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The International Society for Fluoride Research (ISFR) extends a special invitation to you to participate in the 16th Conference. This will be held in the Conference Hall of Zyma at Nyon (30 km from Geneva) Monday, August 31st through Wednesday, September 2nd, 1987. Professor C.A. Baud will host this Conference and he has nominated Christiane Demeurisse as secretary of the Conference. The Fluoride journal will carry information about the Conference in future issues.

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CHRONIC FLUORIDE INTOXICATION: A MAJOR BREAKTHROUGH IN DIAGNOSIS

Recently a major discovery has been reported in the diagnosis of chronic ill effects from drinking fluoridated water. Australian dentist Geoffrey Smith has devised a new method of obtaining biopsy specimens from alveolar bone, the interradicular septum between the roots of molar teeth which is exposed when the tooth is extracted (1). Of twenty-four females in whom F^- in alveolar bone was measured, twelve were suffering from repetitive strain injury (RSI). All subjects were residing in fluoridated Melbourne. Levels of fluoride in alveolar bone of subjects with RSI ranged up to 3300 (average 2737) and were higher than in women of similar age who were not afflicted, namely up to 2200 (average 1687). In another study (2) of patients whose bones contained up to 4720 ppm (mean 3320) all but one of the 17 investigated complained of vague pains, stiffness in lower and upper extremities, shoulder, neck and lower back. Bone changes were shown by x-ray in vertebrae, legs, knees, ankles and, particularly, in forearms and elbows where free bony bodies were found and clear evidence of calcification of tendons and ligaments.

Estimates of dietary intake of RSI subjects revealed a Mg^{2+} deficit and an excessive F^- intake (3). Fluorotic bone has an increased Mg^{2+} content, possibly due to some deposition of MgF_2. A localized Mg^{2+} deficiency could disturb pyrophosphate metabolism and lead to deposition of Ca^{2+} salts in sensitive areas. Through adjustment of dietary intake of the previously mentioned 12 RSI subjects which included more Mg^{2+} and less F^-, eight of the subjects experienced marked relief from previously painful RSI symptoms after a six-week test period.

This new evidence provides another objective criterion of reversible fluoride intoxication. It reinforces and confirms the extensive research based primarily on clinical observation recorded by G.L. Waldbott in more than 80 publications in the U.S.A. and abroad beginning in 1955 (examples and sources: 4-15). In the preskeletal phase of incipient fluorosis, when symptoms are still reversible, objective diagnostic signs are sparse (12,14). In addition to urine analysis and laboratory tests, one of the major diagnostic tools utilized by Dr. Waldbott, was the double-blind test: The pharmacist prepared three one-gallon jugs of distilled water, which he designated #1, #2, and #3. To one of the jugs, he added nine mg of NaF (1 ppm F^-). Neither the patient nor the physician knew which bottle contained plain water or which contained fluoride. The patient was instructed to consume water from one jug for the first few days and from the other two on consecutive days. Invariably, upon recurrence of symptoms, the patient identified the bottle which contained fluoridated water (13).

Through extensive in-depth studies of chronic fluoride intoxication in humans from natural fluoride water, and in humans and animals from fluoride-contaminated air in the vicinity of factories, a definite symptom complex emerged. Although not every individual manifested all the same symptoms, certain salient characteristics recurred almost invariably, namely ulnar nerve palsy, weakness in arms and legs, constipation alternating with diarrhea or both, loss of mental acuity, excessive fatigue, and gastric distress. Other symptoms which occurred often were visual disturbances, backache, and headache, all of which disappeared upon elimination of fluoridated water or removal from the air-contaminated area, provided that the illness was still in its early stages.

From the above, it is conceivable that cases of preskeletal chronic fluoride poisoning constitute an enigma to the diagnostician, especially to the practicing physician. Because recognition requires an in-depth study, it is not unexpected that some cases either remain undiagnosed or are misdiagnosed. This new research by Dr. Smith provides a much-needed objective method for early recognition of the preskeletal phase of chronic fluoride poisoning. It is hoped that it will be widely utilized as a diagnostic tool.

E.M.W.

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FLUORIDE-INDUCED TETRAZOLIUM DYE REDUCTION BY RABBIT NEUTROPHILS

by

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SUMMARY: In rabbit neutrophils sodium fluoride (20 mM) induces a strong increase in reduction of the tetrazolium dye iodonitrotetrazolium (INT) indicating an activation of the metabolic burst. This occurs in the absence of extracellular Ca^{2+}; fluoride-induced INT reduction is little influenced by the presence or absence of Ca^{2+}, Mg^{2+} or Sr^{2+}, but it is strongly inhibited by Co^{2+}, Ni^{2+}, Mn^{2+} and La^{3+}. In the presence of Ca^{2+} or La^{3+} cell damage occurs. Fluoride-induced INT reduction is characterized by a lag time of about 10 min, and is strongly inhibited by Ca^{2+}-complexing and Ca^{2+}-antagonistic drugs. The results suggest the involvement of intracellular Ca^{2+} in fluoride-activation of the metabolic burst. The involvement of phospholipase A_2 in the activation is questionable because inhibitors of this enzyme gave divergent results. There is synergism between fluoride and some other activators of the metabolic burst, especially phorbol myristate acetate, with regard to activation of INT reduction.

KEY WORDS: Calcium; Fluoride; INT reduction; Neutrophils.

Introduction

The neutrophil is the first line defence of the organism against microbial invaders; these are phagocytized and killed by products released by degranulation, and by toxic oxygen products produced during the metabolic burst. The metabolic burst comprises a series of processes, eventually resulting in the enhanced production of toxic oxygen metabolites. Exposure of neutrophils in vitro to certain soluble activators may result in degranulation and a metabolic burst, which enables the study of these processes in the absence of phagocytosis (1).

During activation of the neutrophil to perform a metabolic burst the formation of NADPH is strongly enhanced, and this product is used to convert molecular oxygen into superoxide. The enhanced production of reducing substances makes it possible to follow the metabolic burst with reducable dye molecules. Tetrazolium dyes are well suited for this purpose (1,2). In this investigation indonitrotetrazolium (INT) reduction was used as a measure for activation of the metabolic burst (3.)

Fluoride in relatively high concentrations, is an activator of neutrophils

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(4-12). It induces a strong Ca^{2+}-independent degranulation in rabbit and guinea-pig peritoneal neutrophils, but little or no degranulation in human neutrophils (4-7). Both in human and rabbit neutrophils fluoride induces an activation of the metabolic burst. With human neutrophils, extracellular Ca^{2+} was necessary (11). Diverging results were obtained when human neutrophils were used for fluoride activation, which indicates that fluoride activation is strongly dependent on the cell type used, and on the experimental conditions (7-12).

In this investigation we studied some aspects of fluoride activation of the metabolic burst in rabbit meutrophils, using INT reduction as a measure of the metabolic burst. The absence of a requirement of extracellular Ca^{2+} in fluoride activating of the metabolic burst in these cells presents some advantages. No exocytosis takes place, because fluoride-activated exocytosis in rabbit neutrophils is strictly Ca^{2+}-dependent. There is no possibility of CaF_2 precipitation. An important advantage is the fact that activation of cells in the absence of extracellular Ca^{2+} enables the study about the role of intracellular Ca^{2+}.

Materials and Methods

Neutrophils: Rabbit peritoneal neutrophils were obtained as described previously (11). The cells were suspended in a medium consisting of 140 mM NaCl, 5 mM KCl and 20 mM Tris-HCl pH 7.4. The final cell concentration in the experiments was 3×10^6 neutrophils per ml. All experiments were carried out at 37°C in a shaking waterbath.

Tetrazolium dye reduction: The metabolic burst was measured as an increased iodonitrotetrazolium (INT) reduction. INT reduction was measured by including 0.4 mM INT in the mixture of 3×10^6 neutrophils per ml. In the standard procedure the mixture contained 20 mM sodium fluoride as an activator; 1 mM EDTA was added to remove adherent Ca^{2+} ions, and 1 mM KCN was present to optimize INT reduction. After incubation for 40 min at 37°C the reaction was terminated by adding 5 ml 0.5 M HCl. In those cases where the enzyme release during INT reduction was measured the reaction was terminated by centrifugation, the supernatant was removed for determination of enzyme release, and 5 ml 0.5 M HCl was added to the residue. The HCl suspension was centrifuged and the residue, containing the reduced INT, red colored and insoluble, was taken up in pyridine (2 ml). To dissolve the residue the pryidine solution was warmed for 10 min in a boiling waterbath. After cooling to room temperature the absorbance of the pyridine solution was measured at 510 nm. The results are expressed as nmoles INT reduced per 3×10^6 neutrophils (2,3).

Cell integrity: The release of the cytoplasmic enzyme lactate dehydrogenase (LDH) was determined as a measure for plasma membrane damage. LDH was estimated by measuring the conversion of NADH into NAD^+ during the LDH-catalyzed conversion of pyruvate into lactate. Enzyme release was expressed as a percentage of a maximum value, obtained by treating the cells with 0.2% Triton X-100.

Chemicals: Iodonitrotetrazolium chloride (INT), phorbol myristate acetate (PMA), quin2-AM, chlortetracycline, the cytochalasins A and B and formyl-methionyl-leucyl-phenylalanine (FMLP) were from Sigma Chemical Co; 8-(diethylamino)octyl-3,4-5-trimethoxybenzoate HCl (TMB-8) was obtained from Janssen Chimica, verapamil was a gift from Knoll AG, Ludwigshafen, and prenylamine was from Hoechst Holland.

Results

Fluoride induces INT reduction in the absence of extracellular Ca^{2+}. In the presence of CA^{2+}, Mg^{2+} or Sr^{2+} the fluoride-induced INT reduction is about the same as in the absence of divalent cation. In the presence of extracellular Ca^{2+} LDH release was observed, indicating cell damage (Table 1).

Table 1

The Effect of EDTA, EGTA and Metal Ions on Fluoride-induced INT Reduction

	nmoles INT reduced	% LDH release
-	25.7 ±0.6	4 ±2
1 mM EDTA	25.5 ±1.3	4 ±1
1mM Ca^{2+}	25.2 ±0.7	22 ±2
1 mM Mg^{2+}	24.0 ±1.7	2 ±1
1 mM Sr^{2+}	28.0 ±4.3	2 ±2
1 mM Ba^{2+}	19.4 ±5.6	1 ±1
1 mM Ni^{2+}	7.5 ±0.5	3 ±1
1 mM Co^{2+}	6.6 ±0.4	2 ±2
1 mM Mn^{2+}	4.6 ±0.2	1 ±1
0.1 mM La^{3+}	8.2 ±0.4	36 ±1
Control (no F^- treatment)	4.6 ±0.2	4 ±2

Neutrophils exposed to 20 mM sodium fluoride in presence of given concentration of metal ion, EDTA or EGTA. After incubation for 40 min at 37°C cells were centrifuged. Supernatant was used for LDH determination. To the residue HCl was added and treated as described in Methods. Values given are mean of three experiments ±SD.

Fluoride-induced activation was compared with activation by phorbol myristate acetate. Fluoride-induced activation is characterized by a lag time of 10-15 min which is nearly absent (Figure 1) in PMA activation. Per unit of time fluoride-induced INT reduction is less than PMA-induced INT reduction (Figure 1). Int reduction is a relatively slow process as compared with the reduction of other dyes, such as cytochrome c (2), and it continues for a long time regardless of the type of activator. Individual variations play a role in fluoride-induced activation: there are differences between various batches of cells with regard to fluoride-induced INT reduction.

A number of Ca^{2+}-complexing and Ca^{2+}-antagonistic drugs were studied with regard to their ability to interfere with fluoride-induced INT reduction. In the absence of extracellular Ca^{2+} all compounds tested strongly inhibited fluoride-induced activation (Table 2). No effort was done to see whether this effect could be reversed by extracellular Ca^{2+}, beause of the possible formation of CaF_2 crystals and LDH release which may hamper interpretation of the results.

Whereas there is little difference in fluoride-induced INT reduction in the absence of divalent cations and in the presence of Ca^{2+}, Mg^{2+} and Sr^{2+}, there is some inhibition in the presence of Ba^{2+}. The ions Ni^{2+}, Co^{2+}, Mn^{2+} and La^{3+} strongly inhibit activation by fluoride (Table 1). This is probably not due

Figure 1

Time course of INT reduction in presence of either fluoride or phorbol myristate acetate (PMA) as activator. Values given are mean of three experiments, corrected for the INT reduction of non-activated (resting) cells. INT reduction is represented as nmoles INT as reduced per 3 x 10^6 cells.

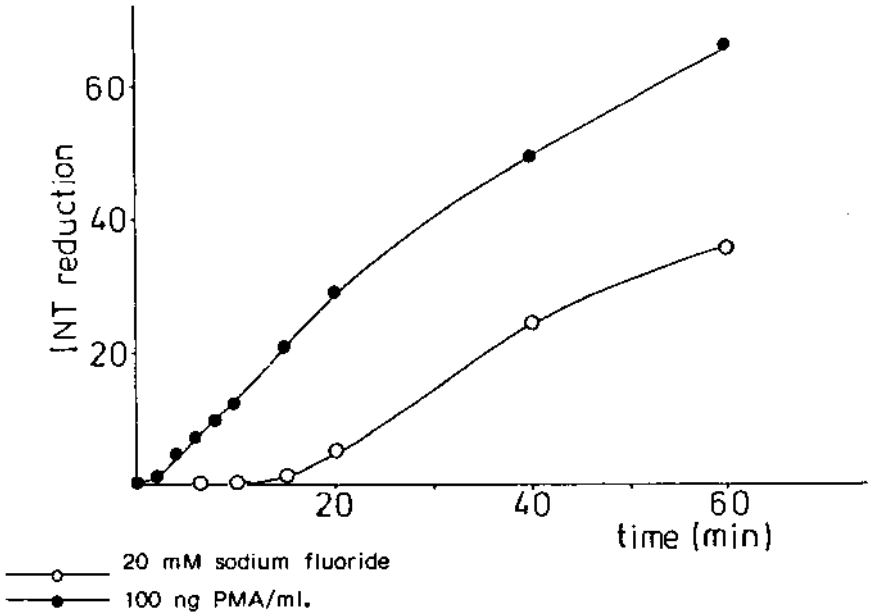


Table 2

Effect of Ca^{2+}-antagonistic and Ca^{2+}-complexing Drugs on Fluoride-induced INT Reduction

Inhibitor	nmoles INT reduced	% inhibition (of activation)
-	41.9 ±1.7	
10 µM quin-2 AM	11.1 ±1.9	92
100 µM chlortetracycline	14.3 ±1.6	82
100 µM TMB-8	18.2 ±1.2	71
200 µM verapamil	25.5 ±0.8	49
10 µM prenylalanine	24.5 ±4.2	52
Control (no F^- treatment)	8.4 ±0.6	

Neutrophils preincubated for 15 min at 37°C with inhibitor, after which INT and F^- (20 mM) added, followed by incubation at 37°C for 40 min. In all experiments 1 mM EDTA was present. Percentage inhibition refers to inhibition of activation, i.e. the difference of INT reduction in F^--activated cells and control cells, which are not exposed to fluoride.

$$\% \text{ inhibition} = \frac{\text{nmole INT reduced}}{41.9 - 8.4} \times 100$$

Values given are mean of three experiments ± SD.

to removal of extracellular fluoride due to precipitation of the metal fluoride, because extracellularly fluoride (20 mM) levels are likely to be far in excess. In the presence of Ca^{2+} and La^{3+} LDH release occurs.

To study the possible involvement of phospholipase A_2 in fluoride activation of neutrophils, a series of inhibitors of this phospholipase was studied. As can be seen in Table 3, there are only a few compounds with a strong inhibiting effect at low concentration (p-bromophenacyl-bromide, chlorpromazine), some compounds with an inhibiting effect at relatively high concentrations (propranolol, chloroquine, mepacrine) and some compounds without an inhibiting effect (hydrocortison phosphate, indomethacine). Some of the compounds tested had a significant potentiating effect at low concentration (p-bromophenacyl-bromide, mepacrine, chloroquine) (Table 3).

Table 3

The Effect of Various Phospholipase A_2 Inhibitors on Fluoride-induced INT Reduction

	INT reduction
-	27.3 ±0.8
p-Bromophenacylbromide, 5 µM	35.9 ±2.0
p-Bromophenacylbromide, 25 µM	8.0 ±0.5
Chlorpromazine, 50 µM	8.1 ±0.4
Propranolol, 500 µM	7.4 ±0.6
Chloroquine, 100 µM	32.8 ±0.8
Chloroquine, 500 µM	20.8 ±0.4
Mepacrine, 10 µM	32.2 ±0.2
Mepacrine, 100 µM	21.3 ±3.1
Hydrocortizonphosphate, 200 µM	26.8 ±0.5
Indomethacin, 100 µM	26.6 ±1.1
Control (no fluoride)	6.6 ±0.4

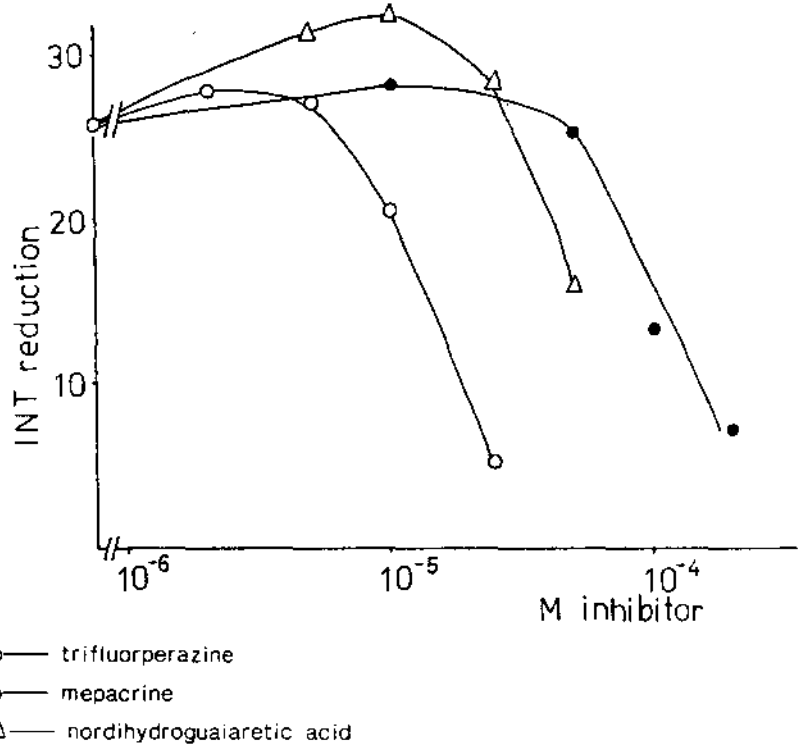
Cells were preincubated in the presence of 1 mM EDTA with the given concentration of inhibitor for 15 min at 37°C, 20 mM fluoride was added followed by incubation for 40 min. Values given are mean of three experiments ± SD.

This potentiating effect, followed by an inhibitory effect, is also displayed by some drugs which are supposed to interfere with other targets: trifluoperazine, which is a membrane-disturbant agent and a calmodulin inhibitor, and mordihydroguaiaretic acid, which is a lipoxygenase inhibitor. All these agents (Table 3, Figure 2) give the same biphasic action: a potentiation of fluoride-induced INT reduction at low concentrations, and an inhibition at higher concentrations.

The effect of the combined action of a suboptimal concentration of fluoride with suboptimal concentrations of other activators is represented in Table 4. The cytochalasins and the chemotactic peptide FMLP, combined with 10 mM fluoride, give only little more INT reduction than the sum of each activator separately. The effect of the combined action of PMA and fluoride is more striking. The activating effect of a suboptimal concentration of PMA combined with 10 mM fluoride is much higher than the sum of each of them separately. When an optimal concentration of PMA (100 ng/ml) is combined with 20 mM fluoride, the fluoride seems to inhibit: the combined activation is much less than the effect of PMA alone.

Figure 2

Effect of some inhibitors on fluoride-induced INT reduction. Cells preincubated in the presence of 1 mM EDTA with the given concentration of inhibitor for 15 min at 37°C; 20 mM sodium fluoride added, followed by incubation for 40 min.



Discussion

Fluoride causes a strong increase of INT reduction in rabbit neutrophils, indicating that it activates the metabolic burst in these cells. INT reduction presents some advantages as an indicator of the metabolic burst over the more commonly used cytochrome c reduction and NBT reduction. INT reduction covers a broader aspect of the metabolic burst than the extracellular release of superoxide, which is exclusively measured during cytochrome c reduction. Though less sensitive than NBT reduction, INT reduction is not accompanied by cell damage, which occurs with NBT reduction (2,3). The time course of INT reduction shows that it is a relatively slow process. With fluoride as activator this is even more pronounced, because the activating effect of fluoride is less than that of PMA, and because of the lag time which is much longer for fluoride-induced than for PMA-induced INT reduction. The lag time probably represents the entry of fluoride into the cells. We have previously shown that this membrane passage is pH-dependent and this makes a penetration of the form of HF likely (5,11,12). The lag time indicates that fluoride activates the

Table 4

Synergism between Suboptimal Concentrations of Fluoride and other Activators in INT Reduction

	INT reduction (Corrected) in the presence of	
	0 F^-	10 mM F^-
-	=0	3.5 ±0.1
0.5 µM cytochalasin A	5.6 ±0.3	9.6 ±0.3
5 µM cytochalasin B	4.5 ±0.2	14.1 ±1.0
10^{-8} M FMLP	4.4 ±0.5	10.4 ±0.3
2 ng PMA/ml	4.8 ±0.2	24.1 ±0.6
5 ng PMA/ml	15.6 ±2.9	37.0 ±2.3

Cells incubated in presence of 1 mM EDTA and the given concentration of activator, without or with 10 mM sodium fluoride, for 40 min at 37°C. From the INT reduction measured (in nmoles INT reduced per 3 x 10^6 cells) the INT reduction of resting cells (being 5.6 ±0.4) was subtracted: INT reduction (corrected) = INT reduction (measured) -INT reduction (resting cells).

Values given are mean of three experiments ± SD.

An optimal concentration of PMA (100 ng/ml) gave an INT reduction of 66.1 ±3.2; 20 mM fluoride gave an INT reduction of 15.5 ±0.4; the combined presence of 100 ng PMA/ml and 20 mM fluoride gave an INT reduction of 42.5 ±1.6.

FMLP = formyl-methionyl-leucyl-phenylalanine;

PMA = phorbol myristate acetate

metabolic burst after penetration into the cell, and that its effect is thus the inner side of the plasma membrane or cell.

In contrast with human neutrophils (7), extracellular Ca^{2+} is not required for fluoride-induced activation of the metabolic burst in rabbit neutrophils. The results indicate, however, that intracellular Ca^{2+} plays a pivotal role in fluoride-induced INT reduction. Quin2-AM is a membrane permeant agent that is intracellularly converted into the water soluble quin2, which cannot leave the cell; high intracellular levels of quin2 are thus attained with low extracellular concentration of quin2-AM (13). Quin2 and chlortetracycline are Ca^{2+}-complexing agents (14), and both strongly inhibit fluoride activation in the absence of extracellular Ca^{2+}.

TMB-8, a reported antagonist of intracellular Ca^{2+} (15), equally inhibits fluoride-induced INT reduction. The same applies to verapamil and prenylamine (14), which have been shown to possess a Ca^{2+}-antagonistic intracellular effect, at relatively high concentrations.

Inhibition of fluoride activation of the metabolic burst by these agents is compatable with the view that intracellular Ca^{2+} plays an important role in this activation.

The effect of the metal ions Ni^{2+}, Co^{2+}, Mn^{2+} and La^{3+} fits well into this picture. These ions are Ca-antagonistic metal ions and prevent Ca^{2+}

movement across the membrane (16). This cannot apply to the inhibition of Ca^{2+} entry into the cells because there is no extracellular Ca^{2+}. It may quite well be, however, that these agents penetrate into the cell, and that they interfere with intracellular Ca^{2+} movements.

There are apparently differences in Ca^{2+} requirements for cells derived from different sources, and for the metabolic burst as compared with exocytosis. In rabbit neutrophils fluoride activation of the metabolic burst may exclusively occur with intracellular Ca^{2+}, whereas for human neutrophils a [partial] dependence on extracellular Ca^{2+} exists. In the same cell type, i.e. rabbit neutrophils, differences exist for Ca^{2+} requirement for fluoride activated metabolic burst and exocytosis: the latter is absolutely dependent on extracellular Ca^{2+}, whereas this is not so with the metabolic burst. This suggests that the Ca^{2+} requirement for the metabolic burst is either lower or that the Ca^{2+} is derived from other stores than in exocytosis.

Phospholipase A_2 has been considered as an important enzyme in neutrophil activation, because of its ability to hydrolyze membrane phospholipids and thus to inititate arachidonic acid metabolism (17). This enzyme could furthermore contribute to differences between rabbit and human neutrophils because there is much more phospholipase A_2 in rabbit than in human neutrophils (18).

Though a role for phospholipase A_2 in prostaglandin metabolism of neutrophils cannot be denied there is no experimental evidence for a rate-limiting role of this enzyme in fluoride activation. Some of the phosphoslipase inhibitors are indeed inhibitory, but mostly in much higher concentrations than required for inhibition of the isolated enzyme (19). Some of the phospholipase A_2 inhibitors as well as the inhibitors of other targets (trifluoperazine (20), nordihydroguaiaretic acid (21)) show a biphasic pattern: potentiation of INT reduction at low concentrations, and inhibition at higher concentrations. This biphasic pattern strongly resembles that of the large class of anesthetic-like membrane disturbing agents, which stabilize membranes in low concentrations, and destabilize in higher concentrations (22). Because all agents we tested, which had a biphasic effect, have less or more hydrophobic parts in their molecules and are thus probably membrane active, it might well be that their effect is not specific, but a general anesthetic-like effect on membrane integrity.

Suboptimal concentrations of PMA and fluoride have a strong synergistic effect. Effects of agents which mobilize intracelular Ca^{2+} have been found to act synergistic with that of PMA, which acts by activation of protein kinase (23). Protein kinase C activation and Ca^{2+} mobilization act synergistically to ellicit the full physiologic response of platelets and neutrophils (23). It may be that fluoride exerts its synergistic effect by mobilizing intracellular Ca^{2+}. Other possibilities however, cannot be excluded, though it is evident that the pathways of fluoride and PMA activation may strongly influence each other.

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SKELETAL FLUOROSIS, SECONDARY TO OCCULT RENAL DISEASE

by

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SUMMARY: Forty-one consecutive patients with skeletal fluorosis were reviewed. Nine of these cases were found to have renal disease; the fluoride content of their drinking water ranged between 0.5-2.0 ppm. It is surmised that these patients developed fluorosis secondary to renal disease.

KEY WORDS: Occult renal disease; Skeletal fluorosis.

Introduction

The development of skeletal fluorosis depends upon intake of fluoride over a prolonged period. In addition, strenuous manual labor and poor nutrition also contribute to the development of skeletal fluorosis (1). The status of the kidneys, too, plays an important role in the development of fluoride toxicity. Normally functioning kidneys can excrete fluoride without significant retention in the body (2). On the other hand, excretion of fluoride is diminished if the person concerned is suffering from chronic kidney disease. He may develop fluorosis even if the fluoride level of water consumed is low (3,6-10).

Since skeletal fluorosis secondary to renal disease appears to be fairly common in our experience, the following report documents this association.

Material

During the past two years, forty-one cases of skeletal fluorosis have been investigated in nine of which renal disease was detected. Five were males and four were females between 25-60 years of age. The fluoride levels of the drinking water ranged from 0.2 ppm to 2.0 ppm. The clinical profile of these cases is given in Table I.

Discussion

The principle means by which 90% of fluoride is excreted from the body is urine. In normal individuals urinary fluoride fluctuates widely between 0 and 1.2 ppm with an average of about 0.4 ppm when the fluoride content of drinking water is 0.3 ppm (2). Fluoride is removed from the circulation by glomerular filtration. Tubular re-absorption of fluoride is less than that of chloride and thus the kidney excretes fluoride rapidly (3). Excretion of fluoride is much less if the person concerned is suffering from chronic renal disease resulting in renal failure, which inevitably leads to high concentrations of fluoride in serum and bone. Hence individuals suffering from chronic renal

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Table 1

No.	Name	Age	Sex	E/NE	C.P.	F Level	B.U.N.	S.C.	R.U.S.	IVP.	Remarks
1.	M.D.	60	M	E	Pain and deformity of spine.	0.2 ppm	60 mg	3.3 mg	L. kidney not seen R. kidney small with irregular margins	Gross delay in excretion	
2.	M.K.	44	M	NE	Pain and restricted spinal movements	0.2 ppm	56 mg	-	L. kidney not functioning. R. kidney normal	-	
3.	K.B.	42	F	E+NE	Pain and restricted neck movements	2 ppm	102 mg	5.2 mg	Bilateral contracted kidneys	-	19 yrs. NE 12 yrs in E again 11 yrs in NE.
4.	C.N.	60	M	NE	Paraparesis	2 ppm	50 mg	2 mg	-	Delayed excretion both sides	
5.	R.M.	50	M	NE	Quadriparesis	0.2 ppm	62 mg	2.4 mg	R. kidney contracted L. kidney normal		
6.	M.S.	25	F	NE	Pain and restricted neck movements	0.2 ppm	74 mg	3.2 mg	Bilateral contraced kidneys with irregular margins	Delayed excretion	
7.	S.R.	46	F	E	Pain and deformity of spine	2 ppm	80 mg	5.2 mg	Bilateral contracted kidneys, left more than right	Grossly delayed excretion	
8.	R.K.	40	M	E	Pain and deformity	2 ppm	60 mg	4.2 mg	Bilateral contracted kidneys		
9.	P.L.	50	F	NE	Neck pain and quadriparesis	0.2 ppm	60 mg	3.6 mg	R. kidney shrunken L. kidney normal	Delayed excretion both sides	

E = Endemic, NE = Non-endemic

C.P. = Clinical Profile
Normal F Level, 1-2 ppm

B.U.N. = Blood Urea Nitrogen
Normal level, 10-20 mg/dl

S.C. = Serum Creatimine
Normal Level, 2 mg/dl

R.U.S. = Renal Ultrasound Scan

IVP = Intra Venus Pyelogram

failure may develop skeletal fluorosis even at a low level of fluoride in drinking water. Patients with renal transplants who subsequently need dialysis developed skeletal fluorosis over a period of 1-2 years when the dialysing fluid contained 1 ppm of fluoride (4). In this study patients developed skeletal fluorosis secondary to renal disease although the fluoride level in drinking water was as low as 1-2 ppm. In case three, the onset of renal disease appears to have precipitated skeletal fluorosis. In 22% of the cases renal disease was responsible for the development of skeletal fluorosis. Hence it is important to investigate renal function in all cases of fluorosis, especially in non-endemic areas.

Conclusion

Although fluoride toxicity on the renal parenchyma leading to the development of skeletal fluorosis should be considered, it is the diseased kidney's inability to excrete the fluoride that appears to be the main determinant in the development of skeletal fluorosis. Hence in the general population, in individuals with apparent or overt kidney disease, drinking fluoridated water may result in disabling skeletal fluorosis.

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STUDIES ON FLUORIDE, PHOSPHORUS AND CALCIUM IN TEETH BEFORE AND AFTER WATER FLUORIDATION IN GUANGZHOU, CHINA

by

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SUMMARY: The fluoride, phosphorus and calcium content of both permanent and deciduous teeth before and after water fluoridation in Guangzhou (1965-1972) was investigated. The whole tooth was crushed and ground to a fine powder which could pass through a 60-100 mesh sieve and the powder was separated into enamel, dentin and cementum by flotation methods. The cementum was discarded. The fluoride in the sample powder (enamel and dentin) was isolated by a diffusion method in a modified Conway cell and determined with a fluoride-ion selective electrode. In the residual solution after diffusion of hydrogen fluoride, phosphate was determined by a molybdovanadophosphoric acid colorimetric method, and calcium was determined by an EDTA titration method after the phosphate was removed by solvent extraction.

Over 80 samples of teeth were analyzed before and after water fluoridation in Guangzhou City in China for 7 years from 1965-1972. Results show a significant increase in fluoride content in deciduous teeth after water fluoridation, but for permanent teeth, the increase was not significant. The weight ratio of calcium to phosphorus was found to be 2:1 approximately.

KEY WORDS: Guangzhou, China; Teeth, fluoride, phosphorus, calcium content; Water fluoridation.

Introduction

Cariostatic action of fluoride may be partly due to the fact that the hydroxyapatite on the outer surface of enamel changes to fluorapatite which is more stable and less soluble than apatite without fluoride (1-4).

$$Ca_5(PO_4)_3OH + H^+ + F^- \longrightarrow Ca_5(PO_4)_3F + H_2O$$

Under normal nutritive conditions, the ratio of calcium to phosphorus in dental enamel is 2:1 (by wt.). This ratio approaches that of calcium to phosphorus in apatite. The fluoride in food and drinking water ingested and the mean values are about 130 and 360 ppm, respectively, if the fluoride concentration in drinking water is 1.1-1.2 ppm (1-4). This fluoride content in enamel may be adequate to convert some of the outer surface of the apatite crystals to fluorapatite which then exerts a protective action on the unsubstituted hydroxyapatite within.

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Guangzhou (in Guangdong, China) is in subtropical zone where the fluoride content in drinking water without fluoridation is 0.2-0.3 ppm. The water supplies have been fluoridated (up to 0.6-1.0 ppm) since 1965. The purposes of this paper are to determine fluoride, phosphorus and calcium in teeth samples at the first and the seventh year after water fluoridation, and to test any relationship between water fluoridation and anti-caries ability.

Material and Methods

Collection and treatment of samples: Natural fluoride concentration is about 0.2-0.3 ppm in drinking water in Guangzhou. In late 1965, water supplies were fluoridated up to 0.5-1.0 ppm. In 1965-1966 and 1971-1972, we collected the tooth samples in our dental clinic from residents who were born and living in Guangzhou. Samples were air-dried, and labeled after washing and drying. Before analysis, the samples were soaked in 3% hydrogen peroxide for 10 min, washed with tap water, and then with deionized water. These samples were dried at 100°C for 15 minutes and placed in a desiccator for 1 day.

The whole tooth was crushed and ground to a fine powder, which could pass through 60-100 mesh sieve in some device such as a ball mill or an agate mortar. By means of a flotation method (5), the whole tooth powder was added to a fluid with a density of 2.70 (bromoform:acetone 91:9). The enamel (density 2.9-3.0) sinks, while the dentin and cementum (2.14 and 2.03, respectively) floats. After separating, the mixture of dentin and cementum was added to a fluid with a density of 2.07 (bromoform:acetone 61:39). The dentin sinks, while the cementum floats. The latter was discarded. Each of these enamel and dentin samples were washed with absolute alcohol 3 times, dried and stored in a desiccator for use.

Isolation of fluoride: 200 mg of enamel or dentin sample powder were weighed accurately, and transferred to a diffusion cell (modified Conway cell) (6) in which was a small boat (instead of the center well of Conway cell) containing a solution of 0.5 ml 2.5 N NaOH. 5 ml of perchloric acid (1:1) was added to the sample. The cell was covered and sealed immediately, and diffused for 22 hrs at 55 ±2°C. After cooling, the NaOH solution that had absorbed HF was transferred to a 50 ml volumetric flask and adjusted to about pH 5 with dilute HCl, diluted to the mark and mixed well. This solution was used for fluoride analyses.

The residual solution in which HF had been removed by diffusion was filtered into a 50 ml volumetric flask, and diluted to the mark and mixed well. This solution was used for phosphorus and calcium analyses.

Determination of fluoride: 10.0 ml of each of 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1} M NaF standard solutions and the above sample solution were transferred to six plastic beakers, respectively. To each beaker was added 10.0 ml of TISAB* solution. Then, a fluoride-ion selective electrode and a saturated calomel electrode attached to a pH meter was immersed in the solution which was

* The total ionic strength adjusted buffer (TISAB) was prepared by dissolving with stirring approximately 57 ml of glacial acetic acid, 58 g of NaCl, and 0.3 g of sodium citrate in about 700 ml of distilled water in a 1000 ml beaker. Cool, and add 5 M NaOH until the solution reaches a pH of 5.2-5.5. Dilute the solution to about 1 liter, mix and store in a stoppered plastic bottle.

stirred magnetically for 3 min. The potentials (mV) of these standards and samples were recorded and a plot of the potential versus the log of the concentrations of the standards constructed. The ppm of fluoride in the sample was calculated.

Determination of phosphorus: A portion of residual solution and a series of phosphate standard solutions were analyzed by a monybdovanadophosphoric acid method for the colorimetric analysis of phosphate (7).

Determination of Calcium: A portion of residual solution was pipetted out and the phosphate removed by extraction with n-butyl alcohol-chloroform (1:1) and sodium molybdate solution. The calcium was determined by titration (7,8).

Results and Discussion

Fluoride, phosphorus and calcium in teeth of Guangzhou residents were determined before and after water fluoridation in the period from 1965-1972. The recoveries of fluoride, phosphorus and calcium were 90 ±5%, 100 ±5%, and 100 ±3%, respectively. The recoveries are suitable for routine analysis.

The physiological role of fluoride in the human body is not yet fully understood. Fluoride is generally considered as a normal chemical material in the body, which promotes the mineralization of tooth and bone. An increase of fluoride concentration in the blood will promote mineralization of teeth and raise the fluoride content of teeth (1-4,9). After tooth eruption, fluoride is absorbed onto the surface layer of enamel. Table 1 shows the analytical results of over 80 samples, and indicates that the fluoride content in enamel and dentin of deciduous teeth after water fluoridation for 7 years increased significantly, because most of the deciduous teeth were minerlized during fluoridation. The fluoride content in permanent teeth increased also, but not significantly, as most permanent teeth had erupted before water fluoridation.

Under normal nutritive conditions, fluoride content in teeth and drinking water is positively correlated. In areas where the fluoride concentration in drinking water is 0.0-0.3 ppm, the fluoride content of enamel and dentin are about 100 and 240 ppm, respectively; in areas where the fluoride concentration in drinking water is 1.1-1.2 ppm (in temperate zone), the content in enamel

Table 1

The contents of fluoride, phosphorus and calcium in teeth before and after water fluoridation in Guangzhou from 1965 to 1972.

Year	F^- in water (ppm)	Tooth sample	F^-, ppm* X ± SD(n)	p value	% Ca	% P	Ca:P by wt.
Enamel							
1965	0.2-0.3	deciduous	103.0 ±40.1 (16)	< 0.05	35.58 ±6.18	17.19 ±2.67	2.07:1
1972	0.6-1.0	teeth	137.1 ±44.2 (14)		34.95 ±4.04	15.52 ±1.81	2.25:1
1965	0.2-0.3	permanent	121.3 ±53.6 (23)	> 0.05	34.28 ±2.13	16.95 ±1.48	2.03:1
1972	0.6-1.0	teeth	138.9 ±69.8 (27)		33.80 ±2.80	16.15 ±1.98	2.10:1
Dentin							
1965	0.2-0.3	deciduous	219.1 ±81.1 (17)	< 0.05	24.72 ±4.59	13.31 ±3.63	1.86:1
1972	0.6-1.0	teeth	320.2 ±128.2 (15)		24.53 ±1.76	14.41 ±2.19	1.70:1
1965	0.2-0.3	permanent	311.5 ±118.1 (23)	> 0.05	25.59 ±2.28	12.64 ±1.79	2.03:1
1972	0.6-1.0	teeth	345.5 ±103.8 (28)		25.99 ±2.84	13.00 ±1.48	2.00:1

*n presents the number of case.

and dentin are about 130 and 360 ppm, respectively (1-4). In Guangzhou, before fluoridation, fluoride concentration in the water supply was 0.2-0.3 ppm. The average content of fluoride in enamel and dentin of deciduous teeth was 103.0 and 219.1 ppm, respectively; and that of permanent teeth was 121.3 and 311.5, respectively. After fluoridation (0.6-1.0 ppm in water) for 7 years, the average content of fluoride in deciduous teeth was 137.1 and 320.2 ppm, and that of permanent teeth 138.9 and 345.5 ppm, respectively. This result conforms with the reports (3,4) elsewhere.

The ratio of calcium to phosphorus in enamel and dentin was about 2:1 by weight (Table 1). This also conforms with other reports. (1-4).

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THE QUALITY OF DRINKING WATER AND HOT SPRING WATER FROM AN ENDEMIC DENTAL FLUOROSIS AREA IN NORTHERN JAPAN

by

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SUMMARY: This study was carried out to compare the quality of the drinking water consumed by school children living in a dental fluorosis area of the Tsugarū Plain in northern Japan and various hot spring water samples in and around the plain. The levels of F^- ions in drinking water, which was supplied from 22 deep wells, were significantly correlated with those of Cl^- ions, indicating that these wells had a common source. Concentrations of Na^+, Cl^-, HCO_3^- ions, and total residue, as well as F^- ions, were higher than those of ordinary underground water. The order of levels of major cations in drinking water agreed with that of hot spring water in Japan. According to 1) the levels of RpH and 8 trace elements, 2) the relationship between the levels of Ca^{2+}, Mg^{2+}, and HCO_3^- ions, and 3) the IR absorption spectra for the total residue, the quality of the drinking water was similar to that of the three hot springs near the wells. These results suggest that these two kinds of underground water passed through a similar stratum. On the other hand, F^-/Cl^- ratios for drinking water were higher than those of the three hot springs, suggesting that the F^- ions in the drinking water did not come from the three hot springs.

KEY WORDS: Deep well; Dental fluorosis; Drinking water; Hot spring water; Northern Japan.

Introduction

Dental fluorosis (mottled teeth) has been reported to be widespread among students at two neighboring primary schools on the Tsugaru Plain in northern Japan whose drinking water was supplied from 22 deep wells (1-3). Concentrations of sodium, chloride ions, and total residue (T-Re), as well as fluoride ions, were higher than those of ordinary underground water uncontaminated by either hot spring water or sea water which indicated the possibility that the hot spring water affected the plain's drinking water. In this study, the concentration of trace elements, as well as the major chemical components, were determined for both drinking water and water samples from the various hot springs in and around the plain. Furthermore, the quality of drinking water was compared with the quality of hot spring water in order to investigate the possibility that drinking water was contaminated by hot spring water.

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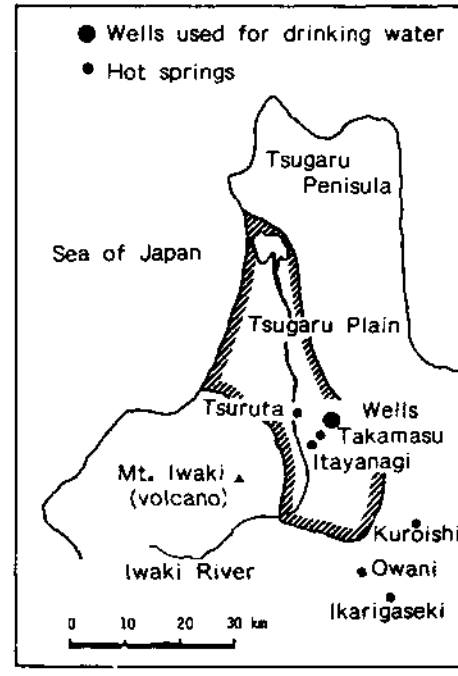
Materials and Methods

In January, 1977, samples of drinking water were taken from 22 wells (150 to 300 m deep) which were distributed within a 3 km radius. Water samples were also collected from 11 hot springs, three of which were located 2-7 km from the wells: others were located outside the plain at a distance of 20 to 30 km from the wells (Figure 1). The F^- ion concentration was determined by the ion selective electrode method (ORION 94-09), Cl^- by Mohr's method, and Na^+ by flame photometry. Both Ca^{2+} and Mg^{2+} were determined by atomic absorption spectrometry (HITACHI 508A), and IR absorption spectra for the total residue were obtained by the KBr tablet method (JEOL LAR-2). In November, 1977, water samples were also collected from 6 of the 22 wells and from the three hot springs near the wells. These samples were analyzed for 8 trace elements including Au, Be, Ge, As, Sb, Bi, Se, and Co, by atomic absorption spectrometry with a carbon tube atomizer after coprecipitation with zirconium hydroxide (HITACHI 170-50, PERKIN ELMER HGA-2100) (4).

Figure 1

Geographical Distribution of the Wells and Hot Springs



Results and Discussion

The results obtained from analysis of the drinking water samples are shown in Table 1. The concentration of F^- ions ranged from 0.30 to 2.27 ppm. Levels of F^- ions in the samples from the wells were significantly correlated with those of total residue ($r = 0.827$, $p < 0.001$) and Na^+ ions ($r = 0.871$, $p < 0.001$), but not with the levels of Ca^{2+}, Mg^{2+}, and HCO_3^- ions, nor total hardness.

Data for various hot spring water samples are also shown in Table 1. The concentrations of F^- ions ranged from 0.41 to 3.57 ppm. The levels in milliequivalents of the Na^+ and Cl^- ions, whose ratios were from 0.9 to 1.2 in almost all hot spring water, were nearly equal ($r = 0.995$, $p < 0.001$), indicating that both ions were dissolved as sodium chloride. For drinking water, although the levels of Ca^{2+}, Mg^{2+} ions, and thus total hardness, were as low as those of average river water in Japan, the levels of F^-, Cl^-, HCO_3^-, and Na^+ ions, as well as total residue of samples from most of the wells, were higher than those of ordinary underground water which was uncontaminated by either hot spring water or sea water.

Order of cation levels: According to Iwasaki (5), the order of levels of major

Table 1

Analysis* of Drinking Water and Hot Spring Water

		Temp. (°C)	pH	RpH	F^-	Cl^-	HCO_3^-	Na^+	Ca^{2+}	Mg^{2+}	Total hardness§	T-Re#
Drinking water												
(22 wells)	mean	23.0	8.3	8.5	0.90	59	154	84	11.7	2.1	38	322
	S.D.	1.7	0.3	0.1	0.48	55	19	38	4.2	1.4	15	99
	min.	21.8	7.4	8.5	0.30	9	125	42	4.8	0.5	14	219
	max.	27.2	8.8	8.8	2.27	246	191	204	23.6	4.8	70	658
Hot spring water												
Itayanagi		48	8.2	8.5	2.81	3724	535	2520	41.2	6.5	130	6871
Takamasu		43	8.2	8.6	2.37	617	266	475	15.9	1.1	44	1382
Tsuruta		85	7.6	8.7	0.41	351	643	418	19.4	32.7	163	1271
Kuroishi (2 sources)	mean	50	7.4	8.2	2.68	309	103	286	32.6	1.0	85	1070
Owani	mean	60	7.0	8.3	3.28	982	160	703	193	7.4	512	2560
(4 sources)	min.	45	6.9	8.3	3.11	613	118	461	149	4.3	389	1790
	max.	67	7.3	8.4	3.57	1205	226	903	221	13.1	606	3125
Ikarigaseki (2 sources)	mean	59	7.3	8.1	1.19	568	51	330	69.9	0.9	178	1260

* Concentrations of ions, Total hardness, and T-Re in ppm. § $CaCO_3$ ppm. based on the content of Ca^{2+} and Mg^{2+} ions.

Total residue after drying at 110°C.

cations in most mineral and hot spring water in Japan is $Na^+ > Ca^{2+} > Mg^{2+}$ regardless of the temperature and the quality of the water. This order, which is thought to depend on the amount of metals in the stratum, is different from that of river water ($Ca^{2+} > Na^+ > Mg^{2+}$) and sea water ($Na^+ > Mg^{2+} > Ca^{2+}$), but is the same as the order obtained for our drinking water samples. This order for the present hot spring water was $Na^+ > Ca^{2+} > Mg^{2+}$, except for the Tsuruta Hot Spring whose order was $Na^+ > Mg^{2+} > Ca^{2+}$.

Ion exchange: In general, the concentrations in mEq/l of Ca^{2+} ions have been found to be equal to those of HCO_3^- or SO_4^{2-} ions when the calcium salts in the stratum are dissolved and the ions added to hot spring water (6). The present data are not in agreement with this finding. However, as shown in

Figure 2

Relationship between the Levels of Chloride and Sodium Ions in Drinking Water

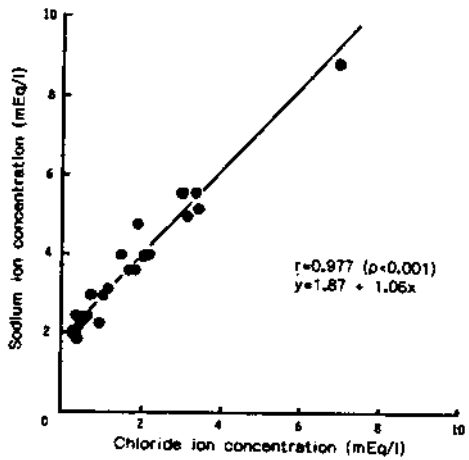
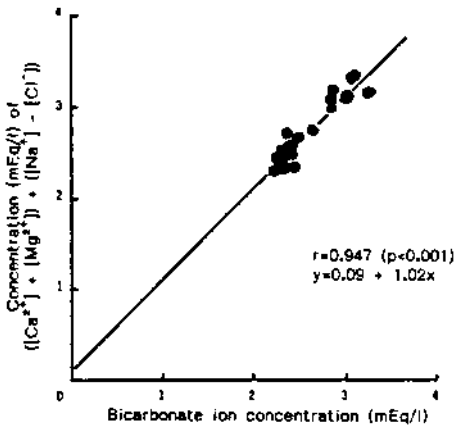


Figure 3

Relationship between the Levels of Bicarbonate Ions and Major Cations in the Drinking Water



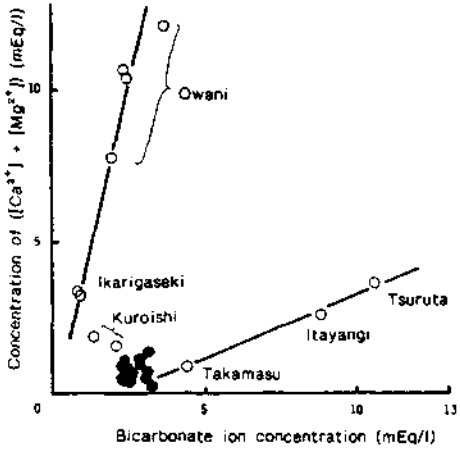
Fluoride

Figure 2, a significant correlation was obtained between the levels of Na^+ and Cl^- ions ($r = 0.977$, $p < 0.001$); the levels of Na^+ ions greater than about 2 mEq/l were equal to those of Cl^- ions. As shown in Figure 3, when the excess of Na^+ ions to Cl^- ions was added to ($[Ca^{2+}] + [Mg^{2+}]$), the total concentrations of ($[Ca^{2+}] + [Mg^{2+}]$) + ($[Na^+] - [Cl^-]$) became nearly equal to those of $[HCO_3^-]$, probably due to the partial replacement of Ca^{2+} and Mg^{2+} ions by Na^+ ions. Thus, an ion exchange phenomenon, generally taking place in hot spring water, was possibly also taking place in the sources for the present drinking water samples. As described above, the quality of drinking water was similar to that of hot spring water. Indeed, according to the following: 1] the relationship between the levels of Ca^{2+}, Mg^{2+} and HCO_3^- ions, 2] RpH, 3] IR absorption spectra for the total residue, and 4] the levels of 8 trace elements, the quality of the water samples from the wells was similar to that of the three hot springs near the wells.

Relationship between the levels of Ca^{2+}, Mg^{2+} and HCO_3^- ions: As shown in Figure 4, analysis of the relationship between levels of $[HCO_3^-]$ and ($[Ca^{2+}] + [Mg^{2+}]$) showed that the samples from the three hot springs near the wells were high in HCO_3^- ions, whereas samples from hot springs outside the plain were high in Ca^{2+} and Mg^{2+} ions. In contrast, samples of drinking water were low in Ca^{2+} and Mg^{2+} ions but were high in HCO_3^- ions; they belonged to the group consisting of the three hot springs near the wells.

Figure 4

Relationship between the Levels of Bicarbonate Ions and ($[Ca^{2+}]+[Mg^{2+}]$) in the Drinking Water (●) and Hot Spring Water (○)



RpH: The values of RpH varied among hot spring groups, although those within each group were almost the same. The level of RpH for the three hot springs near the wells was about 8.5, which is almost the same as that for the drinking water. Thus, it is probable that, except for the carbonate group (CO_2, H_2CO_3, HCO_3^-, and CO_3^{2-}), the factors which affected the pH values were common to the samples from the 22 wells and the three hot springs.

IR absorption spectra: In order to further determine the effect of the stratum on water quality, IR absorption spectra for total residue were obtained. This analysis provided valuable information because the spectra do not reflect substances having ionic bonds (e.g. sodium chloride) which were present in large quantities in the ion rich samples. The IR absorption spectra for the samples from the three hot springs near the wells were similar and almost the same as those for the drinking water from the 22 wells (Figure 5), whereas those for the samples from other hot springs were different (Figure 6).

Trace elements: Concentrations in ppb of trace elements determined for samples from 6 of 22 wells and from the three hot springs near the wells are shown in Table 2. Levels of germanium varied markedly compared with the

Figure 5

IR Absorption Spectra for the Total Residue of the Drinking Water (representative sample of the 22 wells) and the Water from the Three Hot Springs near the Wells.

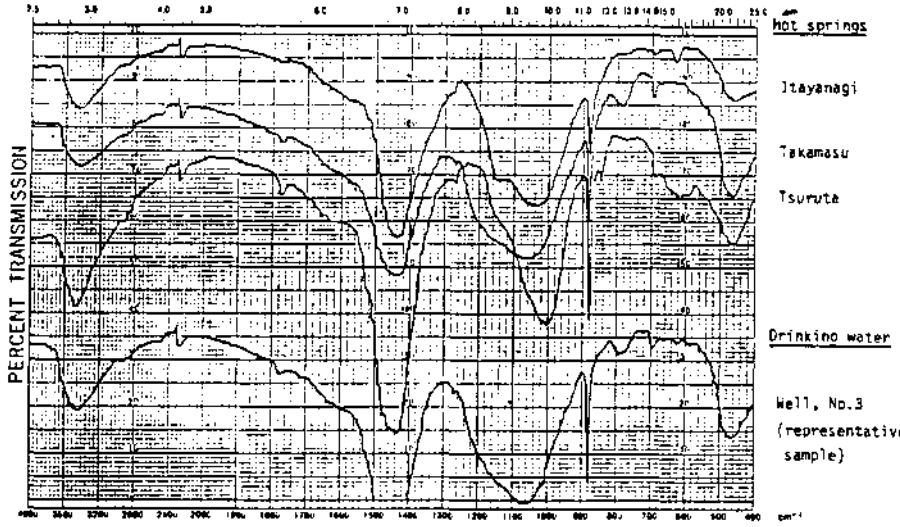


Figure 6

IR Absorption Spectra for the Total Residue of the Hot Spring Water

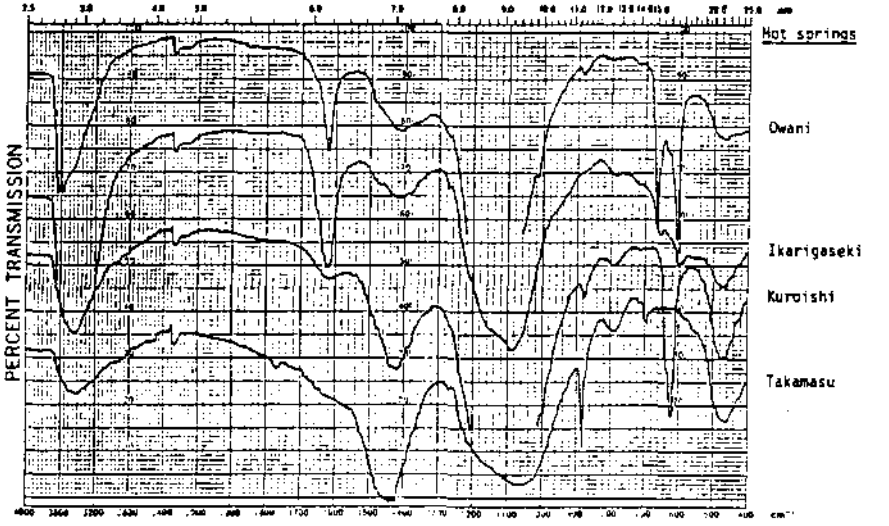


Table 2

Concentrations (ppb) of Trace Elements in Drinking Water and Hot Spring Water

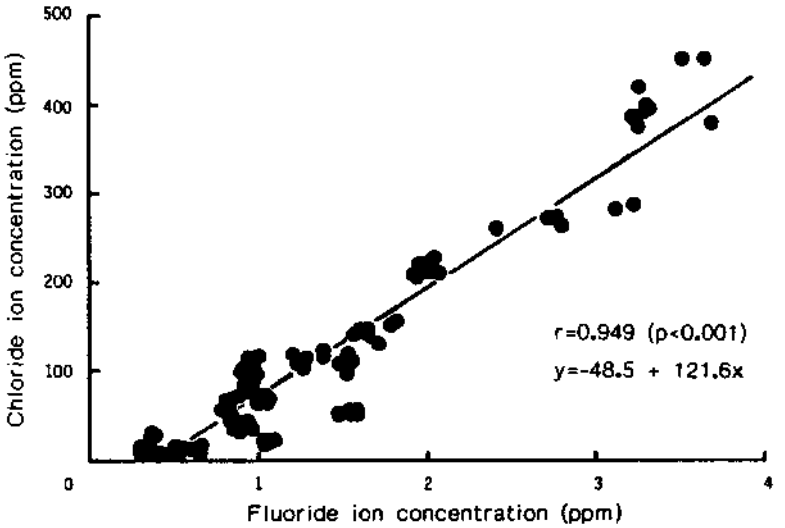
		Au	Be	Ge	As	Sb	Bi	Se	Co
Drinking Water									
(6 wells)	mean	0.33	0.020	1.47	0.82	0.88	0.00	1.69	0.49
	S.D.	0.09	0.002	0.76	0.09	0.06	0.00	0.46	0.06
	min.	0.24	0.016	0.00	0.68	0.82	0.00	1.03	0.41
	max.	0.47	0.022	2.32	0.92	0.96	0.00	2.33	0.57
Hot Spring Water									
Itayanagi		0.37	0.024	5.30	1.17	1.07	0.00	1.71	0.56
Takamasu		0.39	0.028	1.63	0.89	1.07	0.00	2.45	0.55
Tsuruta		0.40	0.029	0.00	0.90	1.13	0.00	2.23	0.55
	mean	0.39	0.027	2.31	0.99	1.09	0.00	2.13	0.55

other trace elements. On the whole, levels of these elements in drinking water were not different from those in samples from the three hot springs. The above described results together suggest that water from these wells and from the three hot springs passed through a common stratum.

F^- and Cl^- ions: Figure 7 shows the monthly variations of levels of F^- and Cl^- ions in drinking water from 23 wells between December, 1974 and August, 1975 (7). Even when the levels of F^- ions varied, the ratios of F^- ions to Cl^- ions were generally constant ($r = 0.949$, $p < 0.001$), indicating that these wells had a common source. As shown in Figure 8, concerning the ratios of levels of F^- ions to Cl^- ions, the drinking water, compared with hot spring water, was rich in F^- ions. This finding suggest that the F^- ions in the drinking water did not result from dilution of hot spring water with circulating underground water having low ion concentrations.

Figure 7

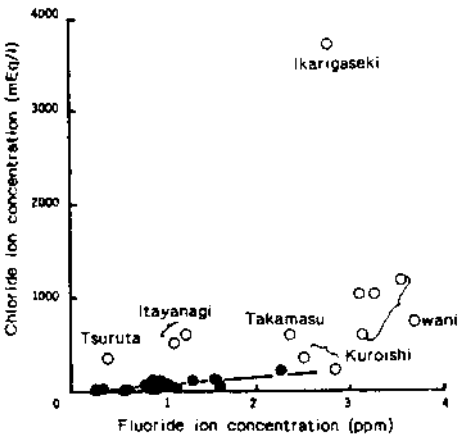
Monthly Variation in the Levels of Fluoride and Chloride Ions in the Drinking Water from the 22 Wells (after Matsuda, K. et al., 1978)



Conclusion

Figure 8

Relationship between the Levels of Fluoride and Chloride Ions in the Drinking Water (●) and Hot Spring Water (○)



As described above, the relationship between levels of F^- and Cl^- ions in drinking water showed a significant correlation, suggesting that the drinking water from 22 wells had a common source. There is a possibility that hot spring water contaminated the drinking water, because its F^- ions level, total residue, etc. were higher than those of ordinary underground water. Indeed, 1) the levels of RpH and trace elements, 2) the relationship between the levels of Ca^{2+}, Mg^{2+} and HCO_3^- ions, and 3) the IR absorption spectra for the total residue all indicate a similarity between drinking water and the three hot springs near the wells. These results suggest that these two kinds of water passed through a common stratum. However, it is highly unlikely that the F^- ions in the drinking water came from the three hot springs because, as shown in Figure 8, the F^-/Cl^- ratios of the drinking water were higher than those of the three hot springs.

Consequently, when drinking water is obtained from deep wells, there is the possibility (and risk) that high levels of F^- ions may be present which can cause dental fluorosis, even if this well water is not contaminated by hot spring water.

Acknowledgement

Gratitude is expressed to Professor Y. Takaesu, Tokyo Dental College (Chiba, Japan) for his support during this investigation; to Professor A. Sato, Seikatsu Gakuen Junior College (Morioka, Japan) for his suggestions concerning the analysis; Professor Ming-Ho Yu, Huxley College of Environmental Studies, Western Washington University (U.S.A.), for his comments on this manuscript; and to Assistant Professor P. Langman, Iwate Medical University (Morioka, Japan) for his assistance regarding English usage.

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LATE RESPONSES IN SKELETAL FLUOROSIS

by

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SUMMARY: In fifteen proven cases of skeletal fluorosis motor nerve conduction velocities of ulnar, median and lateral popliteal nerves were studied electrophysiologically. Electromyography was done in the muscles supplied by C_5 root. Late responses, 'F' wave and 'H' reflex, showed unequivocal evidence of neurogenic lesion, with normal motor conduction velocities and delayed or absent 'F' and 'H' latencies.

KEY WORDS: Electromyography; 'H' reflex; Nerve conduction velocities.

Introduction

There is considerable controversy regarding the single case report of skeletal muscle involvement in skeletal fluorosis in industry. The myopathic and myelopathic features observed were attributed to the direct action of the fluoride ion on these tissues because no evidence of spinal cord or nerve root compression was observed (1,2). Similar myopathic changes, attributable to fluoride, were also reported in experimental studies by Kaul and Susheela (3,4). In earlier studies in human fluorosis, from this department, evidence of neurogenic atrophy was shown. The nerve lesion in fluorosis was thought to be located either in the nerve root or in the peripheral nerve (5,6). The aim of the present study was to determine the location of the nerve lesion in skeletal fluorosis.

Methods and Materials

Fifteen established cases of skeletal fluorosis, 5 females and 10 males (aged 21 to 40 years), were selected at random. Detailed clinical history was recorded and a thorough clinical examination was conducted with special reference to the nervous system.

Electrophysiological investigation: Motor nerve conduction velocities were studied in the median, ulnar and lateral popliteal nerves. Electromyography was done in the muscles supplied by C_5 root, namely deltoid, supraspinatous, triceps and brachioradialis according to the method described earlier (6). Late responses, 'F' wave and 'H' reflex were studied by the Lambert and Daube method (7).

Results

Both peripheral motor nerve conduction velocities in all three nerves studied and compound action potentials were within normal range (Table 1). Electromyographic studies revealed evidence of a neurogenic lesion in the muscles studied in 13 patients. Whereas clinical evidence of muscle involvement

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Table 1

Late Responses in Fluorosis

Abnormal Late Responses Normal NCS*	Normal Late Responses Abnormal NCS	Abnormal Late Responses Abnormal NCS	Normal Late Responses Normal NCS
15	0	0	0

*NCS: Nerve conduction studies

was seen in only 8 patients (Table 2), late responses were abnormal in all patients. 'F' wave responses of the ulnar nerve (Abductor degiti minimi) were not elicitable in 7 patients (Table 3). 'H' reflex was not elicitable in 7 patients on both sides and in 3 the difference in the latencies was more than 2 m sec.

Table 2

Electromyographic Findings

Muscles Tested	Clincial Involvement (Wasting/Weakness)	Electromyographic Evidence of Neurogenic Lesion
Supraspinatus	7	10 (3)
Deltoid	9	13 (4)
Biceps	8	11 (3)
Brachio Radialis	5	8 (3)

Figures in parenthesis indicate number of patients who showed evidence of neurogenic lesion on EMG without clinical involvement.

Table 3

Late Responses in Fluorosis

	F ADM (m sec)	H Soleus (m sec)
Controls	26.6 (21-32)	28.9 (26.8-31)
Patients	31.18 (29-34)*	43.2 (30.6-34.5)**

* Not elicitable in 7 patients. ** Right and left difference more than 2-3-m sec in 3 patients and not elicitable in 7 patients.

Discussion

In recent years, many investigators have been using monosynaptic H reflex and the F response, which is not a reflex, to evaluate the function of the peripheral nervous system (8-10). Whereas conduction only in a motor axon is tested by 'F' response latency, 'H' reflex latency gives information about activity in large afferent (ia) as well as efferent fibers (10,11). These delayed

latencies assess conduction in the proximal segment of both motor and sensory axons as well as excitabilty of the anterior horn cell pool. Abnormal prolongation of minimal late response latencies in entrapment neuropathies is an important adjunct to routine motor and sensory nerve conduction studies. Appropriate abnormalities of late responses have been recorded in surgically proven thoracic outlet syndrome (12), and root compression syndromes affecting S_1/S_2 roots (8,13). In this study electromyographic features are neurogenic in nature. Beause the muscles supplied by one root were tested it can constitute evidence of root lesion.

Conclusion

Thus, from this study, it can be concluded that the nerve lesion is located in the root and that this is the cause of muscle involvement in skeletal fluorosis.

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RELATIONSHIPS BETWEEN IONIC FLUORIDE, TOTAL FLUORIDE, CALCIUM, PHOSPHORUS AND MAGNESIUM IN SERUM OF FLUOROSIS PATIENTS

by

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SUMMARY: The fluoride concentration in serum has been determined by both the AlF method and Ion Selective Electrode method. Ion Selectrode has been used to estimate free fluoride ion (F^-). The AlF method was used to estimate total fluoride TF (ionic fluoride plus non-ionic fluoride) by measuring the molecular absorption intensity of AlF with atomic absorption spectrophotometer. F^-, TF, Ca, P, Mg were measured in serum of healthy human adults and fluorosis patients. Significant differences have been found between the two groups: the ratio of ionic fluoride to total fluoride, F^-/TF, and the ratio of ionic fluoride to calcium, F^-/Ca. A high correlation between two groups: phosphorus and ionic fluoride have also been found. The findings correspond with the clinical symptoms, which might be useful in early diagnosis, in the study of the mechanism of fluorosis and in evaluating therapeutic results.

KEY WORDS: AlF molecular absorption spectrometry; Ionic fluoride; Non-ionic fluoride; Ratio of ionic fluoride to total fluoride (F^-/TF); Total fluoride.

Introduction

To evaluate the effect of fluoride on human health, a number of studies have been done with ionic fluoride in serum. But no reliable method has been available for the determination of ionic fluoride in human serum because of its extremely low concentration. Usually serum ionic fluoride concentration in healthy human adults is less than 10^{-6}M, which is a limit of fluoride electrode detection. Thus, reported values vary over a wide range.

Recently Tsunoda (1), Chiba (2), Fujimori et al (3) reported the total fluoride concentrations in serum (ionic fluoride plus non-ionic fluoride) by AlF molecular absorption spectrometry method, which is currently satisfactory for determination of serum fluoride. By means of this method we (4) have estimated total serum fluoride and compared it with ionic fluoride. We have found the ratio of F^-/TF in patients with fluorosis is larger than in healthy humans and the F^-/TF values related to the degree of fluorosis corrrespond with clinical symptoms (preliminary report).

Methods and Materials

Serum ionic fluoride was determined with an electrode specific for the fluoride ion. Serum total fluoride was determined (4) by measuring molecular

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absorption intensity of AlF with atomic absorption spectrophotometer (Instrumentation Laboratory Video 22 Atomic Absorption Spectrometer, IL Visimax II platinum lamp, IL 655 CTF controlled-temperature furnace), IL 254 FastacII (Flame/Furnace Aerosol Sampling Technique with Automatic Calibration) — Deuterium Lamp background correction (Tables 2, 3, 4).

Table 2

Atomic Absorption Instrumental Parameters

Hollow Cathode Lamp Current:	10 mA
Wavelength	227.5 nm
Bandpass	0.15 nm
Analysis Mode	A - BKG
Integration Time	4 sec., peak height

Table 3

Graphite Atomizer Instrumental Parameters

Purge Gas: N_2 or Ar
Graphite Cuvette: Round Coated

Analysis Program:	Temperature (°C)	Time (sec)
Injection 1		
drying	150	5
ashing	700	15
stop		
injection 2		
drying 2	150	5
ashing 2	700-850	15-25
atomization and measurement	2000	0-5

Table 4

IL 254 (FASTAC) Parameters

Delay	5 sec.
Deposition	20 sec.*
Repeats	as desired
Aspiration Rate	4.0 ml/min

* varies with detection limit demands

Calcium, phosphorus, and magnesium are estimated by absorption spectrophotometry methods. All chemicals are analytical grade. Fluoride standards were established by dissolving sodium fluoride in non-ionic water.

Results

The relationships between F^-, TF, Ca, P, Mg in serum of healthy human adults and fluorosis patients are presented in Table 1.

Significant correlations have been found (F^-, TF/Mg, $p < 0.05$; P, F^-/TF, F^-/P, F^-/Mg, F^-/Ca, Ca/P, TF/P, P/Mg $p < 0.01$). Changes in serum of fluorosis patients besides F^-, P concentrations also affect the ratios of the concentrations of other components. These findings might be useful in early diagnosis, in the study of the mechanism of fluorosis, and in evaluating therapeutic results.

Discussion

It is well known that a large part of serum fluoride is non-ionic fluoride in addition to ionic fluoride. In previous studies it was noted that non-ionic fluoride did not have any biological activity. For this reason only ionic fluoride has been determined in the past. In a current study, a positive correlation has been shown (4) in the ratio of serum ionic fluoride to non-ionic fluoride comparing healthy human adults with fluorosis patients. When serum ionic fluoride

Table 1

Relationship between F^-, TF, Ca, P, Mg, in Serum in Healthy Human Adults and Fluorosis Patients

	Healthy Human Serum			Patients			T	P
	Means	(N)	±S.D.	Means	(N)	±S.D.		
F^-(μg/ml)	0.049	(33)	0.0089	0.081	(21)	0.026	2.122	<0.05
TF(μg/ml)	0.313	(33)	0.069	0.363	(21)	0.134	1.555	>0.05
Ca(mg/dl)	8.946	(33)	0.804	9.266	(21)	0.840	1.391	>0.05
P(mg/dl)	4.226	(23)	0.716	3.004	(21)	0.820	5.270	<0.01
Mg(mM/L)	2.593	(32)	0.405	2.621	(20)	0.783	0.146	>0.05
F^-/TF(%)	16.431	(33)	4.233	24.579	(21)	12.493	2.885	<0.01
F^-/P	0.0118	(23)	0.00248	0.0297	(21)	0.0139	5.788	<0.01
F^-/Mg	0.0190	(32)	0.00338	0.0329	(20)	0.0118	5.116	<0.01
F^-/Ca	0.547	(32)	0.100	0.881	(21)	0.290	5.075	<0.01
TF/Ca	3.531	(32)	0.842	3.872	(21)	1.210	1.264	>0.05
Ca/P	2.224	(23)	0.686	3.343	(21)	1.083	4.049	<0.01
Ca/Mg	3.508	(32)	0.516	3.771	(20)	1.059	1.036	>0.05
TF/Mg	0.123	(32)	0.0309	0.144	(20)	0.0432	2.053	<0.05
TF/P	0.0807	(23)	0.0308	0.129	(21)	0.0522	3.668	<0.01
P/Mg	1.639	(23)	0.359	1.231	(20)	0.580	2.726	<0.01

was increasing, serum total fluoride (F^- plus non-ionic fluoride) was decreasing (Table 5); F^-/TF was increasing. Experiments have demonstrated the existence of an equilibrium between serum ionic fluoride and non-ionic fluoride. Thus using F^-/TF as a reference value might be a better indicator than ionic

Table 5

Serum Ionic Fluoride and Total Fluoride in Healthy Human Adults and in Fluorosis Patients

	Healthy Human Adults			Fluorosis Patients		
	F^-	TF	F^-/TF(%)	F^-	TF	F^-/TF(%)
1.	0.039	0.428	9.1	0.140	0.317	44.2
2	0.042	0.336	12.5	0.100	0.241	41.5
3.	0.056	0.392	14.3	0.070	0.254	27.6
4.	0.051	0.375	13.6	0.100	0.385	26.0
5.	0.043	0.361	11.9	0.130	0.303	42.9
6.	0.041	0.396	10.4	0.130	0.375	34.1
7.	0.048	0.335	14.3	0.140	0.287	48.8
8.	0.047	0.355	13.2	0.100	0.311	32.2
9.	0.048	0.409	11.7	0.170	0.241	70.5
10.	0.048	0.318	15.1	0.170	0.282	60.3

fluoride alone. Comparison of ratios of other elements may also be more useful measures of [potential] injury than quantitative amounts. Positive correlations have been found between total serum fluoride to serum calcium and serum magnesium (Figures 1, 2) in fluorosis patients. Although these mechan-

Figure 1

Relationship between Ca and TF in Serum of Fluorosis Patients.

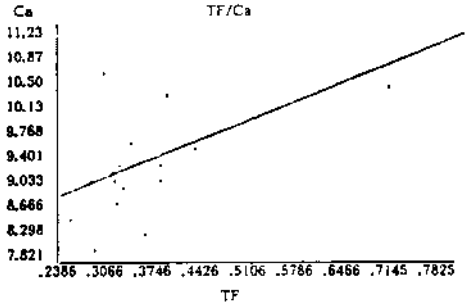
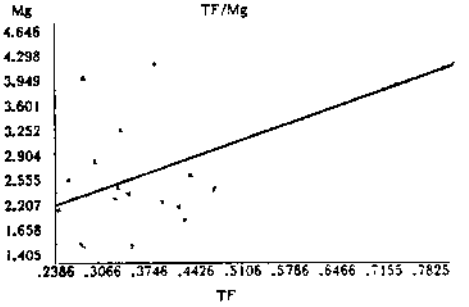


Figure 2

Relationship between Mg and TF in Serum of Fluorosis Patients.



isms are not clear yet, many experiments suggest that these ratios play a role in the incidence and during the course of fluorosis. Further investigation is indicated.

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CORRECTION: In the paper "Dry Deposition of Fluorides on Lime Papers" by L. De Temmerman and H. Baeten (FLUORIDE, 19:3, 124-131, July, 1986), the word "emission" was mistakenly substituted by the editor for the author's word "immission" (ambient concentration). The word "immission" should therefore replace "emission" wherever it occurs in the text and on the figures.

SIMPLE METHOD FOR OBTAINING BONE BIOPSY SPECIMENS FOR FLUORIDE ANALYSIS AND SOME PRELIMINARY RESULTS

by

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(Abstracted from New Zealand Medical Journal 98:454-455, 1985)

Traditionally, bone samples for fluoride analysis have been obtained either from the iliac crest, ribs, vertebrae, femora or sternum in cadavers or in living subjects, from the iliac crest. The interradicular septum, namely the portion of alveolar bone between the roots of molar teeth which are exposed when the tooth is extracted, is a very convenient piece of bone for analysis. It is both easily obtained, and it has a high turnover rate; moreover, fluoride is known to concentrate in areas of active ossification.

To test the new method, fluoride concentrations in alveolar bone of 24 female subjects were measured, 12 of whom were suffering from so-called repetitive strain injury (RSI). All test subjects resided in a Melbourne fluoridated area. Fluoride content (ppm) of 24 alveolar bone ash samples is presented in Table 1. Fluoride levels in bone from subjects with RSI are, on an average, appreciably higher than those found in subjects not afflicted with RSI, namely up to 3300 (average 2737) in the former and up to 2200 (average 1687) in the latter.

Table 1

Body Weight, Age, and Fluoride Content in Alveolar Bone of 24 Female Patients with and without Repetitive Strain Injury (RSI).

Patients with RSI				Patients without RSI			
Patient	Weight (kg)	Age (yr)	F in alveolar bone (ppm)	Patient	Weight (kg)	Age (yr)	F in alveolar bone (ppm)
1	49.1	22	1850	1	53.6	24	1700
2	45.0	27	2600	2	57.7	22	1400
3	52.3	24	2950	3	55.9	22	900
4	51.0	28	2400	4	59.1	28	2200
5	47.3	20	1700	5	62.7	26	1750
6	48.2	31	3700	6	56.8	34	2100
7	51.8	28	3100	7	61.8	37	1900
8	54.5	26	2850	8	56.8	32	2100
9	62.7	25	2900	9	64.5	21	1600
10	56.8	36	3300	10	58.2	19	1200
11	58.2	30	3100	11	59.5	24	1800
12	59.1	28	2400	12	56.4	23	1600
Average	53.0	27	2737 ppm F		58.7	26	1687 ppm F

Of the 17 patients whose bones contained 1350-4720 ppm F (mean 3320 ppm) investigated by another team, none was seriously incapacitated. However, all but one complained of vague pains and stiffness in the lower and upper extremities, shoulders, neck and lower back. X-ray examinations revealed bone

changes in vertebrae, legs, knees, ankles and, particularly, in forearms and elbows where there were free bony bodies as well as clear evidence of calcification of tendons and ligaments.

This preliminary study suggests that fluoride accumulation in bones should be closely monitored, particularly in areas with fluoridated drinking water.

KEY WORDS: Alveolar bone; Bone biopsy; Bone fluoride; Fluoride toxicity (or intoxication); Repetitive strain injury.

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REPETITIVE STRAIN INJURY (RSI) AND MAGNESIUM AND FLUORIDE INTAKE

by

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(Abstracted from New Zealand Medical Journal, 98:556-557, 1985)

Repetitive strain injury (RSI), a "new" clinical syndrome, is characterized chiefly by a severe pain in wrists, forearms, hands and fingers. Although to date, the cause and pathogenesis of RSI is obscure, it is clearly related to frequent physical stresses; it seems to involve, mainly, musculoskeletal structures.

In 12 RSI subjects fluoride (F^-) levels in bone were appreciably higher than 12 appropriate controls. Estimates of dietary intake of RSI subjects, revealed a Mg^{2+} deficit and an excessive F^- intake. Fluorotic bone has an increased Mg^{2+} content possibly due to some deposition of MgF_2. The amorphous phase in bone may act as a "reservoir" of ions available to regulate plasma Ca, PO_4 and Mg^{2+} levels. Fluoride accumulates in bone with age, especially in areas of active ossification. A locally raised F^- concentration in an osteocyte lacunae (during resorption) could interfere with normal functioning of the cell, or trigger the precipitation of crystalline apatite, or lead to the formation of MgF_2. Any one of these reactions might interfere with the passage of Mg^{2+} ions from the bone "reservoir" into circulating extracellular fluid. A localized Mg^{2+} deficiency could disturb pyrophosphate metabolism and lead to deposition of Ca salts in sensitive areas.

Through adjustment of dietary intake of the previously mentioned 12 RSI subjects which included more Mg^{2+} and less F^-, eight of the subjects experienced marked relief from previously painful RSI symptoms after a six week test period.

KEY WORDS: Fluoride-magnesium intake; Repetitive strain injury.

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REPETITIVE STRAIN INJURY, OR INCIPIENT SKELETAL FLUOROSIS?

by

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(Abstracted from New Zealand Medical Journal 98:328, 1985)

Chronic fluoride intoxication may play a role in the etiology of some cases of so-called repetitive strain injury (RSI).

Early bone fluorosis is not clinically obvious; often the only complaints of young adults are vague pains in the small joints of the hands, feet, and lower back. Such cases may be misdiagnosed as rheumatoid arthritis or ankylosing spondylitis. As fluoride continues to accumulate in bone, radiologically detectable changes may be diagnostic. They include fascial calcification along tendons and muscular attachments, particularly the interosseous membranes of forearms and legs. Before x-ray detectable deposits build-up in these tissues, microcrystals of hydroxyapatite must be present. Tendonitis and bursitis are occasionally associated with periarticular deposits of hydroxyapatite. A condition called "calcific perarthritis" often mimics acute arthritis.

It is conceivable that some cases of RSI might result from deposition of apatite crystals in and around synovial sheaths and tendons passing through the carpal tunnel.

KEY WORDS: Arthritic symptoms; Incipient skeletal fluorosis; Repetitive strain injury

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PLASMA FLUORIDE AND BROMIDE CONCENTRATIONS DURING OCCUPATIONAL EXPOSURE TO ENFLURANE OR HALOTHANE

by

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(Abstracted from Acta Anesthesiol. Scand., 29:699-673, 1985)

In seven patients exposed to 200 ppm enflurane, the mean pre-exposure plasma concentration of fluoride was 0.78 ±0.26 μmol/l. The increase after 1 hr was significant. After 2 hrs exposure, the mean concentration was 2.14 ±0.61 μmol/l. Thirty minutes after discontinuation of enflurane, plasma fluoride had decreased. Whereas eight hours after discontinuation, the increase was still significant compared to pre-exposure concentrations, it lasted less than 12 hours. The pre-exposure cerebral spinal fluid (CSF) fluoride concentration was 0.39 ±0.08 μmol/l. Compared to increase in plasma that of CSF was smaller and delayed.

Plasma fluoride concentration is influenced by dietary intake. Fluoride, an end-product of enflurane biodegradation, released after anesthesia, is partly incorporated into bone matrix and partly excreted in urine. Renal clearance of fluoride is influenced by urine acidity.

Thus the present study shows that, even if enflurane is the only volatile anesthetic used, the increase in plasma fluoride due to routine occupational exposure is indistinguishable from normal variations.

KEY WORDS: Blood; Bromide; Cerebrospinal fluid; Enflurane; Environmental exposure; Fluoride; Halothane.

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LEVELS OF FLUORIDE IN SALIVA AND URINE DEPENDING ON TYPE OF ANTICARIES FLUORIDE PROPHYLAXIS AND ON DENTAL CARIES RESISTANCE OF CHILDREN

by

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(Abstracted from Metabolizm Fluoru, 1982, p. 46)

The investigation was to determine the effect of various methods of anticaries fluoride prophylaxis on the levels of fluoride in two body fluids in children, namely saliva and urine, and to determine whether fluid concentrations and occurrence of dental caries are correlated.

Fluoride was determined with a photocolorimetric method based on a very sensitive reaction with a colored complex of zirconium and eriochromocyanine R. The levels of fluoride in saliva and urine of 162 children aged 10 and 11 years (DMF = 0 or DMF > 4) were determined. The children represented three groups which differed in methods of fluoride anticaries prophylaxis.

Statistical analysis of experimental results led to the following conclusions:

Whereas correlation between the level of fluoride in body fluids (especially urine) and dental caries in children is evident, decisive quantitative description is difficult.

Fluoride level in urine depends on the absorption of this element by the system. The level of fluoride in saliva and urine is significantly dependent on the method of fluoride anticaries dental prophylaxis which is applied.

KEY WORDS: Caries; Fluoride; Saliva; Urine.

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PHARMACOKINETICS OF CHRONIC FLUORIDE INGESTION IN GROWING PIGS

by

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(Abstracted from Journal of Dental Research 64:425-430, 1985)

To study the pharmacokinetics of chronic fluoride administration to growing pigs, data were first obtained on plasma levels in relation to oral dose and secondly on bio-availability, biological half-life, and accumulation of the drug. Detailed quantitative investigations of the accumulation of fluoride in bone and its relation to plasma fluoride have not hitherto been reported. Estimation of biological half-life is a prerequisite for calculation of the experimental periods necessary to achieve steady-state plasma concentrations. The study showed that accumulation of fluoride in bone influences plasma fluoride levels during chronic administration of fluoride to growing pigs. The long biological half-life showed that steady-state plasma levels could not be achieved within the six-month experimental period. Thus, for dose-response studies of dental fluorosis in this animal, it is not possible to achieve steady-state plasma concentrations as a basis for correlations to the degrees of pathological change observed in the teeth.

Mean pre-dose plasma fluoride levels increased from 0.014 mg F^-/l (SD 0.002, n = 8), prior to the period of fluoride administration, to 0.242 mg F^-/l (SD 0.038, n = 8) at the end of the experiment. An apparent, mean steady-state plasma fluoride concentration of 0.014 mg/l was observed for controls. Body weight of all animals increased continuously throughout the experiment. Differences between mean weight of experimental and control groups were not significant. Mean fluoride content of cortical bone from fluoride-treated pigs was 1737 mg/kg (SD 309), compared with 129 mg/kg (SD 26) in controls. Corresponding values of trabecular bone were 2836 (SD 200) and 181 (SD 16) mg/kg. This experiment has provided estimates of pharmacokinetic parameters applicable when using the pig for studies of skeletal and dental fluorosis.

KEY WORDS: Chronic F^- ingestion in pigs; F^- plasma levels; Growing pigs

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INHIBITION OF ACID PRODUCTION FROM ORAL BACTERIA BY FLUORAPATITE-DERIVED FLUORIDE

by

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(Abstracted from J. Dent. Res., 65:30-33, 1986)

The inhibitory effect of fluorapatite (FAP)-derived fluoride upon resting cell suspensions of Streptococcus mutans incubated at pH 4.5 and 6.5 was studied using lactic acid production from 0.1% sucrose as an indicator of fermentation activity. Cells incubated with FAP produced significantly less lactic acid than did cells incubated with hydroxyapatite (HAP). Addition of HAP to cell suspensions containing FAP was necessary for inhibition. Incubation with low concentrations of NaF showed significant inhibition in cell suspensions incubated with as little as 0.45 micrograms/mL F at pH 5.0. These results provide further support to the hypothesis that fluoride levels in plaque and enamel, achievable through use of fluoridated water and/or fluoride dentifrices, may produce appreciable inhibition of glycolysis at acidic pH levels readily achieved in plaque. Thus, bacterial acid production may activate plaque and enamel-bound fluoride, resulting in inhibition of further acid production, and thereby contribute substantially to other cariostatic mechanisms of fluoride.

KEY WORDS: Cariostatic mechanism; Fluoroapatite; Glycolysis inhibition; Plaque; Streptococcus mutans.

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TO THE PROBLEM OF TRACE ELEMENTS AND HYDROCARBONS EMISSIONS FROM COMBUSION OF COAL

by

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(Abstracted from J. Hygiene Epidemiol. Microbiol. & Immunol. 28:129-38, 1984)

Coal is the major source of energy in Czechoslovakia and likely to remain so until the end of this century. Apart from the chronic air pollution problems associated with emissions of oxides of sulfur and fly ash, attention is now increasingly centered on atmospheric trace elements emitted from coal-fired power plants and on emission of hydrocarbons that are invariably detectable in the combustion product condensate. In virtually all larger power plants in Czechoslovakia, measurements were carried out between 1975 and 1981 and the data were compared with data reported from the U.S.A.

A broad spectrum of hydrocarbons found in the condensate of combusion products, originating during the coal mass combusion, seemed to indicate the presence of both burning and pyrolitic processes. Apart from organics, the condensate was found to contain a relatively large amount of fluoride compounds (hundreds of mg/l condensate). A long-term analysis of emissions from a power plant in North Bohemia revealed that the fluoride content ranged between 250 and 480 mg/l. Measurements carried out in a power plant in Central Bohemia indicate that waste gas condensate fluorides range from 136-260 mg/l.

According to arithmetic averages of measurements from about 40 power plants during the years 1975-1980, fluoride from combustion of coal in a coal-fired boiler for power generation was 69.4% of the total amount of element supplied; average of 35 measurements from a single power plant in May, 1980 was 52.2.

Large continuous source of emissions are commonly known to have an adverse effect on the biosphere by producing dust aerosols that contain toxic metals. However, relatively little attention is given to the distribution of fluorides and organic compounds whose presence in urban air may constitute one of the major air hygienic problems to the community.

KEY WORDS: Coal combustion; Fluoride emissions; Hydrocarbon emmissions.

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TRABECULAR STRESS FRACTURES DURING FLUORIDE THERAPY FOR OSTEOPOROSIS

by

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(Abstracted from Skeletal Radiol., 14:276-279, 1985)

Osteoporosis is characterized by reduction in bone mass to a level where fractures occur spontaneously or after minimal trauma. Side effects, such as periarticular pain and swelling in the lower limbs reported to occur on an average of 33.2% of patients exclusively in the lower limbs suggest that mechanical factors play a part. The present paper reports bone changes in patients with "fluoride pains." Eight patients suffered a total of 17 episodes of joint pain and swelling in the lower limbs at varying stages of fluoride treatment. Thirteen of the 17 episodes occurred between 7 and 18 months of therapy; the earliest was encountered at 4, and the latest at 36 months.

In a typical episode, the patient complained of spontaneous onset of moderate to severe pain at the affected site. It reached full intensity in one or two days, accompanied by swelling. It was aggravated by weight-bearing but not by passive joint movement, and was relieved by rest.

Clinical examinations revealed swelling and increased warmth of the affected region. All five affected knee joints showed an effusion. Tenderness in the knee region was located either above or below the joint line, in the ankle area on the anteromedial aspect of the tibia 2 or 3 cm above the joint, and in the heel on all aspects of the hindfoot, including the sole. Treatment consisted of discontinuation of NaF therapy in two, temporary increase of vitamin D dosage in one, and immobilization in a plaster cast in three patients.

Radiographs taken at 6-8 weeks showed features suggestive of a healing stress fracture in every case. The affected sites were the distal tibia and posterior half of the calcaneum in six instances each. All five radionuclide scans showed increased uptake at the site of the lesion. The sites of increased uptake were the distal tibia in two and the calcaneum in three instances. Radiographic signs of a stress fracture were unlikely to be present when the patient first complains of pain. In our cases they appeared 6-8 weeks after the onset of symptoms, in two patients radionuclide scans showed increased uptake of the boneseeking isotope at an earlier stage.

The authors urge that special caution be exercised in fluoride treatment of patients with marked cortical osteoporosis.

KEY WORDS: Osteoporosis; Sodium fluoride; Spontaneous fracture

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EFFECT OF LOW LEVELS OF FLUORIDE IN SOLUTION ON ENAMEL DEMINERALIZATION IN VITRO

by

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(Abstracted from J. Dent. Res., 65:23-29, 1986)

To study the effect of low levels of fluoride in solution on in vitro enamel demineralization extracted human teeth were exposed to 0.1 M lactate solutions (at pH 4.3) partially saturated with respect to enamel mineral, which contained between 0.004 and 1 ppm fluoride. Enamel demineralization was monitored by SEM and polarized light microscopy. In the absence of fluoride, rapid enamel demineralization, resulted in formation of cavitations within 72 hours. With the same demineralizing medium containing as little as 0.024 and 0.054 ppm fluoride, a remarkable protection of the enamel surface occurred. Subsurface enamel demineralization was, however, observed under these conditions as well as in a solution containing 0.154 ppm fluoride. When demineralizing solution containing 1 ppm fluoride was used, no mineral loss was detected. The inhibition of enamel demineralization was also associated with a

significant uptake of fluoride by enamel mineral. These observations correlate with an increase in solution supersaturation with respect to fluoridated apatitic species. The results were consistent with the hypothesis that the net rate of enamel demineralization will be reduced in a demineralizing medium supersaturated with respect to less soluble fluoridated phases, due to enhancement of the precipitation rate of fluoridated apatitic phases relative to the rate of dissolution of the original enamel surface.

KEY WORDS: Enamel demineralization; Human teeth; F effect on demineralization

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EFFECT OF FLUORIDE INGESTION ON WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS)

by

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(Abstracted from Journal of Wildlife Diseases, 21:283-288, 1985)

The effects of the addition of 25 or 50 ppm fluoride (F), as sodium fluoride (NaF), to the rations of 5-month-old male white-tailed deer were similar to those observed in domestic cattle fed similar amounts of fluoride. The ingestion of 50 ppm F for 2 yr resulted in the accumulation of over 7,000 ppm F in bone ash. Accumulation of fluoride in antlers was extensive and occurred more rapidly than in skeletal tissue. Fluoride ingestion resulted in lestions on the developing incisors that were similar, but not identical to those seen in other species. Increased molar wear in the deer fed 50 ppm F was minimal, and no gross pathology of the mandible was observed. Only mild hyperostosis of the long bones was evident.

KEY WORDS: Deer; F in antlers, bone ash, skeletal tissues; Hyperstosis of long bones.

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FLUORIDE, TEETH AND BONE

by

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(Abstracted from the Medical Journal of Australia 143:283-285, 1985)

Because fluoride is being increasingly used as a drug, the pharmacological effects of the fluoride ion in humans is of growing concern. 4000 ppm F was found in cancellous bone ash from bones of some women who had consumed 1 ppm fluoridated water for less than 20 years; fluorotic bone changes were detected by x-ray, in bones containing 1350-4700 ppm F particularly in elbows and forearms.

Fluoride, a bone seeker, is cumulative throughout life. To fulfill its mechanical and biochemical functions bone must be in a dynamic state in which there is constant remodelling, both in the growing and in the fully mature skeleton. On the other hand, once tooth enamel is formed, its cellular activity essentially ceases; it becomes a relatively static, non-vital and expendable external surface of the body. Whereas ionic fluoride concentrations in plasma and calcified tissues generally reflect fluoride intake, these concentrations are not related to intake in a simple linear fashion. Fluoride retention is variable, not only among individuals and groups but also within individuals from time to time. Fluoride retention may be increased by decreased efficiency of kidneys in excreting it (renal clearance rate) or by an icnrease in the uptake by calcified tissues.

Fluoride in preformed bone is concentrated mainly in a surface layer of minerals at the border of the osteocyte lacunae and canaliculae, and fluoride in extracellular fluid bone is in slow equilibrium with fluoride in this mineral phase. The interpretation of the osseous lesions in fluorosis rests on the acceptance of osteocytic osteolysis as an important mode of bone resorption. Reliance on osteoclasia as the sole mechanism of bone resorption may be the prime reason for failure of recognition of pathogenesis of osteopathy in chronic fluorosis.

The mere fact that fluoride, like lead, mercury, radium, strontium (including strontium 90) and cadmium, may be incorporated in apatite does not necessarily mean that it is essential for healthy bone formation. Indeed, it might be argued that biological apatite is acting in a defensive manner when harmful ions, such as strontium 90, radium and lead, are removed from the circulating extracellular fluid and incorporated into the crystal lattice of bone salts. Recent evidence suggests that sodium fluoride is genotoxic and that fluoride could completely disrupt the thymidine-adenine link in DNA duplex.

To establish the safety, or otherwise, of fluoride in prevention of caries and in treatment of osteoporosis, the above-mentioned important points require further investigation.

KEY WORDS: Bone pathology, Bone F uptake, Bone remodelling, Osseous lessions, Osteoporosis, Tooth enamel.

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NATURE OF EARLY CARIES LESIONS IN ENAMEL

by

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(Abstracted from J. Dent. Res., 65:2-11, 1986)

Models based on outer surface protection by absorbed agents, the dissolution-precipitation mechanism, and combinations of these two models, as well as models based on porosity or solubility gradients, are discussed in this paper together with their advantages and disadvantages. Initial enamel lesions formed in vivo have no surface layer initially but develop this mineral-rich layer later. The F^- level in solid sound enamel does not determine subsurface lesion formation. Furthermore, that in vitro fluoride ions in the liquid at levels approximately equal to 0.02 ppm determine surface layer formation is difficult to explain.

A kinetic mechanism for surface layer formation in vivo is proposed, based on the assumption that F^- is a main inhibitor in the plaque-covered acidic in vivo situation. The inhibiting fluoride, absorbed onto the crystallite surfaces at OH^- vacancies, orginates from the so-called fluoride in the liquid phase (FL) between the enamel crystallites. Under acidic conditions (plaque), we have, due to influx of fluoride from saliva or plaque as FL, an aqueous phase in the enamel supersaturated with respect to the mineral for a small distance only.

KEY WORDS: Caries mechanism; Enamel fluoride; Enamel surface.

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AUTHOR'S INDEX

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CORRECTIONS

January, 1986, p. 32. Names omitted from Author's Index in October, 1985.

October, 1986, p. 187. Correction of word "immission" (ambient concentration) replacing "emission" in "Dry Deposition of Fluorides on Lime Papers" by L. de Temmerman and H. Baeten (19:3, 124-131, July, 1986).

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SUBJECT INDEX

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