August, reduction of chlorophyll-b in polluted plants was significant. The percentage of deviation of chlorophyll-a was low in April and of chlorophyll-b in August. The percentage deviation of chlorophyll-a was highest in December to March, that in chlorophyll-b, in July.

In all months, total chlorophyll in leaves of species from the polluted area was less than that in the nonpolluted area (Tables 1-4). It

Table 3

Monthly Fluctuation of Chlorophyll-a, Chlorophyll-b and
Total Chlorophyll (mg/g) in C. procera

Species		1978					1979						
		Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.
Chloro- phyll-a	NP	0.51	0.47	0.50	0.47	0.50	0.50	0.46	0.49	0.29	0.61	0.55	0.49
		±	±	±	±	±	±	±	±	±	±	±	±
	P	0.01	0.06	0.06	0.02	0.15	0.02	0.05	0.01	0.06	0.006	0.07	0.01
		±	±	±	±	±	±	±	±	±	±	±	±
Chloro- phyll-b	NP	0.26	0.27	0.26	0.24	0.26	0.26	0.24	0.47	0.26	0.31	0.27	0.26
		±	±	±	±	±	±	±	±	±	±	±	±
	P	0.06	0.03	0.06	0.05	0.02	0.05	0.02	0.02	0.06	0.07	0.05	0.02
		±	±	±	±	±	±	±	±	±	±	±	±
Total Chloro- phyll	NP	0.57	0.51	0.56	0.63	0.50	0.66	0.48	0.56	0.37	0.68	0.77	0.50
		±	±	±	±	±	±	±	±	±	±	±	±
	P	0.08	0.03	0.08	0.13	0.03	0.14	0.03	0.10	0.02	0.10	0.16	0.04
		±	±	±	±	±	±	±	±	±	±	±	±
	NP	0.34	0.27	0.33	0.24	0.27	0.26	0.25	0.53	0.34	0.40	0.69	0.26
		±	±	±	±	±	±	±	±	±	±	±	±
	P	0.02	0.04	0.02	0.07	0.04	0.07	0.04	0.09	0.02	0.02	0.14	0.04
		±	±	±	±	±	±	±	±	±	±	±	±
	NP	1.08	0.98	1.06	1.10	0.99	1.17	0.94	1.05	0.66	1.29	1.32	0.99
		±	±	±	±	±	±	±	±	±	±	±	±
	P	0.01	0.10	0.09	0.14	0.04	0.15	0.08	0.10	0.06	0.10	0.23	0.06
		±	±	±	±	±	±	±	±	±	±	±	±
	NP	0.60	0.54	0.59	0.47	0.53	0.52	0.49	0.99	0.60	0.72	0.96	0.51
		±	±	±	±	±	±	±	±	±	±	±	±
	P	0.05	0.07	0.06	0.14	0.06	0.13	0.06	0.10	0.05	0.06	0.12	0.06
		±	±	±	±	±	±	±	±	±	±	±	±

N - Nonpolluted; P - Polluted

Table 4

Monthly Fluctuation of Chlorophyll-a, Chlorophyll-b and
Total Chlorophyll (mg/g) in Z. nummularia

Species		1978					1979						
		Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.
Chloro- phyll-a	NP	0.54	0.44	0.41	0.40	0.40	0.43	0.56	0.52	0.46	0.49	0.51	0.43
		±	±	±	±	±	±	±	±	±	±	±	±
	P	0.10	0.05	0.02	0.03	0.02	0.02	0.31	0.03	0.02	0.02	0.03	0.04
		±	±	±	±	±	±	±	±	±	±	±	±
Chloro- phyll-a	NP	0.46	0.42	0.43	0.42	0.42	0.45	0.45	0.47	0.44	0.45	0.47	0.40
		±	±	±	±	±	±	±	±	±	±	±	±
	P	0.02	0.02	0.03	0.06	0.03	0.04	0.01	0.04	0.04	0.03	0.05	0.007
		±	±	±	±	±	±	±	±	±	±	±	±
Total Chloro- phyll	NP	0.57	0.53	0.62	0.56	0.61	0.60	0.58	0.65	0.66	0.61	0.70	0.54
		±	±	±	±	±	±	±	±	±	±	±	±
	P	0.02	0.20	0.19	0.18	0.19	0.19	0.20	0.23	0.20	0.22	0.22	0.21
		±	±	±	±	±	±	±	±	±	±	±	±
	NP	0.43	0.39	0.38	0.38	0.37	0.37	0.34	0.46	0.36	0.55	0.34	0.38
		±	±	±	±	±	±	±	±	±	±	±	±
	P	0.07	0.06	0.04	0.06	0.04	0.06	0.04	0.07	0.06	0.07	0.006	0.05
		±	±	±	±	±	±	±	±	±	±	±	±
	NP	1.03	0.96	1.03	0.92	1.01	1.03	0.96	0.97	1.12	1.10	1.21	0.94
		±	±	±	±	±	±	±	±	±	±	±	±
	P	0.20	0.20	0.20	0.17	0.20	0.19	0.20	0.11	0.21	0.23	0.22	0.21
		±	±	±	±	±	±	±	±	±	±	±	±
	NP	0.86	0.81	0.81	0.79	0.79	0.80	0.90	0.93	0.79	0.90	0.78	0.80
		±	±	±	±	±	±	±	±	±	±	±	±
	P	0.08	0.08	0.01	0.11	0.07	0.10	0.35	0.09	0.09	0.08	0.04	0.05
		±	±	±	±	±	±	±	±	±	±	±	±

NP - Nonpolluted; P - Polluted

was more pronounced in C. procera and Z. nummularia. The monthly variation in total chlorophyll in all species from polluted and nonpolluted areas was almost parallel during October-March. In P. juliflora, from the polluted area total chlorophyll fell sharply in April, whereas non-polluted plants showed no considerable variation. Total chlorophyll which increased until July in both polluted and nonpolluted plants, in subsequent months decreased. The lowest percent deviations of total chlorophyll from nonpolluted plants occurred in August, the highest in May. The trend of variation in the total chlorophyll in A. nilotica from the polluted and nonpolluted area was the same during October through April. In polluted plants total chlorophyll fell sharply in November, May and September. From May to August, the increase in total chlorophyll was gradual in polluted plants, but in nonpolluted plants it increased sharply in July and decreased during August and September. The lowest percent deviation in total chlorophyll from nonpolluted plants occurred in September, and the highest in October. In C. procera, total chlorophyll in polluted plants showed less variation during October to April; it increased in May, July, and August, but decreased in June and September. Total chlorophyll in nonpolluted and polluted plants varied little in May and June. The lowest percent deviation of total chlorophyll from the nonpolluted area occurred in August, the highest in May. In polluted plants of Z. nummularia, total chlorophyll gradually decreased from October to February, it increased from February till May and again in July, after a slight decrease in June. In non-polluted plants, total chlorophyll was highest in June, July and August. The lowest percent deviation of total chlorophyll of polluted plants of Z. nummularia, from nonpolluted plants occurred in August, the highest in May.

Discussion

Earlier studies (12-14) and the present findings reveal that three important factors explain the difference in fluoride sensitivity of the plants under study. 1. The location of fluoride (surface or interior), 2. The degree of interchange of fluoride between interior and exterior of the leaf, and 3. The translocation of fluoride to leaf tips and margin.

It has been reported that fluoride inhibits chlorophyll synthesis (12,13,15,16). In the present study, decrease in chlorophyll-b and total chlorophyll was observed in all polluted species. Chlorophyll-a was less affected than chlorophyll-b. The percent decrease in total chlorophyll was significantly higher ($P < 0.05$) in C. procera than in P. juliflora, A. nilotica, and Z. nummularia, whereas in the latter three species, it did not vary significantly. Thus C. procera is more adversely affected than the other three species.

In an industrially contaminated area the fluoride content of vegetation can increase by a factor of 2 to 260, particularly in leaf tissues (15,16). About 40% of the fluoride associated with plant leaves is present on the surface. Particulate fluorides, deposited on the leaf surface, are toxic to plants (12). The toxicity is related to the solubility of the fluoride, because inorganic soluble fluorides

can be absorbed directly through the leaves (13,14).

Deposition of particulate fluorides on the surface of leaves is related to leaf-morphology. Leaves of *P. juliflora* and *A. nilotica* (pinately compound) as well as *Z. nummularia* (simple) retain less particulate fluoride compared to *C. procera* (short petioled and larger). In *C. procera*, particulate fluoride deposited on leaves might have played a major role in reducing the chlorophylls, compared to the other three species.

It has been reported that fluoride inhibits chlorophyll synthesis (17-20), which results in necrosis of leaves, thus affecting the transpiration, photosynthesis and respiration in plants.

Acknowledgement

The author thanks Dr. U.H. Mane, Department of Zoology, Marathwada University for critically evaluating this manuscript and the late Dr. K.M. Desai, Department of Biosciences, South Gujarat University for suggestions during the study.

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Correction: In our Abstract of the report on the "Effect of Fluoride on Collagen Synthesis in the Rat" by B. Uslu (April 1984 issue, p. 139), the ranges of hydroxyproline and protein levels in the callus are given as published in the Results section of that report, but obviously they are reversed and should be interchanged and corrected to agree with the values recorded in Table 2 of the report (Res. Exp. Med., 182:7-12, 1983).

The correct values (in $\mu\text{g}/\text{mg}$) are therefore:

	<u>Hydroxyproline</u>	<u>Protein</u>
Control rats	34 to 61	176 to 480
Fluoride-treated rats	24 to 49	152 to 435

by

Z. Machoy
Szczecin, Poland

The Second National Fluorine Symposium was held in Szczecin on September 26-28, 1983, in the framework of XIX Congress of the Polish Biochemical Society. Eight reports and 38 posters were concerned with the study on fluorine compounds in the various fields of biochemistry, analytics, toxicology, nutrition, prophylaxis, and therapy. The program dealt principally with fluorine metabolism in human beings and animals with emphasis on toxicity of the element, fluoride prophylaxis and therapy.

The two-day symposium opened with a ceremony entirely devoted to the scientific achievements of George L. Waldbott, M.D., who had passed away in July 1982. While a large portrait of Dr. Waldbott was displayed on a screen, Dr. Z. Machoy (Szczecin), paid tribute to the late physician's scientific research, his most important publications, and their significance for world medicine. In Poland the work of Dr. Waldbott is considered a principal source of data on fluoride as well as protagonist of a broad spectrum of studies on fluoride, meriting world-wide interest.

M. Guminska (Cracow), in a report entitled "Effect of Fluorine on Metabolism" discussed the initial fate of the fluoride ion when absorbed into the blood and its effect on health due to excessive accumulation. The consequences of the antagonism between fluorine and magnesium, particularly in the process of bone mineralization, were especially highlighted.

Z. Machoy (Szczecin), in his communication "On Compounds Alleviating the Effect of Fluoride Intolerance" discussed chemical factors attenuating the harmful effects of fluorides which include correct electrolytic composition in body fluids (Ca, Mg, Fe), the presence of microelements (Al, Cu, Mo), vitamin C, certain aminoacids (especially cysteine) and, in animals, boron. The mechanism of action of these substances in the organism is diversified.

A report by G.L. Waldbott (USA), entitled "The Effects of Fluoride on the Human Organism," was read by Z. Machoy. In it Dr. Waldbott described his investigations of people who had been drinking fluoridated water or had resided in the environs of factories emitting fluoride. Intoxication by fluoride is characterized by symptoms of the respiratory, gastrointestinal and urinary tracts and of the neuromuscular system. The fluoride ion penetrates membranes of cells and therefore can reach every organ. Dr. Waldbott concluded that fluoride is one of the most reactive toxic substances in the human organism.

A. Put (Szczecin), in a presentation entitled "Experimental Evaluation of Calcium Carbonate: Influence on the Course of Sodium Fluoride Poisoning," assessed the effect of protracted application of fluoride on

selected parameters, biochemical, hematological, morphological and histochemical changes in bones, teeth and other intrinsic organs in rats. Calcium carbonate decreased the intensity of alterations induced by NaF administration.

Two further presentations dealt with prophylaxis with respect to dental caries. In connection with the use of fluoride for caries prophylaxis, Z. Janczuk (Szczecin) in a report "Fluoride Prophylaxis of Caries in Changing Conditions of the Natural Environment of Man," emphasized the necessity of strict regulation of fluoride dosage.

G. Siebert (Wurzburg), whose subject was "Investigations on Bioavailability of Fluoride in Man," dealt with the level of dietary fluoride. Such work requires exact analytical data and measurements of fluoride absorption from the alimentary tract in the course of digestion, both of which are important in estimating the quantity of fluoride recommended for caries prophylaxis.

E. Czerwinski (Cracow), in a communication under the title "Effect of Fluorine on the Osseous-Articular System in Man" discussed the effects of multi-year exposure of bones and joints in man to fluorine compounds. Selected problems in connection with pathogenesis from the action of fluorine compounds on hard tissues were also discussed.

The final report, by A. Zielinski (Szczecin), entitled "Fluoroderivative Organic Compounds as Components of Blood Substitutes," concentrated on the fundamental conditions which should be met by blood-substitutes. The author also explained his own experiments and achievements in the field of chemistry concerning perfluoro-organic compounds used as blood substitutes.

The majority of the posters were devoted to saliva, tooth enamel, and caries prevention. Some were linked with the biochemistry of fluorine (bioenergetics and enzymology) and analytics as well as diverse aspects of fluoride toxicology in man and animals.

M. Guminska (Cracow) summed up the reports and posters. The well-organized discussion, which followed, was frequently controversial. It was possible, however, to elucidate disputed viewpoints and results and to outline the direction of future investigations. Regarding stomatology, whether and in which regions of the country fluoride should be added to drinking water constitutes a dilemma. In enzymology, the question arose respecting the possibility of predicting which enzymes might react with fluoride compounds. In the field of toxicology, the discussion centered on the necessity of producing a protective diet for inhabitants dwelling in regions where fluoride levels of the atmosphere soil and water are elevated. A small exhibit by foreign firms manufacturing reagents and ion-selective electrodes accompanied the symposium.

The next fluoride symposium will be held in 1985/86 in Szczecin with invited lecturers, including scientists from abroad.

SODIUM FLUORIDE-INDUCED MORPHOLOGICAL AND NEOPLASTIC TRANSFORMATION,
CHROMOSOME ABERRATIONS, SISTER CHROMATID EXCHANGES AND UNSCHEDULED
DNA SYNTHESIS IN CULTURED SYRIAN HAMSTER EMBRYO CELLS

by

Takeki Tsutsui, Nobuko Suzuki, and Manabu Ohmori
Tokyo, Japan

(Abstracted from Cancer Research, 44:938-941, 1984)

Fluoride, an ubiquitous substance found naturally in food and water and utilized extensively for industrial purposes, is being widely recommended for prevention of dental caries by various methods, such as fluoridation of communal water supplies; mouth rinsing with fluoride solutions; toothbrushing with fluoride, gels and pastes; and application of fluoride gels in mouthpieces. Whereas the cytogenetic effect of F^- on mammalian cells in vivo and in vitro is not clear, NaF induced chromosome aberrations in cultured human leukocytes, as well as in cultured ovarian oocytes from mice, ewes, and cows.

Since many chemical carcinogens cause chromosome aberrations and DNA damage, it was the authors' aim to determine whether NaF could induce neoplastic transformation of early-passage SHE (Syrian hamster embryo) cells which are used widely for the study of in vitro carcinogenesis. The assay system of Berwald et al. was used which is rapid, quantitative and distinguishes carcinogens from noncarcinogens with a high degree of accuracy. Present results indicate that NaF induces morphological and neoplastic transformation of SHE cells in culture as well as chromosome aberrations, SCEs (sister chromatid exchanges), and UDS (unscheduled DNA synthesis) in the same cells.

Mass cultures of cells treated with 100 μ g NaF/ml for 24 hrs resulted in about 50% lethality. As concentrations of NaF increased treatment caused morphological transformation which was indistinguishable from morphologically altered colonies induced by benzo(a)pyrene and other chemical carcinogens. Whereas no morphological transformation of SHE cells was observed in untreated cultures, the frequency of transformation increased with increasing NaF doses. NaF also induced neoplastic transformation of SHE cells. When NaF treated cells at 120 to 270 doublings of 35 to 50 passages after treatment were injected into newborn hamsters, one of 2 cultures (I-75 and II-75) treated with 75 μ g NaF/ml and 2 of 2 cultures (I-100 and II-100) treated with 100 μ g NaF/ml formed progressively growing tumors at the site of injection. The latent period for tumor appearance was 28 to 39 days.

Histological examination revealed that the tumors formed in vivo were produced by injection of untreated cells.

Chromosome aberrations, which significantly increased in NaF-treated cells, were induced at the chromatid level, and the incidence increased in a dose-dependent manner. NaF treatment of cells for 24 hrs also caused a dose-dependent increase in SCE frequency, which was elevated

Table 1

Injection of Cells from NaF-Treated and Untreated
Cultures into Newborn Hamsters

Cells	No. of passages	Days in culture	Days of observation after inoculation	No. of tumor-bearing animals/ no. of animals tested
I-K	74	320	360	0/8
I-75	74	316	360	0/10
II-75	34	141	28	3/3
I-100	48	199	35	3/8
II-100	36	141	39	8/8

approximately 2-fold by 80 μ g NaF/ml. When SHE cells were treated for 4 or 8 hrs with 10 to 40 μ g NaF/ml, UDS was not detected.

The mechanisms by which NaF induces transformation of SHE cells are unknown. It is conceivable that, since NaF induced chromosome aberrations SCEs, and UDS in the cells, NaF treatment results in DNA damage which is involved in the initiation of transformation. Emsley et al. have proposed that F^- could play a disruptive role towards DNA through a N-H...F...H-N hydrogen bond in the base pair of thymidine-adenine base pairing.

Whereas UDS induced by NaF in SHE cells was not detected when the cells were treated for 4 or 8 hrs, significant UDS levels were detected in the cells treated more than 12 hrs. The delay in response of DNA repair in NaF-treated cells may be explained as follows: (a) the DNA damaging activity of fluoride may be weak or insufficient to induce detectable DNA damage during the short treatment time of our conditions; (b) inhibition of protein synthesis by fluoride may retard the progression of DNA repair following DNA damage.

The present studies are directly relevant to the carcinogenic potential of NaF, since they provide evidence that NaF induces morphological and neoplastic transformation of cells in culture. Therefore, NaF could be potentially dangerous to humans.

KEY WORDS: Syrian hamster embryo cells; Chromosome aberrations; Sister chromatid exchanges; DNA synthesis; F^- effect on cell cultures; Neoplastic transformation

Reprints: Dept. of Pharmacology, Nippon Dental Univ., Tokyo Faculty, 1-9-20 Fujimi, Chiyoda-ku, Tokyo 102, Japan

ACID REACTIVITY OF CARBONATED APATITES WITH
STRONTIUM AND FLUORIDE SUBSTITUTIONS

by

J.D.B. Featherstone, C.P. Shields, B. Khademazad,
and M.D. Oldershaw
Rochester, N.Y.

(Abstracted from J. Dent. Res. 62:1049-1053, 1983)

The purpose of the present study was to prepare synthetic carbonated apatites in the presence of strontium and/or fluoride, to measure their acid reactivity, and to relate this to chemical composition and structure.

Studies on the reactivity of carbonated apatites are directly applicable to our understanding of one part of the caries process. It is possible that strontium and/or fluoride has a significant effect on the reactivity of the enamel crystals; hence, on caries susceptibility.

Carbonated apatites, containing approximately 3.0 or 6.5% CO_3 were precipitated in the presence of sodium, strontium, and/or fluoride at various concentrations. Almost all available strontium and fluoride was incorporated into the crystals whereas only a portion of the carbonate and sodium present was taken up. When incorporated together, Sr and F^- improved the crystallinity of carbonated apatites and markedly reduced their acid reactivity.

Samples with approximately 3% carbonate are considered to be applicable to dental enamel chemistry. Whereas fluoride present in the crystal at these concentrations does not intrinsically reduce acid reactivity, higher carbonate samples of about 6.5% - which are more relevant to bone or dentin or to the upper limit of enamel carbonate content - did improve crystallinity and did reduce acid reactivity. Although fluoride alone failed to measurably reduce the reactivity of low-carbonated apatites or to improve its crystallinity, the combination of strontium and fluoride in carbonated apatite crystals reduced reactivity by about one-half of that in low carbonated apatites.

Structural properties of carbonated apatites can be markedly changed by incorporation, during synthesis, of such trace metals as strontium and zinc, with fluoride. Unusually low caries levels in some Ohio and Wisconsin towns are apparently associated with naturally occurring combinations of strontium and fluoride in drinking water. Strontium and/or fluoride may have a significant effect on the reactivity of enamel crystals; hence, on caries susceptibility.

Acid reactivity of carbonated apatites, as measured by initial dissolution rates in 0.01 mol/l acetate at pH 4.5, increased markedly in proportion to carbonate inclusion. This reactivity differential, compared with that of noncarbonated hydroxyapatite, could be reduced by

FLUORIDE

half when strontium and fluoride were incorporated together. Crystal defects appear to determine carbonated-apatite reactivity; they can be beneficially modified by inclusion of strontium and fluoride in the presence of carbonate. A direct relationship between acid reactivity as measured by initial dissolution rate, and decrease in crystallinity as indicated by (211) half-widths, has been clearly demonstrated.

The effect of strontium and fluoride together, observed here, is possibly related to substitution of strontium in the triangular positions of calcium deficiency, and subsequent stabilization against acid attack. This phenomenon may partially explain the observed reduction in caries in areas where drinking water contains 5.10 mg Sr/l and F^- at 1-2 mg/l.

Editor's Note: For a recent report on the role of strontium in reducing the incidence of dental caries, see Athanassouli, T.M., Papastathopoulos, D. S., and Apostolopoulos, A.X.: Dental Caries and Strontium Concentration in Drinking Water and Surface Enamel. J. Dent. Res., 62: (9):989-991, 1983.

KEY WORDS: Apatite carbonated; Strontium; Fluoride in apatite.

Reprints: Dept. of Oral Biology, Eastman Dental Center, 625 Elmwood Ave., Rochester, N.Y. 14620

PHARMACOKINETICS OF FLUORIDE GELS IN CHILDREN AND ADULTS

by

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(Abstracted from Caries Res., 15:213-220, 1981)

Plasma fluoride (F^-) concentration and urinary F^- excretion were studied in 8 children aged 5-16 and 5 adults aged 23-26 all of whom were living in an area with 0.2 ppm F^- in water. Three types of gels were tested: (1) Luride topical acidulated phosphate fluoride gel, orange flavor, 1.23% F^- (Hoyt Laboratories); (2) Gel II topical fluoride, acidulated phosphate, chocolate flavor, 1.23% F^- (Pacemaker, Portland, OR) and (3) fluor gel 0.1% F^- (LIC Dental, Stockholm).

The amount of F^- gel applied to each child was 3.33 ± 0.05 g (mean \pm SD). The amount of F^- ingested was 31.2 ± 6.4 mg. The results show that topical application of 1.23% F^- gels may result in plasma F^- levels high enough to cause a decrease in urinary concentration ability.

Most brands have relatively high F^- concentrations, usually ranging from 0.5 to 1.23%. In some cases of high caries activity, home care programs involving 1.23% F^- gel applications as often as twice daily over a period of 1-2 weeks have been reported among both children and adults.

Following topical F^- treatment, varying quantities of the material are retained in the oral cavity and swallowed. In this way, F^- is distributed throughout the body via the blood circulation and may result in systemic effects.

During the first 8 hrs. after gel application, the rate of urinary F^- excretion ranged from 123 to 1006 $\mu\text{g/h}$. The excretion rate fell to $43 \pm 26 \mu\text{g/h}$ during the next collection period (12 h) and variation between subjects decreased. F^- excretion was still elevated during the next 12-hour period ($64 \pm 38 \mu\text{g/h}$). Total urinary F^- output (0-28) ranged between 1283 and 9386 μg . By subtracting this value from the F^- dose ingested, approximately 85% of the total dose, ($26.4 \pm 4.2 \text{ mg } F^-$) was not recovered in the urine. In other words, F^- from the ingested gel was almost completely absorbed. One death has been reported following topical F^- treatment.

The high plasma levels noted in the children can be explained by the high F^- doses ingested per kilogram body weight. For instance, in subject P.U., who received a dose of 1.8 mg/kg body weight, plasma levels were over 1400 ng/m ($36 \mu\text{M}$). These results demonstrate the close relationship, in all children, between ingested dose per kilogram body weight and resultant plasma F^- levels. In adults, although 50-60% of ingested F^- may be retained in the bone pool, this figure varies due to differences in renal F^- clearance.

On a fasting stomach almost all of the F^- swallowed with the gel is absorbed by the gastrointestinal tract. These types of F^- preparation may result in plasma levels as high as 1443 ng/m, ($76 \mu\text{M}$) in children.

Side effects, such as nausea, vomiting and gastrointestinal pain have been reported following F^- gel treatment. Gastrointestinal side effects, from intake of high doses of F^- are due to formation of hydrogen fluoride and sodium hydrogen fluoride in the stomach. Studies in rats have shown a correlation between renal dysfunction and increased serum F^- concentrations caused by polyuric renal insufficiency following NaF injections.

With this background information a certain caution should therefore be observed regarding use in children of high F^- concentration gels. It is also questionable whether there is any significant advantage in employment of high-concentration F^- gels in view of obvious risks of systemic side effects.

KEY WORDS: Fluoride; F^- gel; Plasma; Urinary excretion; Toxicity

Reprints: Dept. of Cariology, School of Dentistry, Karolinska Institutet, Stockholm.

FLUORIDE

FLUORIDE PHARMACOKINETICS DURING ACID-BASE
BALANCE CHANGES IN MAN

by

J. Ekstrand, M. Ehrebe, G.M. Whitford
and P.O. Jarnberg

(Abstracted from Eur. J. Clin. Pharmacol. 18:189-194, 1980)

Five healthy subjects were each given fluoride 3.0 mg (F) as sodium fluoride tablets on two occasions - during production of acid urine, induced by giving NH_4Cl , and during production of alkaline urine obtained by giving NaHCO_3 . Frequent plasma and urine samples were taken up to 12 h and were analyzed with a F^- sensitive electrode. Control experiments without F administrations were also performed to permit calculation of net F concentration in plasma and urine. The urine F excretion was lower during acid than alkaline diuresis. Pharmacokinetic analysis of the net plasma F concentrations showed that the apparent plasma half-life of F was longer when urine was acid (4.3 ± 0.6 h; SD; $n = 5$) than when it was alkaline (2.4 ± 0.4 h). This could be explained by changes in the renal clearance of F, which was always lower with an acid (61.5 ± 8.1 ml/min) than an alkaline (97.8 ± 10.4 ml/min) urine. The apparent extra-renal clearance, which mainly represents clearance to the bone pool, was also significantly higher during production of alkaline (109.2 ± 20.2 ml/min) than of acid (86.3 ± 21.3 ml/min) urine. It is suggested, that increased reabsorption of non-ionic hydrogen fluoride (HF) is responsible for the decreased renal clearance during acidic conditions.

KEY WORDS: Fluoride; Renal clearance, urinary pH, fluoride kinetics

Reprints: Department of Cariology, Karolinska Institutet, Stockholm,
Sweden

Authors' Abstract

FLUORIDE IN WATERS, SOILS AND VEGETATION OF
WESTERN BUENOS AIRES PROVINCE

by

R.S. Lavado, N.B. Reinaudi, and J.A. Vaquero
LaPlata, Argentina

(Abstracted from Ciencia del Suelo, 1:9-14, 1983)

The fluoride content of soils, phreatic waters and vegetation of Carlos Tejedor County were studied by colorimetric and other standard

analytical methods. Fluoride levels were related to clay content. Whereas total fluoride values were low, water-soluble fluoride content was high; the latter was associated with soil alkalinity and salinity, and phreatic water depth. Total fluoride was not correlated with available phosphorus, lime or organic carbon. On the other hand, soluble fluoride correlated with organic carbon but not with available phosphorus or lime. Vegetation was likewise high in fluoride. The highly soluble fluoride content of soils, derived from shallow groundwater, has probably been spread by floods.

Water fluoride ranged between 1.26 and 2.25 ppm, total F^- in soil ranged from 19 to 375 ppm, soil soluble F^- ranged from 0.50 to 9.75 ppm, F^- in plants ranged from 37 to 80 ppm.

KEY WORDS: Soluble and total F^- ; Alkalinity; Salinity; Groundwater

Reprints: PROSAG (Fac. Agronomía - CONICET). An San Martín 4453 - 1417 Buenos Aires, Argentina

MUD-BATH THERAPY OF A PATIENT WITH OSTEOARTHRITIS DEFORMANS EXPOSED TO FLUORINE AND ITS COMPOUNDS

by

L.A. Kozlava, and L.G. Zhukova
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(Abstracted from Voprosy Kurortologii Fizioterapii i Lechebnoi
Fizicheskoi Kultury May-June 1983, pp. 32-34)

The effect of mud application, combined with diet, therapeutic exercises and massage on the course of arthrosis and concomitant toxicological hepatitis - detected frequently in metallurgic workers who had been exposed to the action of fluoride and its compounds - was studied.

The most pronounced therapeutic effect was obtained by means of low-temperature mud application (38 - 40°C) to the affected joints and the lumbar region combined with drinking slightly mineralized water containing organic substances.

KEY WORDS: Industrial fluorosis; Mud-bath therapy

Reprints: Research Institute of Balneology and Physiotherapy, Sverdlovsk, USSR

FLUORIDE

- Alamir, B. 35-41
 Alhava, E.M. 202
 Allmann, D.W. 210-217
 Anand-Srivastova, M.B. 138
 Anand Vandena 14-22
 Anderson, R. 58-59
 Azzouz, M. 35-41

 Batish, K.L. 168-172
 Batish, M.K. 168-172
 Baylink, D.J. 143-144
 Bhatnagar, D.P. 168-172
 Bobyleva-Guarriero, V. 94-104
 Boros, I. 183-192; 217-223
 Bourbon, P. 124-131
 Brunetti, A.J. 53-54
 Bruun, C. 57
 Burk, D. 63-71

 Cantin, M. 138
 Caroli, S. 200
 Cazieux, A. 107-114
 Chandra Shiv 155-159
 Chitra, T. 105-107
 Chomiak, E. 114-118
 Christi, D.P. 55-56
 Clonfero, E. 200
 Colquhoun, J. 234-242

 Daijei Huo 9-14
 DeChateau, P. 204
 Denine, R. 35-41
 Dominok, B. 23-26; 193-196
 Drif, M. 35-41

 Ehrnebo, M. 201; 266
 Ekstrand, J. 59-60; 201; 264-265;
 266
 Elferink, G.R. 72-80
 Elsaid, J. 35-41
 Enriquez, B. 107-114
 Evans, W.H. 142

 Farley, J.R. 143-144
 Featherstone, J.D.B. 263-264
 Fejerskov, O. 57; 59-60
 Forsman, B. 60-61
 Frege, J. 23-26
 Fretwell, L.D. 205
 Fujimora, S. 27-35

 Geetanjali, N. 243-246
 Genest, J. 138
 Ghouini, A. 35-41
 Graham, J.R. 63-71

 Hamrour, S. 35-41
 Hardell, L.I. 204
 Hodge, H. 53-54
 Holm, A.K. 58-59
 Honda, M. 144
 Horiuchi, T. 173-177
 Hughes, P.E. 94-104

 Ichinohe, S. 119-123
 Iino, S. 203
 Iklef, F. 35-41
 Ingram, C.S. 61-62
 Ishizawa, Y. 119-123
 Isozaki, A. 203
 Itai, K. 27-35; 159-167

 Jarnberg, P.O. 201

 Kameyama, H. 144
 Kani, M. 203
 Kani, T. 203
 Kann, J. 57
 Keqin Wu 148-154
 Keszler, P. 183-192; 217-223
 Khademazad, B. 263-264
 Khelfat, K. 35-41
 Kidwai, A.M. 178-182
 Knuuttila, M. 202
 Koch, G. 264-265
 Kozlava, L.A. 267
 Kravitz, E. 54-55
 Krook, L. 199-200

 Lammi, S. 202
 Lappalainen, R. 202
 Lardy, H.A. 94-104
 Lavado, R.S. 266-267
 Leybin, L. 53-54
 Levy, P. 124-131
 Lillie, J.H. 199-200
 Lindgren, L.E. 264-265

 Machoy, Z. 197-198; 259-260
 Maheshwari, U.R. 53-54
 Mane, U.H. 224-233
 Masahiro, Ueda 141

- Maylin, G.A. 199-200
 McDonald, T.J. 53-54
 Merad, R. 35-41
 Milhaud, G. 107-114
 Miszke, A. 114-118
 Mita, M. 119-123; 159-167
 Mohamedally, S.M. 246-251
 Morimoto, M. 173-177
 Motoo Niwa 141
 Mozsik, Gy. 217-223
 Murthy, J.M.K. 243-246

 Naceur, J. 35-41
 Nakashizuka, T. 144
 Nakaya, S. 159-167
 Nash, P.F. 61-62
 Nasu, I. 173-177
 Nath, M. 48-52
 Newbrun, E. 53-54
 Newman, J.R. 41-47

 Ogura, N. 144
 Ohmori Manabu 261-262
 Okada, Y. 144
 Oldershaw, M.D. 263-264
 Otsuba, Y. 144

 Peterson, L.G. 264-265
 Pillai, K.S. 224-233; 252-258
 Poey, J. 35-41
 Pollack, R.L. 54-55
 Post, G.P. 206

 Ramana Rao, J.V. 105-107
 Rammohan Rao, N.V. 243-246
 Rasmussen, P. 131-138
 Ratbour, R.S. 48-52
 Raza Haider 178-182
 Read, I. 142
 Reddy, Raja D. 243-246
 Reggabi, M. 35-41
 Reinaudi, N.B. 266-267
 Richards, A. 59-60
 Rioufol, C. 124-131
 Ritlop, B. 183-192

 Sakuma, N. 203
 Sakurai, S. 159-167
 Sankowski, E. 114-118
 Sato, T. 159-167
 Schaper, R. 183-192

 Schneider, V.S. 53-54
 Shahed, A.R. 210-217
 Shayiq, R.M. 178-182
 Sherlock, J.C. 142
 Shields, C.P. 263-264
 Shimizu, M. 203
 Sidhu, S.S. 168-172
 Siefert, K. 23-26
 Sinclair, H.M. 1-3
 Singh, C.V. 14-22; 48-52
 Singh, D.P. 14-22; 48-52
 Singh, M. 81-93
 Singh, N.P. 14-22; 48-52
 Smith, G.E. 145-147
 Snyder, R. 206
 Spak, C.J. 204
 Suzuki Nobuko 261-262

 Tabet Aoul, M. 35-41
 Takehana, S. 144
 Tatsumi, M. 159-167
 Taves, D.R. 140
 Teotia, M. 14-22; 48-52
 Teotia, S.P.S. 14-22; 48-52
 Thergaonkar, V.P. 155-159
 Thylstrup, A. 57
 Tomar, N.P.S. 14-22; 48-52
 Toshio Imai 141
 Tsunoda, H. 27-35; 119-123; 159-167
 Tsutsui Takehi 261-262
 Tuncel, E. 4-8

 Uslu, B. 139; cn. 258

 Vaquero, J.A. 266-267
 Vegh, A. 183-192

 Wallace, R.S. 199-200
 Walters, C.V. 142
 Wergedal, J.E. 143-144
 Whitford, G.M. 266
 Winiarski, J. 114-118

 Xu, Z.J. 62

 Yumin Li 148-154

 Zhukova, L.G. 267

- Acclimation capacity, 105-107
 Acid phosphatase, 144
 Actinomycetes, 198
 Adenylate (or adenylyl) cyclase, 2
 138, 210-217, 217, 222
 Air pollution, 159-160, 193-196
 Algeria, 35-41
 Alkaline phosphatase, 143, 144, 197
 Alkalinity, 267
 Alveolar bone necrosis, 200
 Aluminum
 factory, plant or smelter, 42,
 164-166, 193, 197, 200
 fluoride molecular absorption
 spectrometry, 27-35
 plant workers, 114-118
 Ambient Air Quality Standards, 42-43
 Amides, 145
 Analyses
 biochemical, 82-84, 89
 cytochemical, 82-83, 86, 89
 Analysis
 of
 bone, 125, 184
 calcium, 133
 plasma, 126
 urine, 126, 133
 ANOVA, 244, 247
 Apatite
 carbonated, 263-264
 crystal growth, 62
 Aquatic
 invertebrates
 bivalve molluscs, 224
 crabs, 224
 vertebrates
 trout, 224
 Argentina, 267
 Arteries, 7
 Arthralgia, 153
 Arthritis, 115
 Arthrosis, 267
 Atomic absorption spectrophotometry, 16, 82, 133, 184
 ATP, 94, 102, 246
 ATPase, 82-83, 86-88, 90, 227-228
 Australia, 145
 Benzene metabolism, 206
 B-glucuronidase, 72-76, 144
 Blood
 fluoride effect on, 27
 urea nitrogen, 244
 Bone
 alveolar necrosis, 200
 clearance to pool, 266
 collagen, 1-3
 density, 148-154, 202
 disease, 14-22, 55-56
 impaired growth, 9-14
 Bovine serum albumin, 212
 Brown coal-fired stations, 193-19
 Cadaver, 246
 Calcium, 15, 17, 53, 72-80, 131-1
 208, 231, 247, 259, 264
 Calcification, arterial, 4
 California, 53, 143
 Calmodulin, 72, 77-79
 cAMP, 178-182, 210-217, 217-223
 Cancer, human, 63-69
 Caries prophylaxis, 205
 Cat, 178
Catla catla, 224-233
 Cattle, 60, 199-200
 Cell growth, 141
Channa punctatus, 105-107, 224
 Chicken, 94-104, 143-144
 China, 9, 13, 62, 148, 206
 Chondrocytes, 2
 Chlorophyll
 a, 252-258
 b, 252-258
 Chromosome aberrations, 261-262
 Chicken
 hepatocytes, 94-104
 metabolism, 94-104
 Circadian rhythm, 173-177
 Clean Air Act, 42
 Collagen, 1, 139, cn. 258
 Compound 1080 (poison), 94
 Congress, International Society
 for Fluoride Research, 1-2
 Copper wedge plates, 149
 Creatine kinase, 1
 Denmark, 57
 Dermatan sulphate, 1
 Dermatoglyphics, 168-172
 Dental
 caries, 57, 62, 260, 261
 Dimethylsulfoxide, 73-74
 DNA, 141, 261-262
 Dog, 176
 Dystrophies, human muscular, 1

- Electrolysis shop, 114-118
 Enamel, mottled, 4, 10, 60-61
 Endangered wildlife species, 45
 England, 61, 142, 246
 Environmental Protection Agency, 42
 Enzyme, 72-73, 78, 81-82, 90-91, 99, 102
 Examinations
 cytologic, 116, 118
 histologic, 116
 Exocytosis, 72-80
 Exostosis, 115
- Factory workers
 aluminum, 207
 fertilizer, 207
- Federal Clean Air Laws, 41-47
 Finland, 202
 Fish, 105-107, 132, 142, 145, 224-233
 Flame spectrophotometer, 174
 Florida, 41
 Fluoremia, 36, 127, 219-130
 Fluoridation-cancer link, 63-69
 Fluoride
 air pollution, 252-258, 259
 anti-carries action, 61-62
 atmospheric, 207
 bone content, 23-26
 calcium, dietary, 15, 17, 20-22
 compounds in work environment, 197
 daily excretion, 243-246
 dietary, 9, 44, 59, 132, 140, 142, 145, 167, 183, 197
 electrode, ion selective, 16, 23, 25, 27-28, 33, 34, 36, 110, 121, 125, 126, 133, 136, 184, 211, 243, 244, 247, 260, 266
 effect on
 aquatic animals, 224-233
 cAMP, 40
 cell cultures, 261-262
 collagen synthesis, 139, cn. 258
 domestic animals and wildlife, 43-44
 mitochondria ATPase activity, 40
 protein synthesis of cells, 141
 renal function, 35-41
 endemic, 14-22
 environmental, 159-167
 gel, 264, 265
 hydrogen, inhaled, 124-131
 impregnation, 107-114
 in
 apatite, 263, 264
 arteries, 246-251
 atmosphere, 125
 blood, 61
 bone, 107-114, 115, 118, 124-131, 140, 143-144, 183-192, 199, 202, 246-247
 cattle (dental), 199-200
 chicken, 54-55
 correlation with radiological grade, 35-41
 dental products, 203, 210, 238-239
 See, also, Fluoride in toothpaste
 diets, 53, 54-55, 60-61
 feces, 53
 feed, 125
 fish, 132, 142, 224-233
 food, 9, 44, 59, 132, 140, 142, 145, 167, 183, 197
 gladiolus, 166
 human tissues, 246-251
 miscellaneous sources, 54
 plasma, 15-17, 124-131, 176, 190
 serum, 136, 140, 183-192
 tooth enamel, 183-192
 toothpaste, 58, 59, 145-146, 238-239, 261
 turkey, 54-55
 urine, 15-17, 36, 53, 116-117, 119-123, 124-131, 131-138, 140, 155-159, 159-167, 173-177, 201, 243-246
 water, 4, 7, 9, 15-17, 20, 35-41, 44, 48-52, 53, 60-61, 63-69, 105-106, 112-113, 117, 125, 132, 140, 144, 145-146, 149, 155-158, 159-160, 164-167, 168, 172, 178, 183-192, 195, 204, 206, 208, 217, 221, 224, 237, 239, 243-246, 259-260, 261, 263-264, 264-265, 267
 intake, 62
 intoxication. See, also
 endemic fluorosis, acute in humans, 201
 cattle, in, 199
 chronic, 4
 ion-selective electrode. See

- fluoride, electrode, ion-selective kinetics, 266
- microdetermination, 27
- minerals, containing, 48
- nonendemic 14-22
- nutritional status, 14-22
- organo, 94
- pharmacokinetic behavior, 136
- plasma, 59-60
- rat, experimentally treated with 226, 230, 258, 260, 265
- residual, after mouth rinsing, 203
- respiratory absorption, 119-123
- sensitive microcapillary electrode, 184
- serum, 27-35
- sodium
 - aerosol, 119-123
 - bone-cell stimulation, 143-144
 - cell-growth inhibition, 141
 - chromosome aberrations, 261-262
 - creatine kinase, increase in, 1
 - cyclic AMP, increase in, 178-182, 221
 - exocytosis induced by, 72-80
 - food, in. See Fluoride in food.
 - glycogenolysis enhancement, 210-217
 - osteoporosis, treatment of, 15
 - oxygen consumption, decrease of, 105-107
- soluble, 267
- standards, 41-47
- tablets, 57, 58-59, 131-138, 266
- technique for measuring in blood and urine, 36
- toothpaste, 58-59, 145-146, 237-238, 261
- toxicity
 - chronic, 48-52, 55-56
 - gel, from topical application of, 264-265
 - prognostic test of, 2
- urinary, factors for individual differences, 207
- workers, retired, 23-26
- Fluorine, industry effluents, 242-251, 252-258
- Fluoroacetate, 94-104
- Fluorocitrate, 94, 95, 97
- Fluorosis
 - bone, 148-154
 - chronic, 94
 - dental, 57, 60-61, 62, 146, 155-157, 165, 199-200, 208, 233-240
 - enamel, 58-59
 - endemic, 4, 14-22, 35-41, 48-52, 56, 155-159, 206
 - eradication of, 48-52
 - experimental. See, also, Rat and Fluoride, rat, experimentally treated, 72-80, 81-93, 94-104, 105-107, 107-114
 - industrial, 148-154, 267
 - injuries caused by, 114-118
 - nonendemic, 14-22
 - radiological changes, 7, 9-14
 - skeletal, 4-8, 9-14, 16, 208, 243-246
- Food, fluoride in, 132, 140, 142
- Fracture, healing of, 139
- France, 107, 124
- Freon, 195
- Fungi, 198
- Gastric secretion, 178-182
- GDR, 23, 193
- Genu
 - valgum, 11, 13, 21, 55
 - varum, 20-21, 55
- Glycogen, 72, 224-233
- Glycogenolysis, 210-217
- Glycolysis, 210-217
- Glyconeogenesis, 94-104
- Glycosaminoglycans, 197
- Groundwater, 267
- Growth, 107-114
- Guinea pig, 72, 124-131
- Hamster, 261-262
- Hepatic chromosomes, 206
- Hepatocytes, 210-217
- Histochemical reactions, 197
- Human milk, 204
- Hungary, 183, 217
- Hydrofluoric acid, 42, 117-118
- Hydrogen fluoride, 193-196, 265
- Hydroxyapatite, 61-62
- Hydroxyproline, 139, 197; cn. 258
- Hypercementosis, 200
- Hyperparathyroidism, 56. See, also Parathyroid hyperplasia
- Impact assessment, 41-47
- Industrial fluorosis
 - aluminum, 149, 159, 164-166, 191

- ceramics, 159, 193
- enamel, 193
- glass, 193
- phosphate fertilizer, 149, 159, 164-166, 193
- steel, 159
- India, 1, 2, 13, 14, 48, 81, 105, 155, 168, 178, 224-233, 252
- Indiana, 210
- Infants, 204
- Iodine, 48
- Ion-sensitive (fluoride) electrode.
 - See Fluoride electrode, ion selective
- Iron, 224-233
- Isopleths, 45
- Isoproterenol, 217, 218, 220-222
- Isotopes, 200
- Italy, 94, 200
- Japan, 27, 119, 141, 144, 159, 173, 203, 261
- Kidney
 - damage, 35-41
 - elevation in cAMP function, 201
 - urinary pH, 201
- Lactate dehydrogenase, 73
- Lamb, 107-114
- Ligament calcifications, 4
- Lipids, 224-233
- Liver, 211, 221
- Lysozyme, 73-76
- MAFF method for fluoride estimation 247
- Memoriam, George L. Waldbott, M.D. 259
- Metabolic respiratory activity, 105-107
- Metabolites, 224-233
- Metallic pollutants, 224-233
- MFP, 210-217
- Mice, 82, 88, 180
- Michigan, 199
- Microscope, scanning, 115
- Mitochondria, 1, 95-97
- Moenickeberg calcifications, 4-8
- Molecular absorption spectrometer, 174
- Monomorphism, 170
- Mucosa, nasal septum, 114-118
- Mud-bath therapy, 267
- Myocardial sarcolemma, 138
- NADH, 73, 101
- $\text{Na}_2\text{PO}_3\text{F}$, 210-217
- NAS, 199
- Neoplastic transformation, 261-262
- Netherlands (The), 72-80
- New Jersey, 206
- New York, 140, 199, 263
- New Zealand, 234
- Noncollagen proteins
 - glycoproteins, 1
 - glycosaminoglycans, 1-2
- Norway, 131
- NRC publication, 90
- Osteoblast increase, 144
- Osteofluorosis, 23-26
- Osteomalacia, 9-11, 13, 16-17, 56
- Osteopenia, disuse, 53-54
- Osteophytosis, 16
- Osteoporosis, 2, 9-10, 13, 14-22, 53-54, 56, 153
- Osteosclerosis, 1, 9-14, 15, 20-21, 24-25, 56, 125, 148, 152-153, 165, 199
- Ovarian oocytes, cultured, 261
- Paradontosis, 115
- Parathyroid
 - bone disease, 16-19
 - hormone, 143
 - hyperplasia, 7
- Parotid amylase, 217-223
- Pennsylvania, 54-55
- Pentobarbital sodium, 121
- Periodontal tissue, 2, 144
- Phagocytosis, 72
- Pharmacokinetics, 201
- Phosphatase
 - acid, 81-82, 84, 86-89, 221
 - alkaline, 15-16, 18, 81-82, 84, 86-88, 90-91, 221
- Phosphate plant, 2, 149, 159, 164-166
- Phosphodiesterase, 210-211, 213-214
- Photo-electric meter, 149
- Pig, F⁻ pharmacokinetics in, 59-60
- Plasma
 - calcium, 16
 - fluoride, 59-60, 264-265
 - Ionic fluoride, 124-131
- Pleiotropism, 171
- Poland, 114, 259
- Polymorphonuclear leukocytes, 72-80
- Polyuria, 40
- Poultry, mechanically deboned, 54-55
- Protein, 1, 15, 17, 22, 141, 224-233
 - in callus, 139 cn. 258

- Pumps, deep bore hand, 48-52
- Quail, 101
- Rabbit, 1-2, 28, 32-34, 72-80, 119-123, 189, 230
- Radiograms, 4-7, 11-14, 15, 20-21
- Radiographs, 55-56, 110, 113, 115
- Radiological
 changes, 9-14
 evaluation, 244
- Radiology, 148
- Rat, 59, 95, 97, 99-100, 138, 319, 141, 144, 178-182, 183-192, 197, 206, 210-217, 217-223, 258
- Renal
 clearance, 266
 function, 35-41
- Rheumatoid arthritis, 72
- Rickets, 16-17, 19-20, 55-56
- Roholm's classification, 5
- Sabre tibia, 55
- Salinity, 267
- Saliva, 27, 197
- Salivary amylase, 217-223
- Second Fluoride Symposium in Szczecin, 259-260
- Secretagogue, 178
- Serum
 alkaline phosphatase, 56
 calcium, 56
 creatinine clearance, 244
 ionic F^- levels, 53
 phosphorus, 56
- SHE cells, 261-262
- Sheep, 108, 189, 199
- Shrubs at Surat, India
 Acacia nilotica, 252-258
 Calotropis procera, 252-258
 Prosopis juliflora, 252-258
 Zizyphus nummularia, 252-258
- Sialic acid, 1
- Silicate factory, 196
- Skeleton, increased density of, 4
- Sodium
 intracellular, 224-233, 247-248
 pentobarbital, 218
- South Africa, 13
- Space food, 173-177
- Stannous fluoride
 fatality from, 205
 mouth rinse, 205
- Symposium, second
 Fluorine, Szczecin, 259-260
- Stress condition, 105-107
- Strontium, 263-264
- Succinic dehydrogenase, 221
- Sweden, 58-59, 59-60, 60-61, 201, 204, 264, 266
- Synthesis
 of
 DNA, 141
 protein, 141
- Tea, 145
- Teeth
 delayed eruption, 200
 hypoplasia, 200
 mottled, 208, 234-235, 239-241
- Theophylline, 211-213
- Total suspended particulates, 43
- Trace elements, 81, 82, 85, 91
- Tricarboxylic acid cycle, 94, 99, 102
- Triton X-100, 73, 96
- Turkey, 4, 139
- United Kingdom, fluoride intake in, 142
- Urinary
 calcium, 131-138
 determinations, 200
 excretion, 264-265
 fluoride. See Fluoride in urine
 pH, 201, 207, 243, 266
- USSR, 267
- Virginia, 205
- Vitamin
 C, 17
 D, 15, 17, 22, 56
- Washington, Seattle, 55-56
- Water, drinking, safe fluoride levels, 49
- Wildlife effects, 41-47
- Winkler's iodometric method, 105-107
- Wisconsin, 94
- X-ray, 2, 107-114, 148

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