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Editor
G. L. Waldbott, M.D.
Detroit, Michigan

Co-Editor
A. W. Burgstahler, Ph.D.
Lawrence, Kansas

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The Fourth Annual Conference of the I.S.F.R. will take place at The Hague, Holland, at the Royal Institute for Engineers Sunday, October 24 to Wednesday, October 27, 1971.

The Program Committee is soliciting abstracts in English, French and German of papers to be presented dealing with any phase of fluoride research. The deadline is August 1, 1971.

Kindly mail abstracts confined to 300 words or less to Dr. Philip Zanfagna, 163 Lawrence St., Lawrence, Massachusetts 01841. Reservations for the conference will be made through the secretary's office.

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EDITORIAL

FLUORIDE INTOXICATION IN HUMANS

The article by Marier and Posen (pages 114-128) calls for a review of various aspects of fluoride intoxication in humans.

Fluoride enters the human system mainly through ingestion with food and water, less through inhalation and to an even more limited extent through the skin as in exposure to hydrofluoric acid (1).

Amounts of F^- taken into the system vary widely. In a non-fluoridated area McClure (2), for instance, estimated the daily F^- intake through food at between 0.3 to 0.5 mg, Oelschlager (3) from 0.3 to 2 mg and above. According to Balazova (4) in the vicinity of a F^- emitting aluminum factory, F^- uptake from food accounts for about 2/3 of the total (49% of which comes from plants, 16% from animal products). The remainder is provided by air and water. The latter supplies only a minor portion, namely 9%. In a fluoridated community, the total F^- ingestion per day by healthy indoor workers is estimated to range between 3 to 5 mg (5).

Fluoride intake through inhalation is highly variable. In a nationwide survey by The National Air Pollution Control Administration (N.A.P.C.A.) (6) 87% of measurements at urban stations showed concentrations of particulate F^- below 0.05 $\mu g/m^3$; 0.2% exceeded 1 $\mu g/m^3$. At non-urban stations, 97% showed no detectable amounts of F^-. This is in contrast to 0.018 ppm (18 μg) as HF reported by Cholak (7) in Baltimore where fertilizer rock phosphate factories are located. Earlier determinations near an aluminum factory were 0.02 to 0.22 mg/m^3 (20 to 220 μg) (8). Continuous exposure to fluoride-laden atmosphere causes considerable increase in F^- content of edible vegetation.

Some of the industries emitting F^- into the atmosphere are those concerned with the production of aluminum, steel, enamel, zirconium, uranium, magnesium, triple phosphate, fertilizer, glass, tile and bricks. Whereas, in general, the amount of sulfur oxides emitted from industries exceeds that of F^-, damage to vegetation and animal life due to airborne F^- is far greater than that caused by sulfur oxides (9).

The strong affinity of F^- to calcium and phosphorus accounts for its deposition in hard tissue (bones, teeth, nails, hair, calcified blood vessels); its affinity to many metals, especially magnesium and manganese, for its interference with the activities of many enzymes.

The toxicity of most F^- compounds is determined by the F^- ion rather than by other elements present in the molecule (10). Highly soluble compounds such as sodium fluoride are more toxic than the less soluble ones.

Acute Intoxication

Through Ingestion: Acute F^- poisoning occurs following excessive inhala-

tion or oral intake of F^- compounds, usually for suicidal or homicidal purposes. Sodium silicofluoride or sodium fluoride in doses 3 to 5 gm, usually mistaken for flour, sugar and bicarbonate of soda, have led to mass poisonings involving, in one case,as many as 75 fatalities (11). Excruciating pain in the stomach and lower abdomen, vomiting of hemorrhagic material, bloody diarrhea, marked dehydration (fluid loss) and epilepsy-like seizures due to low blood calcium are typical symptoms. Rabinowitch (12) reported in a case of F^- intoxication, hypocalcemia as low as 2.6 mg%, probably the lowest value ever found (normal 9 to 11). Convulsions, which usually occur after ingestion of the poison (11), may be delayed several hours.

The acute phase of the disease is of significance in evaluating health effects due to air and water pollution since acute abdominal episodes are not uncommon during the chronic stage of the disease, probably due to temporary consumption of food or water extraordinarily contaminated. Such episodes usually constitute a diagnostic riddle to physicians and are rarely attributed to their cause (13).

Through Inhalation: Another form of acute poisoning follows accidental massive inhalation to F^- compounds, especially silicofluoride and hydrogen fluoride, in workers in various industries. It is characterized by acute respiratory symptoms.

Hydrofluoric acid penetrates the skin or the lining of respiratory organs in an undissociated state (14). The tissue with which it first comes in contact remains temporarily intact. Ulceration begins in the deep layers whence it spreads to the surface (14).

Chronic Intoxication

Chronic intoxication can be classified according to its major causes as follows:

1. Hydrofluorosis due to drinking water, the most common phase of the disease;
2. Industrial fluorosis, the incidence of which has been reduced materially through preventive measures;
3. Neighborhood fluorosis which occurs in populations near F^- emitting industries;
4. Alimentary Fluorosis due to F^- containing food.

The latter two conditions have received little attention in the literature.

The four categories point to the principal source of F^-. However, F^- uptake into the system is never confined to a single source.

Spectrum of Symptoms

In chronic poisoning, the symptoms vary from person to person, depending upon his individual susceptibility, on his state of health and nutrition, food habits, age, sex, duration and extent of former and current F^- intake, on whether the

major portion of F^- is inhaled or ingested, on the simultaneous intake of other chemicals from the atmosphere and on F^-'s interaction with other agents in the intestinal tract (1).

If F^- compounds are inhaled, particularly in conjunction with other irritating dusts and fumes, or if a person is susceptible to respiratory disease, respiratory symptoms, especially bronchitis, emphysema, asthma, conjunctival and nasal irritation are likely to predominate (15).

In India, endemic fluorosis is associated with extensive neurological symptoms such as paralysis of legs and arms (16, 17). On the other hand, in the Sahara desert, only one patient among 148 with advanced skeletal changes had paraplegia (18). In Sicily, where much seafood is eaten, paralysis is rare; instead 45% of the patients with skeletal fluorosis had gastro-intestinal disorders (19), a feature which is rarely observed in India. In Morocco (18) where dust from phosphate mines contaminates edibles, the disease differs materially from fluorosis in the Sahara where maritime fossils constitute the source of water and air contamination. In Spain, wine contaminated by F^- produced a bone disease in chronic alcoholics (20) characterized by osteomalacia which is vastly different from the other forms of fluorosis usually encountered.

Dental and Skeletal Fluorosis

The two conspicuous and most thoroughly studied manifestations on which physicians usually depend for the diagnosis of chronic F^- poisoning are dental and skeletal fluorosis. However, they are not obligatory features of the disease. Dental fluorosis (or mottling of teeth) occurs only in individuals, who have consumed, or have been exposed to F^- during childhood up to age 10 or 12. The skeletal changes of the disease develop only after many years of persistent F^- intake.

In his classical description, Roholm (21) outlined in detail the dental and skeletal changes of fluorosis.

1. Dental Fluorosis, a permanent defect, is characterized by white chalky patches on tooth enamel due to imperfect calcification as indicated by irregular development of enamel rods (rows of cells forming the enamel) and absence of cementing substance. In later years the affected areas may become yellow, brown and even black.

2. In Skeletal Fluorosis, tissue and ligaments about the joints, especially in the pelvic area and spine, become calcified. Osteophytes develop on the surfaces of ribs and of long bones. The bones of arms and legs themselves show excess calcifications in some portions but tend to soften elsewhere. As this condition progresses, serious neurological disturbances develop in the spine. Paralysis of legs, arms, bladder and bowels ensue due to pressure of newly formed bone upon the spinal cord and the nerves as they leave the vertebral column (22). Other sequelae of the bone changes are a rigidity of the chest cage, which interferes with breathing, and spontaneous fractures due to increased brittleness of

the bones. Arthritic changes occur when the capsules surrounding the joints become calcified (20).

Hydrofluorosis

Endemic hydrofluorosis, particularly in India where large sections of the country have been depending on water supplies containing 0.6 to 14 ppm (1), furthermore in Arabia, North Africa, China, the development of the disease has been attributed to excess water consumption because of the excessively hot climates. In a survey of about 46,000 inhabitants of an endemic area (22) in 358 villages of the Punjab province of India, 1,065 cases of skeletal fluorosis were detected by X-rays, 210 of which had no symptoms. One hundred and forty-two cases had crippling deformities, 89 had serious neurological complications as a result of encroachment of newly-formed bone on nerve substance.

More pertinent to conditions in the U.S.A. are reports by Fradà (19) from "natural fluoride" volcanic areas of northern Sicily where the climate is moderate and conditions are similar to those encountered in most of the U.S.A. Fradà reported gastro-intestinal symptoms, especially nausea and vomiting, bowel disturbances, involvement of the liver, headaches, paresthesias, spinal arthritis, muscular pains. The F^- level in water ranged from 3 to 6 ppm.

In the U.S.A. a fatality in a 22 year-old soldier with F^- osteosclerosis who had consumed water containing 1.2 to 5.7 ppm F^- (23) and a similar case of a patient, age 63 (24) whose water contained 2.2 to 3.5 ppm F^-, have been the subject of much controversy. The question arose whether a prevailing kidney disease, and the resultant increased F^- retention in the system, had caused the advanced skeletal changes or whether the pyelitis was due to long-term F^- intake.

Another fatality, a newborn child with advanced calcifications of blood vessels was reported from fluoridated (1 ppm) Ames, Iowa (25). The infant's aorta contained 59.3 ppm F^-. Calcification of arteries, as a manifestation of skeletal fluorosis, is well documented from different parts of the world (18-20, 26-28). The Iowa case points to the fact that F^- passes the placental barrier at high concentrations.

A fourth fatality of a 42 year old nurse was reported from Rochester, N.Y. (29,30) and four additional ones (31) from Ottawa Canada. These patients had advanced kidney disease. Use of artificially fluoridated water for hemodialysis was considered the cause of death. In the Ottawa cases, bones contained up to 22,000 ppm F^- (normal 300 to 500 ppm). In cases of skeletal fluorosis in India reported by Singh and Jolly (32) F^- levels ranged from 600 to 6800 ppm.

Twenty non-fatal cases of advanced fluorosis were recorded by Morris (33) in Arizona where the water contained 1 to 9.2 ppm. Sixteen of the twenty cases had been drinking water from wells most of which, by analysis, contained less than 1.5 ppm. In none of the advanced cases of fluorosis were manifestations other than skeletal reported by Morris. However, his data concerning possible

damage to internal organs, especially to liver and kidneys,were insufficient to eliminate this possibility.

Waldbott (11, 34) reported in persons drinking fluoridated water the same symptoms which Roholm (21) and Fradà had recorded (19). The patients recovered upon eliminating fluoridated water for drinking and cooking and their illness recurred upon its resumption.

Neighborhood Fluorosis

Neighborhood fluorosis was first described in a farmer's household of nine, residing near an ironstone works in South Lincolnshire, England (35). Here too, the symptoms were gastric upsets, stiffness and pains in legs and joints. The subjects had been exposed to F^- air pollution from 3 to 14 years with exception of the farm worker who had been exposed for only one year. The same disease occurred in a farmer's family of three years in the vicinity of an Oregon aluminum smelter (36). In addition to the above-mentioned manifestations, liver and kidney damage was recorded. In neither the British nor the Oregon cases was there any evidence of osteosclerosis. Waldbott (13) reported additional cases among residents near an Ontario fertilizer factory. Balazova (4) found anemia and significantly lower hemoglobin as well as higher erythrocyte values in children residing close to an aluminum factory.

Alimentary Fluorosis

Fluoride intoxication due to chronic intake of F^- contaminated food or drink was first brought to light by Soriano's report (20) of fluorosis in 29 cases of chronic alcoholics who had consumed wine contaminated by 8 to 72 ppm F^-. These patients were observed for at least 15 years before his attention was focussed upon the close resemblence of the bone changes to those of skeletal fluorosis. Eventually, he proved the relationship of the disease with F^- by F^- determinations in bones and urine and by clinical tests. To his series another case of advanced F^- osteosclerosis must be added, that of a man in Hampshire England (37) who developed the same disease, although no obvious source of F^- uptake either from water or from the air was found.

Chizzola Maculae

A new approach to the F^- problem has recently opened up with recognition of bluish-brown maculae on the skin of children and women residing in the vicinity of two Italian aluminum factories (38,39). Recent observations (40) on children and women drinking fluoridated water leave no doubt that the lesions are related to F^- water. They promptly disappear upon eliminating fluoridated water and can be reproduced at will by its resumption. In some of the affected cases, muscular pains and frequent gastro-intestinal upsets are associated with the lesions. Unlike suffusions, they are always round or oval in shape, ranging from a dime to a quarter in size, and they do not change color when they are fading. While they are disappearing at one site they occur in others. The significance of these harmless appearing lesions lies in their pathology: Microscopical-

ly they show accumulation of lymphocytes and basophilic cells around capillaries indicative of a toxic reaction to F^- early in the disease.

As in many other kinds of chronic intoxication, the clinical picture of F^- poisoning varies considerably from person to person because of wide differences in F^- uptake, storage and secretion. When and why F^- accumulates in soft tissue and to what extent such excess storage affects the respective organs constitutes a major gap in our understanding of the disease.

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PHYSIOLOGICAL ROLE OF FLUORINE IN LIVING ORGANISMS

by

C. Mangoni di S. Stefano, F. Gombos and M. Ruggiero
Naples, Italy

SUMMARY: Analysis of several mineral components of bones of 4 mammal species indicates a great homogeneity of the levels of all components with the exception of fluoride. The great variability of fluoride, even in animals nurtured in controlled experimental conditions is interpretated as indicative of contamination of and absence of fluoro-apatite in the normal bone tissue. The hypothesis of a dynamic role of fluoride in activating enzymes of bone formation is advanced.

Introduction

Oral administration of F^- to several animal species is followed by increased F^- levels in blood, dentine and tooth enamel. The largest increase of blood F^- was in the fraction bound to serum albumin (1). These results as well as further investigations suggested that F^- is carried in the blood stream bound to serum albumin. Our data (2-5) indicated that the binding occurs at specific sites and that free blood F^- is present after saturation of the albumin binding sites.

Furthermore, it appeared that the spacial configuration of purified albumin molecule was modified by the F^- binding as indicated by differential spectrometry. In fact, two peaks previously absent in the albumin spectrum, namely at 289 mμ and 244 mμ respectively, appeared in the spectrum of the fluoride-albumin complex (6).

A protein fraction, containing a protein which actively bound F^-, was isolated from dental pulp. Although this protein might have the role of storing F^- locally, we advanced the hypothesis that this protein was an enzyme of the pathway which leads to the formation of bone tissue's conformational charges analogous to those of serum albumin. It might be induced by F^- binding and result in activation of the enzyme at physiological F^- levels or in inhibition at high "toxic" F^- concentrations.

Fluoride then - and this hypothesis is verified by our work now in progress - has a dynamic enzymatic role besides substituting the hydroxyl radical of hydroxylapatite.

In order to determine whether F^- is innocuously incorporated into apatite crystals or whether it also modifies the whole process of calcification, we investi-

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From the Institute of Human Physiology, Physiology of Nutrition and Odontoiatric Clinic, University of Naples, Naples, Italy.

gated whether the bones of F^- treated animals differ from the bones of untreated animals solely in their F^- content or in other constituents as well.

Since the data available from the literature are very contradictory with regard to the "normal" F^- content, we first established the extent of the variability of F^- content in several bones of 4 mammal species.

The object of this preliminary study was, above all, to determine whether the variability in F^- levels was due to the different analytical methods used by the various authors, and secondly to establish guide lines for the selection of homogeneous groups of experimental animals for future investigations.

Material and Methods

Bones of Swiss mice, Wistar rats, of bovines and of humans were used. The laboratory animals which originated from our own animal quarters were inbred for several generations and sacrificed at 5 months. Both rats and mice had identical living conditions and diet. Bovine bones were obtained from the local slaughter house. Human bones were obtained from the Institute of Anatomy.

After removal of cartilages and soft tissue, bones were crushed and dried to constant weight at 105°. Then, they were extracted with ethanol-ether mixture 1.1 (v/v) for 48 hr., in a soxhlet apparatus. After lipid extraction the bones were dried, then ground in a porcelain mortar until the powder passed through a 60 mesh sieve. The powder was thoroughly mixed and aliquots were taken for the different analyses.

Calcium was measured according to Clark and Collip (7), phosphorus according to Fiske and Subbarow (8), magnesium by the method of Orange and Rhein (9), fluoride as already described (6).

Ca, P, Mg and F^- levels were measured on samples ashed at 700° for 5 hr. Sodium and Potassium levels were measured by flame photometry on samples ashed for 12 hr. at 500° as well as on non-ashed air-dried samples. Recovery was measured by international standards and the results corrected accordingly. Chloride was measured as described by Carr (10), Citrate according to Zipkin and McClure (11), Nitrogen as described by Nessler and Carbonate as reported by Pregl (12). The last-mentioned analysis was carried out on bone powder treated in the following manner: 10 grams of bone powder was extracted by continuous stirring for 24 hours at 37° with 100 ml of isotonic saline solution containing a few crystals of thymol. The insoluble residue was repeatedly washed with double distilled water in order to eliminate the sodium chloride used for the extraction.

Results

As shown in Table 1, the levels of various bone components were constant with the exception of F^-. They were similar for specific bones even when different animal species were compared. The absence of correlation between

TABLE 1

Chemical Composition of Several Bones of Different Mammals

	MOUSE		RAT		OX		MAN	
	% ± SD	mEq/g	% ± SD	mEq/g	% ± SD	mEq/g	% ± SD	mEq/g
CATIONS:								
Calcium	26.30 ± 0.18	12.75	26.40 ± 0.16	13.10	26.70	13.32	26.56 ± 0.19	13.30
Magnesium	0.442 ± 0.007	0.40	0.437 ± 0.008	0.38	0.436 ± 0.009	0.358	0.450 ± 0.08	0.364
Natrium	0.746 ± 0.013	0.38	0.750 ± 0.017	0.41	0.731 ± 0.015	0.318	0.735 ± 0.019	0.323
Kalium	0.059 ± 0.001	0.02	0.061 ± 0.001	0.02	0.055 ± 0.001	0.014	0.060 ± 0.001	0.013
ANIONS:								
Phosphorus as PO_4^{---}	12.50 ± 0.015	12.10	12.45 ± 0.017	12.09	12.47 ± 0.013	12.06	12.63 ± 0.016	12.12
Carbon dioxide as CO_3^{--}	3.53 ± 0.030	1.50	3.47 ± 0.025	1.48	3.48 ± 0.022	1.58	3.50 ± 0.032	1.50
Citric acid as cit^{---}	0.875 ± 0.007	0.15	0.868 ± 0.009	0.13	0.863 ± 0.004	0.138	0.883 ± 0.008	0.18
Chloride	0.079 ± 0.009	0.023	0.081 ± 0.011	0.024	0.077 ± 0.004	0.022	0.080 ± 0.006	0.028
Fluorine	0.058 ± 0.032	0.031	0.070 ± 0.029	0.033	0.072 ± 0.035	0.038	0.065 ± 0.028	0.042
Cations mEq / Anions mEq		0.98		1.01		1.01		1.02
Calcium mM / Phosphorus mM		1.630		1.659		1.656		1.660
Nitrogen %	5.0 ± 0.07		4.7 ± 0.06		4.92 ± 0.05		5.0 ± 0.09	

Data are expressed as milliequivalents and as % of the mean ± SD of all constituents of each bone. Each value represents an average of at least 15 determinations.

amounts of F^- and those of other bone components indicated that the chemical composition of bones was not modified by their F^- content.

Discussion

Within the framework of one hypothesis these results indicate that the bone composition did not change regardless of whether or not our hypothetical enzyme was activated. Probably only the rate of formation of bone tissue was accelerated. The amount of F^- was extremely variable from one bone to another in the same animal, between animals of the same species and from one species to another. In view of the heterogeneous origin of human and bovine bone samples, the results for these two species could be an indication of different diets followed by the individuals within these two species. This interpretation, however, was invalidated by similar results obtained with mice and rats. In these animals, likewise, the F^- values, even when analogous bones were compared, varied widely, although a regular distribution pattern was noted.

Our results confirmed those found in the literature. They proved that variability of the data reported for F^- was not due to different analytical techniques. They cast serious doubt on the hypothesis that these differences were due solely to different diets. A possible interpretation is that F^- in bones is present only as a contaminant and fluoroapatite formation is incidental. If this interpretation is correct, our tentative hypothesis for a dynamic role of F^- in bone formation appears to be more appealing because variability between individuals of one species may be greater than that between different species.

A corollary to our results is that, for meaningful studies on F^- function and metabolism, it is necessary to work on genetically homogeneous laboratory animals obtained by selective inbreeding starting with genetically homogeneous groups. We are attempting to obtain such groups.

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RENAL OSTEODYSTROPHY IN PATIENTS ON LONG-TERM HEMODIALYSIS WITH FLUORIDATED WATER

by

G. A. Posen, J. R. Marier and Z. F. Jaworski
Ottawa, Canada

SUMMARY: Serum and bone fluoride concentrations of ten patients maintained on long-term hemodialysis with fluoridated water (1 ppm, i.e., 50 μM) were correlated with duration of treatment and the occurrence of clinical, radiological, and histological manifestations of bone disease. Two patients had symptomatic renal osteodystrophy when accepted on the program, whereas six others developed the disease within a year of fluoridated dialysis. However, in all patients, the disease progressed despite recommended therapy (including high doses of vitamin D). The mean pre-dialysis serum fluoride level was 16 ± 4 μM which rose to 28 ±3 post-dialysis. The bone fluoride content ranged from 800 to 22,500 ppm on a dry fat-free basis. Toxic effects have been reported at these levels and could complicate underlying renal osteodystrophy. Further studies are required to delineate the role of fluoride in this condition.

In our experience, renal osteodystrophy has been a common and disabling complication of maintenance hemodialysis. Some investigators (1, 2, 3) have reported improvement of this condition by treatment with various doses of vitamin D, calcium supplements, and phosphorus-binding gels. However, the bone lesions in our patients have developed or continued to progress despite these measures.

In our hemodialysis center, opened in April 1964, fluoridated dialysis began with the fluoridation of the city water supply in November 1965. Our subsequent therapeutic failure was completely unexpected and a possible explanation was suggested by the observation of Taves et al. (4, 5) that the serum fluoride (i.e., F^-) levels in patients chronically hemodialysed with fluoridated water are comparable to those that cause fluorotic bone disease (6). Thus, the study of fluoride levels in our patients became of particular interest because several of them had been on fluoridated dialysis for much longer periods than those patients reported by Taves et al. (4, 5).

From the Dept. of Medicine, Ottawa General Hospital, and University of Ottawa; also, Division of Biology, National Research Council, Ottawa, Canada.

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During hemodialysis with fluoridated water, serum fluoride uptake comes about because fluoridated water contains about 50 μM (1.0 ppm) of fluoride, whereas human serum normally contains only about 1 μM (0.02 ppm) of free fluoride ion (5). Thus, there can be a 50:1 gradient favoring diffusion of fluoride ion from the dialysis water into the patient's blood, and ensuing deposition of fluoride into bones increasing with the duration of fluoridated dialysis.

Materials and Methods

The study group comprised ten patients (Table 1) dialysed with fluoridated water for periods ranging from one to thirty-one months. Three of the patients (G.B., R.N., and C.W.) were treated for ten to twelve months with non-fluoridated dialysate prior to fluoridation of the Ottawa water supply. The patients ranged in age from 16 to 61 years; four were female and six were male; eight had glomerulonephritis as their primary diagnosis and two had polycystic disease.

TABLE 1

Patient	Age	Sex	Diagnosis	Duration Dialysis (months)	
				Non F	F*
1. G.B.	28	F	G.N.	10	30.5
2. C.W.	50	M	P.C.	10	31
3. R.N.	36	M	G.N.	12	30
4. L.C.	42	M	G.N.	0	28
5. W.B.	25	M	G.N.	0	26
6. Y.L.	17	F	G.N.	0	16
7. R.V.	40	F	P.C.	0	12
8. D.C.	16	F	G.N.	0	9
9. H.R.	48	M	G.N.	0	1
10. A.D.	61	M	G.N.	0	1

* = When clinical tests were made
G.N. = Glomerulonephritis
P.C. = Polycystic disease

The patients were dialysed on a single-pass Kiil system. The duration of each dialysis varied from eight-to-ten hours, three times a week, to twelve-to-fourteen hours twice a week. Thus, the program involved a minimum of twenty-four hours of fluoridated dialysis per week. The dialysate contained 3 meq calcium and 1.5 meq magnesium per liter. The fluoride content of the dialysis water was between 0.9 and 1.0 ppm, i.e., 50 μM, after November 1965.

In September 1967, all patients began receiving phosphorus-binding gels, 800 mg of elemental calcium, and 5,000 units of vitamin D per day. In four

patients (G. B., R. N., R. V., and C. W.) who were becoming disabled by their bone disease, vitamin D was increased in 50,000-unit increments to a total of 200,000 units daily.

The skeletal status of our patients was studied before the initiation of dialysis and then serially by biochemical, histological and radiological methods. Serial radiological bone surveys, including skull, clavicles, ribs, vertebrae, pelvis, hands, and long bones, were performed before the patients were placed on the hemodialysis program and at 6-month intervals thereafter.

Serum and dialysate calcium was measured by atomic absorption spectrophotometry, serum phosphorus by a standard photocolorimetric procedure, and serum alkaline phosphatase by the Bessy-Lowry method.

Serum ionic fluoride was measured by Taves' diffusion method (7, 8) modified by releasing fluoride directly from serum by means of hexamethyldisiloxane in HCl (9). The fluoride was trapped in sodium bicarbonate, and measured by a decrease in fluorescence of a morin-thorium complex. Serum fluoride levels were determined before and after dialysis treatments. The approximate uptake of fluoride during a dialysis was determined by averaging the change in serum fluoride levels entering and leaving the dialyser, and multiplying this by the blood-flow and the length of time of the dialysis. The approximate value multiplied by 0.8 gives the fluoride uptake. The value of 0.8 is the correction found necessary when applying this method to patient D. M. (5), where a more accurate graphical integration method could be applied. A correction is needed because the arterio-venous difference is a non-linear function with time.

Trephine biopsies of the iliac crest were obtained under local anesthesia in 7 patients and prepared for histological examination in the undecalcified state by the method of Bohatirchuk (10).

Bone fluoride levels were estimated by the Marier and Rose Zr-SPADNS method (11), but with the volumes scaled-down to permit determination of fluoride in the range of 0 to 4 μg per test. This modification was necessary because of the small size of the bone-biopsy samples (1.0 to 3.5 mg), and involved the use of a 2.0 ml aliquot of test solution along with 0.4 ml additions of the two reagents; in all other respects, the procedure was as previously described (11). The procedure involves drying and defatting of the tissue, ashing in the presence of 200 mg of MgO fixative (containing 0.003% F), microdistillation, then colorimetric estimation. With each group of samples, known fluoride standards containing the MgO fixative were processed simultaneously through all phases of the procedure, so as to correct for any variation in the "baseline" fluoride correction substracted from the total result. Preliminary studies had indicated an accuracy ranging between 87 and 123%, averaging 99%. Although the small size of the bone samples precluded duplicate ashings etc., the 15 ml volume of distillate was large enough to allow replicate colorimetric determinations, and each value reported in this paper represents an average of (at least) triplicate determinations.

Results

The patients' serum calcium, phosphorus, alkaline phosphatase, and Ca x P products, obtained before and after the initiation of the dialysis program, are shown in Table 2.

TABLE 2

Patient	Calcium(Mg%) B	Calcium(Mg%) A	Phosphorus (Mg%) B	Phosphorus (Mg%) A	Alk. Phos.(Bl) B	Alk. Phos.(Bl) A	Post-treatment Ca x P Product
1. G.B.	8.4	9.1	6.3	6.8	2.5	2.4	61.9
2. C.W.	7.0	10.1	5.7	5.0	1.2	3.5	60.6
3. R.N.	7.2	8.6	12.7	9.0	2.5	2.4	77.4
4. L.C.	8.9	10.0	9.0	6.6	1.2	2.7	66.0
5. W.B.	6.7	9.5	7.0	5.0	1.8	3.6	47.5
6. Y.L.	3.5	9.5	13.5	7.9	1.6	3.1	75.1
7. R.V.	7.6	9.4	7.5	4.5	5.6	4.5	42.3
8. D.C.	7.2	8.9	8.5	7.9	1.9	2.7	70.3
9. H.R.	9.8	9.5	3.8	4.9	3.5	2.8	46.6
10. A.D.	9.0	9.6	5.6	5.5	2.6	2.3	52.8

B = Before dialysis program
A = After dialysis program and treatment with Ca, Vit. D. and P-binding gels
Normal values: Ca 8.9 to 10.3 mg%; P 2.8 to 4.5 mg%; alkaline phosphatase 0.8 to 2.3 BU (Bessy-Lowry Units); Ca x P Product = 24.9 to 46.3.

Prior to dialysis treatment, the serum calcium concentrations were subnormal in all except two patients (A. D. and H. R.). These levels became normal within six months after starting treatment, except for R. N. whose calcium level, although increased, was below the lower limit of normal. The serum phosphorus levels, which were elevated prior to treatment in all except patient H. R., fell after starting the dialysis therapy; however, only in R. V. did it reach the normal range. The alkaline phosphatase values were normal in five of the ten cases at the beginning of the dialysis program; but after one year of treatment all except one were above the upper limit of the normal range. Similarly, all but one of the Ca x P products remained higher than normal after dialysis therapy.

On radiological examination (Table 3), eight of the ten patients showed varying degrees of hypomineralization, seven had looser zones, two had subperiosteal reabsorption, and one had osteosclerosis. These radiological changes were associated with bone pains, arthralgias and fractures in seven of the patients.

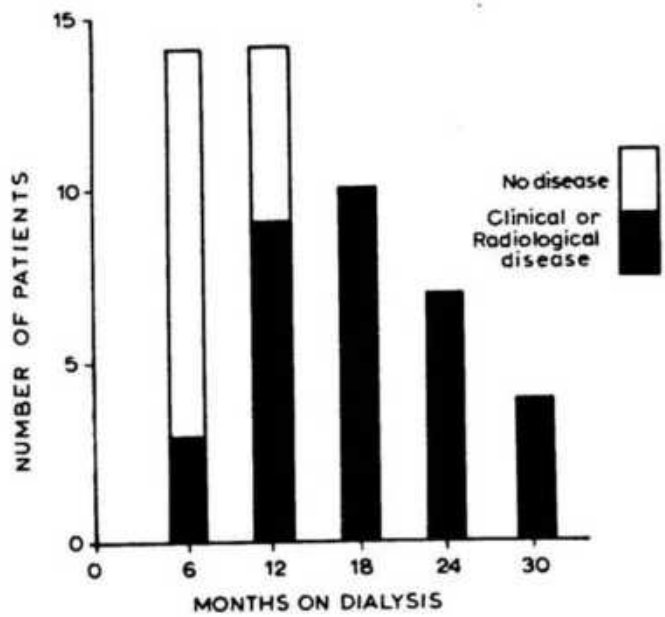
TABLE 3

Patients	Radiological Disease				Clinical Features		
	Hypomineralization	Looser Zones	Sub-Periosteal Reabsorption	Osteosclerosis	Bone Pain	Arthralgia	Fractures
1. G. B.	+	++	-	-	++	++	++
2. C. W.	++	+++	-	-	+++	+++	+++
3. R. N.	++	+++	-	-	+++	+++	+++
4. L. C.	++	+	-	-	++	++	+
5. W. R.	++	+	-	-	++	+++	+
6. Y. L.	++	++	-	-	+++	+	+++
7. R. V.	++	++	+	-	+++	+	+
8. D. C.	-	-	++	+	-	-	-
9. H. R.	-	-	-	-	-	-	-
10. A. D.	+	-	-	-	-	-	-

- Absent; + mild; ++ moderate; +++ severe

Fig. 1

Frequency of Bone Lesions in the Dialysis Program
General Hospital, Ottawa, Canada

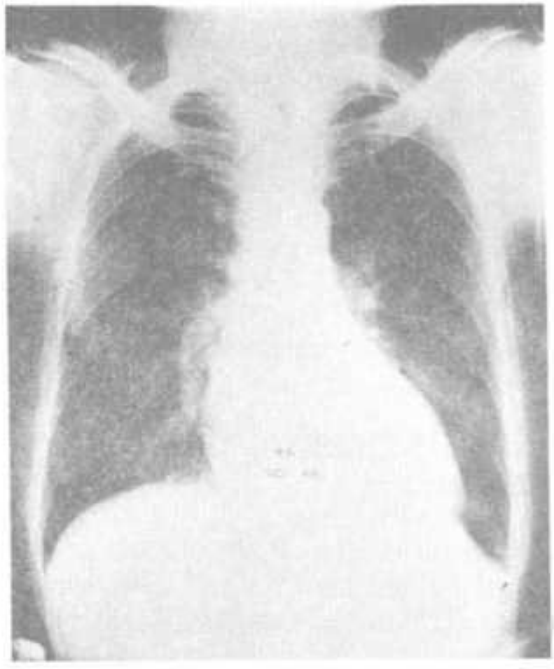


The frequency of clinical and radiological evidence of bone disease at various times after starting dialysis is depicted in Figure 1. There was a progressive increase in the frequency of bone disease with the passage of time until, after eighteen months of dialysis, evidence of bone disease was present in all patients.

Figures 2 and 3 show the radiological progression of the bone disease in patient R. N., who was on dialysis since November 1964. The patient was dialyzed for approximately two years before he developed signs of bone disease (Fig. 2).

Fig. 2

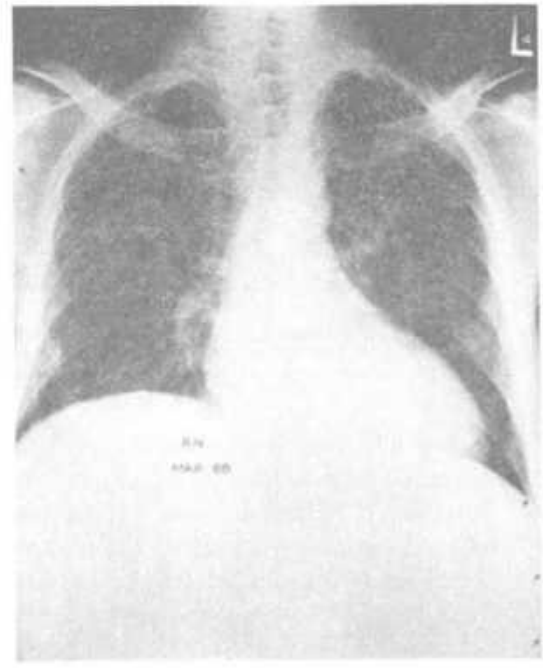
Chest Film, Patient R.N. (Nov. '66)



Early looser zone in 9th lt. rib, but no loss of alignment, no periosteal reaction at site of the radiolucent band.

Fig. 3

Same Patient (March '68), Progression of the Osteodystrophy with Looser Zones in Numerous Ribs, Including Lt. 6th, 8th (2 lesions), 9th and 10th Ribs.

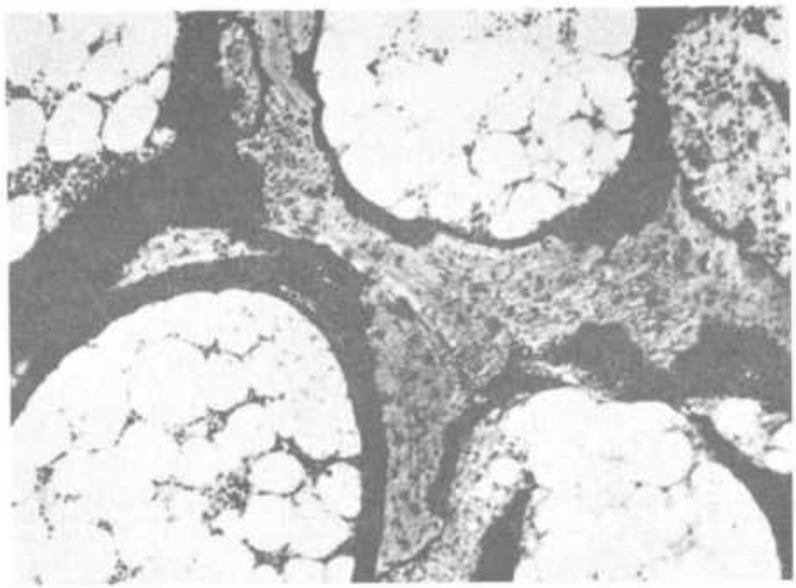


Several ribs show slight displacement, indicating true fracture through the looser zone, but no evidence of healing with periosteal new bone although granular calcification is present adjacent to bone lesion. Calcification in the right rotator cuff.

However, Ottawa water was not fluoridated until November 1965, so that patient R. N. was dialyzed with fluoridated water for only one year. Significantly, unlike our other patients who developed bone disease after only one year of dialysis, he did not show signs of disease for two years. The patient was placed on vitamin D 50,000 units per day, 800 mg of elemental calcium per day, and phosphorus-binding gels. In November 1967, because of progression of the disease, vitamin D was increased to 100,000 units daily, but with no benefit (Fig. 3). Iliac crest biopsy taken in November 1967 (Fig. 4) demonstrated wide uncalcified osteoid seams and areas of bone resorption.

Fig. 4

Undecalcified Iliac Crest Bone Histology of Patient R. N. (Nov. '67)

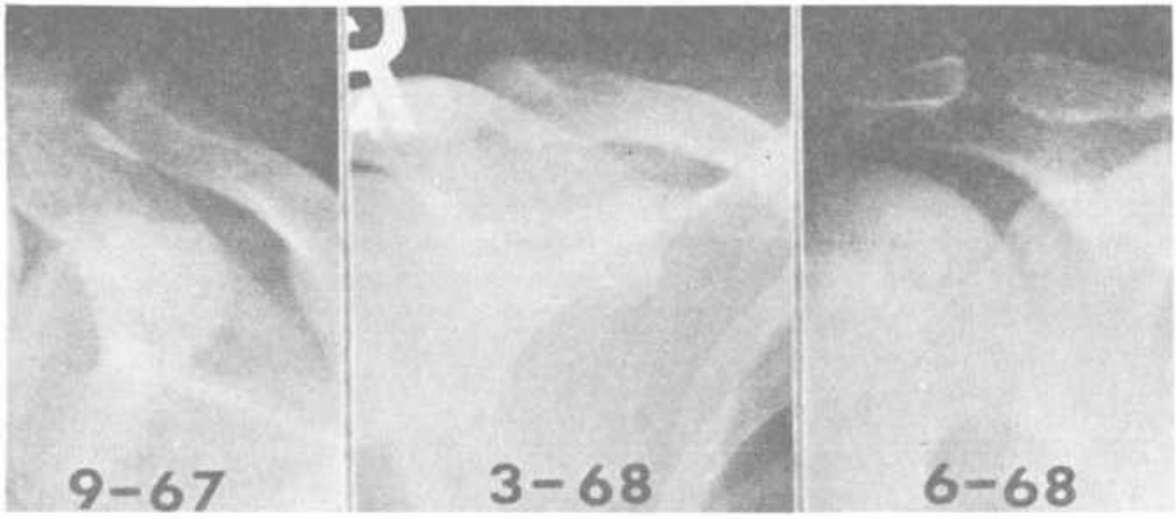


The radiological features of patient D. C. are shown in Fig. 5. When the patient started treatment, her serum calcium was 7.2 mg per 100 ml and the lateral ends of her clavicles showed resorption, a sign of secondary hyperparathyroidism. After six months of dialysis, vitamin D and calcium supplements, and phosphorus-binding gels, her serum calcium rose to the lower limit of normal and her clavicles had undergone remineralization. After continuing the same therapy for an additional three months, she again showed resorption of the lateral end of her clavicles.

The increasing severity of histological bone changes with the duration of fluoridated dialysis is shown in Fig. 6. Vitamin D and calcium therapy had no apparent effect on the bone disease.

Fig. 5

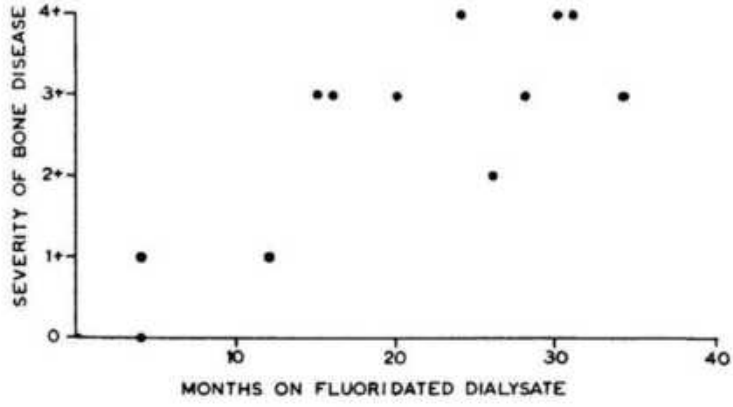
Localized Shoulder View of Patient D. C. in Sept. '67, Outer End of the Clavicle is Cupped and Irregularly Mineralized Due to Subperiosteal Bone Resorption



March '68: Following therapy, the mineralization of outer end of the clavicle has returned to normal.
June '68: Irregularity of mineralization again apparent, not as marked as in initial film.

Fig. 6

Severity of Bone Lesions After Maintenance on Fluoridated Hemodialysis

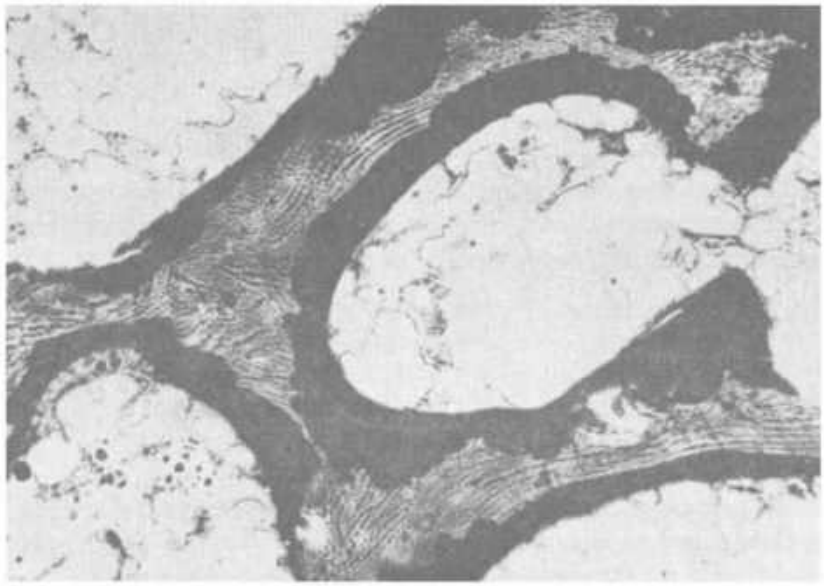


0 = No radiological or histological disease, no clinical symptoms.
1 = Mild radiological and histological disease, but no clinical symptoms.
2 = Moderate radiological and histological disease, with mild bone pains.
3 = Severe radiological and histological disease, with severe bone pains.
4 = Incapacitating disease, with multiple fractures.

The characteristic appearance of an undecalcified section of iliac crest sample is shown in Fig. 7. It was obtained from patient G. B. after six months of vitamin D in daily doses of 100,000 units. Large amounts of uncalcified osteoid and areas of bone resorption are representative of bone biopsies from our patients maintained on fluoridated hemodialysis, and the bones appeared most affected in those dialysed for the longest periods.

Fig. 7

Undecalcified Iliac Crest Bone Histology (Patient G. B.). After Six Months of Vitamin D Supplementation, 100,000 Units Daily



The pre-dialysis fluoride levels were elevated in all patients (Table 4), compared to the 1 μM found in humans not unduly exposed to fluoride (13). The levels rose as blood passed through the dialyser and, at the end of dialysis, the serum levels of arterial blood had increased markedly above their pre-dialysis levels. The estimated uptake of fluoride during a single dialysis ranged from 10 to 29 mg. Fig. 8 shows that the patients' serum fluoride levels increased as a function of time on fluoridated dialysis; the Rochester levels (5) are included for comparison.

The concentration of fluoride in the iliac crest biopsies of individual patients ranged from 800 to 22,700 ppm (Table 4), and were related to the length of time the patients had been maintained on fluoridated hemodialysis (Fig. 9). The two lowest values were found in patients just starting on the program; the lower value was in H. R. who, prior to treatment, had resided in a non-fluoridated community, whereas the higher value was obtained in A. D. who had resided in a fluoridated community for three years prior to treatment.

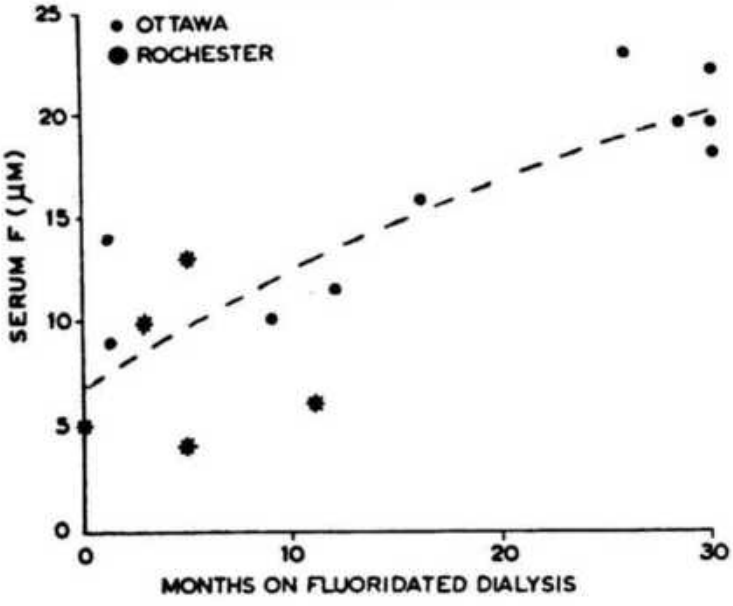
TABLE 4

Patient	Serum Fluoride (Micromoles/Litre) Pre-Dialysis A	Pre-Dialysis V	Post-Dialysis A	Post-Dialysis V	Fluoride Uptake* (mg)	Fluoride Iliac Crest ppm dry fat free
1. G.B.	19	33	32	37	17	22,700
2. C.W.	18	34	29	36	23	19,700
3. R.N.	22	32	29.5	33	10	-
4. L.C.	19.5	33	28	35	12	-
5. W.B.	23	36.5	30	37.5	14	-
6. Y.L.	16	24.5	28	30	12	13,300
7. R.V.	11.5	25.5	23	33	29	20,900
8. D.C.	10.5	20	22.5	28	17	15,900
9. H.R.	8.9	-	30	-	-	800
10. A.D.	14	27	30	34	16	9,500

A = Blood entering dialyzer; V = Blood leaving dialyzer
*on a "per dialysis" basis, and excluding fluoride uptake from foods and beverages, i.e., ingestion of 2 to 5 mg F per day (11,12).

Fig. 8

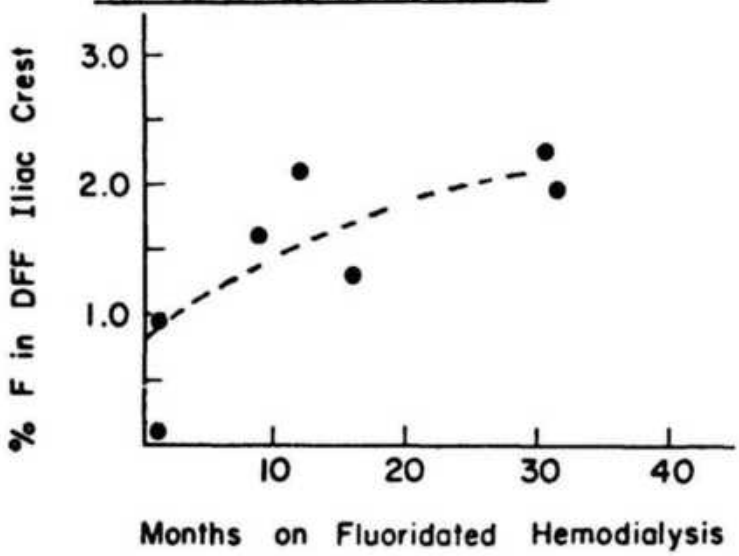
Serum fluoride (F^-) Levels as Related to Time on Fluoridated Dialysis



Each point represents a value in a different case. Dotted line indicates trend obtained by calculating a coefficient of variability (R^2). According to animal experiments pathological changes in bone appear when serum F^- approaches 15 μM (5,19).

Fig. 9

Dry Fat-Free Iliac Crest Fluoride Levels as Related to Time on Fluoridated Dialysis.



Each point represents a value in a different patient. Dotted line indicates trend obtained by calculating a coefficient of variability (R^2). A fluoride content of 1% is equivalent to 10,000 ppm.

The effect of fluoridated hemodialysis on the observed bone and serum fluoride levels in the Ottawa patients was statistically evaluated by calculating a coefficient of determination (R^2) for the data shown in Fig. 8 and 9. This calculation of "best fit" accounted for 65% of the variability in the bone fluoride data and 70% of that in serum fluoride results, and indicated that fluoridated hemodialysis has a comparable effect on bone and serum fluoride increments (Table 5).

TABLE 5

Months of Fluoridated Hemodialysis	Bone Fluoride ppm (estimated) dry fat-free	Bone Fluoride cumulative increment factor	Serum Fluoride µM (estimated)	Serum Fluoride cumulative increment factor
0	8,260	---	7.09	---
10	14,550	1.8	12.54	1.8
20	18,730	2.3	16.84	2.4
30	21,500	2.6	20.23	2.8

Comment

Clinically, radiologically, and histologically, the disease seen in these patients was indistinguishable from uremic osteodystrophy, although the manifestations of bone disease tended to appear sooner and in more severe form in our patients maintained on fluoridated dialysis. Uremic osteodystrophy is characterized by two well-recognized defects: the first is osteitis fibrosa (increased areas of bone resorption and marrow fibrosis) ascribed to secondary hyperparathyroidism; the second is osteomalacia (increased amounts of non-mineralized osteoid) ascribed to the acquired resistance to the action of vitamin D. These two features may be found in various amounts and combinations in individual cases. Stanbury (14, 15) states that when osteomalacia is predominant, uremic osteodystrophy should respond to vitamin D in the appropriate dose. Kay (1) has found histological osteodystrophy in practically all of his patients maintained on non-fluoridated hemodialysis, but prevented them from reaching the symptomatic stage by maintaining a calcium concentration of 3 meq per liter in the dialysis fluid, supplementary dietary calcium, proper usage of phosphate binders, and small doses of vitamin D. Our patients were treated in the same manner and given increasing doses of vitamin D as they became symptomatic, but without improvement.

The failure of our therapeutic efforts suggested that there may have been other factors complicating the disease. The possibility that fluoride was involved was raised by the observations of Taves et al. (4, 5) that the serum fluoride (i.e., F^-) levels in patients regularly hemodialysed with fluoridated water are elevated and comparable to those producing fluorotic bone disease in various mammalian species (6). Our study has confirmed these observations and, in addition, has demonstrated that the basal serum fluoride levels (i.e., arterial values at the beginning of each dialysis) are related to the duration of exposure to fluoridated hemodialysis. An increase in the basal serum levels would be expected as the more reactive bones become increasingly saturated with fluoride, and thus, less able to clear fluoride from the serum (5).

The "zero time" values estimated for bone and serum fluoride (Table 5) are much higher than those normally observed in adult humans who have not been unduly exposed to fluoride (6, 13). However, Taves et al. (5) have reported a serum F^- value of 5.1 uM in a patient not previously dialyzed, but residing in a community with fluoridated water. In the present study, one of the patients had a bone fluoride level of 9,500 ppm (dry fat-free) after only one month of dialysis; this patient also resided in a fluoridated community. The fact that these patients had little-or-no kidney function should be borne in mind, especially as Call et al. (16) have demonstrated that humans with certain types of bilateral kidney disease accumulate more bone fluoride than do humans who do not have these kidney ailments.

Our bone fluoride concentrations, however, must be interpreted with caution for three reasons. First, they are higher than any previously-reported values by as much as 50% (17). Second, the samples were too small to permit duplicate ashings, even though replicate analyses were done on the single distillates.

Third, subsequent analysis (with larger samples) on three of these patients at autopsy, and in patients biopsied four months after defluoridation of the dialysis water, showed only a fraction as much fluoride. (Note: This data will be reported in detail when the effects of defluoridated dialysis, introduced in our center in the fall of 1968, are known). The uptake (and presumably, the loss) of fluoride is less than 30 mg per dialysis (Table 4), so that only a small fraction of the skeleton could have changed as much as indicated in the present study. We have not, however, been able to find a reasonable technical basis to question the analytical procedures; therefore, we tentatively postulate that the fluoride content of these patients' bones may vary markedly with time and with sampling site in a particular bone (18).

Another point that needs to be resolved is why these patients showed no histological or radiographic evidence of increased bone production, as often seen in endemic fluorosis. The serum fluoride concentration may have been too high for this in some patients; but, presumably, earlier in the course of their dialysis, their serum fluoride content was in the range expected to produce osteosclerotic fluorosis (19). Several explanations may be advanced. First, the accumulation of fluoride may have been too rapid (6). Second, since these patients usually have histological osteodystrophy when accepted on the dialysis program, and since osteodystrophy is characterized by a very slow bone-turnover rate (20), their bones could not respond to fluoride levels stimulating bone formation. The third possible explanation is the synergistic effect noted in senile osteoporotics treated with both vitamin D and fluoride (21). In those patients, vitamin D was used to correct the fluoride-induced increase in osteoid seams; but, instead of helping, vitamin D appeared to contribute to the widening of osteoid seams. Our findings of worsening bone disease after combined vitamin D and fluoride exposure may thus be the same phenomenon noted in the study of senile osteoporosis.

Histologically and radiographically, these patients showed features of uremic osteodystrophy instead of the fluorosis characterized by exostoses and osteosclerosis. Nevertheless, the observed changes (osteomalacia, osteitis fibrosa and osteoporosis) were similar to those induced by high doses of fluoride in humans and experimental animals, in which widened osteoid seams have been observed (6, 22-27), and where increased areas of resorption due to secondary hyperparathyroidism may be seen (28). Therefore, it seems likely that fluoride was aggravating the underlying renal osteodystrophy in our patients, and that this effect was enhanced by concomitant administration of high doses of vitamin D.

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EXPERIMENTAL ACUTE FLUOROACETAMIDE POISONING IN SHEEP AND DOGS

I. SYMPTOMATOLOGY AND PATHOLOGY

by

M. N. Egyed* and J. L. Shupe
Veterinary Dept., Utah State Univ., Logan, Utah

SUMMARY: Clinical observations and lesions are described in acute, fatal experimental fluoroacetamide (FAA) poisoning in sheep. In one of the sheep ventricular fibrillation was demonstrated.

Secondary FAA poisoning was produced in two dogs by feeding them poisonous skeletal muscle of sheep. Clinical symptoms and lesions described in both, sheep and dogs, are practically the same as in sodium fluoroacetate poisoning. The pathological findings are more striking in the dog than in the sheep. The lesions described are non-specific and not pathognomic.

In 1965 Egyed and Brisk described the features of experimental FAA poisoning in sheep, mice and rats (1). Circumstantial evidence and laboratory observations (2) indicate that accidental FAA poisoning occurs not only in sheep but in cattle, horses and dogs. In 1964 Papworth (3) reported suspected FAA poisoning in dairy cattle, due to contaminated effluents from a factory manufacturing FAA. The affected herd had to be slaughtered. Detailed information on clinical history, pathology, as well as the confirmation of the diagnosis by laboratory methods of this episode was published only recently. It concluded definitely that organic fluorine (FAA) induced the illness and the death of the cows (4, 5).

Material and Methods

In order to obtain further knowledge on the mechanism of this kind of poisoning we carried out the following experiments:

Six crossbred white faced sheep were given 20 mg/kg chemically pure FAA ** in gelatin capsules.

Feeding trials were carried out in two female Labrador dogs. They were fed skeletal muscle (meat) from the sheep to which FAA had been fed. Clinical

**Manufactured by Aldrich Chemical Co. Inc., Milwaukee, Wisconsin.

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*Permanent address: Kimron Veterinary Institute, Bet Dagan, Israel.

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observations, heart action (ECG**) as well as gross and microscopic pathology were carried out in the sheep. The same examinations were carried out in the dogs with the exception of ECG.

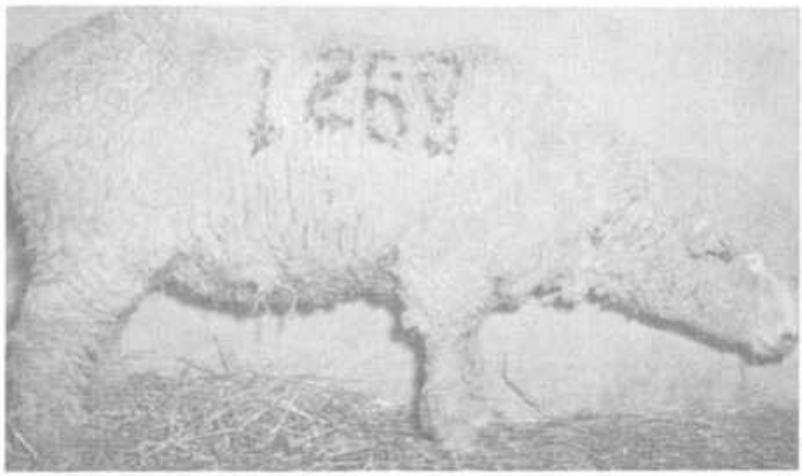
A. EXPERIMENTS IN SHEEP

Doses of 20 mg/kg FAA given orally proved to be lethal in every instance. Five of six sheep died 7 to 8 hours after the administration. The sixth, which had obviously chewed the capsules and spilled out some of the poison, died 20 hours after administration of FAA.

a) Clinical Observations: The first symptoms which appeared after 1 to 2 hours were increased respiratory rate, and superficial and labored breathing. Two to four hours after the administration of the compound, grinding of teeth, drooping and leaning of the head against the wall was observed (Fig. 1). As the

Fig. 1

Sheep in the Early Stage of FAA Intoxication



depression gradually became more obvious and the animals were reluctant to move, they stood with abnormal extension of the forelimbs forward and of the hindlimbs backward. At this stage, frequent urination was a characteristic feature.

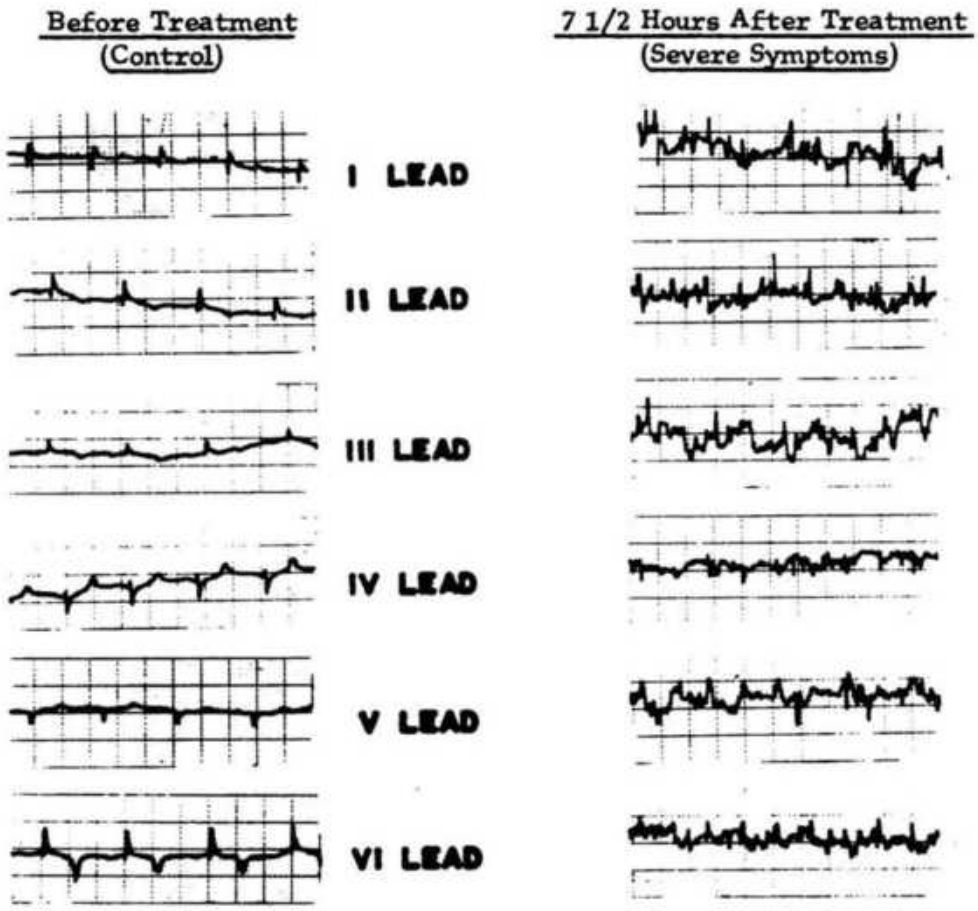
In the more advanced stage, 4 to 6 hours after the ingestion of the compound, the sheep laid down and their eyelids were partially closed. They got up only when approached.

**Sanborn Viso Cardiette, Model 51 (Sanborn Company, Waltham, Mass.) used

In the terminal stage, a short excitement, characterized by running-like movements of the limbs, preceded the sudden collapse. This was followed by extremely rapid labored breathing, accompanied by abnormal moaning. Frothing from the mouth and external nares,

Fig. 2

Electrocardiogram of Sheep (1269) in Fluoroacetamide Poisoning



a common finding, was a sign of ensuing death. The extreme terminal distress, in general, lasted 20 to 60 minutes.

b) Heart: Most herbivorous animals respond to fluoroacetate with a distinct cardiac effect. The sheep's heart rate which was 90 to 125 per minute prior to administration of FAA had increased to 150 to 250 per minute 4 to 5 hours afterwards. Following collapse, the heart rate was so rapid that it was impossible to make an accurate count by stethoscope. It was obviously well above 400 per minute.

Attempts were made to take electrocardiograms from 4 poisoned sheep at the terminal stage of poisoning. Breathing was so labored and rapid that the electrocardiogram yielded an adequate recording in only one sheep. The recor-

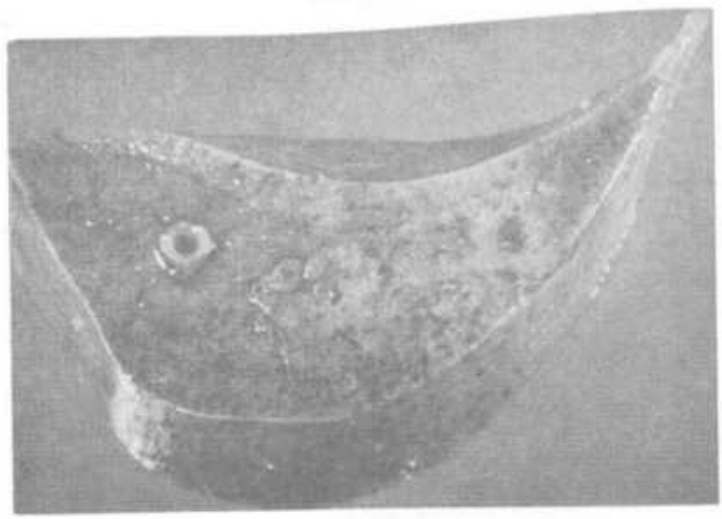
ding was taken 30 minutes before the sheep died i. e. 7 1/2 hours after administration of the compound. It was compared with the electrocardiogram of the same sheep taken before FAA application. All six leads revealed ventricular fibrillation (Fig. 2).

c) Necropsy Findings: Congestion and hemorrhages in various organs and tissues of the body were the principal findings especially in the lungs, stomach, intestines, liver, kidneys, heart and adrenals.

Gross examination showed frothy colored mucus around mouth and nose and a bloody discolored area around the anus. Subcutaneous petechiae and frothy pink mucus in the trachea, bronchi and bronchioli were observed. The liver exhibited rounded edges. It was congested, enlarged and purple in color. The lungs contained areas of dark red and purple discoloration and pits on pressure. Pink, frothy mucus oozed out from their surfaces (Fig. 3). Petechial and Ecchymotic areas were also noted on the diaphragm, especially in sheep which died 20 hours after administration of the poison (Fig. 4), as well as on the epicardial and myocardial surfaces and on the mucosae of the stomach and intestines. Congestion scattered diffusely throughout the cortex and medulla of the kidneys and focal hyperemic areas in the cortex of the adrenal glands were seen.

Fig. 3

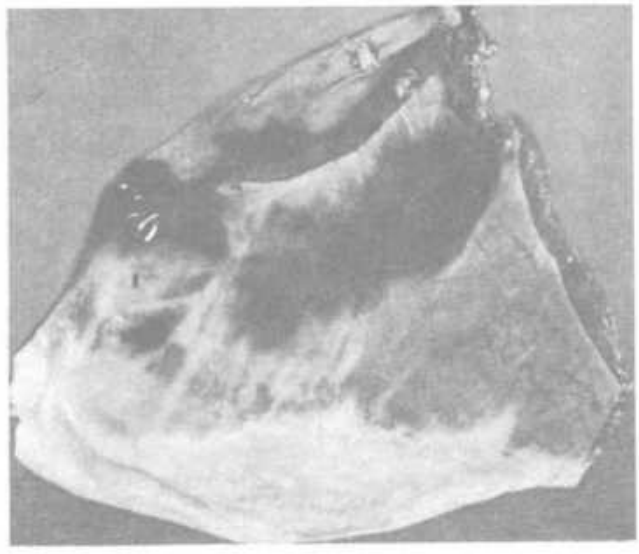
Lungs



Histologically, pulmonary congestion with acute intra-alveolar hemorrhages and edema of the lungs were found. Congestion and hemorrhage, and slight superficial coagulative necrosis of the gastro-intestinal mucosae was observed. Congestion with equivocal to slight degeneration of hepatic cells and congestion, focal hemorrhages and degeneration of tubular epithelium was found. The cortex of the kidneys exhibited hyperemia and vascular engorgement.

Fig. 4

Diaphragm



B. EXPERIMENTS IN DOGS

a) Clinical Observations

Experiment 1: A dog weighing 20 pounds was fed 380 g of thigh muscle from one of the poisoned sheep. Nine hours from the time of feeding, the dog appeared to be depressed and two hours later, tonic-clonic seizures occurred. These initial convulsions with short intervals lasted about 20 minutes. Later the dog was able to get up and walk with a staggering gait. Following the second round of convulsive seizures which lasted about 15 minutes, the dog died.

Experiment 2: A dog weighing 90 pounds was fed 150g of skeletal muscle from a sheep that had died 1½ months previously and 150 g from another sheep that had died 2½ months previously. The meat had been kept frozen on dry ice throughout the entire time. It was thawed just prior to the feeding experiment. Nine hours following the administration of the meat, the dog was offered another 100 g of meat from the second sheep. One hour later (10 hours after the first feeding) the dog appeared to be distressed and was reluctant to come out of her cage as she had done previously. Later, frothiness around the mouth and internal nares, labored breathing, tetanic convulsions resembling strychnine poisoning, and limb motions similar to swimming and running were observed. The dog was unable to get up. After a short interval, convulsions started again, and large amounts of saliva exuded about her mouth and face. Bloody fecal material was excreted. The intermittent convulsions persisted for some time. The dog died 13 hours after the beginning of feeding.

On gross examination, the heart was almost round and covered with epicardial and endocardial hemorrhages. The lungs were dark red, blood-tinged and pitted on pressure. Frothy mucus was noted in the trachea and bronchi.

The liver was enlarged, congested and purplish in color. The spleen was enlarged, congested and nodular. Ecchymotic hemorrhages were found on the serosal surface. The gastric mucosa showed diffuse mucosal congestion. The kidneys were purplish red and congested and the mucosal surface of the bladder was slightly hyperemic. On the muscular part of the diaphragm petechiae and ecchymoses were noted.

The histological findings were similar to those of the sheep.

Discussion

The symptomatology and pathology of Dichapetalum cymosum (gifblaar) poisoning, a highly toxic South-African plant, was first described about 37 years before the identification of the toxic principle (6), which turned out to be fluoroacetic acid (7). Clinical symptoms were similar in the bovine, sheep and goat (6, 8): restlessness and continuously lying down and getting up with feet lifted high and staggered gait were the most characteristic features. Increased heart rate, quick and shallow respirations, diuresis and frequent micturition were also observed (6). Moreover, partial or complete heart block in potassium monofluoroacetate poisoning in sheep and rabbit was found (9). Acacia georginae (gidyea) a poisonous plant in Australia likewise accumulates fluoroacetic acid and is responsible for heavy losses among cattle and sheep. The most striking feature of the disease is the suddenness with which death occurs both in cattle and sheep (10). In cattle, the terminal symptoms are due to acute heart failure (11), the excessive heart rate progressing to ventricular fibrillation. Experimental sodium fluoroacetate poisoning in sheep caused death about 2¾ to 4½ hours following its administration, when the dose was 10 and 2.5 mg/kg respectively. Restlessness, frequent changing from standing and reclining position, fast, weak pulse and collapse were the characteristic symptoms (12). Chronic FAA poisoning in cattle revealed listnessness, intermittent loss of appetite, lack of coordination, accelerated heart and respiratory rate (4).

There are no characteristic post-mortem lesions (6). The heart is always in a flabby dilated state (13). Similar findings were reported in FAA poisoning in cattle (4), and in Acacia georginae poisoning (11). Subepicardial and subendocardial hemorrhages, hyperemia and endema of the lungs, hyperemia and sometimes degenerative changes in the liver, kidneys and myocardium, as well as catarrhal gastro-enteritis was described in Dichapetalum poisoning (8). Pulmonary edema was found in experimental fluoroacetate (12) and in experimental FAA poisoning in sheep (1). Whitten and Murray (14) described significant myocardial lesions in acute natural and experimental Acacia georginae poisoning in cattle: irregular areas of pallor of the underlying muscles. On section, the myocardium itself may occasionally be mottled. In some hearts from the poison area, careful slicing of the heart reveals occasional gross lesions sometimes extending through the muscle wall. Microscopically, an acute multifocal injury to the myocardium followed by a cellular response in the interstitial tissues was found to be characteristic. The authors conclude that the

the myocardium is the most susceptible target organ as indicated by the pathological lesions. In fluoroacetate poisoning - depending on the species - cardiac or central nervous cells are the first to demonstrate the energy source of depletion, since they require the highest rate of oxidation.

In experimental methyl fluoroacetate poisoning, ventricular fibrillation was found in rabbit, goat, horse, some pigs and two species of monkeys (rhesus and spider) (15). In the experimental goats, once ventricular fibrillation developed, no return to sinus rhythm was noted. The mechanism of ventricular fibrillation in methyl fluoroacetate poisoning is similar to electrically induced ventricular fibrillation (16). It is of interest that auricular fibrillation does not occur in fluoroacetate poisoning in any species.

Although the biologic test i. e. feeding trial, in dogs is a cruel method for the diagnosis of fluoroorganic poisoning, in questionable (forensic) cases it may support the time-consuming and sophisticated chemical method. Secondary fluoroacetate poisoning was produced (17) in one dog which consumed the heart muscle of an experimentally poisoned horse, whereas two other dogs did not suffer ill-effects after having eaten the skeletal muscle and liver of the same horse. In our experiment, two dogs were fatally poisoned by the feeding of FAA poisoned sheep skeletal muscle. It may be that in the horse experiment the poisoned meat did not contain the actual toxic principle i. e. fluorocitric acid in toxic or lethal concentration, although this consideration is not in complete accordance with the theoretical possibility: It is suggested that in FAA poisoning lower fluorocitric acid concentration should be expected than in fluoroacetic acid poisoning (18), and that therefore the latter is more toxic.

However, the clinical aspects, especially the effect on the heart and pathological changes, were much more striking in FAA poisoning in dogs than in sheep. The lesions appeared to be acute; they were mostly vascular with equivocal to slight coagulation necrosis of parenchymal cells in some organs.

Some of the above-described changes may be associated with terminal convulsive seizures prior to death.

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EXPERIMENTAL ACUTE FLUOROACETAMIDE POISONING IN GUINEA PIG AND SHEEP

II. BIOCHEMISTRY

by

M. N. Egyed[1] and G. W. Miller[2]
Veterinary and Botany Dept., Utah State Univ., Logan Utah

SUMMARY: Tissue 0_2-uptake, tissue and blood citric acid and blood sugar were studied in experimental fluoroacetamide (FAA, chemically $FCH_2CO\ NH_2$) poisoning. In the poisoned guinea pig and sheep tissues, the 0_2 consumption was slightly decreased. When FAA (or sodium fluoroacetate, FAC) was added to normal guinea pig tissue slices, or especially when FAA (or FAC) was added to guinea pig kidney mitochondria, the degree of inhibition was more striking. Elevated blood and tissue citric acid was found in FAA poisoned guinea pig and sheep; furthermore, significantly increased blood sugar was demonstrated in FAA poisoned sheep.

INTRODUCTION

Fluoroacetamide (FAA) is a simple derivative of fluoroacetic acid (FAC, chemically $FCH_2\ C00H$). Both compounds are potent rat poisons, and the latter is a toxic agent of a number of poisonous plants. FAA is hydrolysed in animal tissues to FAC (1) and from this state FAA obviously follows the same metabolic pattern as the parent acid: From FAC fluorocitric acid is formed. This compound is the actual active principle in FAC or FAA poisoning. It acts by blocking the enzyme aconitase, which catalyses the interconversion of citrate and isocitrate in the tricarboxylic acid (Krebs) cycle. Although the effect of FAC on tissue 0_2 consumption was studied by a number of authors (see below), no information is available on how FAA affects tissue respiration.

Citric acid accumulation was demonstrated both in vitro (2) and in vivo (3) in FAC poisoning, and also in rats and sheep in FAA poisoning (4). The pattern of citric acid accumulation in FAA poisoning has not been studied before. Some discrepancy exists in the literature concerning the effect of FAA on blood sugar concentration. Reinvestigation of this point is warranted.

MATERIAL AND METHODS

1. 0_2 - Uptake Experiments

a) Guinea pigs, mostly male weighing 300 to 500 grams, were poisoned

1) Permanent address: Kimron Veterinary Institute, Bet Dagan, Israel;
2) Present address: Huxley College, Western Washington State College, Bellingham, Washington, U.S.A.

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by injection intraperitoneally with 20 mg FAA/kg body weight. The FAA was dissolved in 0.5 ml physiological saline. This dose proved to be lethal in every instance. The animals were decapitated at the terminal stage of poisoning, which in most cases was 5 to 7 hours after the injection. The tissues were sliced in a humid chamber, where the humidity was maintained by aeration of water. The slices which were rapidly weighed varied between 0.10 to 0.20 g. They were immediately placed into reaction vessels containing 3 ml of medium. The details are presented in Table 1. Normal (control) guinea pigs were also

TABLE 1 (Guinea Pigs)

Inhibition of 0_2 Consumption in FAA (20 mg/kg i.p.) Poisoning Under Various Conditions in Guinea Pig

Tissue	Technique	Buffer	Substrate	Added Poison	Inhibition in %
1. Poisoned heart	Slice	Bicarbonate (pH 7.25)	No	None	16.3
2. Poisoned liver	"	"	"	"	12.1
3. Poisoned spleen	"	"	"	"	10.7
4. Poisoned kidney	"	"	"	"	17.5
5. Normal kidney	"	"	"	10 mM FAC	29.8
6. "	"	"	"	10 mM FAA	23.0
7. "	"	Reinforced with Mg^{++} and ATP	Sodium Succinate	10 mM FAC	44.0
8. "	"	"	"	10 mM FAA	37.3
9. "	Mitochondria	"	Sodium L-Malate	20 µM FAC	54.7
10. "	"	"	"	"	66.3*
11. "	"	"	"	20 µM FAA	42.6
12. "	"	"	"	"	62.3*

*After 2 hrs' incubation

sacrificed and studied for comparison. For the measurement of 0_2 consumption, a Gilson Medical Electronic Differential Respirometer was used. The reaction vessels were incubated in a shaking water bath at 37.5°C for one hour following 15 minutes of equilibrium. One experiment was allowed to continue for two hours. Vessels containing the complete reaction mixture with the exception of tissues were used as controls. The 0_2-uptake was calculated in ml

of oxygen extrapolated to 1 gram of tissue (wet weight). For each series of experiments, four poisoned and two control guinea pigs were used, and from each guinea pig tissue duplicate experiments were run and the average value was calculated. Guinea pig mitochondria were prepared according to the method of Peters and Wakelin (5) with a slight modification. In one experiment sodium succinate was used as a substitute for trisodium citrate.

The method of Camp and Farmer (6) was used for blood citric acid analysis. Citric acid in the tissue was determined by the method of Taylor (7).

b) Cross-bred white faced sheep were poisoned orally with 20 mg FAA/kg body weight. The dose was lethal in every case. For 0_2 uptake experiments, the sheep were sacrificed at the agony stage and the tissues were treated as in the guinea pig experiments. Citric acid was measured as indicated above. Blood (plasma) sugar was estimated in sheep blood plasma by a O-toluidine* method. The number of sheep used is given for each series of experiments.

The experiments demonstrated that:

1. Inhibition of 0_2 consumption caused by acute FAA poisoning occurred in both guinea pig and sheep tissue (Table 1 and 2).
2. The degree of inhibition was slightly higher in sheep than in guinea pig tissue.
3. In both species, 0_2 uptake was inhibited to a greater extent in heart and kidneys than in spleen and liver.
4. The decrease of the 0_2 uptake was more significant when FAA (or FAC) was added to normal guinea pig kidney slices.
5. The same concentration of FAA (or FAC) resulted in greater inhibition of 0_2-uptake in reinforced buffer containing sodium succinate as buffer than with the buffer alone. It is interesting to note that in a F^- treated plant (Pelargonium zonale) only succinate was able to cause inhibition among all the Krebs-cycle dehydrogenase enzymes, as shown by histochemical studies (8).

TABLE 2 (Sheep)

Inhibition of 0_2 Consumption in FAA Poisoned (20 mg/kg orally) Sheep Tissue Slices in Bicarbonate-Buffered Medium, After 1 Hour of Incubation

Tissue	Inhibition in %	Remarks
1) Heart	18.7	(average of 2)x
2) Liver	11.2	"
3) Spleen	11.5	"
4) Kidney	20.3	"

x = Two healthy (control) sheep were sacrificed for comparative studies

*Commercial reagent manufactured by Hartmann - Leddon Co., Inc. Philadelphia.

6. The 0_2 consumption was most strikingly inhibited when FAA (or FAC) was added to kidney mitochondria prepared from normal guinea pigs. Similarly decrease of 0_2 uptake was observed when FAC was added to kidney slices (9) or to washed rabbit kidney cortex (10).

2. Citric Acid Metabolism

The accumulation of citric acid in the blood and various organs was demonstrated in acute FAA poisoning both in guinea pigs and in sheep (Table 3, 4). The rate of increase was more striking in guinea pigs, especially in the guinea pig kidneys than in sheep. Some correlation seems to exist between the increased citric acid concentration and lowered 0_2 consumption of kidney and heart. The citric acid accumulation, as referred to previously, is due to blockage of citric acid oxidation in the Krebs cycle. The large build-up of citric acid is considered to be a symptom of poisoning and not the cause of death (11).

TABLE 3

Tissue Citric Acid Concentration in Normal and FAA Poisoned (20 mg/kg) Guinea Pigs (Figures in μg/g wet weight)

Tissue	Control (Average of 4)	Poisoned (Average of 6)	Increase
Blood (plasma)	42	116	2.8 fold
Heart	90	610	6.8 "
Spleen	73	337	4.6 "
Kidney	34	925	27.2 "

TABLE 4

Tissue Citric Acid Concentration in Normal and FAA Poisoned (20 mg/kg) Sheep (Figures in μg/g wet weight)

Tissue	Control (Average of 3)	Poisoned (Average of 4)	Increase
Blood (plasma)	36	68*	1.9 fold
Heart	54	290	5.4 "
Kidney	60	180	3.0 "

*The results of the blood citric acid of another sheep is presented in Table 6.

The higher inhibition in the mitochondria is probably due to the fact that interfering constituents, which are present in tissue slices, have been elimina-

ted. However, the possibility of greater permeability of the mitochondria to FAA than of tissue slices, cannot be excluded.

Since the aerobic respiration takes place in mitochondria, in vitro effects of poisons on metabolic processes could logically involve mitochondrial studies.

In all experiments, FAC resulted in a greater inhibition than did FAA. This observation is in accordance with the metabolic pattern of FAA, since FAA is hydrolized to fluoroacetate before fluoracitrate formation takes place. However this difference was more or less eliminated in the experiment with mitochondria after 2 hours of incubation. This indicates a similar mode of action for the two compounds.

3. Glucose Metabolism

Blood sugar determinations were carried out in five sheep poisoned with 20 mg FAA/kg body weight. From each sheep, blood was taken before the administration of FAA for blood sugar and citric acid analysis (Table 5).

TABLE 5

Blood (plasma) Sugar in Experimental FAA Poisoning in Sheep

Control (Before FAA administration)	Poisoned (6-7 hours after poisoning)	Increase
58 mg%	248 mg%	4.3 fold

TABLE 6

Blood (plasma) Sugar and Citrate Levels in Experimental FAA Poisoning in Sheep

Time	Blood (plasma sugar)	Increase	Blood (plasma) citrate increase (%)
Before FAA administration	52 mg%		2.0 mg%
1 hour after "	53 "		1.9 "
2 hours " "	58 "		1.8 "
3 " " "	57 "		1.6 "
4 " " "	56 "		1.9 "
5 " " "	55 "		2.0 "
6 " " "	70 "		4.0 " 2.4 fold
7 " " "	225 "	4.3 fold	6.0 " 3 fold

In this experiment, the animal demonstrated ventricular fibrillation (12) and died some 45 minutes after the last bleeding. Although orally given (50 mg/kg) FAA in rats has caused marked hypoglycemia with convulsions (13), our experiments did not produce them. Nevertheless, the rate of increase in blood sugar was less in rats than in sheep (3). Whereas through the whole course of poisoning the blood sugar remained normal, it rose dramatically only a relatively short time before death. The mechanism of FAC diabetes is not completly understood.

Experiments with perfused rat heart relate impaired carbohydrate metabolism to inhibition of phosphofructokinase by the increase in tissue citrate resulting from experimental FAC poisoning.

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WATER EXTRACTS OF SUPERPHOSPHATE AS LOW FLUORIDE SOURCE OF PHOSPHORUS FOR DOMESTIC ANIMALS

by

J. Tusl and J. Dedek
Pohorelice, Czechoslovakia

SUMMARY: Superphosphate which is used as a fertilizer and as a food supplement for cattle was extracted with water for 1, 3, 6, 12 or 24 hours in order to obtain solutions rich in phosphorus and low in F^-. During three hours over 70% phosphorus was extracted compared to about 5% of F^-. Since the amount of F^- thus extracted is below the usually accepted daily threshold value for domestic animals, including cattle, the present technique can remove toxic surplus of F^- from phosphates before it is fed to cattle.

Mineral nutrition of domestic animals sometimes presents problems. Whereas calcium is present in sufficient amounts in feed, usually phosphorus must be supplied artificially. The use of rock phosphate or some industrial products is limited due to their high F^- content which may cause fluorosis, especially in cattle (1). Only supplements with a low F^- content are therefore desirable. However, the most suitable feed containing 100 ppm F^- or less are not usually available in sufficient amounts. Thus, it is necessary to utilize supplements with a higher F^- content, e.g. superphosphate.

Special low F^- feed products up to 1.2% F^- are not believed to have an adverse effect on health, efficiency and reproduction of dairy cows (2). Also, the level of urinary F^- (2) did not indicate any adverse effect when compared with typical values (1). These findings warrant wider application of low F^- (1.8%) superphosphate. To provide an inexpensive fertilizer such as superphosphate, phosphate materials were treated with cold or hot water to prepare extracts. These solutions were expected to be low in F^- because most F^- compounds in superphosphate are only slightly water soluble. The above procedure is currently being used but quantitative data on the concentration of minerals in resulting extracts is lacking. The aim of this study was to determine whether or not the above-mentioned simple procedure can decrease the toxicity of superphosphate due to F^-.

Method

The experiments were carried out with superphosphate fertilizer and a low F^- feed. Both materials were supplied by Chemical Works, Prerov, CSSR.

From the Research Institute for Animal Nutrition, Feed Science and Technology, Pohorelice, Czechoslovakia.

TABLE 1

The Content of Mineral Nutrients in Superphosphate and in their Aqueous Extracts

Sample	F		P		Ca		Mg		Na		Mn		Cu		Zn	
Fertilizer	16.2		92.6		180.7		0.42		2.21		400		950		3800	
Feeding SPF	10.5		85.7		192.8		0.90		4.07		250		1120		5100	
Extracts:	C	H	C	H	C	H	C	H	C	H	C	H	C	H	C	H
Fertilizer																
1 hour	0.29	0.21	14.5	18.7	4.0	5.2	0.04	0.04	0.10	0.27	27	35	8.5	9.2	4.5	3.7
3 hours	0.28	0.23	21.6	22.4	6.4	7.7	0.03	0.06	0.17	0.27	38	39	6.5	8.0	5.0	4.7
6 hours	0.28	0.23	22.0	22.0	6.4	6.5	0.03	0.05	0.17	0.28	45	40	9.2	8.0	7.5	5.0
12 hours	0.26	0.24	20.0	19.6	6.9	6.9	0.04	0.04	0.18	0.27	40	43	8.5	8.3	7.5	5.0
24 hours	0.24	0.21	24.1	23.5	7.5	7.6	0.04	0.04	0.17	0.29	39	44	8.0	8.0	7.5	6.2
Feeding SPF																
1 hour	0.09	0.09	17.2	20.5	7.5	7.5	0.07	0.11	0.54	0.66	25	22	9.2	9.2	32.0	31.5
3 hours	0.11	0.09	20.3	20.5	7.7	7.6	0.06	0.09	0.50	0.56	23	18	10.5	9.2	32.5	33.7
6 hours	0.14	0.10	21.0	18.3	7.4	7.5	0.07	0.10	0.50	0.57	27	23	8.8	9.2	34.5	31.2
12 hours	0.10	0.08	18.9	22.0	7.7	7.8	0.08	0.09	0.51	0.57	25	25	10.0	8.5	32.0	31.0
24 hours	0.11	0.06	22.0	22.0	8.0	8.0	0.09	0.10	0.52	0.60	27	27	9.2	10.2	33.5	35.7

[a] Results are present in Mg/g (mg/ml) for F, P, Ca, Mg and Na and in ppm for Mn, Cu and Zn.
SPF = Superphosphate, C = cold water, H = hot water

To prepare extracts, 100 g of each superphosphate were treated with 300 ml of cold (18°C) or warm (55°C) distilled water. The extraction took place for 1, 3, 6, 12 or 24 hours. Samples were shaken repeatedly in order to insure complete extraction of solubles.

The extracts obtained as well as solid samples of superphosphate dissolved in diluted perchloric acid were analyzed for substances which are important for animal nutrition. Fluoride was determined by employing the F^- ion activity electrode in citrate buffer (3), phosphorus by molybdovanadic method (4) and calcium, magnesium, sodium, manganese, copper and zinc by atomic absorption spectrophotometry (5).

Results and Discussion

The content of important mineral nutrients in both kinds of superphosphate as well as in water extracts are recorded in Table 1. The values for F^- and phosphorus of solid materials are in good agreement with the usually accepted values. Typical values for low F^- feeding phosphate are 12 and 96 mg/g respectively (6).

The mineral content of extracts remained practically constant after 3 hours (Table 1). Also the pH values of 4.5 and 4.8 for fertilizer and feed superphosphate respectively did not change with time. In some cases, e.g. F^-, Mg, Na, the maximum of the yield was reached during 1 hour. The treatment with hot water did not change the mineral content of extracts significantly but produced lower values for extracted F^- at higher temperatures. Upon comparing the extracts, depending on the kind of superphosphate, significant difference were seen in most elements corresponding to values for the original solid materi However, with regard to extracted F^-, the higher the temperature the lower we the F^- values.

Table 1 shows that all obtained extracts are rich in phosphorus, but poor in F^-. It indicates that the treatment of superphosphate is a satisfactory method for eliminating F^- prior to utilizing it as feed. Extraction for 3 hours with cold water seems to be the most rapid and economical technique. Overnight treatment is desirable because solid particles settle to the bottom and the yield of minerals is maximal.

The absolute yield of mineral nutrients may be easily calculated from Table 1. As much as 70.0 to 72.5% phosphorus are extracted from both phosphate materials during 3 hours, but only 2.5 to 6.4% F^- appeared in extracts. The obtained relationship between F^- and phosphorus after 3 hours and use of cold water is 1:62.5 for fertilizer and 1:180 as a phosphate supplement to feed. The values are more favorable when the extraction is performed at 55°C than by cold treatment. But even the latter produced much better relationships than the original values for solid superphosphate namely 1:5.7 and 1:8.1 respectively.

With respect to other elements, the extraction of calcium yielded surprisingly low values namely, 10.5 to 12.8%, but plant feed or limestone contain sufficient amounts of this element for supplementary animal feeding. Extracts of superphosphate supply considerable amounts of magnesium, sodium and manganese namely 21 to 43%, 23 to 41% and 23 to 29% of the original amount respectively. It should be noted that elements extracted in water are most readily available for animals. Therefore actual recovery is higher than stated. Copper and zinc are supplied in negligible amounts in the extracts. Water solutions have an iron content of about 10 ppm.

100 g of superphosphate taken for preparing extracts represent daily additional supplements of phosphorus required by an adult cow weighing 500 kg. Since only 70% of phosphorus is extracted from superphosphate into water, about 150 g of phosphate materials should be used for preparing the extract. Even in this case the amount of F^- supplied is well below the threshold limit which is 750 mg for natural phosphates (6) or 300 to 500 mg for sodium fluoride (1, 6). The amount supplied is 126 mg for fertilizer and 50 mg for feed superphosphate. The amounts of F^- remaining in the material extracted by means of the suggested technique cannot be dangerous for animals.

The results of this study will make possible a wider use of less expensive industrial phosphates as phosphorus supplements for domestic animals particularly for cattle which are susceptible to fluorosis.

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EFFECT OF A SHORT PERIOD OF FLUORIDE INGESTION ON DENTAL FLUOROSIS IN CATTLE

by

J. W. Suttie and E. C. Falton
Madison, Wisconsin

(Abstracted from American J. of Veterinary Research 32:217-233, Feb. 1, 1971)

The authors determined the exact stage of the tooth's development at which each tooth is sensitive to dental fluorosis in cattle, a condition which is due to a disturbance of the ameloblasts, the enamel-forming organs. In the presence of increased concentrations of plasma F^-, the matrix laid down by such cells is faulty and abnormally calcified.

Method: Instead of the usual procedure of administering F^- continuously for an extended period, the authors added F^- to the daily ration in doses of 2.5 mg/kg of body weight during the 13th, 14th and 15th months of age in one group of three heifers; during the 16th, 17th and 18th months of age in a second group of three. NaF was administered for periods of 2 weeks to 3 months mixed with a standard 16% protein concentrate and alfalfa grass. Fluoride content of plasma, of the 11th coccygeal, and of the enamel surface of each right incisor crown was studied.

When the heifers were 4 years old, the mottling of teeth was scored from 1 to 5 according to the degree of damage. Number 5, the most severe category encompassed general mottling, hypoplasia, greater wear of the incisor tooth than for score 4 and erosion of the enamel from dentin.

The enamel surface of the crown of each right incisor was divided into five approximately equal horizontal sections. They were then ground from the dentin with a dental tooth drill and analyzed for F^-.

Results: The amount of F^- in plasma of the calves increased promptly following administration of F^-. The level did not vary during the three months' exposure period. The values rapidly decreased to normal at termination of exposure. Thus, the teeth were only subjected to increased F^- concentrations for a short period. Even though the F^- concentration in the blood in the two groups was similar, retention of F^- was greater in the vertebrae of heifers in group 1 which were exposed at an earlier age (13th to 15th months).

In these same group 1 animals which were exposed during the period of calcification of the first part of the crown of the 2nd incisor, only the first and second incisors were affected. Exposure during 16th to 18th months of age caused substantial damage to the second and third incisors, and in two heifers to the fourth incisor, but did not affect the first incisor. The F^- content of the dentin of the 1st incisor was about the same in both groups of heifers. However, a high F^- concentration was found in the dentin from the second incisor of group 2 heifers. Presumably in all but one of group 1 heifers

little second incisor dentin had formed prior to F^- administration. The F^- concentrations of the second incisor dentin in group 1 was generally lower than that in the 1st incisor.

Teeth, which were considered normal, contained less than 200 ppm F^-. Those with the most extensive changes (group 5) showed more than 750 ppm.

The data indicated that the enamel is capable of retaining high concentrations of F^- during a limited period during its development. Wide variations in F^- concentrations in different parts of the enamel of the same tooth were noted. The calcification of the enamel procedes from top to the bottom of the crown of one incisor before calcification begins at the top of the next one.

The experiment indicated that the period of tooth formation during which the tooth is subject to F^- damage is somewhat longer than may have been thought. It is further concluded that determination of enamel F^- content can provide an accurate estimate of the period during which an animal was exposed to increased F^- uptake and the duration of that exposure.

This research was performed using grants from 17 metal corporations.

THE EFFECT OF FLUORIDE UPON THE UPPER RESPIRATORY TRACT AND THE EARS IN ALUMINUM WORKERS

by

Jerzy Rybicki
Gdansk, Poland

(Abstracted from Medycyna Pracy, 21:192-195, 1970)

This paper deals with the health of workers at a Polish smelter which produces aluminum from bauxite by thermal electrolysis with the use of cryolite and other F^- compounds. During the process hydrogen fluoride escapes into the air at concentrations which surpass the Maximum Allowable Concentration (MAC) value.

In an attempt to assess any possible F^- hazard particularly to the upper respiratory tract and the ears of workers, age, working period and positions of the workers in the factory were recorded.

The staff of the electrolysis hall consists of 170 men, 20 to 45 years old nearly half of whom are 25 to 30 years of age. Twenty-nine percent were employed for less than one year; 48%, for one to two years; 20%, for two to three years and only 3% for more than ten years.

The group of workers was divided into two sub-groups according to the degree of their known exposure to F^-. One hundred workers operating the electrolyzers were exposed to hydrogen fluoride concentrations surpassing the MAC values 7 to 11 times. They were present in the polluted area for more than 6 hours a day. The second group of 70 workers was exposed to hydrogen fluoride for only 3 to 4 hours daily at concentrations 4 to 6 times surpassing the MAC value.

Only 1% to 3% of the workers had complaints of intermittent headaches and respiratory distress, but were otherwise free of symptoms.

The examination of the workers revealed catarrhal and hypertrophic changes of the upper respiratory tract depending on their age and the period of their employment. Subjects working more than two years in the electrolysis hall exhibited evidence of atrophy of the nasal mucous membranes. Nose bleeds and acute episodes of inflammation of the upper respiratory tract, especially among the younger group of workers, were noted. Most older workers were afflicted with chronic respiratory and ear infections.

With respect to prophylaxis and treatment, the author made the following suggestions:

1. Persons with defects and tendency to frequent upper respiratory tract and ear infections should not be employed at the electrolysis halls.

2. Because of the hazardous working conditions in the electrolysis hall, only men aged 20 to 40 should be employed.

3. Inhalation of alkaline solutions (sodium bicarbonate) in low concentrations may be of prophylactic and therapeutic value. A device described in Hungary, enabling simultaneous inhalation of several workers, is advocated.

Jerzy Krechniak

FLUORIDE CONTENT OF KIDNEY STONES

by

E. Auermann and H. Kuhn
Karl-Marx-Stadt, B. D. R.

(Abstracted from Deutches Gesundheitswesen 24:565-69, 1969)

The purpose of this study was to determine 1) whether F^- is a constituent of kidney stones, 2) whether or not there are characteristic differences in the F^- levels of various kinds of kidney stones and 3) whether or not the F^- content of kidney stones is related to the stone's calcium and phosphorus content.

Kidney stones consist of an organic ground substance and the crystalline minerals which form the stone. The ground substance which consists of a high molecular weight protein, amounts to approximately 2 to 4% of the total structure of the stone. The crystalline stone minerals are deposited on the fibrils of the ground structure.

According to former reports F^- levels in kidney stones vary widely. Williams (1946) reported a range from 0.4 to 156 mg% F^- (4 to 1560 ppm), Zipkin et al. (1962) between 0.02 and 1.11% with a mean of 0.31%.

The authors assayed 56 kidney stones obtained from adults residing in or near Karl-Marx-Stadt prior to fluoridation of this city when its water supply averaged 0.25 mg/l F^-. After the stones were dried, pulverized and ashed, the double distillation procedure was used and F^- determinations were made by the zirconium-xeriochromcyanin method.

Of the 56 specimens, 28 were calcium oxalate stones, 11 a mixture of calcium oxalate and phosphate, 14 uric acid stones, 2 oxalate uric acid mixture and 2 were a composition of oxalate phosphate and uric acid.

The F^- values of the oxalate stones showed a very wide distribution ranging between 21 mg% and 1065 mg% with a mean of 113 mg% (1130 ppm). In ap-

proximately 75% of all stones, the F^- content ranged between 21 and 134 mg% (1340 ppm). Compared with F^- levels of other organs, the authors considered values up to 10,650 ppm unusually high.

Among the oxalate phosphate stones, the F^- values ranged from 27 to 140 mg% with a mean of 96 mg% (960 ppm). There was no indication that calcium phosphate or the apatite structure accounted for higher F^- accumulation than the calcium oxalate.

As was to be expected, the 13 uric acid stones showed the lowest F^- values. They ranged between 7 and 43 mg% F^- with a median of 20 mg% (200 ppm).

The authors do not believe that F^- plays a primary role in the crystallization of the calcium oxalate and phosphate stones, although it is likely that F^- favors and, perhaps accelerates, the formation of these stones. In the formation of apatite compounds, F^- is likely to be preferred. Therefore the stone is harder, more dense and less soluble. The F^- concentration of these stones seems to depend upon the F^- levels of the surrounding fluid. An increase of F^- in drinking water is bound to increase the urinary F^- values. Therefore higher F^- intake through drinking water may have a bearing on the increase of F^- in kidney stones. The authors intend to make a follow up study after 5 to 6 years of fluoridation.

FLUORIDE LEVELS IN DRINKING WATER IN TEXAS

by

I. L. Shannon
Houston, Texas

(Abstracted from Texas Medicine 66:48-53, Aug. 1970)

The author sampled 262 water supplies throughout Texas for their F^- content. Fluoride concentrations ranged from 0.07 ppm at Hemphill to 5.0 at Midland with a mean for the state of 0.84 ppm. Fluoride levels in drinking waters of western Texas were much higher than those in the central and eastern portions. 52% of the waters sampled in western Texas showed F^- levels at 1 ppm or less; 39.8% ranged from 1.01 ppm to 3; 8.2% from 3.01 to 5 ppm.

In commenting on these results, the author quoted various statistical studies pointing to F^-'s alleged beneficial effects. On the other hand, advanced dental and skeletal fluorosis has been reported from western Texas as well as from other endemic areas at the above-mentioned concentrations.

From Veterans Administration Hospital, 2002 Holcombe Blvd., Houston, Texas.

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