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The 13th conference of the International Society for Fluoride Research will convene in New Delhi, India between November 14-17, 1983. The program committee is soliciting abstracts (up to 300 words) of papers to be presented at the conference dealing with fluoride action on (1) Ecology and Environment (2) Geology and Geochemistry and (3) Health aspects.

Final date for receipt of abstracts in the prescribed format is Aug. 1, 1983. Authors will be notified of acceptance of papers for presentation by Sept. 15, 1983. Kindly send abstracts to Dr. A.K. Susheela, Organizing Secretary of the 13th ISFR conference, Department of Anatomy, All India Institute of Medical Sciences, New Delhi - 110029 India.

AIR India has been appointed official carrier for the conference. General information and forms for registration, hotel reservations and sightseeing are now available through the conference secretariat, Dr. Susheela (see address above).

EDITORIAL

A CLINICIAN'S DILEMMA

Physicians are frequently faced with clinical situations which they are unable to explain on the basis of our current knowledge. Oftentimes, thorough medical histories, extensive examinations, numerous laboratory tests and biopsies fail to give a clue concerning the cause of the illness. Even after a patient's demise, pathologists are often at a loss concerning the nature of the disease and the cause of death.

New illnesses and new clinical syndromes are being described but usually there is a total lack of understanding with respect to their causation. It is true, advances in virology, in epidemiology, biochemistry, as well as new, sophisticated laboratory procedures in recent years have aided us considerably in our diagnostic efforts. However, certain newly arising epidemics as, for instance, the Legionaires disease remain a mystery to the medical profession for many years. Even concerning diseases which have plagued mankind for centuries and occur daily in our practice such as colitis, arthritis, psoriasis, arteriosclerosis, the collagen diseases, and many more, today's medical profession is still very much in the dark.

This situation is much more complicated when we are dealing with ailments which, in contrast to an acute infection or severe gastrointestinal upset, do not single out an individual organ or group of organs but cause vague, nonspecific symptoms such as general malaise, headaches, joint pains, etc. The so-called Minamata disease which decimated a part of the population of a Japanese seacoast and was finally attributed to the mercury effluents from a factory affected newborn children. Their mothers had hardly any complaints but transmitted the mercury to their offspring. Similarly, attention to the vague and subtle symptoms of the initial stage of lead poisoning, has disclosed virtual epidemics in children, of a creeping, progressive disease, long before the characteristic signs of lead poisoning such as the leadline of the gums or the radial nerve palsy became noticeable. A slowly developing stage of hypertension and kidney disease is being recognized as the result of low-grade cadmium poisoning. The most striking example of such a slowly progressive, eventually fatal disease, is the development of lung cancer from smoking. It required literally hundreds of years before the medical profession realized its cause.

Unfortunately, in their efforts to seek an adequate explanation for the initial stage of many such disorders, physicians often resort to explaining them on a psychosomatic basis and thus add further to the patients' discomfort.

Attention to environmental causes has paved the way to the solution of some of these problems. Toxic agents, either airborne or ingested, are often responsible for low-grade, inconspicuous poisoning. They affect either the whole body or involve mainly certain organs as, for instance, asbestos the lungs and abdomen, carbon monoxide the brain and the heart, the two organs most susceptible to low oxygen supplies. Others powerful enough to penetrate cell membranes can enter every organ of the body and

thus account for a wide spectrum of symptoms.

Such a low-grade, gradually deteriorating disease involving numerous organs has been recognized in recent years as being due to fluoride which has become one of the most widely used industrial chemicals and also the source of great environmental pollution. As the consequence of ever expanding industrial use of fluoride and its wide distribution in the biosphere there has been increasing recognition of low-grade chronic fluorosis which is now being encountered in many areas throughout the world. Indeed, the discovery of fluoride and its recognition by the profession has already solved the mystery of widespread illness. In India, according to a conservative estimate some 1/2 million persons suffer from crippling arthritis due to fluoride in water and food.

There is reason to believe that fluoride will supply the answer to numerous heretofore clinical phenomena and to the explanation of certain illnesses, the cause of which has in the past eluded the efforts of many clinical investigators.

G.L.W.

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SUBACUTE FLUOROSIS DUE TO AIRBORNE FLUORIDE

by

G.L. Waldbott
Warren, Michigan

SUMMARY: Three additional cases are described in which prolonged intake of airborne fluoride induced a chronic illness without the skeletal changes which are characteristic of skeletal fluorosis. In one patient, inhalation of sodium fluoride as a fungicide used for preservation of old records, was responsible; in the two others, handling drums of hydrofluosilicic acid from which fumes escaped and leaking containers of uranium fluoride, respectively. Except for the respiratory symptoms, the multisymptomatic clinical picture, due to airborne fluoride, is identical with the preskeletal phase of chronic fluoride intoxication.

KEY WORDS: Airborne fluoride; Subacute fluorosis; Respiratory symptoms; Preskeletal fluorosis

Introduction

Since the classical description of industrial fluoride poisoning by Kaj Roholm in 1937 (1) most reports in the literature have been concerned with acute fluoride intoxication and with skeletal fluorosis, the advanced stage of chronic intoxication due to inhaled fluoride. However little information is available on intoxication from exposures to airborne fluorides extending over periods of time insufficient to cause the development of the skeletal changes.

Murray and Wilson (2) observed seven members of a farmer's family with gastrointestinal and arthritic symptoms which they attributed to fluoride intake from a nearby ironstone factory. Carnow and Connibear (3), in a survey of 1242 aluminum workers, described a variety of musculoskeletal symptoms such as back or neck pain, stiffness in the back and joints, pains and swelling in joints prior to the development of the typical x-ray changes of skeletal fluorosis. Waldbott and Lee (4) reported similar symptoms in an oil refinery worker who was exposed to hydrogen fluoride gas daily during his work for 10 years.

Here three additional cases are presented which, although due to different occupational exposures to airborne fluoride, are remarkably similar.

Case #1 - S.M., a 45-year old female, secretary at the County Office of Honolulu, Hawaii, seen on 5-27-80, complained mainly of stiffness and pains in arms and hands, frequent sore throats, hoarseness, and swelling of submaxillary glands (Table 1). Her illness started early in 1978 with dryness and irritation in the throat, "sores" in mouth and throat, intermittent hoarseness, frequent nosebleeds, excessive thirst, and peeling of

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the skin of the hands. She also had frequent episodes of abdominal pains, especially in the right upper quadrant, a tendency to diarrhea with three to four bowel movements daily which alternated with constipation lasting usually for 2-3 days. She complained of pains in arms, legs and buttocks, of arthralgia in wrists and the left knee, especially on arising, and of paresthesias in hands and legs to such an extent that she frequently dropped things from her hands. Moreover, her legs often "collapsed under her" so that she fell down. About 3 to 4 times a month she had episodes of sharp retrosternal pains lasting for 2-3 days. Her vision was often blurred, and she had scotomatas in both eyes. Minor exertion elicited dyspnea persisting for several hours. On one such occasion her blood pressure was 174/110. The clinical picture was dominated by progressive general weakness which obliged her to stop work on March 31, 1978.

She had always enjoyed good health until December 1977 when she was assigned to do some research work for 5-6 weeks, which entailed handling bound documents eight hours daily in a windowless, non-ventilated room. She noticed a white powder on the shelves, on the books and between the pages inside the books. In turning the pages, she often moistened her fingers in her mouth. Occasionally she took some of the books home with her and the dust from the volumes contaminated the desk at her home.

On April 3, 1978, a biopsy revealed inflammatory changes in the larynx. In October 1978 she experienced two episodes of severe pain, swelling and redness above both ankles and in the left knee. This condition cleared up spontaneously after one or two days. X-rays showed no evidence of arthritis.

In December 1978, the white powder was identified at the Industrial Analytical Laboratory in Honolulu as sodium fluoride containing traces of sodium silicofluoride. At that time, in April 1980, at the University of California, San Francisco, the disease was diagnosed as fluoride intoxication although extensive laboratory tests including electromyographic studies were unrevealing. Intermittent hoarseness and dryness in the throat and muscular pains in buttocks and thighs became more intense and frequent, requiring medication. The abdominal pains, mainly in the epigastric area, and abdominal distention persisted. Gastrointestinal x-rays, barium enema and gallbladder studies in March 1979 revealed a diaphragmatic hernia, gallstones and a duodenal ulcer. A cholecystectomy on April 10, 1979 revealed the gallbladder somewhat inflamed containing three gallstones. However the abdominal symptoms persisted after the surgery. At that time the pains in the wrists were suggestive of bilateral carpotunnel syndrome.

In December 1979 and April 1980 the patient had episodes of respiratory distress, mainly laryngitis, dyspnea and chest pains, one of which required hospitalization at the Straub Hospital in Honolulu. These conditions developed while she was on short visits to fluoridated San Francisco. Honolulu's water supply is not fluoridated (0.05 ppm). In June, July and August 1979 she had short episodes of swelling of the left parotid glands. The pains and paresthesias in the arms and legs, which had persisted since Feburary 1978, gradually worsened.

The patient's past and family histories were unremarkable except that

she had had an allergic reaction to penicillin in 1964 and, in March 1979 urticaria following administration of iodized dye for gallbladder x-rays. She had been habitually drinking between 3 and 4 cups of tea daily and smoking about 10 cigarettes a day.

The positive examination findings were: Blood pressure 150/110; sensitiveness on pressure of the facial and occipital nerve exits bilaterally: slight cloudiness of the frontal sinuses; pain was elicited on palpation of the epigastric area below both costal margins and on palpation and flexion of the dorsal surface of the left wrist, of the left knee and in the lumbar and cervical portion of the spine. However, there was no loss of muscular power.

X-rays showed minor anterior osteophyte formation and early degenerative disc changes in the lumbo-sacral and cervical spine. An arthritic profile was negative.

The significant laboratory findings are presented in Table 1. Electromyographic studies on November 30, 1979 revealed bilateral damage of the median nerves of the wrists, more pronounced on the right. The 24-hour urinary fluoride, on May 27-28, 1979, was 1.09 mg, the plasma fluoride 1.1 µM or 0.021 ppm.

Comment: This patient had been in good health until her exposure to sodium fluoride 8 hours daily for about 6 weeks. The diagnosis of serious fluoride intoxication was established at two clinics; it subsequently turned into a chronic condition. The x-ray changes in the spine (osteophyte formation and degenerative disc changes) of the kind usually interpreted as evidence of "old age" are typical of preskeletal fluorosis.

Case #2 - W.J., a 50-year old employee of the Fremont, Ohio waterworks plant was seen on July 15, 1980 complaining of increasingly severe general malaise which had developed gradually over the past few years to such an extent that it impaired his ability to carry on his work. At the slightest exertion, he became so fatigued that he had to rest. Paresthesias in both arms caused him to drop such items as tools and keys from his hands. He complained of pain and numbness in feet and toes which were somewhat relieved by exercise. His legs tended to "collapse under him" because of "weakness in the knees." He had constant pain and stiffness in the lower back and involuntary fibrillation of muscle. When climbing stairs with a heavy load, he experienced severe pains in the knees.

In addition, he was subject to frequent nasal congestion, occasional nosebleed, and intermittent episodes of vertigo. His vision was impaired and failed to improve with glasses. He noticed, frequently, what he called "pinpoint lights" in both eyes. In recent months he experienced fecal incontinence and occasional diarrhea; his abdomen was often bloated. He complained of frequent urination, occasional dysuria and often had to drink up to two gallons of water a day. He noted a distinct decline in his mental acuity. While teaching tool and die work, "his mind often stopped" in the middle of answering his students' questions. When inter-

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Table 1

Symptomatology

Category	S.M. 45, F.	W.J. 50, M.	I.J. 38, F.	K.M. 57, M.
Source of fluoride:	Insect powder, sodium silicofluoride	Handling barrels with fluosilicic acid	Uranium fluoride	Atmospheric HF
Respiratory	Epistaxis; sinusitis; laryngitis	Epistaxis	Rhinitis; bronchitis; laryngitis; tonsillitis; dyspnea, pneumonitis	Cough; dyspnea
Musculoskeletal	Pain in arms, buttocks; arthralgia	Back pain; fibrillation	Pain in back, shoulder, fingers, r. metacarpal joint	Pain in back, leg; hand tremors
Gastrointestinal	Stomatitis; abdominal pains; diarrhea; parotitis	Abdominal distension; pain; fecal incontinence; diarrhea	Nausea; vomiting; parotitis; hepatitis	Diarrhea; fecal incontinence
Gastro-urinary	Polyuria; polydipsia	Polyuria; polydipsia; dysuria	Polyuria; polydipsia; dysuria	Bladder; diverticulae
Neurological	Paresthesias, arms, legs; blurred vision	Paresthesias, arms, legs; scotomas; mental deterioration; blurred vision	Paresthesias; headaches	Headaches; mental deterioration; blurred vision
Others	Hypertension; cholecystectomy	Vertigo; F^- toothpaste	"Bruises"; pruritus; hypertension; cholecystectomy	"Burns" of skin

rupted during a conversation, he could no longer recall what he was talking about. He also had marked insomnia with only 1 to 2 hours of sleep a night. In addition, he noted a near complete cessation of his sexual power.

The past history was unremarkable. In 1955 his gums and lips were inflamed which was cured promptly when, at his dentist's suggestion, he discontinued the use of fluoride toothpaste. He smoked one pack of cigarettes a day. He drank water from his own well but often consumed the fluoridated water (1 ppm) at the water plant.

From Dec. 1970 to June 1980 he had been working steadily at the water plant except for occasional intervals of 1-2 days. His workday was comprised of 8-hours but, at times, he worked up to 16-hours a day. He had to maintain the flow of hydrofluosilicic acid into the local water supply, make hourly recordings of the chemical analysis of the water, make adjustments on the fluoride feeders and handle barrels containing the acid. When opening the barrels, escaping fumes often obliged him to go outdoors. The window glass of the door appeared etched and the metal on scales was corroded.

During the first 3 years of his employment he had not been given any instructions as to how to handle the barrels. He wore no respiratory mask nor was he informed about the danger of fluoride toxicity until 1976. The work place was poorly ventilated. Storage of empty barrels, containing traces of fluoride, at his work place contributed materially to his discomfort. For about 2 years the vent motor had been burnt out. He had to open the door frequently for ventilation or go outdoors for fresh air. In February 1979 after changing the fluoride barrels three times daily, he had to stop working for three days.

The positive examination findings were cloudiness of all sinuses on transillumination, multiple scars on the back suggestive of former acne lesions, limitation and painful movement of shoulder joints and of the cervical spine. Bending the knees elicited severe pain.

CBC, sedimentation rate, thyroid function, a 12 test serum profile, urinary and blood assays for copper and zinc were unremarkable (Table 2). X-rays revealed evidence of sinusitis and hypertrophic changes in the cervical spine. The 24-hour urine specimen on 7-27-80 showed a fluoride content of 1.4 mg.

On September 10, 1980, the patient was re-examined after being on leave since June 18, 1980. He stated that his vision had improved. He had been strictly avoiding food high in fluoride and fluoridated water. He continued to have pains in joints, fecal incontinence, nasal irritation, diarrhea and occasional bloating of abdomen, stiffness in the lower spine, numbness in arms and legs and muscular fibrillation. On two occasions after he had inadvertently eaten shrimp and chicken - both are "high" fluoride foods - stiffness in back and general weakness were distinctly exacerbated.

Another 24-hour urinary assay for fluoride yielded 1.2 mg. The plas-

Table 2

Laboratory Findings in 4 cases of Nonskeletal Fluorosis

	Normal	W.J.	I.J.	S.M.	K.M.**
Glucose	65-110 mg%	80	150	103	127
Total Protein	6.0-8.0 gm%	7.3	7.1	8.4	7.0
Ca	8.5-10.7 mg%	9.7	9.4	9.5	
Inorg. Phos.	2.5-4.5 mg%	3.4	3.6	4.0	
Bun	10-26 mg%	14	10	13	
Creat.	0.6-1.5 mg%	0.9	1.1	0.9	1.3
Total Bili.	0.1-1.2 mg%	0.8	0.5	0.4	
Alk. Phos.	30-115 IU/L	67	176*	97	56
LDH	90-250 IU/L	209	190	261*	150
SGOT	5-50 IU/L	25	13	19	26
Album.	3.5-5.2 gm%	4.5	4.4	5	
Choles.	150-300 mg%	252	214		251
Uric Acid	2.0-6.4 mg%	4.1	6.3	4.4	8.7*
Triglyco.	30-150 mg/100 ml	67	166*		
Total lipids	450-850 mg/100 ml	600	1182*		
Na	135-150 Meq/L		138		
K	3.6-5.2 Meq/L		4.1		
CL	95-107 Meq/L				
CO_2	25-30 Meq/L				
Iron	46-150 mg%		21		
Urinary F^-		1.17-1.42		1.01-1.09	

* - High

** - Published in Clinical Toxicology, 13:391-402, 1978

ma fluoride on 7-15-80 was 0.59 µM (0.011 ppm).

On a subsequent visit, November 18, 1980, the patient had recovered completely. However on October 31, 1980 another exacerbation with cramps in legs, diarrhea, pain and ankle edema lasted for approximately one week. He learned subsequently that he had consumed coffee made with fluoridated city water within an hour prior to the onset. On November 12, 1980 a similar episode followed drinking two glasses of beer which, on subsequent analysis, was found to contain 1.2 ppm fluoride. A major relapse occurred in February 1982 after exposure to welding fumes (HF) at a plant where he had found new employment.

Comment: This patient enjoyed good health prior to exposure to fluoride fumes and to possible ingestion of minute amounts of fluoride practically daily throughout his 10 years' employment. Also he had been drinking fluoridated water at work. The toothpaste episode prior to his employment at the waterworks points to a low tolerance to fluoride. A slow but distinct improvement ensued after he stopped working and was a-

voiding fluoridated water and "high" fluoride food. Like in Case 1, inadvertent uptake of minimal amounts of fluoride precipitated intermittent recurrences of the disease.

Case #3 - I.J., a 38-year old female seen on 8-18-81 complained of persistent pains in the lower spine, which made it difficult for her to rise after sitting down. She also experienced pain in knees, shoulders, finger joints, particularly in the right metacarpal joint. Numbness in the left arm extending into the hand and fingers impaired her ability to grasp and caused her to drop things from her hands. Weakness in the right hand interfered with her ability write. Also she reported a tendency to bruises on the skin which were indolent, round and never larger than a twenty-five cent coin. Swelling of both parotis glands was continuous. Bilateral headaches, mainly in the occipital area, occurred about once every two weeks. Almost daily she had episodes of nausea and vomiting, usually after eating, but also at night on an empty stomach. She had occasional dysuria and a tendency to cough and hoarseness.

From July 1969 to March 1979 she was employed analyzing samples of uranium fluoride (UF_6) in order to determine the concentration of ^{235}U. During this work she was frequently exposed to small amounts of hydrogen fluoride (HF) which escaped from the vials containing uranium fluoride. Some of the samples were encased in metal tubing that was applied directly to an apparatus where the gas was opened and allowed to enter a machine that ran the data necessary for the calculations. Other samples were placed in plastic tubings with metal clips at both ends which, in most instances, did not prevent gas from escaping. On many occasions the sample clasps were actually placed on top of the sample contained in this plastic tube without being closed off from either end, thus allowing the sample to escape as though it had been opened. Approximately 6-8 of these plastic tubes had to be reworked daily in order to get the sample to flow into the machine. This required opening the tubing and sliding the clasps down which automatically allowed parts of the gas to escape. At least once a month she had to work with 24 of these samples within an 8-hour work period.

Within a few months after she started working she became subject to bronchitis, nasal irritation and laryngitis occasionally associated with slight fever. The sore throat was more or less persistent throughout her employment, often accompanied by diarrhea and abdominal pains. Throat cultures were negative. The patient was hospitalized at the East Tennessee Baptist Hospital in January 1974 because of generalized pruritis. At that time she had a cholecystectomy which was followed by a protracted period of hepatitis confirmed by a liver biopsy on 5-16-74.

On July 7, 1975 while working, she suddenly experienced a severe cough, choking, dyspnea, and vomiting. A defective valve of a tube behind her desk permitted vapors from the tube to escape. Two hours later, while still coughing almost continuously, she was given positive pressure oxygen and a cough syrup. The chest x-ray was negative. This condition was followed by pains along the spine and of the chest, dyspnea, dysuria, polyuria, and polydipsia, and extreme weakness. On July 7 and 8, 1975

three urine samples showed 4.4, 10.3 and 1.0 uranium disintegrations per 100 ml per sample which averages less than 0.5% of the exposable allowable dose under the ERD radiation protection guides. The patient's badge film which records external radiation, recorded 20 millirems penetrated dose and 62 millirems skin dose for July, August, and September 1975 which did not exceed the allowable values.

In December 1975,a bronchoscopy revealed evidence of diffuse bronchitis. The chest x-ray disclosed pneumonitis in the right mid lung zone. At that time the alkaline phosphatase was high (135 and 154 units) and the hematocrit 41.6%. There was a slight increase in triglycerides suggestive of hyperlipemia of 1182 (normal up to 850). The GOPT was 144, serum amylase 110, SGTO 147 (Table 1).

In August 1981, 2-1/2 years after cessation of exposure, the urinary fluoride was 4.92 mg/day which indicates that her former exposure to fluoride must have been unusually high.

A sialogram of the right parotid gland revealed a stretching of the small ducts in the superior portion of the gland. Because of a positive tuberculin skin test, she was placed on an antituberculous drug.

The examination revealed normal vital signs with a blood pressure of 142/74. The positive findings were marked bilateral swelling of both parotis glands, not painful on pressure. The nasal mucosa appeared to be hypertrophic, and the frontal sinuses were cloudy. On the right lower arm the sensibility to sharp and dull was impaired. There was an indolent macula similar to a suffusion on the inner aspect of the right upper arm. Pain was elicited on motion of the right wrist and on pressure of the sacral bone. To arise from a chair was painful. Pain and tenderness were elicited on palpation of the right upper quadrant, the liver was somewhat enlarged.

Uranium hexafluoride, a most volatile uranium compound, sublimes at 1 ATM at 56.5° and hydrolyzes rapidly to give HF and uranyl fluoride (UO_2F_2). HF is promptly absorbed through the alveolar-capillary bed into the bloodstream where it can give rise to systemic symptoms.

Comment: The illness began gradually with respiratory symptoms associated with gastrointestinal and lower urinary tract disease, and musculoskeletal neurological features. There was objective evidence of damage to the liver and parotis gland. Since the July 7, 1975 episode, the patient had required medical attention. According to the report of the factory's health physicist, her badge for radiation and the urinary assay for uranium indicated that no excessive radiation was at play in her illness. Therefore radiation damage can be excluded.

Discussion

There are three phases of poisoning due to inhaled fluoride:

1. The acute, sometimes fatal phase, is characterized at first by respiratory symptoms, mainly cough, dyspnea and nasal irritation, followed

by such systemic symptoms as muscular pains, pains in the spine, vomiting, gastric pains and diarrhea and urinary symptoms. Eventually cardiac or pulmonary failure ensues (5).

2. Following less extensive exposure over prolonged periods of time (5 to 20 years) a condition termed skeletal fluorosis develops characterized by abnormal new bone formation and calcification of ligaments and arteries, joints and muscle insertions.

3. The nonskeletal phase of fluoride poisoning represents a protracted form of acute poisoning prior to the development of bone changes. The multisymptomatic aspect of this condition was first outlined in the classical book by Kaj Roholm in 1937, and follows essentially the pattern of acute intoxication in an attenuated form. Only in recent years has this disease been reported in detail (6-9).

The three patients reported here and the patient reported previously (10) had been in good health prior to daily exposure to fluoride during periods of time ranging from 8 hours for two months to 10 years (10).

They were exposed daily during their working hours to varying amounts of atmospheric fluoride. Its irritating action affected at first the respiratory tract and induced predominantly such symptoms as cough, laryngitis, wheezing, frequent sore throats which were followed by, or associated with, the systemic symptoms known to result from absorption of fluoride into the bloodstream. As in acute intoxication (11), fluoride may affect any organ of the body, but primarily the gastrointestinal tract, mimicking gallbladder disease, the musculoskeletal and central nervous system and the lower urinary tract (11).

Whereas our cases showed general improvement after cessation of daily exposure, inadvertent uptake of minimal amounts of fluoride precipitated recurrences. During these episodes, the original target organs again became involved. In case 2, subsequent fluoride intake was clearly linked to fluoride-containing food.

Three of the four patients developed hepatitis. In two of them gallbladder surgery failed to improve the condition. Chronic parotitis, an otherwise rare disease, which occurred in two of the four cases has been experimentally produced by sodium fluoride (12). Otherwise, the illness is identical with what has been encountered following fluoride ingestion from food and water under well controlled conditions (13). Gastritis and gastric ulcer, which are major characteristics of acute fluoride intoxication, as well as intestinal disorders have been described by Czerwinski, Franke, Zhavoronkov in conjunction with skeletal fluorosis and are often encountered in the treatment of osteoporosis with large doses of fluoride.

Urinary fluoride is generally considered an indicator of fluoride intake, but wide variations from person to person exist in the amount of urinary fluoride excretion. My own observations, however, demonstrate that doses of fluoride generally considered harmless can elicit adverse health effects. In case #3, three months after cessation of daily exposure, the

Table 3

Properties of Fluoride	Clinical Effects
1. Affinity to Calcium	Calcification of bones, teeth, arteries, joint capsules, ligaments; Hyperparathyroidism
2. Affinity to Metals	
a. Magnesium	Interference with many enzyme activities
b. Manganese	Impairment of mitochondrial functior
c. Iron	Anemia
d. Aluminum	
e. Molybdenum	Genu valgum
f. Copper-Zinc	Counteracts fluoride toxicity
3. H-Bonding	
a. Interference with protein and RNA synthesis	Damage to cells Possible carcinogenicity
b. Formation of HF in acid medium	Ulceration in stomach, mouth; lower urinary tract disease
c. Interference with the serotonin pathway	Mutagenicity; carcinogenicity
d. Effect on enzyme activity	Involvement of many organs, especially liver and kidneys
4. Effect on Immune Mechanism	
5. Miscellaneous Properties	
a. Activation of adenyl cyclase	Regulation of cell function
b. Its small electronegative size	Penetration of cell membrane
c. Effect on carbohydrate metabolism	Damage to liver, pancreas

urinary fluoride excretion was still 0.5 ppm, which indicates continued sequestration of fluoride from bones and other tissues which had accumulated in the body. Other positive laboratory data are sparse and suggest that, at this stage of the disease, damage to liver,kidneys and other organs is not sufficiently advanced to produce consistent laboratory findings.

Many clinicians consider fluorosis to be a systemic disease, not confined to pathology of bones and teeth. Fluoride has been found to accumulate in nearly every organ of the body (14). Its ability to penetrate cell membranes causes it to interfere with the functions of many organs (15). Its inhibition of calcium phosphorus metabolism (16), of glycolysis due to inhibition of inolase, and disruptive amide hydrogen bonding are responsible for interference with protein metabolism (17).

Table 3,above,lists some of the properties of fluoride which explain its multisymptomatic action.

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NEIGHBORHOOD FLUOROSIS WITH SKELETAL MANIFESTATIONS

by

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SUMMARY: Forty-four persons (about 1% of the population) of a small Saxon town showed x-ray evidence of skeletal fluorosis. Three fluoride-emitting factories which produce hydrofluoric acid, aluminum and graphite are located in this town. The affected persons, although exposed for decades to these emittants, have had no occupational contact with fluoride. Atmospheric fluoride emissions have been high and fluoride levels in water due to industrial wastes have increased. The subjects have suffered only minor complaints. Their life span is normal. Renal fluoride excretion had increased and, in several persons, bone ash upon analysis showed high fluoride levels. For many years cattle fluorosis, dust damage to plants and dental fluorosis of children was not uncommon in the town. A change in the municipal water system and better technological conditions in industry has led to improved sanitation and hygiene in the area.

KEY WORDS: Fluorosis, neighborhood; Skeletal fluorosis in Saxony

Introduction

Fluoride damage to plants and animal fluorosis have been observed in the vicinity of fluoride-emitting facilities for decades, as well as damage to humans residing in the environs of the fluoride emissions. Reports concerning the neighborhood of aluminum smelter and rock phosphate fertilizer factories originated in the Soviet Union (1), Czechoslovakia (2), Hungary (3), the U.S.A. (4), Canada (5), Scotland (6), Italy (7) and Belgium (8). Decreased hemoglobin levels, dental fluorosis and skin affections had been recorded.

In 1946 Murray and Wilson (9) noted increased renal fluoride excretion and dental fluorosis in a farmer's family near an ironstone calcination plant. The subjects suffered from stiff joints, headaches, bronchitis, loss of appetite and stomach complaints. The authors differentiated this type of fluorosis from industrial and endemic fluorosis and, for the first time, used the term "neighborhood fluorosis". Waldbott and Cecilioni (10) also employed this new term for the description of complaints in 32 residents in the vicinity of a fertilizer plant in Canada. Symptoms of these subjects involved the gastrointestinal and respiratory tract, as well as the skin. None of the above-named authors found x-ray evidence of skeletal fluorosis. In 1967 Herbert et al. (11) described a case of osteopetrosis in France in a 46-year old male not associated with occupa-

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pational fluoride. He had been residing at a distance of 10 km from an aluminum smelter in the main wind direction. Cattle fluorosis had occurred frequently in the man's village. He suffered from lumbago. The x-rays showed a marked increase in bone density of the spine and ribs. This is the first case of genuine neighborhood skeletal fluorosis described in the literature.

Material and Methods

The discovery of single persons with osteosclerosis from a circumscribed area led to additional investigations in the diagnosis and differentiation of the disease. Full x-ray examinations were made on all persons who showed a high bone density on chest x-rays. In addition we made radiographs of the thoracic and lumbar spine, of the pelvis and both the right forearm and lower leg.

Laboratory tests were possible on 34 affected persons. Fluoride content in the bone ash was determined in 10. After drying, pieces of bone or cylinders were ashed for two hours at 450° C. The fluoride determination was made by distillation with perchloric acid and formation of a complex of circoneriochromecyanine. Histological investigations were made in 11 cases.

In order to obtain information about the incidence of this disease in the residential area,we reviewed the available radiographs of a routine x-ray survey on 2482 residents of the area in question. Furthermore we investigated 20 persons by means of x-ray who were residing in the environs of the aluminum smelter. These persons were receiving compensation for damage to their plants and animals due to fluoride emissions from the factory. In exploring the possibility of industrial origin of the environmental fluoride pollution, we carried out numerous investigations both in connection with drinking and river water by using a Czechoslovakian fluoride-sensitive electrode of the firm Monokrystaly Turnov.

Observations

Among the patients of our hospital we found, on x-ray , persons with unusually increased skeletal density. At first we believed that they were workers with industrial fluorosis due to the fluoric acid factory situated nearby. However several persons had never worked in a factory using fluorides; all had been residing for decades in the vicinity of such plants. Thus we detected, at first spontaneously, but later as the result of a systemic search, 44 persons affected with typical x-ray signs of fluorosis at stages I, II and III who had never had any occupational fluoride exposure. In addition, we observed the disease among 30 persons who had been hospitalized and x-rayed because of other diseases. The diseases for which these patients were hospitalized are summarized in Table 1. Additional cases were found upon re-examining the above-named x-ray survey of the population (12). Increased bone density of ribs and clavicles was detected in 36 cases (1.4%) out of 2482 small radiographs. In 20 of the 36 cases we were able to obtain additional x-rays. By this method we discovered one person whose skeletal fluorosis was in x-ray stage III, 3 in

stage II, 3 in stage I-II, 1 person in the first stage and 5 in the intermediate stage 0-I (13). None of these subjects had ever had any occupational contact with fluoride. However they had been residing for about 50.5 years in the fluoride emission area. Another person, a 73-year old man whose fluorosis was in stage I-II, discovered by our investigation, had always resided in the vicinity of the aluminum smelting plant (14).

Table 1

Diseases of 30 Patients with Osteosclerosis Admitted to the Hospital

Cancer and leukemia	4
General atherosclerosis	9
Chronic ischemic heart disease	17
Hypertonia	6
Mat. onset diabetes	3
Urinary stones	2
Chronic pyelonephritis with renal failure	7
Gallstones	5
Stomach ulcer	1
Psoriasis	1
Lumbago	1

Several patients suffered from more than 1 disease.

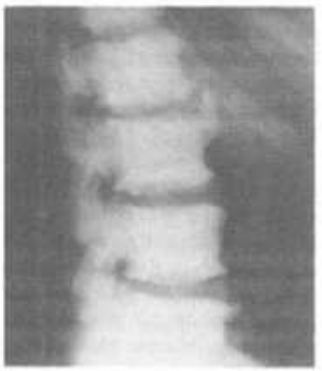
Of the 44 persons with osteosclerosis, 34 were male and 10 were female, aged 44 to 86 years (average 70.9). The affected persons, representing 0.95% of the district's population, had resided in this area from 10 to 80 years (average 46). The skeletal x-rays showed typical fluorosis,

Figure 1

Osteosclerosis of the Thoracic Spine

Figure 2

Osteopetrosis of the Lumbar Spine



osteosclerosis with thickening of the bone trabeculae of the spine, pelvis and ribs, periostosis with saw-like appositions on the bones of the forearms and lower legs, partial ossification of ligaments of the spine and pelvis as well as exostoses on the pelvis and the thigh bones.

Figure 3

Periostosis and Exostoses of the Pelvis

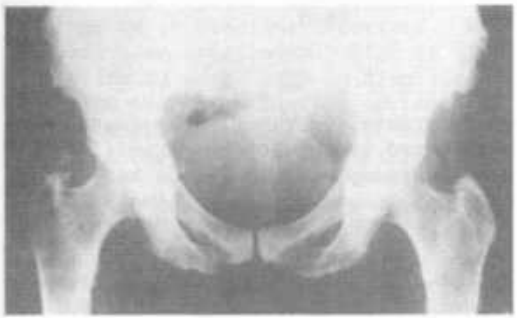


Figure 4

Ossification of Pelvic Ligaments

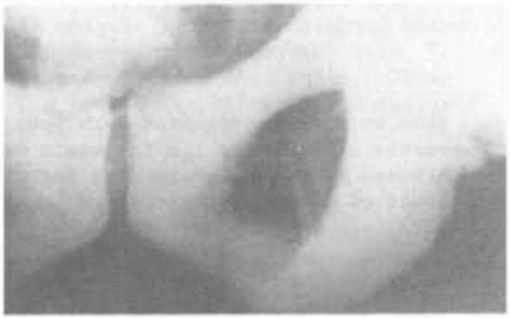


Table 2 shows the distribution of the roentgenological stages, according to Roholm (15) and Fritz (16), related to age and duration of residence in the fluoride-polluted area. The laboratory tests in 34 persons with osteosclerosis can be summarized as follows: The alkaline phosphatase exceeded the normal value of 60 units/l in 16 cases, namely 68 to 780 u/l (average 115.4 u/l). Creatinine and plasma urea were above normal in 34.5% and 39.4% respectively. In 10 subjects fluoride analyses were made. Bone ash of the ribs ranged from 3430 to 10,090 ppm, of the pelvic bone 5700 to 10,060, of the thoracic spine 4850 to 10,310 ppm. The lumbar spine bones contained 3900 to 11,300 and the sternum 12,300 ppm fluoride. No anemia was found.

Histological investigations in bones were made in 11 cases of various stages of fluorosis. Material was derived from a pelvic crest needle biopsy in 4 cases and from a dissection in 7. The analysis showed: 1.Thickening and spongiosation of the corticalis. 2. Mosaic structure of the cement lines. 3. Subperiostel osseous new formation as described in industrial fluorosis by Franke and Auermann (17).

During our search for the fluoride origins,we found three fluoride emittants in the heart of the residential area: a hydrofluoric acid factory, an aluminum smelting plant and a graphite mill. The first two are widely recognized fluoride emitters. In the third one,fluoric acid was required for the production of graphite with highest purity. After use

Table 2

Distribution of the Roentgenological Stages in Comparison with Age and Duration of Residence

Roentgenological Stage	n	Age (average)	Yrs. of Residence (average)
0 - I	16	66.2	42.1
I	6	71.5	42.0
I - II	10	73.4	51.8
II	6	76.0	58.3
II - III	5	69.4	43.2
III	1	73.0	48.0

this acid, in neutralized form, was discharged into the river. The dust, vapors of industry and its waste waters affected the whole ecosystem. Increased fluoride levels were detected in the atmosphere, in leaves and fruit of trees, in vegetables, grass (18), cattle and domestic animals (19).

The fluoric acid factory has existed at this place since 1905. In recent decades it has emitted a large amount of fluorides because of old fashioned technology which worked in the so-called "open-system". In the surroundings of this factory, plants and trees failed to grow, and windows became etched in a short time. Unfortunately all emittants within the town were situated together in a valley where the emanation acted intensively upon the residential area (20).

In the beginning of our investigation, we considered the atmospheric fluoride pollution and the ingestion of food high in fluoride the only source of the osteosclerosis in residents. Soon, however, drinking water turned out to be one of the main sources. At first we investigated water at three points of the river which flows through the town. Upstream of the emittants, 36 measurements showed an average fluoride content of 1.4 ppm (0.6 to 5.8 ppm). In the center of the town we found, in 25 tests, increased fluoride levels from 4.6 to 125 ppm (average 41.9 ppm). Downstream, of all emittants similar values (6.2 to 138 ppm) were seen (mean content of 20 measurements was 47.4 ppm). Drinking water showed results similar to river water. In 103 samples, the fluoride level ranged from 2 to 30 ppm (mean value 9.0 ppm). The water samples were collected from widely separated points of the residential area at various seasons and times of day. The river and the water pipe system were closely connected in an indirect way since the wells of the waterworks, which also showed an increased fluoride content, are situated within 5 meters distance of the bank of the river.

Discussion

In the past, the affected district has been the subject of intensive fluoride research. In 1941 fluorosis in cattle near the fluoric acid

plant was reported (21) and in 1944 the first description of industrial fluorosis in Germany in 34 workers was reported by Peperkorn and Kaehling (22). In 1953 Hoffmann-Axthelm (23) described dental fluorosis in children residing close to the fluoric acid factory which he attributed to fluoride waste waters. In 1958 Fritz (16) published a thorough investigation on 67 workers of the same factory with severe industrial fluorosis and in 1980 dentists reported on their findings in children with dental fluorosis (24). About 84% of children residing in the polluted district were found to be affected. (Degree I, 26.1%; degree II, 30%; degree III, 27.9%). On the other hand,50.6% of the children were free of dental caries, a common finding reported in the literature.

We consider the occurrence of osteopetrosis,without occupational exposure to fluoride,important. Whereas,in endemic fluorosis,drinking water has been continuously high in fluoride for decades, our subjects were drinking water high in fluoride originating from industrial waste. Thus this man-made fluoride content had varying levels depending on the degree of pollution. The result was true skeletal neighborhood fluorosis in contrast to endemic fluorosis.

Life expectancy in the population appeared to be normal and in the male population exposed to fluorides we found a significantly lower ratio of bone fractures than in an unexposed group (28). All patients attended the hospital because of other diseases. The skeletal changes were found either by accident or by means of screening the healthy population. As do other authors, we noted a marked individual disposition to the incorporation of fluorides into the skeleton (16, 25, 26).

Regarding the diagnosis (27), the typical x-ray changes, extensive nonoccupational exposure to fluoride and increased fluoride content of bone ash prove the existence of neighborhood fluorosis. Laboratory results did not contribute much to the diagnosis. Like other authors, we found an elevation of the alkaline phosphatase.

In compliance with our suggestions, the following measures have been introduced. 1. Storage of fluoride-containing smelting wastes on the soil near drinking waterwells is no longer permitted. They must be stored in covered concrete bunkers. 2. The town's drinking water system is being shut down. 3. A new water supply with a low fluoride level is being piped in from a reservoir. The author believes that, through these measures, the danger of fluoride intoxication for the district's population has been abolished.

Acknowledgement

We are grateful to Prof. Rath and Dr. Franke, University of Halle/Saale, to Dr. Auermann, Institute of Hygiene,Karl-Marx-Stadt, to Mr. Funke, District Hospital Zittau and to the two Pathological Institutes in Dresden for their support.

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CLINICAL RADIOLOGICAL OBSERVATIONS AMONG WORKERS OF FLUORIDE PROCESSING INDUSTRY

by

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SUMMARY: A fluorspar processing facility which began operations in 1965 producing freezing gas, HF acid, cryolite, NaF, etc., is situated on the West Coast of India, North of Bombay. Four hundred-thirty eight male workers, the majority of whom were employed for more than 9 years without interdepartmental rotation, were interrogated and examined clinically. Two hundred-twenty six random urine samples were analyzed. One hundred-six x-rays (rt. forearm A.P. view) were taken. The overall mean urinary fluoride level was 1.96 ppm. In 11.9% the urinary fluoride level was higher than 4.5 ppm. Although clinical signs suggestive of fluorosis were absent, 34.0% of the workers had complaints. Dental and radiological changes suggestive of fluorosis were present in 9.6% and 21.8% workers respectively. The overall mean urinary value ± 2 SE was used to categorize the department according to the degree of exposure risk. The mean urinary fluoride level and complaints were significantly higher whereas the dental and radiological changes were not higher in the high risk groups than in the low risk. Urinary complaints, dental and radiological changes in workers with more than 9 years employment were not higher than in those with less than 9 years employment. In workers of the lower socio-economic group, the mean urinary fluoride level and complaints were significantly higher than in the higher socio-economic group. Under prevailing conditions in the factory, the degree of exposure seems to act independently of duration of exposure and socio-economic status.

KEY WORDS: Fluorosis, industrial; India, industrial fluorosis in; Fluorspar processing; Fluorosis, radiological studies; Fluorosis

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Introduction

The problem of endemic fluorosis has been extensively studied in India. Rapid industrial development in the country and increasing use of fluoride compounds in industries leads to high fluoride content in dust and volatile waste products and creates health problems for industrial workers and for the neighborhood. The problem of fluorosis among workers of industries using fluoride-based compounds as raw material has been studied in the past by several scientists. This study, the first of its kind carried out in India in 1976-77 was undertaken in an industry processing fluorspar as raw material. Situated on the West Coast of India, North of Bombay, it produced refrigeration gas, hydrofluoric acid, synthetic cryolite, sodium fluoride and other fluoride-based compounds as the final products. This study was planned to identify the nature and magnitude of the problem of industrial fluorosis and to prepare baseline information. An attempt was also made to identify the factors which increase the risk to health among workers exposed to industrial environment.

This study embraced 438 male workers, 85% of whom were between 25-50 years of age and more than 70.0% had been employed for more than 9 years in the same department. An attempt was made to identify the pattern and magnitude of the problem with the help of signs, symptoms, urinary fluoride level and radiological observations. We also tried to identify the factors which increase the risk of an industrial environment on the health of workers.

Material and Methods

Out of 496 workers employed in eight different departments, 438 (88.3%) were interrogated and examined clinically. Spot urine samples of 226 (51.6%) examined workers collected during working hours were analyzed. For measurement of fluoride level in ppm, urinary fluoride estimation was done with the help of Orion Ion specific electrode. One-hundred-six (41.6%) randomly selected workers were x-rayed (right forearm, A.P. view). The observations were analyzed according to the degree of exposure risk, duration of employment and socio-economic status of workers. Among the 438 workers examined, spot urine samples from 61.5% workers were analyzed; 41.6% randomly selected workers were x-rayed.

In this study, the level of fluoride in drinking water of the factory (working place) and the nearby city and surrounding villages (residence of workers) was less than 1 ppm; the workers had been residing in the area for more than 2 years. The presumed average intake is one liter of water. Therefore an equal quantity, less than 1 ppm, of urinary fluoride excretion can be expected. Distribution of workers, according to their urinary fluoride level, is shown in Table 1. In the majority of workers, urinary fluoride excretion was between 0.6-1.5 ppm. The mean urinary fluoride level was 1.96 ppm. In 46% of the workers, the urinary fluoride level was more than 1.5 ppm and, in 11.9%, more than 4.5 ppm.

Urinary fluoride excretion, as an index of total exposure is useful and significant in industrial fluoride exposure. In this study, correlation

Table 1

Distribution of Urinary F^- Level in Industrial Workers

Urinary F^- ppm	No. of Workers	Proportion	
0.5	51	22.7	
0.6-01.5	71	31.4	
1.6-02.5	40	17.7	
2.6-03.5	19	8.4	
3.6-04.5	18	8.0	
4.6-05.5	12)	5.3	
5.6-06.5	9)	4.0)	
6.6-07.5	3)	1.3)	
7.6-08.5	1)27	0.4)11.9	
8.6-09.5	1)	0.4)	
9.6-10.5	1)	0.4)	
TOTAL	226	100.0	

Table 2

Complaints in Industrial Workers

Complaints	Prevalence Rate (%)
Pain in cervical and lumbar spine	14.1
Pain in knee and ankle joint	12.1
Myalgia, myasthenia, paresthesia	3.8
Spasticity in extremities	2.2
Headache	1.8
Visual disturbances	0.5
Hearing disturbances	1.3
Muscular twitchings	1.1
Nausea, vomiting, epigastric pain	2.7
Diarrhea, distension, constipation	7.3
Nasal, conjunctival irritation and secretion	4.3
Emphysema, asthma, chest pain	3.4
Skin rash	1.1
Urinary complaints	1.5
Loss of weight	2.5

Out of 438 workers, 149 (34.0%) had complaints

No. of complaints per worker = 1.78

of urinary fluoride with dental ($v = 0.9977$, $t = 38.93$, d.f. = 6, $P<0.01$) and radiological changes ($v = 0.8464$, $t = 4.18$, d.f. = 8, $P<0.05$) was significant which is positive evidence of fluorosis. The Hodge and Smith (1)'

belief, that fluoride analysis of spot samples constitutes reliable averages for industrial hygiene control, is contradicted by others (2). Whereas to measure fluoride ingestion and inhalation, spot urine samples are not as reliable as 24-hour specimens they have been used in this study to gauge workers' health because 24-hour samples could not be obtained.

Abnormal physical signs or disabling effect of chronic exposure to industrial fluoride have rarely, if ever, been observed and reported by other workers (3-7). In chronic fluoride poisoning the action of fluoride, after its entry into the human body, is not confined to one or two target organs. Therefore many additional manifestations accompany dental and skeletal fluorosis. In his classical description of fluoride intoxication, Roholm (8) has outlined a number of manifestations which are associated with skeletal fluorosis mainly, gastrointestinal and neuromuscular symptoms as well as such features as tachycardia, polydipsia, and allergic skin lesions. The pattern of health complaints in this study closely resembles that described by Waldbott (9-21) in both neighborhood and hydrofluorosis. In this study, also, no worker had abnormal physical signs of skeletal disease or disabling effect. The most common health complaints of workers were backache (14.1%) and joint pains (12.1%) (Table 2).

Fluorotic dental changes are more relevant in children. In this study 9.59% of workers employed after 18 years of age in this factory had fluorotic dental changes such as chalky white teeth, pitting and mottling.

Table 3

Fluorotic Dental Changes in Industrial Workers

Dental Changes	No. of Workers	Proportion %
Chalky white	23	54.76
Pitting	5	11.91
Mottling	4	9.52
Chalky white and mottling	2	4.76
Chalky white and pitting	2	4.76
Mottling and pitting	1	2.38
Chalky white, pitting and mottling	5	11.91
TOTAL	42	100.0

The majority of workers (67%) were residing in nearby cities or villages. Of those who emigrated from other areas, the majority were not from a known endemic fluorosis area. Of the 42 with dental changes, 31 were factory workers, the others held administrative or security positions. The

majority had chalky white teeth. A positive association between fluorotic dental and radiological changes was significant ($x^2 = 7.03$, d.f. = 1.$P<0.01$). In the absence of available references for dental changes in industrial workers it is difficult to explain the relevance of this finding.

Of 183 workers x-rayed, 40 (21.86%) had radiological changes such as increased bone density, thickening of cortex, irregular bony margin, calcification of interosseous membranes etc. Positive radiological changes among industrial workers, reported by other workers, were 84% (4,6,9).

The extent of fluoride uptake in different parts of the skeleton and dentition depends on the amount ingested and absorbed, the duration and type of fluoride exposure and the nutritional status. The symptoms of the preskeletal phase vary from person to person depending on individual susceptibility, state of health and nutrition, food habits, age, sex, duration and extent of former and current fluoride intake as well as whether fluoride is inhaled or ingested as indicated by Waldbott (20). Attempts, therefore, have been made to analyze the effect of fluoride exposure on factory workers' health in relation to degree of exposure and socio-economic status.

On the basis of ± 2 SE deviation from mean urinary fluoride values of all workers, cases were grouped according to degree of exposure. In the high risk group, the mean urinary fluoride level was 2.22 ppm. In the other departments hydrofluoric acid was 2.70 ppm, cryolite 2.37 ppm and sulfuric acid 2.38 ppm. In the moderate risk cases, the mean urinary fluoride level ranged from 1.7 to 2.22 ppm, which includes workshop (1.97 ppm) and security (1.75 ppm) departments. Low risk, mean urinary fluoride level was less than 1.7 ppm. Included are freezing gas(1.4 ppm), quality control (1.45 ppm) and administration (1.48 ppm).

Fluoride levels of spot urine samples collected during working hours show the effect of cumulative exposure as well as immediate exposure in working environment. The mean urinary fluoride level was highest (2.42ppm) in high risk department and lowest (1.39 ppm) in low risk department and the difference was statistically significant. In 21.43% of the workers in the high risk group and in 2.38% of the workers in the low risk group, the urinary fluoride level was above 4.5 ppm (Table 4).

Major complaints, namely joint pains and backache, were analyzed further. A higher proportion of workers had complaints in high risk department (29.24%) than in low risk department (9.87%). The difference was statistically significant (Table 5).

Fluorotic dental changes were recorded in 11.69% of workers in the high risk and in 7.24% in low risk department; radiological changes in 23.53% of the workers in the high risk and in 20.0% in the low risk department. These differences were not statistically significant (Table 5). In Roholm's 1937 study (8) and in the Medical Council's Study (22), radiological changes were also related to dust and inhalation and the degree of exposure.

With no interdepartmental rotation in the factory under study, all workers were exposed to the same environment throughout the years of their employment. Initially workers were grouped in four categories according to duration of employment as shown in Table 6. By statistical analyses those with more than 9 years employment were compared with those employed less than 9 years because the number of workers in groups employed less

Table 4

Mean Urinary F^- Level and Proportion of Samples with > 4.5 ppm Level

Level of Risk in Department	No. of Samples	Mean Urinary F^- Level (ppm)	% Sample with > 4.5 ppm
High	84	2.42 ± 2.00	21.43
Moderate	58	1.78 ± 1.97	12.07
Low	84	1.39 ± 1.94	2.38
Total	226	1.96 ± 1.94	11.95

	Z Value	
High/Moderate	- 1.85	X^2 = 14.536
High/Low	- 3.85	df = 2
Moderate/Low	- 1.30	P < 0.01

than 3, 3-6, 6-9 years was very small in comparison with those employed more than 9 years.

Among workers whose urinary fluoride was more than 4.5 ppm, 10.5% had been working more than 9 years and 16.36% less than 9 years (Table 6).

The proportion of workers having complaints, dental and radiological changes, was higher (19.8%, 10.0% and 25.4% respectively) in the group employed more than 9 years than for the group of workers employed less than 9 years (12.4%, 8.2% and 14.75% respectively). However, these differences between the group employed more than 9 years and that less than 9 years were not statistically significant (Table 7). In Roholm's study (8) workers' radiological changes were related to duration of exposure whereas in the present study duration of employment had no significant effect.

The socio-economic status of workers is related in two ways to the effect of the factory environment on their health. Workers of lower socio-economic group, unskilled or skilled laborers, are more at risk to direct exposure in the process and their diet is likely to be deficient in calcium and vitamin C compared to the higher socio-economic group.

Workers were grouped according to Prasad's 1970 socio-economic clas-

Table 5

Workers with Complaints, Dental Fluorosis and Positive X-rays

Department	Complaints	Dental Fluorosis	Positive X-rays
High	50/171 (29.24%)	20/171 (11.69%)	16/68 (23.53%)
Moderate	14/114 (12.17%)	11/115 (9.56%)	16/75 (21.23%)
Low	15/152 (9.87%)	11/152 (7.24%)	8/40 (20.00%)
Total	79/438 (18.03%)	42/438 (9.59%)	40/183 (21.86%)
X^2 Value	24.5	1.84	0.025
df	2	2	2
P	<0.05	>0.05	>0.05
High/Low d Value	4.57	1.37	0.43

Complaints - Lumbar, and Cervical Spine, Knee and Ankle Joint Pain

Note: No. of workers with positive find / Total No. of workers

Table 6

Mean Urinary F^- Level and Urine Samples with > 4.5 ppm Level

Employment (Years)	No. Urine Samples	Mean Urinary Fluoride ppm	% Samples with > 4.5 ppm Level
> 9	171	1.94 ± 1.89	10.5
6-9	21	1.76 ± 2.16) 2.08	19.1)
3-6	28	2.25 ± 1.97) ±	10.7) 16.36
< 3	6	1.83 ± 2.48) 1.99	33.4)
Total	226	1.96 ± 1.94	11.95
			X^2 = 3.98 df = 3 P > 0.05
	>9/< 9 Years	Z = 0.848	X^2 = 0.85 df = 1 P > 0.05

Table 7

Workers with Complaints, Dental Fluorosis and Positive X-rays According to Duration of Employment

Employment (Years)	Complaints		Dental Fluorosis		Positive X-rays	
> 9	67/337 (19.8%)		34/337 (10.0%)		31/122 (25.4%)	
6-9	4/46 (8.6%)	12.4%	4/46 (8.6%)	8.2%	3/25 (12.0%)	14.75%
3-6	7/42 (16.6%)		3/42 (7.1%)		3/28 (10.7)	
< 3	1/9 (11.11%)		1/9 (11.11%)		3/8 (37.5%)	
Total	79/438 (18.0%)		42/438 (9.59%)		40/183 (21.9%)	
X^2 Value	3.01		5.41		5.50	
df	4		4		3	
P	> 0.05		>0.05		>0.05	
>9/ < 9 Years						
X^2 Value	2.853		0.296		2.703	
df	1		1		1	
P	>0.05		>0.05		>0.05	

sification (23), based on per capita income per month. The highest proportion of workers were in the middle (III) socio-economic group. The proportion of workers in higher (I) and lower (IV) socio-economic group was the same (Table 8).

Mean urinary fluoride level was highest (2.66 ppm) in lower (IV) socio-economic group and lowest (1.62 ppm) in higher socio-economic group (I), and this difference was statistically significant (Table 8).

The proportion of workers with complaints was highest (13.6%) in (IV) the lower socio-economic group whereas it was lowest (0.9%) in the higher (I) socio-economic group. This difference was statistically significant. Dental and radiological changes in workers of the lower socio-economic group were 12.1% and 21.87% respectively whereas the corresponding changes in the higher socio-economic group were 8.6% and 23.07% respectively (Table 9).

In the present study only the urinary fluoride level and complaints are significantly higher in high risk group than in the low risk group and

Table 8

Mean Urinary F^- Level and Samples with > 4.5 ppm Level According to Socio-Economic Status

Socio-Economic Group	No. Urine Samples	Mean Urinary F^- Level ppm	% Samples >4.5 ppm
I	38	$\pm$ 1.62 1.67	7.89
II	69	$\pm$ 1.85 1.67	13.04
III	83	$\pm$ 2.08 1.89	10.84
IV	34	$\pm$ 2.66 2.4	23.53
Not known	2	-	-
TOTAL	226	$\pm$ 1.96 1.94	11.95
	I / IV	Z Value =2.12	d Value = 1.84 X^2 3.10 df 1 P> 0.05

in the lower socio-economic group than in the higher socio-economic group. This indicates that these two parameters are more suggestive of early effect of exposure to fluoride compounds on workers' health. Bowler (4), in his 1947 study, found that radiological changes appear after seven years of exposure in workers exposed to fluoride compounds. In this study it seems that nine years' exposure is not enough to produce statistically significant effects on radiological changes resulting from cumulative effect of exposure to fluoride compounds under the existing situation of the factory.

Distribution of workers with less than 9 years employment and more than 9 years employment and that of higher socio-economic and lower socio-economic group was not statistically different in the high and low risk group. This suggests that a significantly high urinary fluoride level and number of complaints in the high risk group can be attributed mainly to continuous exposure throughout the years of employment.

Conclusion

According to this study, under the prevailing factory conditions, the degree of exposure risk seems to be independent of the duration of exposure

Table 9

Workers with Complaints, Dental Fluorosis and Positive X-rays According to Socio-economic Status

Socio-economic Group	Complaints	Dental Fluorosis	Positive X-rays
I	6/70 (0.9%)	6/70 (8.6%)	6/70 (23.07%)
II	35/126 (27.8%)	8/126 (6.3%)	16/59 (27.11%)
III	28/172 (16.3%)	20/172 (11.6%)	11/65 (16.92%)
IV	9/66 (13.6%)	8/66 (12.1%)	7/32 (21.87%)
V	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)
Not known	0/3 (00.0%)	0/3 (00.0%)	-
TOTAL	79/438	42/438	40/183
I / IV Z_2	2.90	0.66	0.10
X^2	18.03	4.03	2.009
df	4	4	3
P	<0.01	>0.05	>0.05

Note: No. of workers with positive find / Total No. of workers

and socio-economic status. Workers in a high risk area suffer more without routine interdepartmental rotation.

Acknowledgement

The authors are grateful to ICMR for financial support of the project and to NIN Hyderabad for their collaboration. We thank the Dean of Medical College, Surat, for his permission to carry out this work and acknowledge the cooperation of management and workers of the factory.

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ENAMEL FLUOROSIS IN HUMAN TEMPORARY AND PERMANENT TEETH FROM A HIGH FLUORIDE AREA NEAR DRESDEN

by

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SUMMARY: Macroscopically varying degrees of enamel mottling, including pitting, were recorded in teeth from the high fluoride area of Dohna. Previous descriptions focused attention on primary changes, i.e. the ones that presumably most directly reflect ameloblast activity. The altered morphology, most often confined to the outer parts of the thickness of fluorosed enamel, is explained as representing a predominantly secondary - postformative - change, that does reveal but not constitute the primary change. Moreover it seems that pitting, staining and ridging of sheep enamel and perhaps in many published instances of severe dental fluorosis are largely the result of the post-eruptive effect of oral environment on the soft, poorly calcified fluorotic enamel.

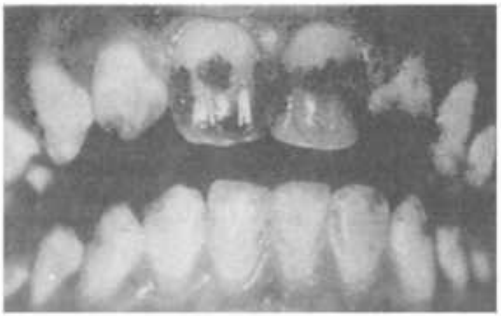
KEY WORDS: Fluorosis, dental; Dresden, high F^- near

Introduction

The local fluoride level in water of Dohna ranges between 0.9 and 4.5 ppm. Figures 1 - 8 show the appearance of different fluorotic degrees in permanent and primary teeth and some statistical data on the distribution of fluorosis.

Figure 1

Medium Dental Fluorosis



Permanent dentition, age 3 yrs.

Figure 2

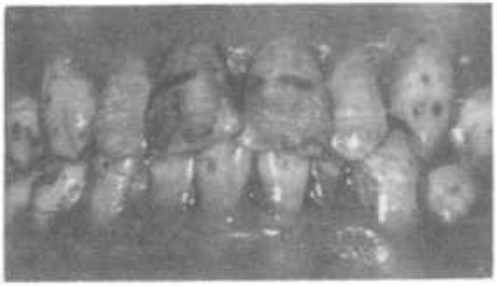
Severe Dental Fluorosis

Permanent dentition, male 16 yrs.

From the Department of Conservative Dentistry and Dentistry for Children Medical Academy "C.G. Carus", Dresden, GDR. Presented at the 11th I.S.F.R. Conference, April 8-10, 1981, Dresden, GDR.

Figure 3

Severe Dental Fluorosis



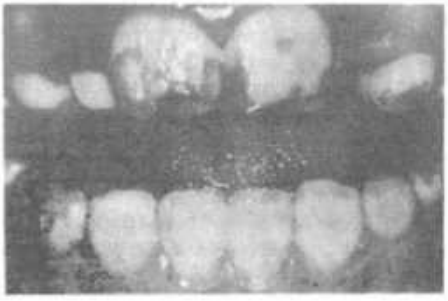
Permanent dentition, female 5 yrs.

Figure 4

Enlargement of Fig. 3
(Cut-out)

Figure 5

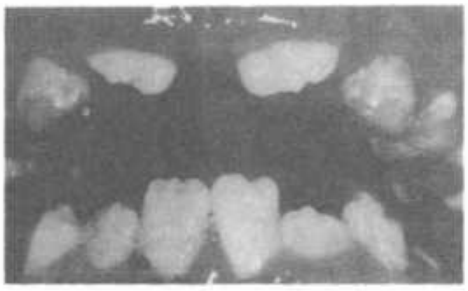
Medium Dental Fluorosis



Primary dentition, female 5 yrs.

Figure 6

Severe Dental Fluorosis

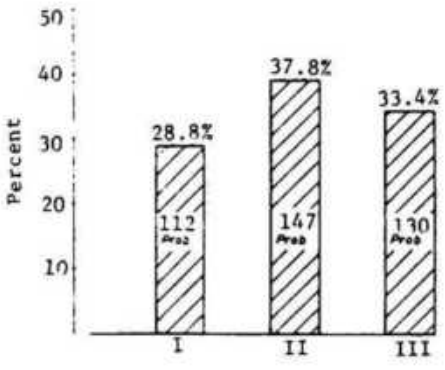


Primary dentition: permanent teeth seem not involved.

Early studies on dental fluorosis in 1916 (1) state frequently that, with rare exceptions, primary dentition does not seem to be involved and in 1965 Zipkin (2) stressed that the primary dentition seemed to be affected solely where the fluoride content exceeded 4.5 ppm. Similar findings have been reported from India (3) and Sweden (4). Several studies deal with structure of enamel from fluorosed human permanent teeth but few data are available concerned with the enamel of primary teeth. This sparcity of reports on fluorosis of primary incisors has been ascribed by Gedalia to the placental barriers which protect these teeth prenatally during mineralization. Primary teeth are usually whiter than permanent teeth because the total layer of enamel is thinner. Therefore, in

Figure 7

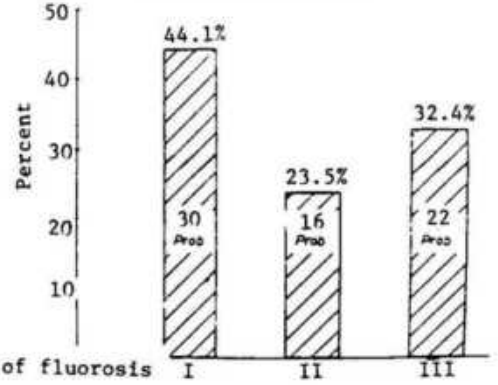
Dental Fluorosis Among Children Aged 7 to 16 Years in Dohna



Degrees: Mild (I); Medium (II); Severe (III)

Figure 8

Dental Fluorosis-Degrees in the Primary Dentition



Children aged 3 to 6 years

clinical studies when the internal pores remain filled with fluids, minor degrees of dental fluorosis may often be overlooked.

Our study shows that primary teeth from an area where food and drinking water contain considerable fluoride display varying degrees of mottling which, in ground sections, are microscopically hypomineralized, porous areas. The subsurface localization of the defective lesion and the maintenance of normal prism structure are in accordance with the changes described in fluorosed permanent teeth.

In a previous review of the conflicting evidence regarding the extent of placental transfer of fluoride, the author concluded that fluoride does pass through the human placenta. Moreover, the human placenta may be unable to maintain a difference in fluoride concentration between the maternal and fetal body fluids. It can therefore be assumed that prenatal and postnatal enamel in children residing in a high fluoride area such as Dohna is formed under similar environmental conditions.

Microscopically, the widening of the inner microporous zone, which corresponds with a zone of lower mineral content may thus be the result of the prenatal influence of fluoride. Similar changes have never been described in permanent fluorosed teeth. In the present material of dental hard tissue, the inner microporous zone was most often separated from the subsurface lesion by an enamel zone of normal porosity, which may indicate that various stages in normal amelogenesis may be affected in different ways.

In human amelogenesis, the growth of crystals is continuous while new enamel matrix is still being layed down superficially onto it. This is of

particular importance in the first formed cusp where crystal growth continues for a far longer period before the ameloblasts cease their secretory activity than in the cervical and outer half of the enamel which may explain why the innter cusp enamel appears less affected than the outer enamel in unerupted teeth.

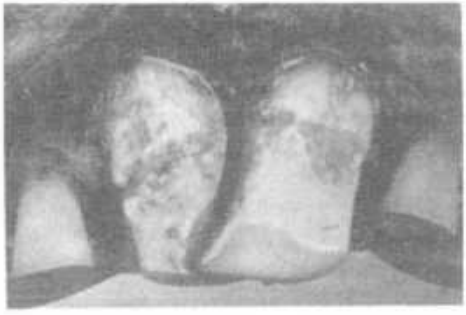
Increased fluoride levels might interfere with the complex cellular activity in the enamel organ and capillary layer, which is necessary for removal of protein and water in order to obtain a sufficient crystal growth in the final stages of enamel formation. This would produce a hypomineralized subsurface lesion similar to that described here. This description of fluorosed human enamel focuses attention on the primary alterations, which presumably reflect ameloblast activity most directly.

Subsequent attention was directed towards investigating that part of the fluorosed enamel which underlies the outer alteration, or to what has been discussed so far. This area had an appearance that was easily judged to be normal in those teeth where the outer alteration showed a minimal extension. However in teeth where the outer alteration was more advanced, the inner enamel was also changed. The conventionally indentified changes in the outer enamel are, however, also regarded as secondary and postformative.

Further studies may clarify to what extent the secondary changes occur pre- or posteruptively. They have been reported in unerupted, fluorosed teeth, but it should be noted that these reports, in emphasizing pre-eruptive changes, might be better described as evaluating impacted (retained) teeth. Unerupted teeth show considerable variation in enamel structure and mineralization even when nonfluorosed. The present choice of the term postformative should, intentionally, leave this matter open.

Figure 9

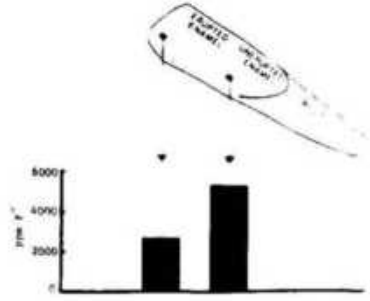
Teeth of fluorotic sheep in jaw. Tissue, fixed in formaldehyde, visibly shrunk.



Above site of gingival margin which can be seen, the teeth are visibly fluorotic.

Figure 10

Fluoride content (calculated from F/p ratios) of the enamel



Unerupted tissue contained large amounts of fluoride.

In addition, recent diagnosis of severe fluorosis in first permanent incisors of sheep (Fig. 9) has shown that, whereas both erupted and unerupted enamel contained very high concentrations of fluoride (Fig. 10), only the erupted enamel of the same tooth generally appeared normal(Fig. 11).

Figure 11

Gross changes in enamel of extracted tooth are associated solely with erupted part of tooth

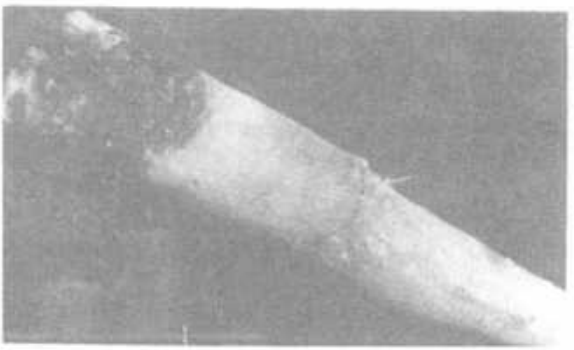
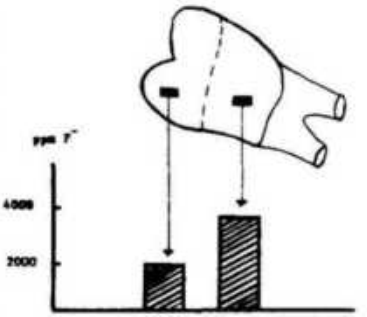


Figure 12

Fluoride content,calculated from F/P ratios, of lower first molar with visible fluorosis in erupted part



Consequently, the above interpretation is confirmed because the pitting, staining and ridging of the sheep enamel and,perhaps,in many of the published instances of severe dental fluorosis are largely the result of the post-eruptive effect of oral environment on the soft,poorly calcified fluorotic enamel. This statement is in accordance with the results obtained by fluoride analysis of a human fluorotic third molar (Fig. 12) which remained in a half erupted position.

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EXPERIMENTAL FLUOROSIS IN RATS: NaF INDUCED CHANGES OF BONE AND BONE MARROW

by

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SUMMARY: According to recent studies, excess fluoride intake can cause both osteosclerosis and osteoporosis. The effect of fluoride can be influenced by several factors. In our experiments the aim was to demonstrate the alterations in bone and bone marrow caused by sodium fluoride, and to define the relation between the bone lesion and fluoride dosage.

Of 20 white female rats, body weight 200 gr., 10 were administered 0.5 mg NaF, the other 10, 5 mg intraperitoneally for two months. NaF-induced bone changes were analyzed on ribs, vertebrae and femur. The decalcinated specimens were fixed in 8% neutral formalin, imbedded in paraffin, serially sectioned and stained with HE, or investigated by polarization optic methods. Bone and bone marrow alterations were evaluated by the morphometric method.

KEY WORDS: Fluorosis in rats; Bone changes; Rats, fluorosis in; Bone marrow

Introduction

Prolonged ingestion of excessive fluoride induces osteosclerosis, subperiosteally newly formed bone, vertebral osteophytes and calcification of paravertebral ligaments resembling the changes of ankylosing spondylitis, but the classical radiological changes in the sacroiliac joints are absent (1). The osteosclerotic effects of fluoride have been explained on the basis of secondary hyperparathyroidism (2-5). The effect of fluoride can be influenced by several factors such as vitamin D, ascorbic acid, etc.

The aim of our experiments was to study the changes in bone and bone marrow caused by sodium fluoride and to determine the degree of changes related to the dose of the fluoride compound.

Material and Methods

Ten white female rats weighing 200 grams were given 0.5 mg and 10, 5 mg of sodium fluoride intraperitoneally daily for two months. Ten rats served as controls without treatment. The ribs, vertebrae, tibia and femur were removed, fixed in 8% formaldehyde solution and decalcinated in EDTA (0.1%, pH7, 4). 5 µm thick serial sections were cut from the paraffin-embedded samples and processed for hematoxylin-eosin staining.

From the National Institute of Rheumatology, Budapest, Hungary. Presented at the 11th I.S.F.R. Conference, April 8-10, 1981, Dresden, G.D.R.

Results

In treated rats, the number of osteoclasts decreased in proportion to the dose of NaF whereas the number of osteoblasts increased (only locally, not generally), or did not change. The decrease in the number of osteo-

Figure 1

Figure 2

Remodeling Zone of Tibia, HE 12.5 x 4

0.5 mg NaF

5 mg NaF

With greater dose of NaF, osteoclast number decreased, corticalis sclerosed, subperiosteal border less "lacy."

Figure 3

Figure 4

Femur Head, HE 2.5 x 4 Magnification Refers to 24 x 36 mm Negative

Extended chondral areas in epiphysis a sign of inhibited enchondral ossification caused by greater NaF dose.

clasts was significant, particularly in the growth zone of the tibia (Figs. 1-2) and femur and in the area of enchondral ossification (Figs. 3-4). Osteoclerosis, following the treatment developed in proportion to the dose of NaF (Figs. 1-4).

Degenerative changes in proportion to the dose were noted in the epiphyseal cartilage: the chondrocytes lost their regular arrangement (Figs. 5-6).

Figure 5 Figure 6

Epiphyseal Cartilage from Tibia, HE 12.5 x 4

0.5 mg NaF 5 mg NaF

With greater NaF dose bone tissue sclerosed, osteocytes focally necrosed, arrangement of chondrocytes in some places irregular, chondral framework of primary spongiosa summarized in white line broadened as sign of inhibited ossification.

In some places the bone tissue became necrotic and the osteocytes lost their nuclear staining (Figs. 6-11). We have seen secondary fractures within the necrotic areas.

The hemopoietic tissue mass decreased in proportion to the dose of NaF (Figs. 7-8) and, in some places, the marrow was replaced by fibrotic connective tissue (Fig.10).

Osteophytes occurred mainly on the vertebral body, mostly in animals treated with larger doses. Only in a few cases were the osteophytes localized marginally (Fig. 9). In the majority of cases the osteophytes were present in the middle third corticals of the vertebral bodies. We have seen newly formed bone subperiosteally as well (Fig. 10).

Figure 7

Ribs, HE 12.5 x 4

0.5 mg NaF

Figure 8

5 mg NaF

Hemopoietic tissue mass decreased, bone tissue sclerosed in proportion to NaF dose.

Figure 9

Tibia - Osteophyte HE 12.5 x 4

5 mg NaF

Figure 10

Subperiosteal New Woven Bone Formation HE 12.5 x 4

5 mg NaF

Focal calcifications were seen in paravertebral ligaments, in intervertebral discs and in the cortical Haversian canals (Fig. 12).

The articular cartillage and particularly the lower calcified zone became enlarged in proportion to the dose of NaF.

Figure 11

Femur Spongiosa, HE 12.5 x 4

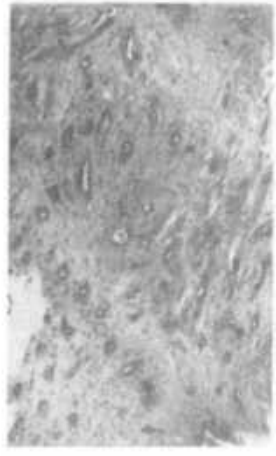
5 mg NaF

According to greater NaF dose cancellous bone sclerosed, osteocytes focally necrosed, marrow replaced by fibrotic connective tissue

Figure 12

Vertebra Corticalis, HE 12.5 x 4

5 mg NaF



Mottled calcification in Haversian canals.

Discussion

The results of our experiemnts suggest that increased doses of NaF cause more extensive osteosclerosis due to the decrease in number and/or activity of osteoclasts. Therefore osteosclerosis is caused primarily, not by increased bone formation but, by the inhibition of bone resorption. This view is supported by the fact that fluoride inhibits acid phosphatase activity more than alkaline phosphatase (6). The acid phosphatase activity of osteoclasts is of greater intensity than that of osteoblasts and the alkaline phosphatase activity of osteoblasts is of greater intensity than that of osteoclasts. So fluoride inhibits the osteoclasts more than the osteoblasts.

In addition, the metabolism of osteoclasts is of greater intensity

than that of osteoblasts (7,8). Therefore osteoclasts are more sensitive to fluoride poisoning, than osteoblasts.

Osteosclerosis, caused by fluoride, is really the result of a toxic effect which is reflected by the dose dependent decrease of osteoclasts and of the hemopoietic elements, the irregular arrangement of the epiphyseal cartillage chondrocytes and also by skeletal necrosis.

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PRODUCTION OF FLUOROACETATE BY CALLUS TISSUE FROM LEAVES OF Acacia georginae

by

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SUMMARY: Callus cultures of Acacia georginae were initiated from leaf discs from young leaves. Growth of callus was slow but predictable with tissue volumes up to 2.2 cm^3 being formed. Fluoride concentrations up to 80 ppm in the medium produced no adverse effect on callus growth. Reversible growth inhibition occurred at 160 ppm, whereas apparent death occurred at higher concen-

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trations. Fluoroacetate was detected by gas chromatography in the callus treated with 40, 80, and 160 ppm fluoride.

KEY WORDS: Callus, Acacia, fluoride, fluoroacetate

Introduction

More than 25 species of plants worldwide metabolize inorganic fluoride into various organofluorine compounds, especially fluoroacetate (1). Fluoroacetate may accumulate to concentrations which are extremely toxic to herbivorous animals, including cattle. Attempts to study the conversion of inorganic fluoride to organofluorine compounds and their subsequent metabolism have been hindered by several problems. These include shortage of tissue, presence of many highly differentiated cell types, and non-uniformity of cell types. Peters et al. (2) found that seedling and small plants of gidyea (Acacia georginae F.M. Bailey), grown under laboratory conditions, produced fluoroacetate from inorganic fluoride. They found a wide variation in the extent to which individual plants took up inorganic fluoride and in the extent to which they produced fluoroacetate. A genetic origin for the variations was suggested. To circumvent possible variations, Preuss et al. (3) grew callus of A. georginae from stem sections of seedlings of young plants. When dosed with inorganic fluoride, the callus produced fluoroacetate which appeared both in the tissue and in the growth medium. They concluded that, contrary to previous work by Peters et al. (2), the root system was not, or was potentially not, the only site of fluoroacetate production.

Callus tissue culture offers several advantages: First, within a given clone derived from some original tissue, there is a uniform "genotype." Hence, variations in synthesis of fluoroacetate due to genetic differences are minimized. Second, intact plants or macerates of intact plants necessarily consist of numerous highly differentiated cell types. Callus consists of uniform, and undifferentiated cells. Third, considerable quantities of callus may be grown within a reasonable time.

Acacia georginae used in the present study, has become the model plant for studies of the metabolism of organofluorine compounds. Our primary objectives were to establish callus cultures of A. georginae derived from leaf discs and to ascertain whether, when administered inorganic fluoride, they produced fluoroacetate.

Materials and Methods

Acacia georginae plants, grown from seeds from the Alice Springs area of Australia and grown in our greenhouse for several years, served as a source of tissue to be tested for callus induction. Leaf discs containing some midvein tissue and approximately 1 cm in diameter were punched from young leaves on new twigs. The discs were surface sterilized by washing with sodium laural sulfate (1%) with stirring for 1 hr. The wash solution was changed three times at 15 min. intervals. The final wash was removed

by aspiration and replaced with a 10% solution of Purex (Tm) and slowly stirred for 20 min. The Purex solution was removed by filtration and the materials washed three times with sterile distilled water. Finally, the tissue was soaked for 3 min. in 70% ethanol. The alcohol was filtered away and the discs were placed on sterile towels and blotted dry. The discs were placed on the surface of 20 ml of 0.9% agar containing incubation medium in 50 ml Erlenmeyer flasks. The medium was a modification of that of White (4) supplemented with extract of garden pea (Pisum sativa), coconut milk, kinetin and inositol (hereafter referred to as WPC medium). Cultures were incubated on a laboratory shelf with ambient lighting and a temperature of approximately 25°C + 2.5°C.

The influence of various levels of inorganic fluoride (as NaF) was tested with callus grown on filter paper bridges in contact with 10 ml of WPC medium lacking agar. The culture tubes were Corning 16 x 150 mm disposables. Small explants of third generation callus were placed aseptically on the surface of the bridges and incubated, under the conditions previously described, for three weeks. Callus growth was scored visually using an arbitrary system reflecting relative growth as compared to controls. Scoring of callus growth follows: Maximum growth, +++, same as control with no added fluoride; good growth, ++, less than control but still vigorous; fair growth, +, growth much less than control, but tissue increasing or maintaining a healthy appearance without noticeable growth; no growth, 0, zero growth, tissue failing to maintain a healthy appearance relative to control. All tubes were scored at the end of three weeks.The concentrations of fluoride used were 0, 10, 20, 40, 80, 160, 320, 640,and 1280 ppm. All treatments were replicated 10 times.

Organic acids were extracted from callus tissue by the methods of Oelrichs et al. (5), Yang et al. (6), and Yu et al. (7). The acids were esterified with diazomethane by the McKeown et al. method (8). Diazomethane was prepared from N-methyl-N-nitroso-p-toluene sulfonamide (9). For comparison, two grams of fresh leaves were extracted by the same method as described previously.

Methyl fluoroacetate was prepared from sodium fluoroacetate (Sigma) by dissolving the latter in water and passing the solution through Dowex 50W-X (H+form) to convert it to the acid form. The eluate was evaporated to dryness at 39°C under reduced pressure. The residue was dissolved in anhydrous methanol and methylated with diazomethane. A water control was prepared in a similar fashion using deionized distilled water.

The methyl esters of the organic acids were analyzed by gas chromatography. Two columns (2 mm x 2 m) were packed with Tenax 60/80 (Applied Science Lab., Inc.) and conditioned overnight at 325°C. The gas chromatograph (Hewlett-Packard 5830A and 18850A GC Terminal) was isothermal for the first 2 min. at 75 C, followed by a programmed increase of 2 C/min to 150 C and thereafter at 30C/min to 250 C. The carrier was nitrogen flowing at 13 ml/min. The component gases were detected by a flame ionization detector. Normally, 1 ul of the sample was injected for analysis.

Results and Discussion

Leaf discs of Acacia georginae, not more than 2 weeks old, were successfully induced to form callus on WPC medium. Earlier attempts using older leaves were unsuccessful. Callus formed within 2-3 days along the region of the midvein and subsequently from the margin of the disc. Cell proliferation was relatively slow but continuous, ultimately obscuring the original leaf disc. The callus itself was typical friable and had a cream-like color. No green pigmentation was observed. Older callus (several weeks old) produced a dark pigment. The largest calli typically formed were approximately 2 cm in diameter and weighed approximately 2 g. Growth ceased as the medium became visibly desiccated.

Callus was subcultured up to 5 times without obvious loss of vigor. When subculturing was performed, small pieces of callus surface, approximately 1 mm^2, were aseptically excised with a scalpel and placed on fresh WPC medium and incubated as previously described. Growth began immediately and the growth appeared to be undiminished during successive transfers.

Callus growth on WPC liquid medium was excellent for a period of about 3 weeks. At that time the experiment was terminated due to apparent evaporation of water from the medium. Growth scores are presented in Table 1. Increased concentrations of fluoride appeared to have no effect on growth rate and appearance up to 80 ppm of fluoride. At 160 ppm no growth was apparent. The calli, however, maintained a healthy appearance and began to grow when removed from the fluoride-containing medium,washed and placed in fresh medium without added fluoride. Calli incubated on media containing 320 ppm or more of fluoride showed significant browning

Table 1

Effect of Increasing Concentrations of F^- on Growth of Callus Tissue of Acacia georginae

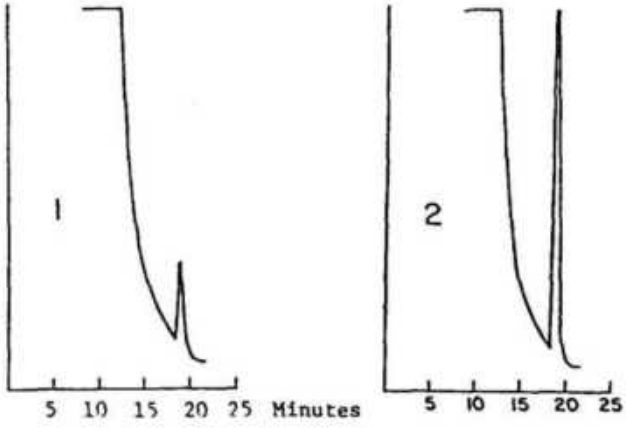
F^- Concentration (ppm)	Growth of Callus Tissue
0 (control)	+++
10	+++
20	+++
40	+++
80	+++
160	+
320	0
640	0
1280	0

within a week and failed to grow. They also failed to resume growth when subsequently transferred to fresh, fluoride-free medium.

Extracts from fresh leaves produced a pronounced gas chromatographic peak at approximately 19.5 min(Fig.1). An equal volume of fresh leaf extract with added methylfluoroacetate produced a single large peak at the same retention time (Fig. 2). Methylated extract from callus treated

Figure 1 Figure 2

Gas Chromatogram of Methylated Extract
Fresh Leaves, A. georginae



Cochromatographed with known methyl fluoroacetate.

with 40 ppm fluoride (Fig. 3) gave a peak at the same retention time. A similar peak was found for 80 to 160 ppm fluoride. Pure methylfluoroacetate gave a sharp peak between approximately 18.6 and 19.3 min. (Fig. 4). Methylated extract of callus grown with no fluoride showed no peaks in the 15-24 min. interval (Fig. 5); likewise for the water control (Fig.6).

Calli grew equally well on 0-80 ppm fluoride in WPC medium. At 160 ppm fluoride, growth was reversibly inhibited whereas at higher concentrations callus death occurred. A toxic threshold around 160 ppm fluoride is clearly indicated. The uniformly good growth at lower concentrations rather than a gradation of growth reduction suggests that some mechanism was operative which protected the growth processes.

Qualitatively, fluoroacetate was detected in the callus exposed to 40 ppm fluoride. Cells derived from leaf tissue, therefore, can produce

Figure 3

Gas Chromatogram of Methylated Extract A. georginae

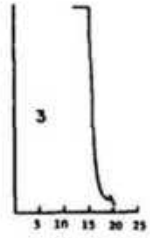
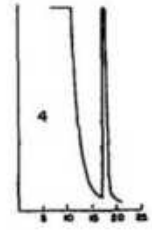


Figure 4

Gas Chromatogram of Known Methyl Fluoroacetate



Callus treated with inorganic F^-

Figure 5

Gas Chromatogram of Extract of Callus A. georginae

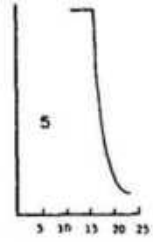
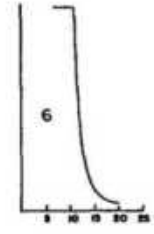


Figure 6

Gas Chromatogram of Water Control



Without treatment with inorganic F^-

fluoroacetate and, by inference, the differentiated leaf cells had at least the potential to do so. Peters et al. (2) suggested that the roots were the site of production of fluoroacetate. Preuss et al. (3) showed that stem tissue cultures of A. georginae could produce fluoroacetate. In this study we have shown that leaf-derived callus tissue can produce fluoroacetate. One may infer that essentially all plant organs have at least the potential to do so. The problem of autotoxicity remains unresolved.

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EFFECT OF FLUOROACETATE ON GLUCOSE SYNTHESIS IN RAT LIVER

by

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SUMMARY: In order to get an insight into the increased glycemia in rats intoxicated with fluoroacetate (FAc), the effect of this poison on the gluconeogenesis in isolated hepatocytes was studied.

FAc (10 mM) inhibited the synthesis of glucose from pyruvate during the initial period of incubation, whereas the glucose synthesis from lactate in the same period was unimpaired and sometimes activated. This activation could in part explain the increased glycemia in intoxicated animals.

It was suggested that FAc acts at the level of the malate shuttle. In fact, the decrease of gluconeogenesis from pyruvate might be due to the

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inhibition of this shuttle, with a consequent decrease of supply to the cytosol of both NADH and carbon skeleton compounds. The decrease in transport of NADH to cytosol could also explain the initial activation of gluconeogenesis from lactate. Under these conditions the optimal (lactate)/(pyruvate) ratio is reached earlier. In a more prolonged incubation period, the lack of malate shuttle function would lead to an inhibition of glucose synthesis from lactate also.

Experiments were done with chicken hepatocytes, where there is no requirement for transport of oxaloacetate out of the mitochondria, which seems to confirm the proposed hypothesis.

KEY WORDS: Gluconeogenesis, fluoroacetate, isolated hepatocytes

Introduction

In 1953 Peters et al.(1) reported that fluoroacetate is converted in the organism to fluorocitrate,which is a powerful inhibitor of the enzyme aconitase,(EC4.2.1.3.)thus causing a block of the tricarboxylic acid cycle and a subsequent increase of citrate concentration in tissues. This is a major effect of fluoroacetate; but the pathological and metabolic effects of this poison are various (2-5) and,to date,not all of them have been explained. One of them is the increase of glucose concentration in blood of fed and fasted animals (4,6,7) after intoxication. Most probably this increased glycemia is hormone dependent (4,8). However, the source of the increased glucose in the blood has not been clearly identified. In the case of fed animals, the high glycemia may be due to glycogenolysis; but in the case of fasted animals, where low levels of hepatic glycogen are observed, the mechanism of glucose level increase in blood remains to be explained. As *de novo* synthesis is the possible source of blood glucose in fasted animals, it may be that fluoroacetate poisoning has some effect on gluconeogenesis.

In the present work, gluconeogenesis was studied in hepatocytes isolated from animals intoxicated with fluoroacetate and in normal hepatocytes treated in vitro with fluoroacetate. In this system cellular integrity is preserved but hormonal and nervous influences are excluded.

Materials and Methods

The following animals were used: Wistar female albino rats weighing 200-250 g; Hubbard strain male white chicks weighing 300-400 g and Marley strain female guinea pigs weighing 250-300 g. The animals were fasted for 48 h before the experiment and were allowed water ad libitum. Liver parenchymal cells were isolated essentially according to the method

described by Berry and Friend (9) as modified by Krebs et al. (10), excluding hyaluronidase. Isolated hepatocytes were incubated as described by Krebs et al. (10). Substrates were added in concentrations indicated below. Aliquots of cell suspensions, corresponding to 50 - 80 mg wet weight tissue, were added to the incubation medium in a final volume of 4 ml. Incubation was interrupted by addition of 0.14 ml of 70% $HClO_4$. The reaction mixture was centrifuged at 5,000 xg for 15 minutes; the supernatant was neutralized with 20% KOH; and then glucose, ATP, lactate and pyruvate levels were determined according to the methods of Slein (11), Strehler (12), Hohorst (13), and Bücher et al. (14) respectively. Oxygen uptake was determined according to the method of Berry and Kun (15).

Fluoroacetate was supplied by Calbiochem AG, Switzerland; Collagenase by Worthington, USA. All the enzymes used for metabolite determinations were supplied by Boehringer, Mannheim GmbH. All the reagents were of analytical grade.

Results

At first, parenchymal cells isolated from rats intoxicated with a lethal dose of fluoroacetate were used. As it is shown in Table 1, the rate of glucose synthesis from lactate was greatly increased in these hepatocytes (+78%), while the gluconeogenesis from pyruvate in the same preparation was inhibited. In order to exclude completely either the possibility of hormonal influence during *in vivo* intoxication or the

Table 1

Gluconeogenesis in Hepatocytes Isolated From Rats Intoxicated with Fluoroacetate

Substrate Added	Rate of Glucose Formation (μmoles/min./g wet wt. cells)	
	Hepatocytes from Untreated Rats	Hepatocytes from FAc Intoxicated Rats
None	0.132±0.017 [18]	0.104±0.026 [3]
10 mM Pyruvate	0.622±0.054 [7]	0.219±0.039 [3] Δ = -65%
10 mM Lactate	0.480±0.071 [9]	0.857±0.085 [3] Δ = +78%

Experimental animals were injected with 10 mg NaFAc/kg body weight i.p. After 30 min. liver parenchymal cells were isolated by the method described in Materials and Methods. 10 mM FAc was added to perfusion medium. Incubation of both control and experimental hepatocytes was carried out for 30 min. at 37°C in the absence of fluoroacetate. Mean values ± S.E.M. are given. Values in brackets indicate the number of replications.

interaction with metabolism of other organs and cells, parenchymal cells isolated from liver of untreated animals were used subsequently.

Preliminary results demonstrated that fluoroacetate had no effect on the integrity of hepatocyte membranes as shown by the equal amount of lactate dehydrogenase (EC1.1.1.27) released from the cells during incubation, both in the control and FAc treated samples (unpublished observations).

Results obtained in vitro (Table 2) agreed with those obtained in vivo, although the increase of gluconeogenesis from lactate was not so high as that in vivo. It must be stressed that the results are correlated with the viability of cells: when freshly prepared cells were used immediately after isolation, the increase in glucose production from lactate, as an effect of fluoroacetate, was higher and its inhibition from pyruvate was lower.

Table 2

Effect of Fluoroacetate on Glucose Synthesis in Isolated Rat Hepatocytes

Substrate Added	Rate of Glucose Formation (μmoles/min./g wet wt. cells) Control	10 mM FAc
None	0.132±0.017 [18]	0.125±0.028 [17]
10 mM Pyruvate	0.6222±0.054 [7] $p<0.01$	0.252±0.032 [7] $\Delta = -59\%$
10 mM Lactate	0.480±0.071 [9] $p<0.2$	0.572±0.044 [9] $\Delta = +19\%$

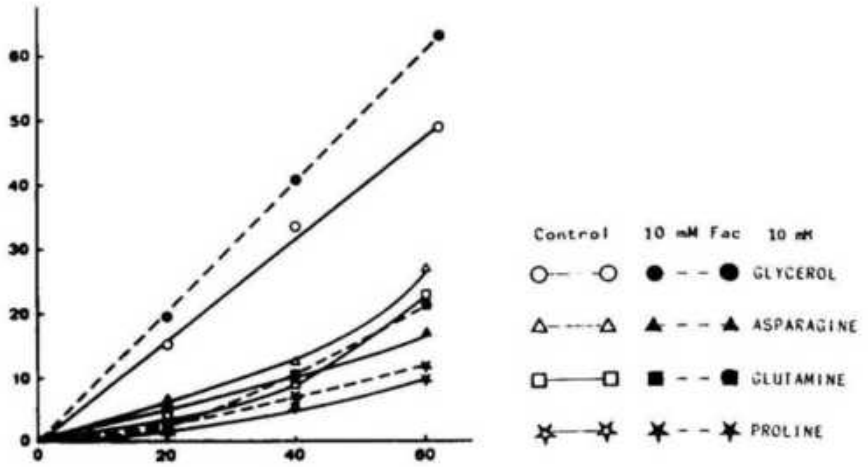
The hepatocytes were isolated from 48 h starved rats. Incubation time was 30 min. at 37°C. Mean values ± S.E.M. are given. Values in brackets indicate the number of replications.

In order to elucidate the point of action of fluoroacetate, substrates which do not involve pyruvate carboxylase (EC 6.4.1.1.) (proline, asparagine, glutamine) were also used. In the case of glycerol, the enzyme 3-phosphoglyceraldehyde dehydrogenase (EC 1.2.1.12) was excluded too. Glucose synthesis from these substrates was not significantly affected after 30 min. incubation. After 60 minutes, only the gluconeogenesis from asparagine showed a moderate inhibition, while it was enhanced in the case of glycerol (Fig. 1).

The amount of ATP (as an index of the tricarboxylic acid activity

Figure 1

Effect of Fluoroacetate on Glucose Synthesis from Different Substrates in Rat Liver Cells



and of the integrity of oxidative phosphorylation) was measured in the cells incubated with fluoroacetate (Table 3). The level of ATP did not

Table 3

Effect of Fluoroacetate on the Respiratory Activity of of Rat Liver Parenchymal Cells

Substrate Added	Oxygen uptake (µl O_2/9 wet wt/1hr.) C	10 mM FAc	Δ%
None	1755	1386	-21
10 mM Pyruvate	2058	1956	- 5
10 mM Lactate	2040	1860	- 9

Hepatocytes were prepared as indicated in Material and Methods. Oxygen uptake was measured by standard manometric techniques as indicated by Berry and Kun (15), incubating the cell suspension at 37°C in an atmosphere of air for 60 min. Data represent the mean of two experiments.

change in fluoroacetate treated samples after 60 min. incubation. The concentration with and without substrates or FAc was about 2.6 umoles/g wet wt. cells.

The results of experiments in which the respiration of isolated cells was measured support the previous data (Table 4). The presence of fluoroacetate, in fact, does not appreciably modify oxygen uptake. The present data agree with the results obtained by Buffa et al. (5), who reported that the liver slices and whole liver homogenate, prepared from starved rats poisoned with fluoroacetate, did not show inhibition of respiration, but rather consumed oxygen at a faster rate than controls. Moreover, as was observed by Gal et al. (2), very little synthesis of fluorocitrate takes place in the liver of starved rats intoxicated with fluoroacetate and therefore no inhibition of the tricarboxylic acid cycle should be expected.

It seems that the highest degree of gluconeogenesis inhibition took place whenever reducing power was required, as in the case when pyruvate was used as substrate. It is known, in fact, that the rate of gluconeo-

Table 4

Effect of Ethanol on the Gluconeogenesis from Pyruvate in Presence of Fluoroacetate

Ethanol Added	Rate of Glucose Formation (μmoles/min./g wet wt. cells) Control	10 mM FAc
None	0.580	0.322 Δ = -45%
5 mM	0.817	0.778 Δ = -5%
10 mM	0.793	0.711 Δ = -10%
20 mM	0.853	0.761 Δ = -11%

Hepatocytes were prepared as described in Materials and Methods. Incubation of hepatocytes in presence of 10 mM pyruvate was for 20 min. at 37°C. Determination of glucose was made as indicated in Materials and Methods. Mean values of two experiments are presented.

genesis is controlled by the NADH/NAD ratio (16). It may be that fluoroacetate exerted its inhibitory effect by interfering with this ratio. As shown in Table 4 the addition of ethanol enhanced the rate of gluconeo-

genesis from pyruvate in control samples. Our results agree with those obtained by Krebs et al. (17) with perfused liver, and those of Woitczak et al. (18) with isolated hepatocytes. In the presence of ethanol and inhibitory effect of fluoroacetate was negligible.

It is known that the rate of gluconeogenesis is also dependent on the efficiency of fatty acid oxidation (19-21). Table 5 shows that the addition of 1 mM oleate to the incubation mixture increased the glucose production from pyruvate in control samples and partially removed the inhibition due to fluoroacetate.

Table 5

Effect of Fluoroacetate on the Gluconeogenesis from 10 mM Pyruvate in the Presence of Oleate

Addition	Control	10 mM FAc	Δ%
	μmoles of glucose/g wet wt./min.		
None	0.637±0.067	0.457±0.060	-28
1 mM Oleate	1.193±0.139	1.078±0.153	-10

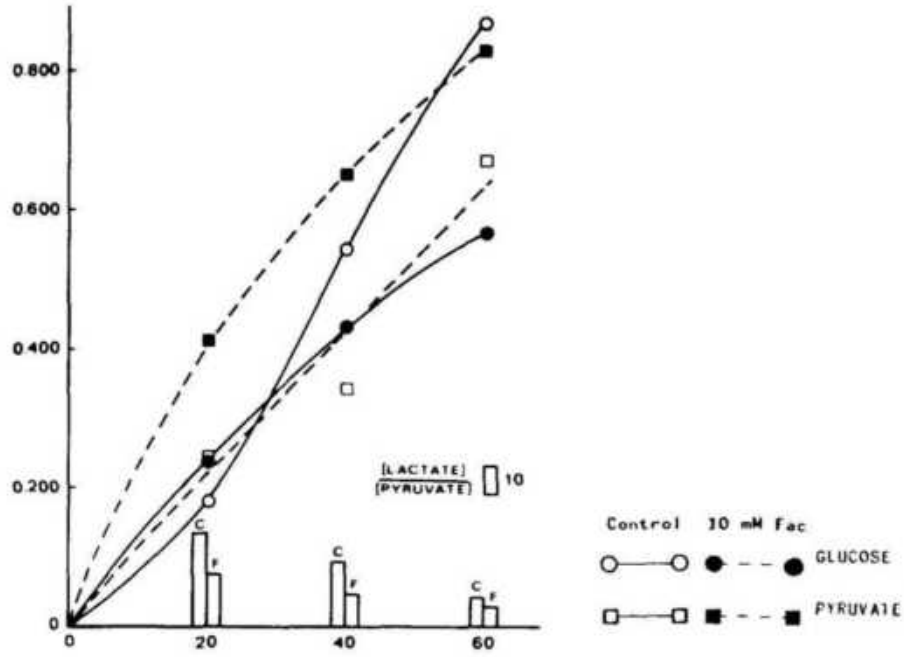
The parenchymal rat liver cells were prepared and incubated as indicated in Materials and Methods. Incubation time was 20 min. Mean values of four experiments ± S.E.M. are presented.

When the hepatocytes were incubated for more than 30 min. (Fig. 2), it was noted that during gluconeogenesis from lactate, fluoroacetate increasingly inhibited glucose formation. Pyruvate production, residual lactate and glucose accumulation were determined in the same samples. During incubation the pyruvate concentration in fluoroacetate samples was always higher than in controls and the (lactate)/(pyruvate) ratio at the same time was always lower. However, it must be noted that the differences between these ratios at the end of incubation became minimal. The addition of oleate to the incubation mixture containing lactate(Fig. 3) decreased glucose production in the first 20 min., while it was increased in the subsequent time period. The addition of fluoroacetate enhanced glucose synthesis (+77%) during the first 20 min., while after 40 min. the amount of glucose in the fluoroacetate treated samples was lower than in controls. The addition of oleate decreased the pyruvate content both in control and fluoroacetate samples; the (lactate)/(pyruvate) ratio in fluoroacetate samples was always lower than in control samples.

When the cells were preincubated for 40 minutes with fluoroacetate and then lactate was added, glucose synthesis was lowered from 21.6

Figure 2

Effect of Fluoroacetate on Gluconeogenesis from Lactate



μmoles/g wet wt/20 min. to 14.4 μmoles/g wet wt/20 min. (results of 4 different experiments). At the end of incubation, the (lactate)/(pyruvate) ratio in fluoroacetate samples was about 40, while in control samples it was about 24.

It is known that the intracellular distribution of phosphoenolpyruvate carboxykinase (EC 4.1.1.32) is species-dependent (22). The effect of fluoroacetate on gluconeogenesis in chicken hepatocytes, where this enzyme is intramitochondrial (23), and in guinea pig hepatocytes, in which this enzyme has a dual distribution between the mitochondrial and cytosolic compartments (24), was studied. As is shown in Table 6, the inhibition of glucose synthesis from pyruvate in guinea pig hepatocytes was lower than in the rat liver cells (see Table 2), where this enzyme is localized in the cytosol (22); while no inhibition of gluconeogenesis from pyruvate in chicken hepatocytes was observed.

Discussion

The results presented in Table 1 show that an activation of glucose synthesis from lactate takes place in the liver of fluoroacetate intoxi-

cated rats. A much lower activation of gluconeogenesis from lactate was observed in vitro (Table 2). This difference may be due to some interference present when the whole organism is considered.

Glucose synthesis from pyruvate was inhibited by fluoroacetate in hepatocytes from the beginning of incubation and, in the case of lactate after 20-40 min. (Table 2 and Fig. 2). This inhibition does not appear to be due to a decrease of aconitase activity (Table 3 and 4), in response to fluorocitrate. It seems that very little or no fluorocitrate in the liver of fasted animals is formed (3,5).

Table 6

Effect of Fluoroacetate on Glucose Production in Chicken and Guinea Pig Hepatocytes

	umoles of glucose/g wet wt./min.		
Animal	Control	10 mM FAc	Δ%
Guinea pig	1.69 [2]	1.32 [2]	-22
Chicken	1.45 [4]	1.74 [4]	+20

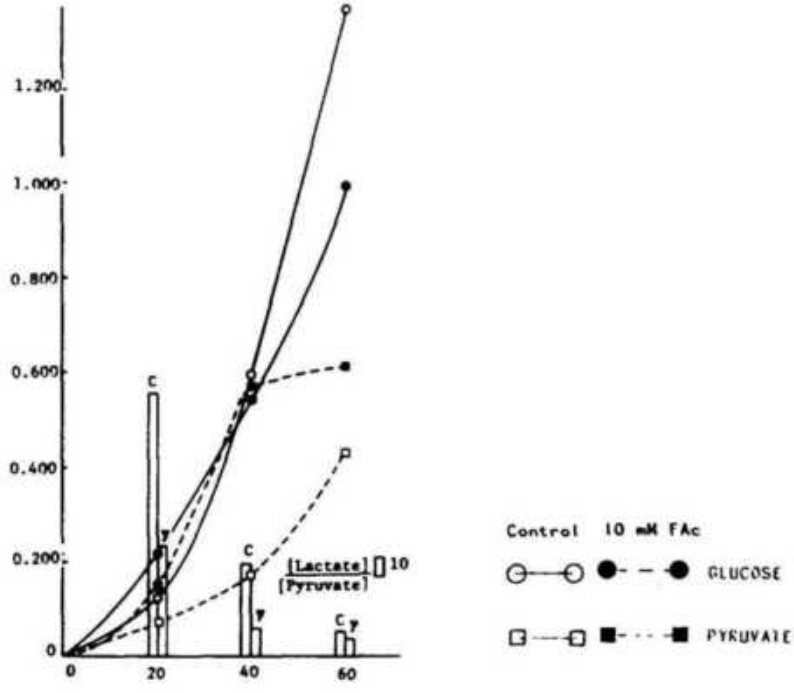
Parenchymal cells were prepared from livers by collagenase perfusion method and incubated as indicated in Materials and Methods. Incubation time, in case of guinea pig hepatocytes was 40 min. and in chicken hepatocytes 20 min. Values in brackets indicate the number of experiments.

Results obtained with substrates which do not require the participation of pyruvate carboxylase (glutamine, asparagine and proline) or glyceraldehydephosphate dehydrogenase (glycerol) indicate that fluoroacetate induced inhibition of glucose synthesis is correlated in some way with the utilization of pyruvate, either added as such or formed from lactate (Table 2 and Fig. 1).

Although the gluconeogenetic pathways from lactate and pyruvate are apparently similar, they differ in the mode of transport of oxaloacetate from mitochondria to cytosol (20, 22). In the case of pyruvate, the malate-oxaloacetate shuttle is mainly used for transporting oxaloacetate and reducing power out of the mitochondria, simultaneously. When glucose synthesis starts from lactate, the glutamate-aspartate shuttle is mainly utilized. Only when NaDH formed during lactate oxidation accumulates in

Figure 3

Effect of Fluoroacetate on Gluconeogenesis from Lactate in the Presence of Oleate



excess, does the malate-oxaloacetate shuttle become important, in order to transport malate into mitochondria (25). If fluoroacetate inhibits the malate shuttle function in some way, transport of both carbon skeleton compounds and reducing power out of the mitochondria would be decreased, (when pyruvate is used as the substrate), and the inhibition of glucose synthesis is observed in the early period of incubation. The removal of inhibition of gluconeogenesis pyruvate from the addition of ethanol indicates that the malate shuttle plays a prominent role in the transport of NADH rather than in the supply of carbon skeleton compounds, as these can be transported by the aspartate-glutamate shuttle. On the other hand, partial restoration of fluoroacetate-inhibited gluconeogenesis by the addition of oleate (Table 6), suggests that another shuttle transporting reducing power from mitochondria may exist as already suggested by Berry and Kun (15).

The decrease of NADH transport into the cytosol, as a consequence of inhibition of the malate shuttle by fluoroacetate, leads to a higher

production of pyruvate from lactate (Fig. 2). In this situation the optimal ratio of (lactate)/(pyruvate) (25) is reached more rapidly and glucose production is increased (Table 2, Fig. 2). The addition of oleate, which at first inhibits glucose production from lactate, probably by supplying an excess of NADH, increases activation produced by fluoroacetate (Fig. 3). In the latter 10 min. period of incubation with lactate, a higher level of oxaloacetate is reached, requiring the intervention of the malate shuttle, since the glutamate-aspartate shuttle is rate limiting (25). During this period, the inhibition of glucose synthesis by fluoroacetate is observed.

The decrease of gluconeogenesis from lactate in the last 10 min. of incubation might also be explained by the inhibition of transport of excess cytoplasmic NADH into the mitochondria by the malate shuttle coupled with the glutamate-aspartate shuttle. The diminishing differences between the (lactate)/(pyruvate) ratios of control and fluoroacetate treated samples during incubation may be the result of this situation. The results obtained with hepatocytes preincubated without lactate confirm this hypothesis. Under these conditions the glutamate-aspartate shuttle reaches the maximum of activity producing an optimal (lactate)/(pyruvate) ratio (25); when the malate shuttle was inhibited by fluoroacetate, gluconeogenesis was inhibited as a consequence of an elevated (lactate)/(pyruvate) ratio (see Results).

The results obtained with chicken hepatocytes show that fluoroacetate interferes with the malate shuttle function. In birds, phosphoenolpyruvate carboxykinase is situated in the mitochondria, and there is no necessity to obtain oxaloacetate from the mitochondria, no fluoroacetate induced inhibition of glucose synthesis from pyruvate was observed.

In conclusion, the data of the present research indicates that the inhibition of the malate shuttle function, resulting in a consequent modification of the NADH/NAD ratio, may partially explain the increased level of blood glucose obtained in fasted animals intoxicated with fluoroacetate. It is very likely, however, that in vivo some other factors, such as hormonal regulation or interaction with other organs and systems, may interfere with this effect, further complicating the situation.

Acknowledgement

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Abstract

FLUORIDE, AN INTERNALLY ACTING PLATELET ACTIVATOR, CAUSES PHOSPHORYLATION OF PLATELET PHOSPHATIDIC ACID AND 20K AND 47K PROTEINS

by

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(Abstracted from Thrombos. Haemostas. 46:149, 1981)

The effects of fluoride, which is transported into platelets in order to induce secretion, are compared with known effects of thrombin, which acts via external sites. Thus, the changes related to transmission of signal through the platelet membrane will not be common to the two activators, only those changes which are subsequent to the internal triggering of platelet activation. Human platelets were prepared by collection in EDTA and washing in saline-EDTA or by gel filtration of citrated platelet-rich plasma. The two methods gave similar results. Platelets prelabeled in plasma with ^{32}P and then separated were incubated at 37°C with 10 mM fluoride at pH 7.4, and samples removed at intervals. The protein was precipitated with $HClO_4$, then solubilized by sonication with SDS buffer and the protein bands separated by acrylamide slab gel electrophoresis. The 20K and 47K bands showed 100 to 200% increase in label, with maximum at 8 min. incubation (50% secretion). A great increase was seen already at 3 min. incubation, where little secretion is observed. Samples were extracted with chloroform-methanol, evaporated to dryness under N_2, redissolved in chloroform and applied on thin layer silica gels on aluminum plates. Two different systems for separating phosphatidic acid (PA) were used. No significant increase in ^{32}P radioactivity was seen in PA the first 3 min. The label at 20 min. was 3x that at 8 min. Thus the labeling related to contractile events, a late step in secretion, precedes the labeling of PA, suggesting that the major part of this labeling is not related to the initial phase of platelet activation.

KEY WORDS: Fluoride, platelet activation by; Platelet phosphorylation

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Authors' Abstract

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EFFECT OF INTRAPERITONEALLY INJECTED FLUORIDE ON PLASMA CALCIUM IN SUCKLING AND ADULT RATS

by

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(Abstracted from Calcif. Tissue Int. 33:541-544, 1981)

Sodium fluoride 10, 20, or 40 mg/kg body weight was given intraperitoneally to rats (6-11 days old and 90-95 days old). Blood analyses showed an initial increase in plasma fluoride concentration. The subsequent decrease in fluoride was paralleled by a decrease in total plasma calcium. These plasma concentrations were normal at blood collection 4 days after fluoride injection. The baby rats differed from the older rats in that their initial plasma calcium was higher and that the drop in plasma calcium concentration was less pronounced than in the old rats. A diet low in calcium and phosphate enhanced the effects of fluoride on total plasma calcium. The data indicate that the effect of large doses of fluoride on lowering the plasma calcium level is modified by the calcium intake.

Authors' Abstract

KEY WORDS: Fluoride; Plasma calcium

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EFFECTS OF FLUORIDE, CALCIUM, AND PHOSPHATE ADMINISTRATION ON MINERALIZATION IN RATS

by

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(Abstracted from Calcif. Tissue Int. 31:225-230, 1980)

Seven days before a fluoride injection of 20 mg sodium fluoride per kg body weight, 3-month old rats grown on a standard pellet diet containing 0.8% calcium and 1.4% phosphate were given a diet of rice with only 0.025% calcium and 0.1% phosphate. Microradiographs of the continuously growing incisors showed a hypermineralized and a subsequent hypomineralized zone. Blood analysis demonstrated a decrease and a subsequent reestablishment of plasma calcium concentration. In some experiments calcium and phosphate were administered to compensate the hypocalcemia which prevented the hypomineralized zone from arising. A delay of calcium and phosphate administration led to formation of a mineralized band within

the hypomineralized zone. The results are discussed with reference to calcium homeostasis.

Authors' Abstract

KEY WORDS: Fluoride metabolism; Calcium metabolism; Mineralization; Dentin

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THE EFFECT OF SODIUM FLUORIDE ON EGG PRODUCTION, EGG QUALITY, AND BONE STRENGTH OF CAGED LAYERS

by

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(Abstracted from Poultry Science 60:771-776, 1981)

The development of bone fragility in caged layers is a major problem in the poultry industry. The incidence of bone breakage during the processing of spent hens substantially reduces the economic return from these birds. In two trials, a commercial strain of White Leghorn pullets was used. In each trial, one-day-old chicks were divided into two groups, wing banded, and placed in floor pens. The treated group received fluoridated water at levels up to 300 ppm. The control group of birds received only well water. At 20 weeks of age, birds from each group were transferred to one of two laying batteries. One battery of birds received fluoridated water (100 ppm F^-). The other served as a control and those birds received only well water. Production rate, egg weight, shell strength, shell thickness, and Haugh units were determined for each bird. At 45 weeks of age, the humeri and tibiae were removed, and bone strength, percentage of bone ash, and fluoride content were determined.

Combined data from both trials showed that the fluoride treatment increased the breaking strength of humeri from 6.86 to 13.35 kg and that of tibiae from 6.61 to 13.10 kg. The fluoride treatment also significantly ($P < .01$) increased the percentage of bone ash. Egg quality and rate of production were not reduced by the fluoride treatment.

Author's Abstract

KEY WORDS: Hens, caged, bone strength, fluoride, eggs

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METABOLIC ASPECTS OF THE SECRETION OF STORED COMPOUNDS FROM BLOOD PLATELETS

by

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(Abstracted from Biochem. J., 194:187-192, 1981)

The purpose of this study was to investigate the response of human blood platelets to fluoride at different pH. The results were as follows: (1) Fluoride induced secretion faster and a lower concentration when pH was lowered. (2) Platelets exposed to 2 mM fluoride at 0°C at pH 5.3 underwent secretion when first pH and then temperature was raised, although no secretion was seen at 2 mM fluoride concentration in the absence of the preincubation at low pH. (3) The concentration of (^{14}C)ATP in platelets decreased steeply in response to fluoride before induction of secretion. Addition of antimycin blocked or partly inhibited secretion. Fluoride thus exerts an inhibitory effect on platelet glycolysis before induction of secretion. (4) Fluoride accumulated in the platelet pellet by a time course that preceded secretion. The accumulation was faster and greater at pH 6 than at 7.4. These four points are taken as indirect evidence that fluoride has to penetrate to the interior of the platelet to induce secretion. The activation takes place over a wide range of acid pH in contrast with induction of platelet function via the outside of the plasma membrane. In addition evidence is presented that the salvage pathway may under special circumstances play an important role in the re-synthesis of platelet adenine nucleotides.

Authors' Abstract

KEY WORDS: Blood platelets, fluoride effects, secretion by

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HISTOLOGICAL AND HISTOCHEMICAL APPEARANCE OF LIVERS AND KIDNEYS OF RATS AFTER LONG-TERM TREATMENT WITH DIFFERENT CONCENTRATIONS OF SODIUM FLUORIDE IN DRINKING WATER

by

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Sao Paulo, Brazil

(Abstracted from Acta Anat. 108:288-294, 1980)

Livers and kidneys of 48 albino rats were studied histologically and histochemically. The animals received a diet containing 1 ppm NaF in demineralized drinking water, to avoid the effect of other ions especially calcium and magnesium, or 1, 10 or 100 ppm NaF in tap water during 90 or 180 days. The rats treated with fluoride did not present any abnormalities with respect to weight gain, morphology, behavior, and macroscopic appearance of the livers and kidneys compared to controls. Histochemically, the polysaccharides, the proteic reactive groups and the acid and alkaline phosphatases presented no visible alterations.

Morphologic alterations in either the cells or the mitochondria of the livers and kidneys were revealed microscopically. Lipids, kidneys and livers of the rats treated for 90 days showed no fat deposition, whereas those treated for 180 days showed zones of deposition of lipids in the livers and kidneys with different frequencies when compared with controls. The association between lipid infiltration and the presence of fluoride in the drinking water, however, was statistically significant only in the livers. The authors state that "the hypothesis that fluorinated water accelerated this process in the treated animals cannot be excluded."

KEY WORDS: Histology; Histochemistry; Liver; Kidney; Fluoride

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EFFECT OF SODIUM FLUORIDE ON THE VIABILITY AND GROWTH OF STREPTOCOCCUS MUTANS

by

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(Abstracted from J. Dent. Res. 59:159-167, 1980)

A fluoride-sensitive (FS) strain of Streptococcus mutans and laboratory-induced fluoride-resistant (FR) offspring were compared for the

effects of sodium fluoride on viability and growth. A significant fluoride-related loss of viability in resting cell suspensions of the FS strain during a 47-hour exposure to fluoride levels above 75 ppm occurred that was not encountered with the FR strain. The addition of 300 ppm F^- to actively growing six-hour broth cultures almost totally arrested the growth of the FS strain, whereas that of the FR culture was only slightly reduced. The addition of 600 ppm F^- immediately terminated FS growth, and greatly reduced the rate and maximum growth of FR cultures.

KEY WORDS: Streptococcus mutans; Fluoride sensitivity; Fluoride resistance

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IS THE CURRENT REDUCTION IN CARIES IN OUR SCHOOL CHILDREN REAL OR IMAGINARY?

by

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(Abstracted from Tandlakartidningen ARG 14:138-141, 1982)

When a program of regular mouth rinsing with fluoride by school children was introduced, the incidence of caries fell considerably. Through use of fluoride toothpaste and fluoride topical application, as well as other individual measures the incidence of caries was further reduced. Subsequently a continual reduction in caries, which has been reported, may be only apparent, the result of a change in the method for determining caries incidence and an altered method of treating teeth.

Both from the economic and scientific point of view, it is necessary to distinguish between real and apparent reduction in caries incidence. Both tooth care centers and patients are being misled by reports which solely take into consideration visible defects and conventional fillings.

Formerly, during the 1950s and 1960s, it was customary to fill the most inconspicuous beginning caries; even prophylactic fillings to forestall caries were not unusual. At the end of 1960 and in the 1970s, occlusal areas without caries were receiving prophylactic treatment, including fillings, which were recorded as filled (F) surfaces.

At the end of the 1970s prophylactic fillings were less often used. Instead, occlusal surfaces and beginning caries were treated with fluoride

lacquer. Such treatments were neither recorded as caries nor as fillings in the DMF system of tabulation. Thus, although the condition of teeth had undergone no appreciable change, reduction in caries incidence was indicated by the DMF scores.

In Sweden, children from age 3 through 19 receive free annual dental examinations and treatments including necessary prophylaxis and information about dental health. Prophylaxis implies not only painting with fluoride but information about regular cleaning of the teeth and good diet.

Dr. Forsman stresses the importance of continued prophylaxis throughout adult life so that the "imaginary caries reduction does not become a caries explosion."

KEY WORDS: Caries reduction; DMF scores; Prophylactic measures

Reprints: Dr. Britta Forsman, Dental Care Unit, Vaxjo, Sweden. Published in Swedish, abstracted into English by S.H. (Licensed in Dental Surgery).

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EFFECTS OF FLUORIDE ON HUMAN ENAMEL AND SELACHIAN ENAMALOID IN VITRO A HIGH-RESOLUTION TEM AND ELECTRON DIFFRACTION STUDY

by

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(Abstracted from Calcif. Tissue Int. 33:9-13, 1981)

The effects of fluoride on human enamel and selachian enameloid in vitro were visualized in TEM and analyzed with electron diffraction. It is demonstrated that, under precise pH conditions inducing concentration balance between F^- ions and apatite, calcium fluoride is no longer formed. Crystalline changes occur instead. A secondary growing process, inducing a twofold increase in crystal size, involves all crystal faces, altering the hexagonal symmetry. It is suggested that the mechanism involved is not a dissolution precipitation process but rather a secondary growth of residual crystallites induced by apatite dissolution.

Authors' Abstract

KEY WORDS: Fluoride; Enamel; Enameloid; TEM; Electron diffraction

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