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CONTENTS

The Fifth Annual Conference of the I. S. F. R. will be held at Oxford University, Oxford, England, Sunday, April 8 to Wednesday, April 11, 1973.

The program committee is soliciting reports of original research or review articles limited to 20 minutes. Authors need not be members of the I. S. F. R. Abstracts of 250 words in English in triplicate should be mailed prior to December 1, 1972, to the Secretary Dr. G. L. Waldbott, P. O. Box 692, Warren, Michigan 48090.

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MANUSCRIPTS for publication should be submitted in English, double-spaced with generous margins. References should be arranged according to the order in which they are cited in the text, and written as follows: Author, title, journal, volume, pages and year. Each paper must contain a summary of not more than 20 lines.

Contributors will receive 10 copies of the issue of FLUORIDE containing their paper, free of charge.

FLUORIDE is listed in
Current Contents Agricultural
Food and Veterinary Sciences

EDITORIAL

CAN FLUORIDE CAUSE LUNG CANCER?

The etiology of cancer still remains obscure in spite of considerable advances in research during recent years. The wide variety of agents which enter into the causation of cancer, their interaction with each other, the slow, insidious onset of the disease, the varying response of animals and humans to carcinogenic agents and the necessary limitations on studies to which a human can be subjected, are some of the factors which account for the slow progress in identifying the cause or causes of cancer.

Only little information is available on the possible carciongenic role of the fluorine ion. In 1954 Taylor (1) showed in experiments involving 645 mice that the life-span of cancer-prone mice was shortened, on an average, by about 9% when fluoride in concentrations of 1 ppm was added to their drinking water.

Subsequently in 1964 and 1965 Taylor (2, 3) established that the two halogens, bromide and fluoride stimulate the growth of cancer, the latter at a concentration as low as 1 ppm. In 54 experiments with 991 mice bearing transplanted tumors and 58 experiments utilizing 1817 eggs which contained implanted mouse cancer tissue, he found that cancer growth was stimulated by more minute amounts of sodium fluoride than of sodium bromide. This observation held true in mice regardless of whether the halogens were added to drinking water, introduced by subdermal injection or added to suspensions of cancer tissue prior to inoculation.

Fluoride when incorporated into the molecule of dimethylaminoazobenzene, a powerful carcinogenic enchanced its cancer-producing ability seven times as much as did other halogens (4).

With respect to observation on humans, in the fluorspar (CaF_2) mining community of St. Lawrence, Newfoundland, 21.8% of all employees and 36.2% of underground miners died of lung cancer during 1933-1961 (5). In addition to 62% fluorspar, the dust contained 19% quartz. Radiation, with an average alpha energy between 2.5 and 10 times higher than the suggested working level, was mairly implicated by investigators. The role of fluoride in producing cancer was not given the attention it warranted. During the latter part of the nineteenth century a similar situation has been reported from fluorspar mines of Joachimsthal and Schneeberg in Czechoslavakia and Germany (6).

In the environs of two Russian aluminum factories, Litvinov et al. (7) encountered a higher mortality from cancer than in a less contaminated control area, seven kilometers distant. In addition to hydrogen fluoride and 3,1,4-benzpyrene, other major pollutants were resinous substances and silicon dioxide. No evidence of excess radiation was reported in that area. A correlation was established between the rate of smoking and the susceptibility of the population to respiratory ailments. In 1970 in the industrial Rhein and Ruhr area of Germany, Hettche recorded (8) the highest number of deaths - 96 per 100,000 - in the cen-

ter of the industrial city of Essen, the lowest - 14 - in the residential area.

In this issue (page 172) Cecilioni established the fact that the mortality from lung cancer in the proximity of steel mills in the city of Hamilton, Ontario, is significantly higher than in outlying districts and is also higher than in Ontario and Canada as a whole. Cecilioni also demonstrated the existence of widespread contamination by fluoride in the environs of the steel plants. In the bones of six workers who had died of lung cancer he found 3 to 5 times higher fluoride levels than in bones of the controls. These levels exceed even those recorded in chronic skeletal fluorosis (9).

It is true the number of Cecilioni's cases is far too small to permit final conclusions. Similarly, the mere fact that contamination by fluoride is widespread near the steel mills does not necessarily indict fluoride as the only possible source of the excess mortality rate from lung cancer since numerous other cancer-producing agents emanate from the factories namely nickel carbonate, chromium, beryllium, silicates and certain organic agents (10). No data on the possible organic carcinogens which emanate from industry have been obtained from the area.

Nevertheless Cecilioni's observations must be considered significant. Even if fluoride were not solely responsible for the development of lung cancer in conjunction with other carcinogens it might act as a synergist. For instance, beryllium combined with fluoride has a much greater carcinogenic potency than either element exerts individually (11). Similarly the fluorine ion in radioactive cerium fluoride, which is known to produce cancer in mice, might conceivably be a potent synergist in the production of lung cancer (12).

Fluoride is also a constituent of asbestos at levels of the order of 70 to 579 ppm (13). Asbestos is recognized as a carcinogenic agent. Recently Duffey et al. (14) found in the bone marrow smear of fluoride-treated persons a certain kind of giant cell which had not been described previously and which they considered possible indicators of a malignancy.

As a practicing physician in Hamilton, the tools at Cecilioni's disposal to carry out extensive experimental or epidemiological research are relatively limited. Nevertheless his observations are valuable. They should be followed up by the use of a larger sampling and more sophisticated statistical methods.

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LUNG CANCER IN A STEEL CITY
ITS POSSIBLE RELATION TO FLUORIDE EMISSIONS

by

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Hamilton, Ontario

SUMMARY: During 1966 to 1968, 300 deaths from primary lung cancer occurred in the industrial steel city of Hamilton, Ontario. This represents an annual death rate of 34 per 100,000 population. A breakdown of the city into zones revealed three distinctly different rates. A direct relationship between the rate and the proximity to the main heavy industry section of the city was noted namely a high of 65 per 100,000 in the north-east end of the city close to the steel mills, a low of 12 in the section of the city most distant from the factories and 23 in the intermediate zone. The last-mentioned rate is about the same as that for the Province of Ontario and for Canada as a whole. A marked rise in steel production in Hamilton and a corresponding increase in the use of fluorspar flux occurred during the same period. Characteristic fluoride damage to vegetation was established especially in the north-eastern part of Hamilton. Analyses of vegetation, dust and human bones yielded high levels of fluoride, sulfur and silica.

One hundred years ago primary lung cancer (bronchogenic carcinoma), was practically unknown and 50 years ago, it was a rare disease. It is now one of the most frequent causes of death and heads the list of mortality from cancer (1). In cities the death rate from lung cancer is about twice as high as in the country (2).

Epidemiological studies on death rates from lung cancer suggest a close relationship between the incidence of cancer and steel making industrial centers such as the Midlands (England), the Ruhr Valley (Germany), La Plata City (Argentina) and some of the large steel-producing cities of the United States of America (3).

In a survey of deaths from lung cancer in the Ruhr and Rhine districts of Germany during the period 1960 to 1968, Hettche (4) established an "urban factor" in the prevalence of death from lung cancer on a local geographic basis. The highest mortality rate - 96 per 100,000 deaths - was found in the center of the industrial city of Essen; the lowest, 14, in the northern residen-

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From the Hamilton General Hospital, Hamilton, Ontario

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Presented at the Fourth Annual Conference of I. S. F. R., The Hague, 10/24-27/71.

tial part of the city. Iron and steel workers and professional motorists (truck, bus and taxi drivers) showed the highest incidence. Suspecting that airborne gases and dusts were responsible for reduction in life expectancy, Hettche suggested that similar studies in industrial cities be carried out based on a division into regions or zones according to the distance from the main sources of emissions.

Kotin (5) also linked the size of a city, especially if highly industrialized, with the incidence of lung cancer. He demonstrated that a broad spectrum of respiratory irritants, including polluted air and cigarette smoke, retard the flow of mucus from the bronchi and thus account for prolonged retention of these agents in the air passages. Despite a significant lack of data on the effect of hydrocarbons, the overall evidence "implicates the atmosphere as one dominant factor in the pathogenesis of lung cancer" (3).

The current study was prompted by the author's personal experience with a striking increase of lung cancer in general practice in the steel city of Hamilton, Ontario. In the late thirties and early forties, the author encountered approximately one patient with lung cancer every five or six years. This contrasts with three to five per year during the past five years. Most of these patients had been employees of the local steel companies.

Another observation suggested the initiation of the current study: The number of deaths from lung cancer in Hamilton over the past thirty years, as recorded in Vital Statistics for Ontario was much higher in Hamilton than in other communities of comparable size in the province of Ontario.

Method

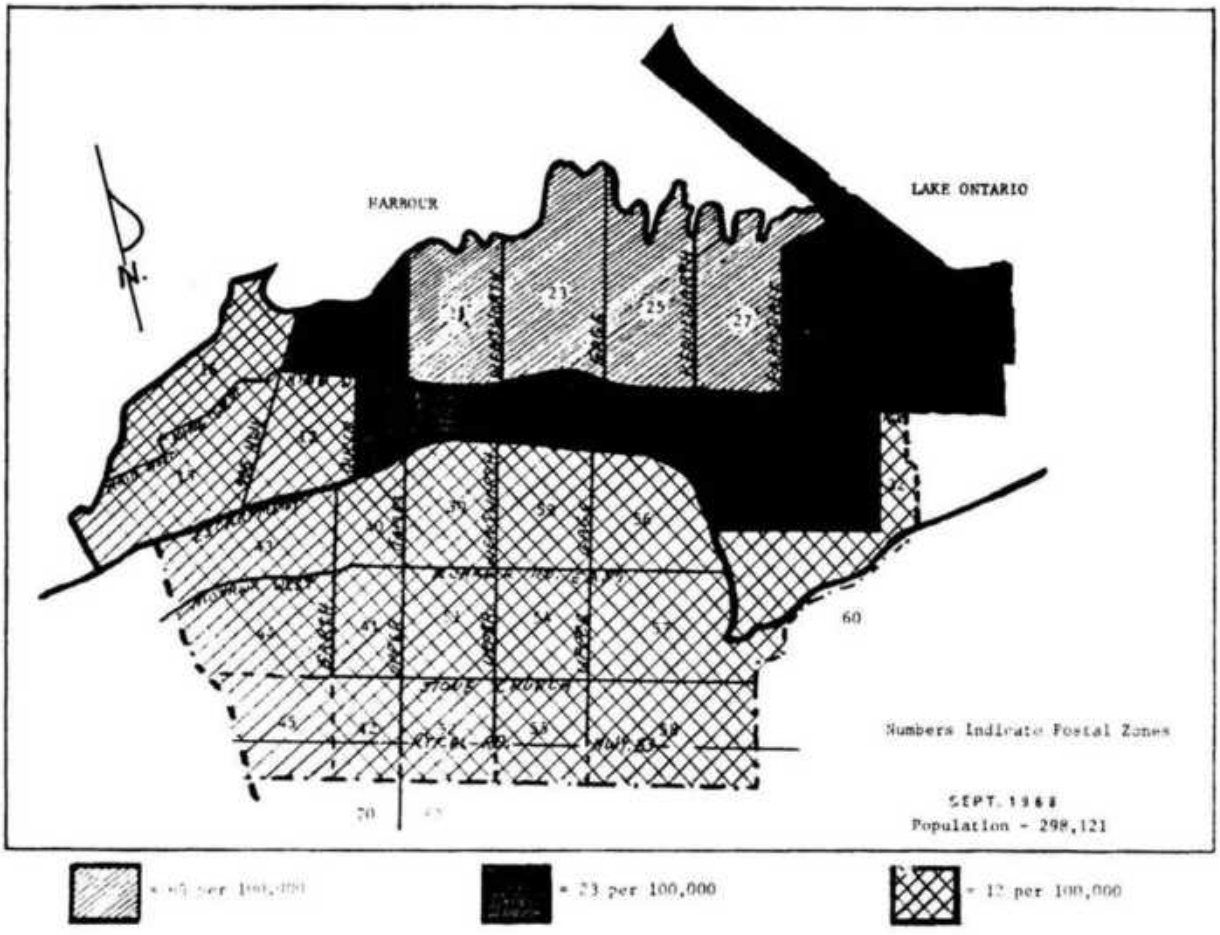
Three hundred* medical case histories of all deaths from lung cancer in Hamilton's four hospitals and the Cancer Clinic were reviewed for the years 1966, 1967 and 1968. The patients' places of residence and employment - both before, and at the time of the last admission to the hospital - their age, sex, nationality, country of origin and smoking habits were reviewed.

For the purpose of this study the city was divided into three areas as indicated in Fig. 1. The cases were allocated to the proper postal zone within the city according to their last place of residence.

*According to the Vital Statistics of the Province of Ontario, there were 286 deaths from primary lung cancer for the three years - fourteen less than the 300 covered in this study obtained from the medical records of Hamilton's four hospitals and the Cancer Clinic. This discrepancy is explained by the fact that the autopsy findings in some cases differed from the cause of death recorded on the death certificate, which is usually made out before the final results of the post-mortem examination are available.

Fig. 1

Three Sections of Hamilton, Ontario
Arranged According to Distance From Steel Plants



Results

A preponderance of the cases had worked and/or resided in the northeast section of the city, namely in Postal Zones 21, 23, 25 and 27. The mortality for lung cancer in this industrial area was 65 per 100,000 people. The distribution of the cases and the total number of deaths from lung cancer according to sex and age is presented in Table 1.

The four postal zones have a population of nearly 60,000 people or about 1/5 of the total population of the city. These zones accounted for 2/5 of the total number of deaths from lung cancer during the three year period. The

TABLE 1

Incidence of Lung Cancer in Hamilton, Ontario 1966-1968

Year	Number of cases		Sex	
	Total	per 100,000	male	female
1966	94	65	85	9
1967	111	71.8	103	8
1968	95	60	80	15

rate was only 12 per 100,000 in the south-western section of the city, and 23 per 100,000 in the intermediate area. The last-mentioned incidence is about the same as that for the Province of Ontario and for Canada as a whole whereas the incidence of 65 per 100,000 is equal to that of the midlands of England.

Most of the cases belonged to a low socio-economic level. The average age for males was 65.5, for females 60.5. A striking feature was the sex distribution of the 300 cases as outlined in Table 2.

TABLE 2

Sex Distribution of Lung Cancer Deaths
(ratio of male to female)

Hamilton	8.4: 1
North East end	14.7: 1
Other zones	7.3: 1

The incidence of the disease was inversely related to the proportion of women but no relationship was noted between the cancer ratio and the total cigarette consumption. The male/female ratio in the incidence of bronchial carcinoma varies widely in different parts of the world. It ranges from a low of 6% in the women of Holland and Finland, to a high of 51% in Nigeria (6). This wide variation could be due to differences in religious and social customs and in smoking habits. Racial differences may be involved as suggested by the fact that in most European countries the ratio is about 6:1 whereas in the Far East, Africa, South America and Iceland it is 2:1 or even less. This ratio has remained constant in spite of a dramatic rise in the total incidence of the disease.

It was apparent in this study that cigarette smoking is a significant factor in the cause of lung cancer. Scrutiny of the smoking habits of the cancer cases revealed that only 7% of the males and 22% of the females in the northeast section of Hamilton smoked less than their counterparts in the less polluted zones. A small percentage of non-smokers was found in the industrial

section.

Variations in individual susceptibility were exemplified among immigrants from such industrial countries as Great Britain and the central European countries among whom a much higher incidence of lung cancer was encountered than among Canadian-born individuals or other ethnic groups. This greater risk factor applies to English immigrants to New Zealand and to South Africa as reported by Eastcott (7) and Dean (8).

Histological examination of most of the lung cancers, or biopsies in the study revealed that nearly one half of the cases from the industrial area were of the small, undifferentiated "oat cell" type. This kind of cancer usually constitutes 20% or less of primary disease in male smokers. The more common epidermoid or squamous variety occurs in 40 to 50% of all lung cancer cases elsewhere (1). "Oat cell" cancers also predominate in St. Lawrence fluorspar mines (9).

Discussion

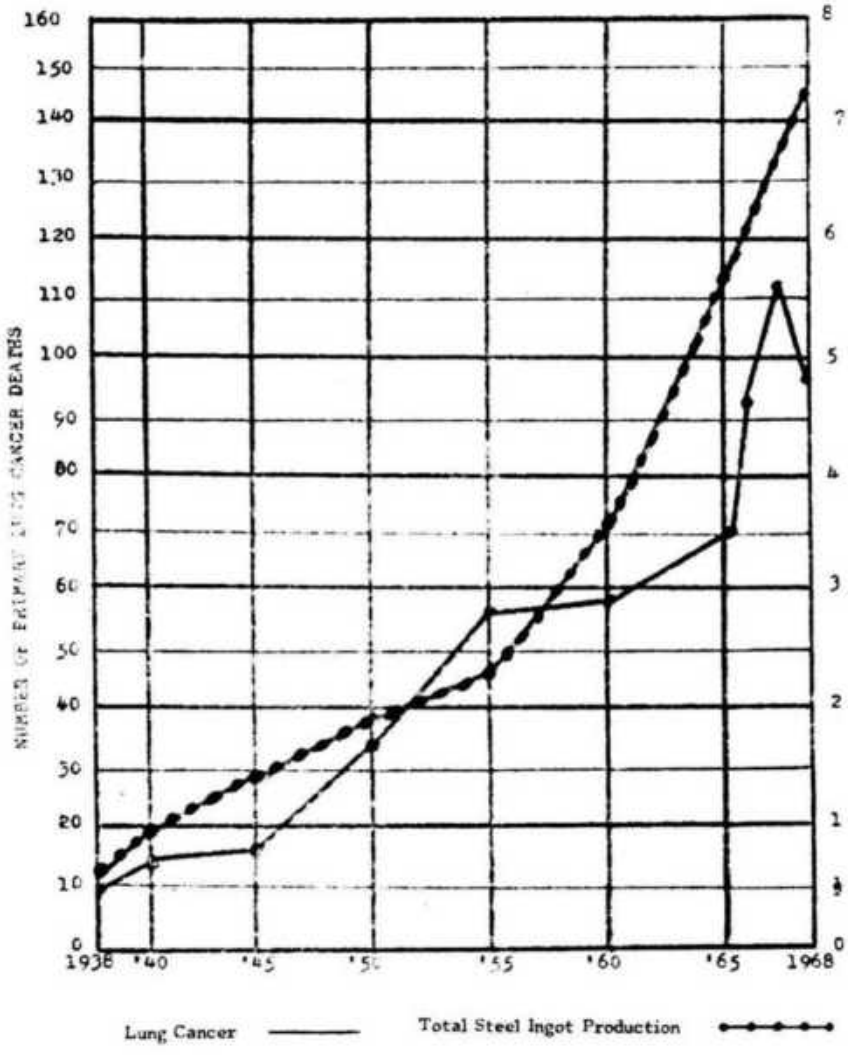
In the thirty year period from 1938 to 1968 the population of Hamilton has failed to double but iron and steel production has increased fifteen fold and the morbidity for lung cancer tenfold (Fig. 2). During the same period, the use of cigarettes and employment of fluorspar as a flux in steel making, have also risen sharply. In Hamilton the incidence of respiratory diseases increases during episodes of high air pollution, especially in the north-east industrial area in spite of the prevailing westerly and south-westerly winds. The incidence of chronic respiratory illness also increases during and after high pollution-peak levels in the industrial section of Hamilton where excess lung cancer was encountered.

The Polluted Area: Hamilton is not situated in a valley but an escarpment, with an elevation about 200 feet runs through the southern side of the city with water (Lake Erie, Hamilton Bay and Lake Ontario) along the northern side. This unusual topography accounts for frequent local weather inversions and smog which may last for several days at a time.

Emissions from the Steel Mills: With respect to the specific agent or agents which may account for the high cancer mortality, the gaseous emissions from iron melting in electric arc or electric induction furnaces emanate from two sources - the burning or vaporization of combustible materials which are in the charged raw materials, and the burning of the carbon electrodes and some of the charged metals during meltdown (10). Gaseous pollutants include carbon monoxide, carbon dioxide, nitrogen dioxide, sulphur dioxide, hydrogen fluoride. The major components of particulate emissions from the steel-making industry are iron oxide, silicon dioxide, oxides of magnesium, manganese, lead, aluminum, zinc, calcium, nickel carbonate (carbonyl) and combustibles. The magnesium oxide and iron oxide particles are less than one micron in diameter and are difficult to collect (10). They are not as readily removed from the upper respiratory tract as are the larger-sized particles.

Fig. 2

Lung Cancer Deaths and Ingot Steel Production
in Hamilton - 1938 to 1968



Sources of Lung Cancer

In the well known incidents at the fluorspar mines of Schneeberg and Joachimsthal (Germany and Czechoslavakia) during the latter part of the 19th century, 70% of the miners developed pneumoconiosis and subsequently died of lung cancer. Such irritants as cobalt, silicates, nickel, arsenic, alum, calcium, magnesium, chlorides and radium emanations were involved. In other cases of lung cancer, dust of all types including tar and exhaust fumes from motor vehicles and airplanes combined with chemically polluted air have been indicted (1).

Cigarette smoking has been established as a dominant cause of lung cancer even though the specific chemical or chemicals in tobacco smoke have not as yet been irrefutably identified. The risk of developing lung cancer increases among individuals with a history of chronic bronchitis, independent of cigarette smoking. A heavy cigarette smoker, who works and/or lives in a polluted industrial environment, has a greater chance of developing lung cancer than a non-smokers or a person residing in a non-polluted area (1).

Possible Role of Fluoride

Fluorspar (calcium fluoride - CaF_2),which is used as a fluxing agent in the production of steel, contains 48% fluorine when pure, less than 6% silica and 0.3% sulphur. In the basic open-hearth process of steel-making, this mineral is usually added at the rate of 5 to 8 pounds per ton of steel produced. However, when used in electric steel-smelting the consumption of fluorspar varies from 14 to 40 pounds per ton of steel produced (11). Thus, up to 30,000 tons of fluorspar may be used annually to produce over seven million net tons ingot (raw) steel.

In the steel-making process, fluorides appear in the atmosphere largely as hydrogen fluoride (HF) gas. Upon contact with moist air and with the moisture of the respiratory mucous membranes, HF immediately forms hydrofluoric acid. It has been demonstrated that gaseous hydrogen fluoride, one of the most common air pollutants is associated with several large-scale industrial processes. It has a mutagenic effect on plants and fruit flies (Drosophila Melanogaster) (12). Although a Royal Commission in 1968, and a survey by Villiers et al. (13) in 1971 implicated radiation as the major cause of lung cancer in St. Lawrence, Newfoundland miners, the effect of fluoride either as the major or a contributing cause has not been ruled out. Samples of dust taken from the mines revealed significant amounts of calcium fluoride, silicon, iron and magnesium, as well as traces of copper, aluminum, chromium, manganese, titanium, cobalt and barium. Some of these inorganic metallic dusts are potential carcinogens (14). Underground rock and ore samples contained nickel and beryllium, both of which have also been documented often as pulmonary carcinogens. When inhaled, hydrogen fluoride attacks the larynx and trachea giving rise to burning pain behind the sternum, to expectoration and even hemoptysis (15). The ultimate result is slow ulceration of the gums, nasal mucosa, larynx, bronchi and conjunctivae (15). Furthermore,fluoride interacts with most of the above-mentioned agents. Its synergistic effect with beryllium in the production of the malignant lung tumors has been demonstrated by Schepers (16).

Environmental Fluoride in Hamilton

The findings in this study point to a correlation between the emissions from steel mills and excess lung cancer. The levels of atmospheric fluorides monitored in Hamilton during April and May 1970, proved to be eight times those which are considered damaging to vegetation. At several stations in the industrial area, levels of more than 300 µg F/100 m per 30 days were found (17). More recent monitoring in 1971, has revealed even higher atmospheric levels for fluoride

TABLE 3

Fluoride Content of Dust and Vegetation

	Fluoride (ppm)
White Birch leaves [1]	33.0
Prune "	86.3
Apricot "	96.3
Norway Maple "	112
Swiss Chard " [2]	146
Gladiolus "	168; 109
Endive " [3]	269.0
Red Rose Tea 6 bags	91.0
Nestle's Instant Tea	371.0

Sulfur Content: [1] 2300 ppm, [2] 8500 ppm, [3] 9300 ppm
Analyses by Warf Institute, Inc., Madison, Wisconsin

TABLE 4

Fluoride Content of Bones

Lung Cancer Case		Fluoride ppm	Controls Case		Fluoride ppm	Causes of Death
1	Rib	1010	7	Rib	215	Coronary Occlusion and Emphysema with Cor Pulmonale*
	Vertebra	1882		Vertebrae	198	
2	Rib	1520				
	Vertebra	1690	8	Vertebra	256	Coronary Occlusion and Emphysema with Cor Pulmonale
3	Rib	1534				
4	Rib	948	9	Rib	477	Cancer of the Larynx*
5	Rib	1580				
6	Rib	1247				

*Had never worked in steel mill.

12,000 feet (3658 meters) from the emitting source (18) namely up to 74 ppb at ground level, and 91 ppb at an elevation of 350 to 400 feet (107 to 122 meters).

In the Hamilton area many samples of plants, leaves and garden vegetables were analyzed for their fluoride and sulphur content during the past three years. Typical fluoride damage was observed in most of the vegetation, some of which occurred several miles downwind from the main sources of pollution. Samples of garden vegetables showed concentrations of fluoride 20 to 30 times higher than the maximum permissable levels (Table 3).

Samples of particulate matter, 'Red Dust' (ferrous oxide) and clinker dust emitted from local blast furnaces, openhearths and a sintering plant contained iron oxide and other ingredients. Their total fluoride content reached levels as high as 863 ppm, their silicon content 5,000 ppm (0.5%) in one sample.

Samples of rib and vertebrae were analyzed (Table 4). The fluoride content normally ranged below 300 ppm of fluoride (19) (one sample of lung tissue contained only 0.57 ppm of fluoride and rib cartilage 7.2). Bone samples from six cancer patients averaged 3 to 5 times the normal amount of fluoride. At such levels skeletal fluorosis has been described by Soriano (20) and by Singh et al. (21).

Thus, there is sufficient evidence to show that not only vegetation and the atmosphere are contaminated by fluoride but that this contamination has made its impact upon the residents of the area. Further studies are in progress to determine whether or not the high intake of fluoride has contributed to the causation of lung cancer.

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HISTOLOGICAL CHANGES OF HUMAN FLUOROSIS, EXPERIMENTAL FLUOROSIS IN ANIMALS AND OSTEOPOROSIS FOLLOWING SODIUM FLUORIDE THERAPY

by

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SUMMARY: The histology of the bone changes in a case of severe industrial fluorosis and in other less advanced cases is described. The findings are compared with those in rats fed 10 and 20 mg NaF/kg/body weight daily during a 2 year period. After 2 to 4 months, new endosteal and periosteal bone showed incomplete calcification and irregular matrix. After 6 to 8 months the cortical bone became spongious and porotic. Nine individuals with osteoporosis received sodium fluoride treatment for 14 to 24 months. In 8, doses of 30 to 60 mg NaF per day produced a distinct increase in bone mass. The various phases in the development of the new bone are described. Endosteal apposition with transition of the newly formed fibrous bone into lamellar bone formation of osteophytes and thickening of the trabeculae of the spongiosa are demonstrated. Excessive resorption in the form of spongiosation of the corticalis and formation of atypical new bone was observed.

The histology of skeletal fluorosis in humans and animals has shown widely varying findings, especially in the latter. Whereas the histological changes of advanced human industrial and hydrofluorosis have been described (1-12) reports of the incipient stages are sparse. Two cases of incipient fluorosis (13) were complicated by osteoporosis of old age.

On the basis of an autopsy report and of iliac crest biopsies among patients with fluorosis, the histology of the early and advanced stages of skeletal fluorosis is presented. Franke et al. (14-18) have recorded the clinical, roentgenological, pathologic-anatomical and histological changes in aluminum factory workers as well as microanalytical determinations of fluoride in these bone specimens.*

*The pathology was evaluated by Prof. Coutelle, Director of the Pathology Institute, Martin-Luther-University, of Halle.

From the Orthopedic Hospital of the Martin-Luther-University.

* * * * * * * *

Presented at the Fourth Annual Conference of I. S. F. R., The Hague, 10/24-27/71.

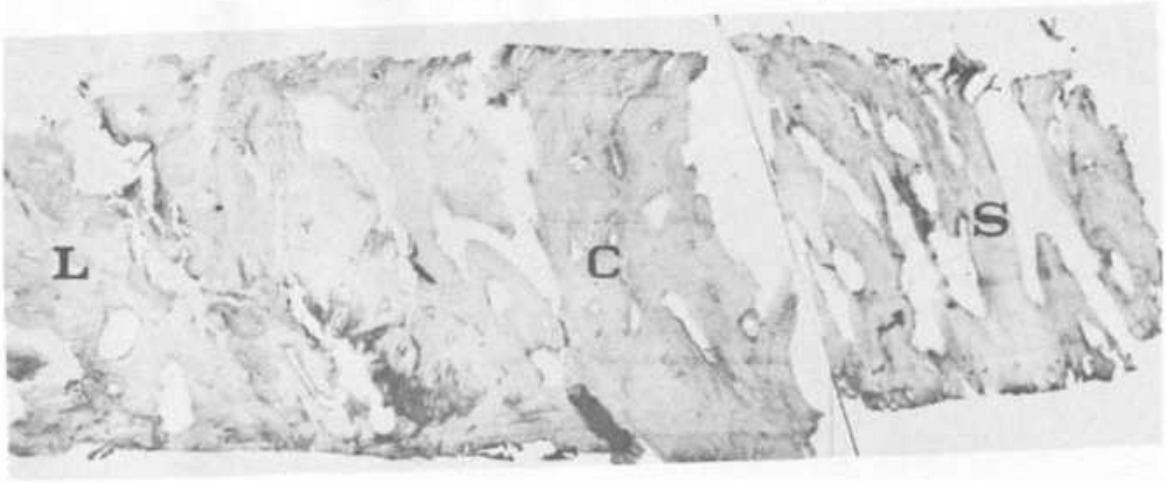
I. Human Fluorosis

Figure 1 presents the iliac crest of a patient with third degree skeletal fluorosis, according to the classification by Roholm (1) and by Fritz (13). The fluoride content of the ashed bone was 1.15% (11,500ppm). We recognize three layers namely, coarse spongy bone in the central portion, compact bone in the midsection and, at the outer part, the lamellar bone showing irregularly organized trabeculae. This specimen demonstrates two typical histological features of fluorosis: Coarse, highly hyperostotic, thickened spongiosa and subperiostial formation of osteophytes. The higher magnification illustrates particularly the subperiosteal irregularly formed trabeculae, foci of fibrous bone and of atypical bone apposition in the form of lamellae derived from defective matrix.

In 1970 we described, for the first time in skeletal fluorosis, foci in

Fig. 1

Iliac Crest; Fluorosis III (x5)



Bone: Coarse spongy (S); Compact (C); Lamellar (L)

the subperiosteal area which, upon staining with hematoxylin eosin, shew a characteristic bluish-gray color (Fig. 2). The fibrous structure of these foci is particularly coarse and appears to be independent of the surrounding osteones. No cavities of bone cells are recognizable in these areas. These lesions cannot be easily cut; they are PAS positive and exhibit a bluish color upon staining with alizarin blue. Such large foci were found only in one patient with first to second degree fluorosis; the bones in this case contained 0.87% (8700 ppm) fluoride (ashed) (Fig. 3). Besides the coarse lamellar structure and fibrous degeneration of the bone structure calcium deposits which we found in these areas became deep blue due to hematoxylin eosin stain. These areas are usually smaller in other cases.

Fig. 2

Same Case as in Fig. 1 Bluish Gray Focus (↑) (x 102)

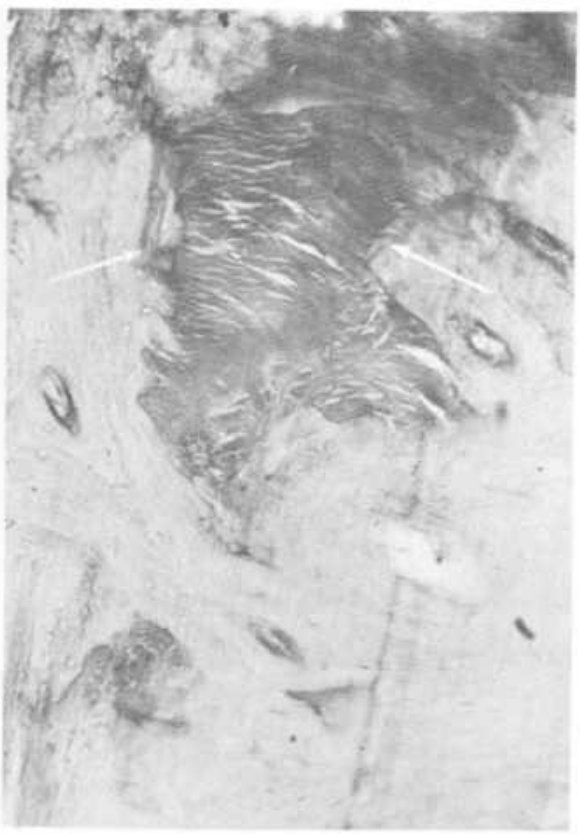


Fig. 3

Similar Fibrous Focus; Fluorosis I-II (x42)

The thickened sclerotic bone is especially distinct in a cross section of the skull (Fig. 4). Here the lamina externa is thickened and flows without demarcation into the diploë which consists of markedly thickened trabeculae. The lamina interna is well delineated. On both surfaces of the skull we find layers of subperiosteal, newly formed fibrous bone of varying thickness. Higher magnification (Fig. 5) reveals an irregular bone structure consisting of a mosaic of pagetoid cement lines with focal, bluish-gray bone defects, osteoid seams at the Haversian canals, enlarged canals, enlarged areas of osteocytes, lamellar bone transformed into fibrous bone and minute bluish-black granules. Similar findings were encountered at the compacta of the femur. Both specimens were derived from the first patient with fluorosis III who had expired following an automobile accident.

Another phenomenon of fluorosis is illustrated in Fig. 6 derived from a case of fluorosis II with a fluoride content of 0.77% in bone ash, namely the

Fig. 4

Cross Section of Skull;
Fluorosis III (x 12)

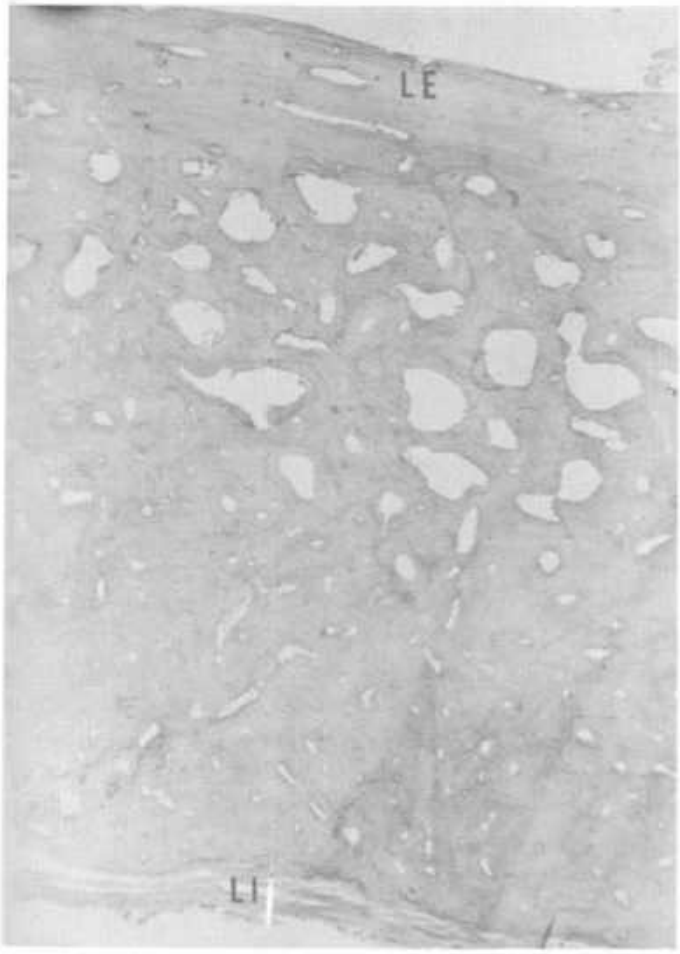
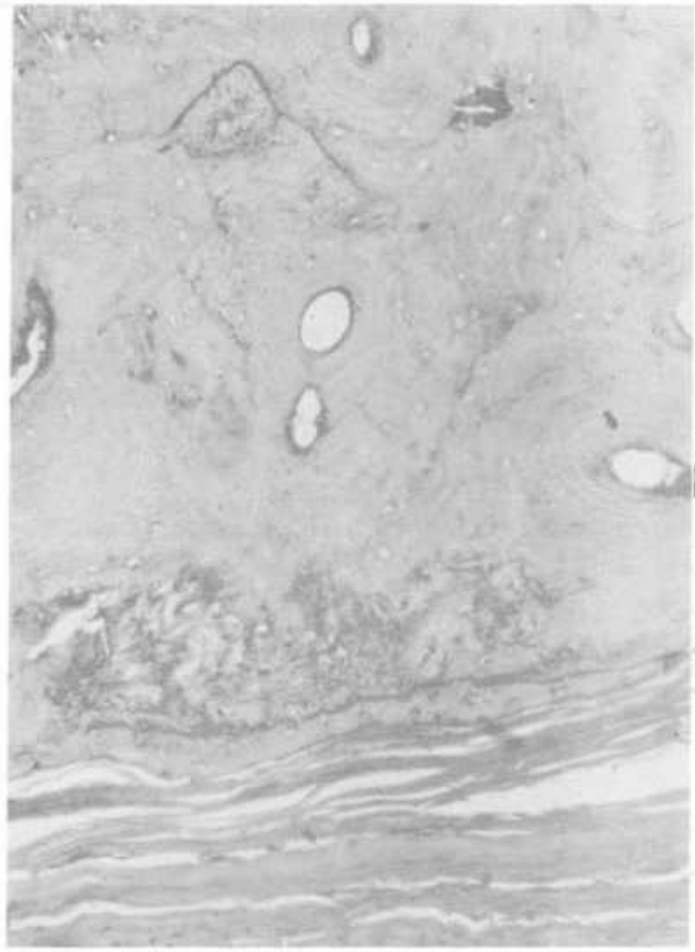


Fig. 5

Same as in Fig. 4 (x 120)



Lamellar Externa (LE)
Lamellar Interna (LI)

loosening up of the cortical layer by a large space containing bone marrow called the spongiosation of the cortical layer. The same findings were also encountered in a patient with fluorosis I whose bone ash contained 0.60% fluoride.

In only two specimens did we observe the granulae which are frequently reported in the literature. They had been decalcified by trichloracetic acid (Fig. 7). These patients had fluorosis I to II.

Fig. 6

Spongiosa of Iliac Crest
Fluorosis II (x 5)

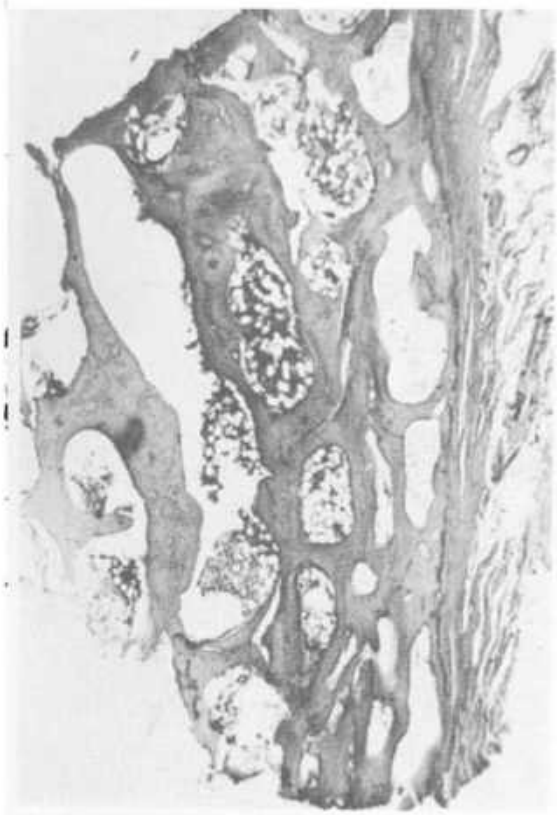
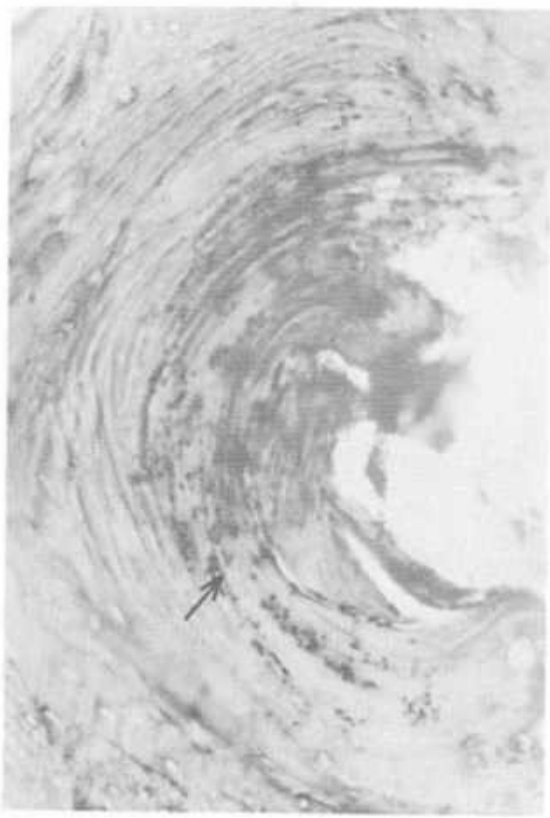


Fig. 7

Formation of Granules (↑):
Fluorosis I-II (x 163)



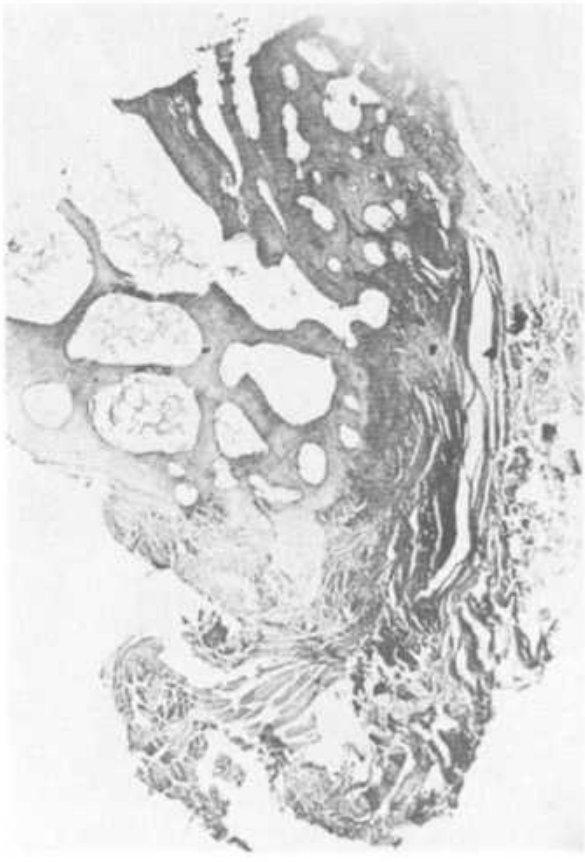
In cases exhibiting the earliest X-ray changes which are termed "Schwach" (faint) signs by Fritz we found the following:

1) The bone biopsy of a 32 year-old patient with X-ray evidence of incipient fluorosis and 0.43% fluoride in the bone ash (Fig. 8) revealed: Thickening and spongiosation of the cortical layer, thickening of the trabeculae of the spongiosa by lamellar bone. Atypical bluish-gray defects are noted in these areas.

2) In a 31 year-old patient with an identical type of fluorosis and practically the same fluoride content of the ash (0.41%) the corticalis is thin and the spongiosa delicate. However sites of subperiosteal bone also show new fibrous bone formation, again with replacement by lamellar bone (Fig. 8 and 9).

Fig. 8

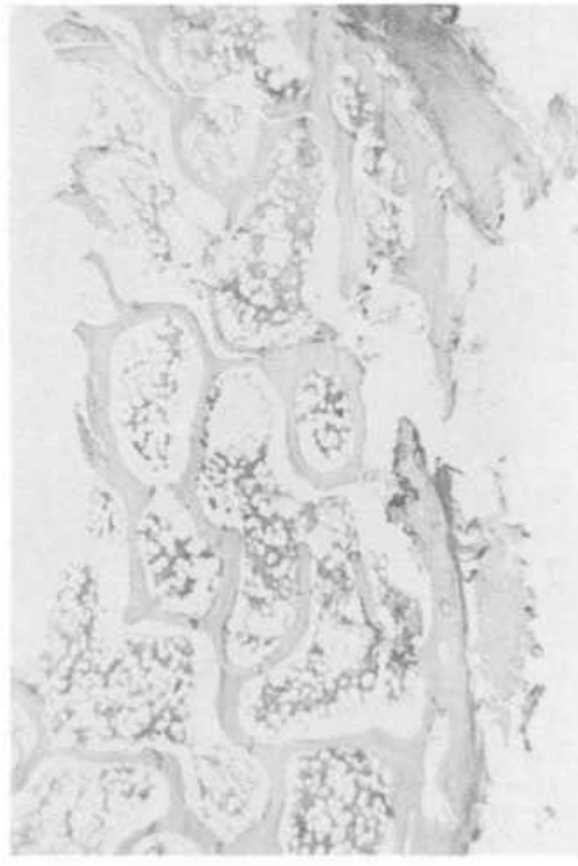
Iliac Crest; Fluorosis I (x 5)



Periosteal Bone Replaced by Lamellar Bone

Fig. 9

Iliac Crest;
Similar to That of Fig. 8 (x 5)



Thin Cortex; Delicate Spongiosa

II. Animal Experiments

Simultaneously with our studies on human skeletal fluorosis we carried out experiments on rats, the details of which were reported elsewhere (16, 17). Young and old albino rats were divided into four groups of 20 animals each. They received, in their drinking water, 10 and 20 milligram NaF daily per kilogram of body weight for as long as one year. Every second month, two animals of each group were sacrificed. Three animals were given the fluoride supplement for a period of two years.

After two months we found, among the old animals which had received 20 milligram, periosteal and occasionally endosteal newly formed bone (Fig. 10 and 11). The structural changes of the corticalis which occurred in the fourth month consisted of incomplete calcification, changes of fibrous bone into la-

Fig. 10

Lumbar Vertebra; Senile Rat (x 160)

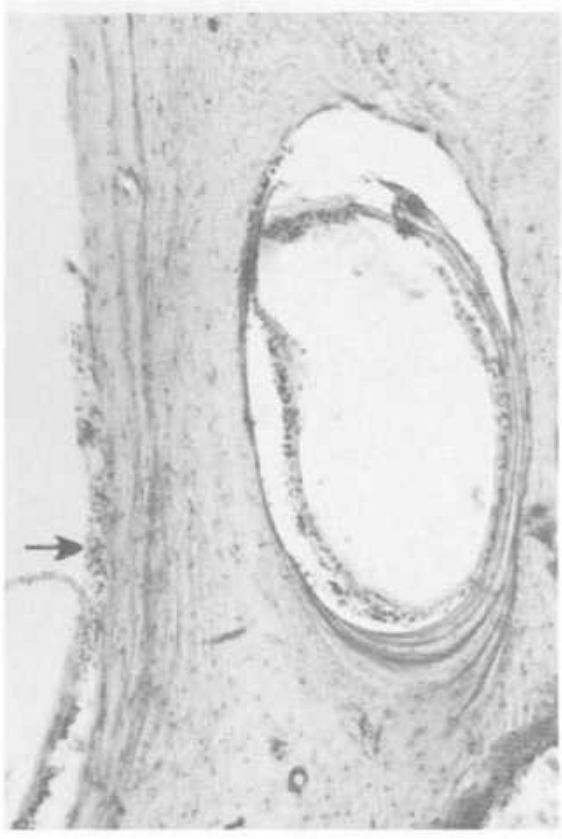


Fig. 11

Same Rat; Bone Apposition (↑) (x 160)

Periosteal Apposition of Bone After
20 mg NaF/kg/day for 3 Months

mellar bone and irregularity of the matrix. After six months, spongiosation of the corticalis took place. Thinning of the cortex occurred after eight months and of the trabeculae after 10 months (Fig. 12).

In the group which received ten milligram, the changes occurred two to four months later and were not as pronounced as in the 20 mg group. Excess calcification at the sites of muscle attachment at the ilium was seen in both groups.

Young animals showed widening of growth lines which was noticeable throughout the entire experimental period. Structurally, however, these zones did not appear to be much disturbed. The other changes were similar to those of the older animals. After 12 months, the bone ash contained 0.91% (9100 ppm) fluoride.

In reviewing the above data we found initially bone apposition of perios-

Fig. 12

Spongiosed Cortex of Femur of Young Rat; 20 mg NaF/kg for 12 months (x 40)

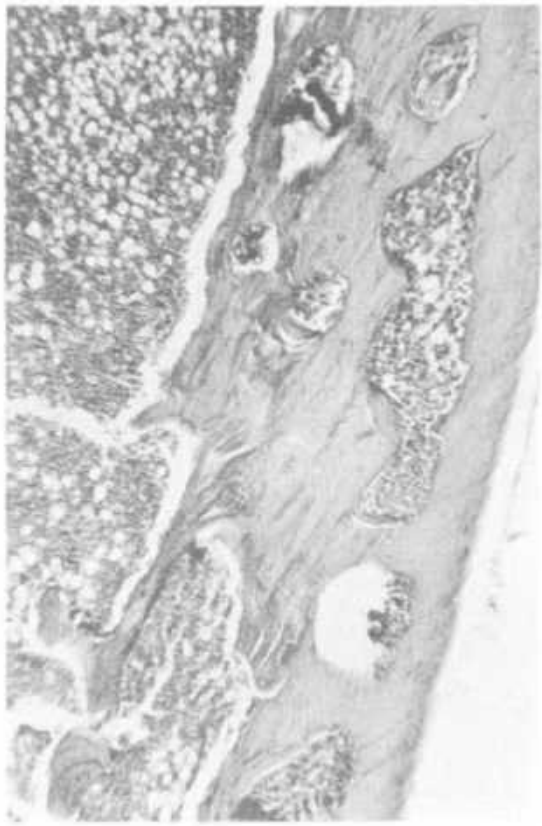


Fig. 13

Cortex of Lumbar Vertebra (x 160)

Three Layers of Cement Lines After Three Interruptions of NaF Ingestion During Two Years

teal and endosteal bone, beginning thickening of the spongiosa, increased formation of exostoses at the sites of the attachments of muscles and atypical bone structure. In a later stage of the disease, however, the clinical picture was dominated by resorption of bone. Since new bone apposition was very limited, the clinical findings of osteoporosis emerged. In general, the histological findings were very distinct, apparently because rats react less intensively to fluoride than do cattle, rabbits and dogs. Furthermore the alterations of the bones were not marked to such an extent that changes in the X-ray picture or in the fragility of the bone could be identified as measured by breaking strength.

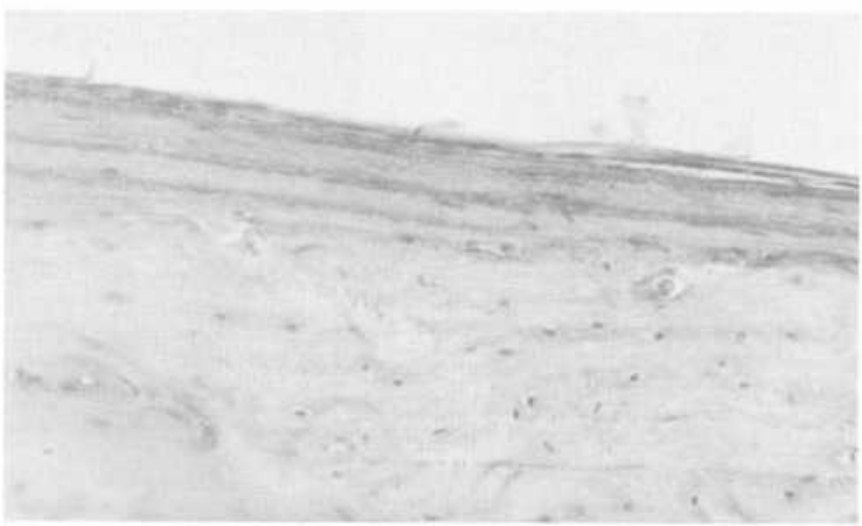
In the three rats, which were subjected to the experiment for more than 24 months, we instituted three intervals of four weeks each during the second experimental year when they were fed no fluoride. We believe that these intervals

without fluoride were reflected in the three layers of the cement lines in the corticalis of the femur and the vertebrae (Fig. 14). This finding corresponds to observations by Cohrs (19) who noted annual rings in bones of cattle which pastured during the summer months near a fluoride-emitting factory but received fluoride-free forage during the winter months. Ramberg and Olson (20) observed the same phenomenon in experimental fluorosis of cattle.

A second experiment under the same conditions which have been described above with considerably smaller doses - namely 1 milligram NaF per kilogram body weight - revealed, after four months, more pronounced apposition of both endosteal and periosteal bone (Fig. 14) without material disturbances of the bone structure.

Fig. 14

Femur of Rat; 1 mg NaF/kg/day
for 4 Months (x 320)



III. Sodium Fluoride Treatment In Osteoporosis

In 1971 Mattner and Franke (21) reported the results of sodium fluoride treatment in osteoporosis. Thirty-three patients with idiopathic osteoporosis and with osteoporosis due to prednisone received 20 to 60 mg NaF per day for 12 to 24 months. Twenty-four individuals i. e. more than 2/3 improved subjectively with this treatment; some became completely symptom-free. Four patients showed no changes; in five the pains became worse. In two patients the X-rays showed considerable remineralization. With the aid of a comparable X-ray densitometry an increase of bone density was noted in 15 of 17 patients.

Nine patients had biopsies of the iliac crest before and after 14 to 24 months of NaF therapy.

Eight patients exhibited a distinct increase of the bone mass in spite of relatively small doses of 20 to 60 milligram NaF per day (Fig. 15). Histomor-

Fig. 15

Iliac Crest in Osteoporosis (x 64)

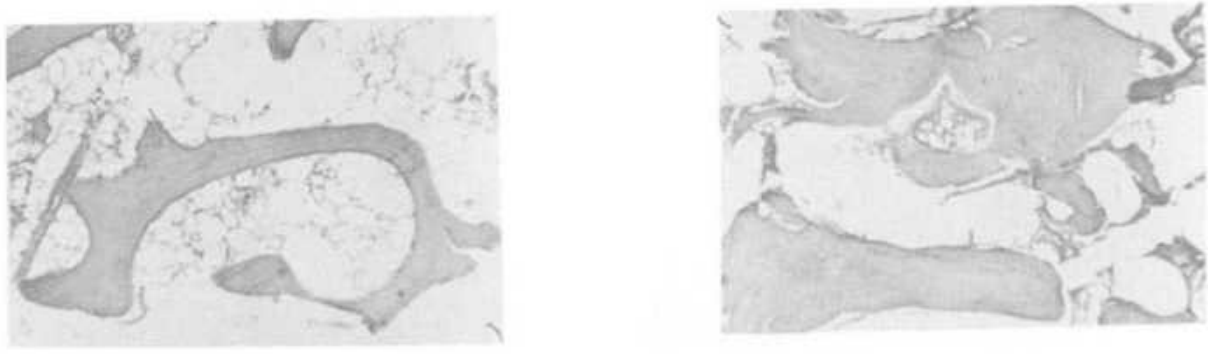


TABLE 1

Thickness and Density of Trabeculae After NaF Treatment

Patient	(mg NaF)	Duration (Months)	Thickness			Density		
			Before Treatment	After Treatment	Increased Thickness	Before Treatment	After Treatment	Increased Thickness
K. K.	40-60	24	98.0	168.5	+	30.4%	38.9%	+
K. Kh.	20-40	18	109.3	120.2	-	27.1%	34.3%	-
E. H.	20-30	18	89.8	135.7	+	23.5%	36.8%	+
H. P.	30-40	14	—	168.0	—	—	36.6%	—
F. E.	40	18	115.4	115.6	-	21.6%	29.5%	+
J. J.	20-60	14	119.0	179.5	+	26.2%	30.6%	-
G. R.	40-53	14	125.0.	171.7	+	26.1%	32.5%	+
E. B.	35-40	18	90.4	113.9	+	15.1%	25.0%	+
M. H.	30-40	15	98.6	98.9	-	25.8%	21.7%	-

phometrically we found an increase up to 50 to 70 μm by measuring the average thickness of the trabeculae. Furthermore, there was an increase of the bone mass up to 13% as measured by the point counting method. The increase in thickness and the bone density in all nine patients was significant in the T test namely 2 d for thickness $<10^{-10}$ and 2 d for density $<10^{-8}$. Subperiosteal new bone formation took place (Fig. 16) in the form of new fibrous bone with beginning alterationsinto lamellar bone and apposition of new bone at the formerly existing trabeculae (Fig. 17). The newly formed bone is atypical and irregular, especially in areas of the compacta. Figure 18 demonstrates, for instance, increased density of compacta with irregularly arranged osteones and with enlargement of osteocyte cavities. In between lies the osteoid. Enlarged osteoid seams and the so-called mottled bone (Fig. 19) noted by Johnson (22) were encountered less often. Following fluoride therapy the so-called spongiosation of the corticalis which

Fig. 16

Subperiosteal Fibrous Bone Formation (x 100)

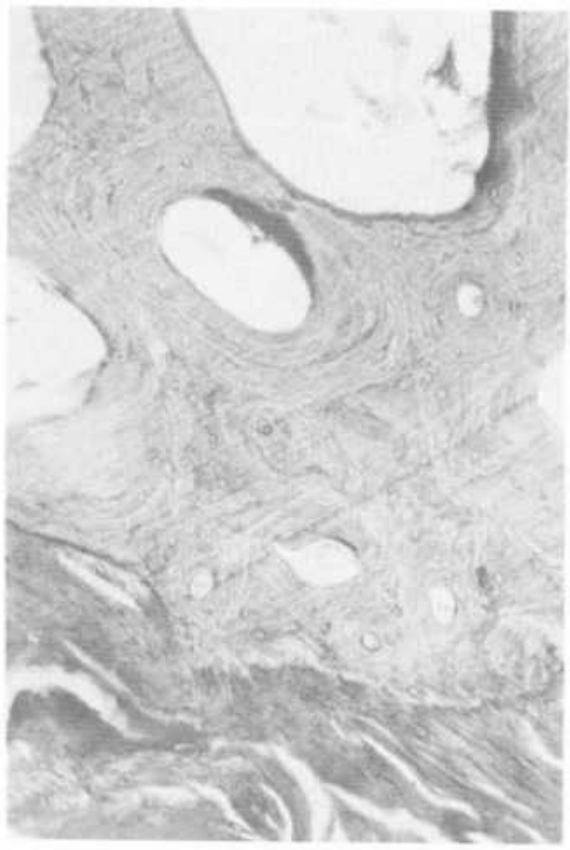
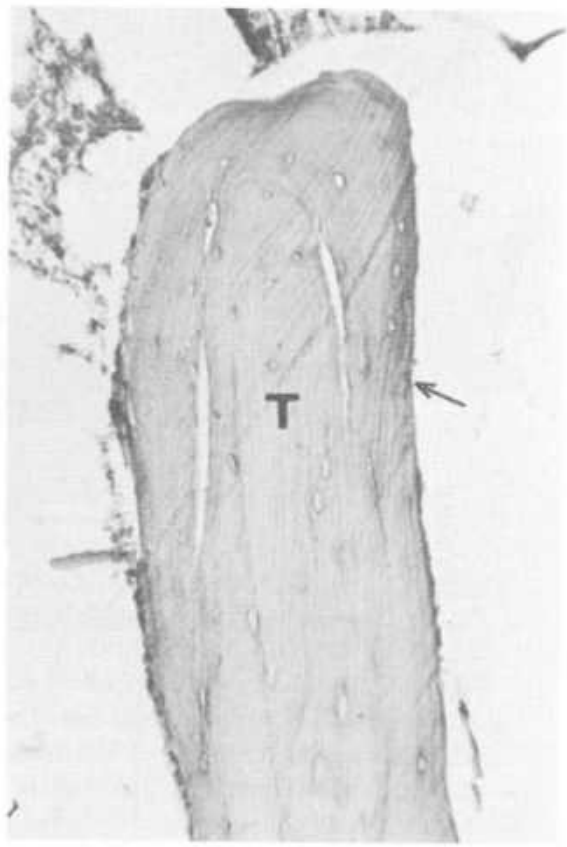


Fig. 17

New Bone Apposition (↑) at Trabecula (T) After NaF Treatment (x 160)



Beginning Lamellar Bone Formation After NaF Treatment

Fig. 18

Iliac Crest Thickened Compacta; Enlarged Osteocyte Cavities; Osteoid Between Osteons (x 100)

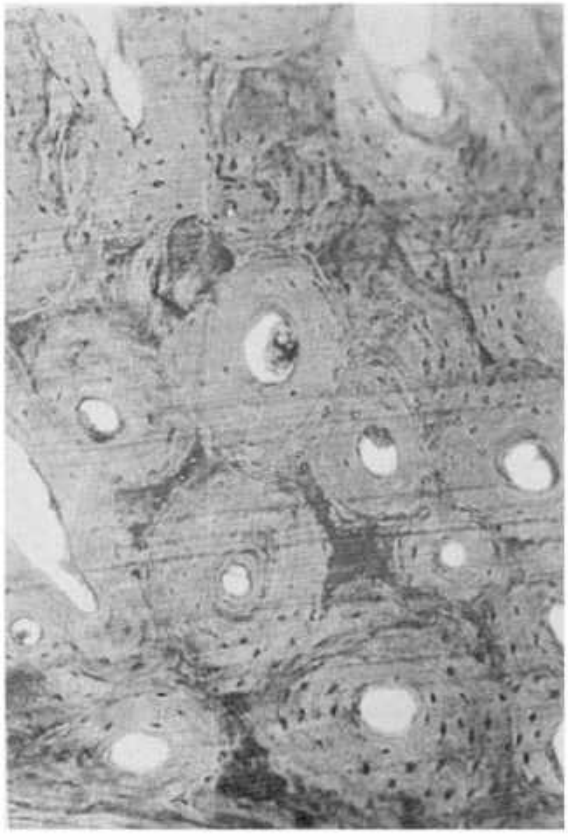
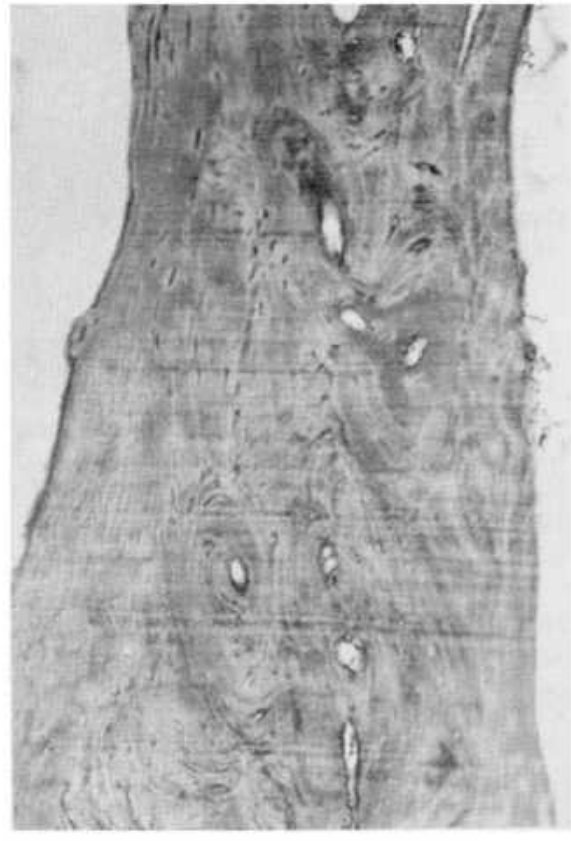


Fig. 19

Mottled Bone Following NaF Treatment (x 100)



we and other authors (23) had observed in humans and in experimental fluorosis were demonstrated (Fig. 20).

Here too, distinctly new bone formation was noted in the subperiosteal layer and in the spongiosa. However, these atypical alterations of newly-formed bones, especially formation of osteoid, were at no place as pronounced as had been demonstrated by other authors (24-33) who administered doses of 50 to 200 milligrams of NaF per day.

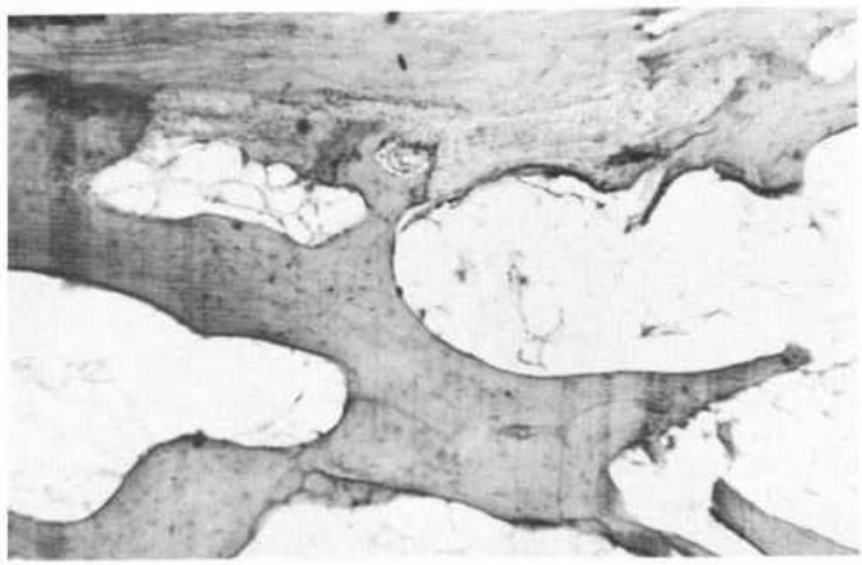
Discussion

As demonstrated by these illustrations three phenomena persistently occurred in the different types of fluorosis:

1) Increasingly new bone formation through periosteal and endosteal ap-

Fig. 20

Iliac Crest; Osteoporosis After NaF Treatment (x 64)



Spongiosation of Cortex Following NaF Therapy in Osteoporosis

position with alteration of the newly formed fibrous bone into lamellar bone, with formation of osteophytes and thickening of the trabeculae of the spongiosa.

2) Excessive resorption in the form of spongiosation of the corticalis,

3) Formation of new bone which is frequently atypical.

These three phenomena, however, occur in varying degrees. Presumably such factors as dose, duration of fluoride intake and individual reactivity play an important role.

The action of fluoride upon the bone can be visualized as follows:

1) Fluoride stimulates the formation of osteoblasts (34, 30, 25, 26). Indeed we could even consider fluoride a "whip" for osteoblasts. Small doses lead mainly to newly formed bone, larger ones to disturbance of the function of osteoblasts probably because fluoride interferes with certain enzymes. Small doses also lead to formation of irregular and poorly calcified matrix. It is possible that the calcification cannot keep pace with the excess production of matrix. For instance, the greatest disturbances in newly formed bone were demonstrated in the first case of fluorosis III where the histology showed a completely irregular bone.

On the other hand, among our patients with osteoporosis who received doses as low as 20 to 60 milligrams NaF per day we observed relatively little osteoid. This is also borne out by recent observations of Schenk et al. (35) who

recorded in one patient a reduction of the number of osteoids following lowering of the doses. Furthermore, the results of our first and second experiments with rats with the low dose of 1 mg NaF/kg body weight/day supports this fact.

Other authors (36-45) have emphasized the significance of the size of the doses.

2) Simultaneously, direct stimulation of the osteoclasts seems to occur (35). In our animal experiments the resorption (spongiosation) of the compacta was particularly striking with the larger doses.

The activity of osteoclasts, however, hardly reaches the level of that of osteoblasts since the physical and chemical alterations of the bone crystals through formation of fluoroapetite (46-49) block the activity of the osteoblasts. Rich (44-45) also considers the possibility of blockage of osteoclastic activity by high concentrations of fluoride at the areas of resorption.

3) This inhibition of resorption through disturbance of the calcium homeostasis is likely to lead to a stimulation of the parathyroid glands and, therefore, to a limited degree of secondary hyperparathyroidism. Increase of parathyroid hormone during fluoride therapy (25) hyperplasia of the parathyroid glands at post-morten and surgery following NaF therapy (25, 32) and in animal experiment (41) indicate that such stimulation of the parathyroid glands occurs. The elevation of serum calcium levels in the blood in our fluoride-treated individuals also point up the role played by the parathyroid glands, a phenomenon which has not been clearly established in the past. In our experiments with rats we observed, by means of the point-counting method, a significant increase in the mass of cell nuclei of the parathyroid glands as compared with those of protoplasm.

4) Another, heretofore little observed, factor is the individual susceptibility of the patient as indicated by the following observations:

a) of two aluminum workers employed for 15 to 20 years in the same place, one developed fluorosis III, the other showed only slight incipient changes,

b) Spongiosation of the compacta is not noticeable on all individuals histologically,

c) In certain individuals, NaF therapy failed completely to bring about the slightest improvement,

d) In our experiments the reaction of each animal was different to the same doses.

Further studies are needed concerning the role of nutritional habits and the varying thresholds of absorption and excretion, especially that of the kidney.

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See Discussion on next page.

Discussion

Dr. Teotia: We have made many tests on human bones with fluorosis. Our consistent findings were disorders of the compact bone, thickening of the cortex with irregular Haversian canals, with osteocytes and irregular bone formation. Newly formed bone after NaF treatment differs materially from normal bone when vitamins, calcium and other bone building minerals are not administered simultaneously. In our experiments, treatment with NaF in doses of 30 to 60 mg daily for 14 to 24 months did not correct osteoporosis.

Dr. Franke: Our patients with osteoporosis showed an increase in bone formation in both the spongy and the compact areas. Although irregular and atypical, it was strong and functional.

Dr. Jolly: I attended a symposium on osteoporosis in Switzerland recently. Dr. Clayton Rich of Seattle, U.S.A., originator of fluoride treatment for osteoporosis was present. He too, is no longer optimistic about administrating fluoride in these cases. Along with osteoporosis and osteosclerosis, osteomalacia can result from this type of treatment. Both of us felt that the new bone appears osteosclerotic and that it is not strong under stress. Regardless of whether or not fluoride is combined with calcium, we do not know what the long-term results will be.

Dr. Franke: The treatment of osteoporosis is still in the experimental stage. Basically I agree that, when one sees the histological changes, one tends to doubt the efficacy of fluoride treatment. However, our low dosage and the administration of fluoride in powder form does not appear to produce much osteomalacia. We experimented with rats, using 10-20 mg NaF per kilo bodyweight (40 mg killed the rats). Their bones, especially the femurs, had good flexibility and tensile strength. In a severe case of industrial fluorosis, an iliac crest biopsy revealed a cortex so thick that the tip of the canula broke off upon attempting to do a sternal puncture. In a man with fluorosis III and in three control individuals, we determined the resistance of the body of the first lumbar vertebra to pressure. We found a threefold increase of the resistance in the fluorosis case.

Dr. Teotia: But these treated patients still have osteomalacia, do they not? Our fluorotic patients showed an increase in serum alkaline phosphatase levels usually from 12 to 17 K/A units. We have not found any changes in their calcium balance.

Dr. Franke: The presence of pronounced osteoid formation is different when this treatment is used for humans than in experimental animals. Our serum alkaline phosphatase levels were normal, ranging from 3 to 8 K/A units compared to 3 to 12 K/A units in the control group.

Dr. Jolly: Calcium balance is often below normal i.e. Ca is excreted in excess in the urine and faeces. I am surprised that you found no change in magnesium content. The magnesium balance does not return to normal in our cases because they live in an endemic area. Excessive excretion of magnesium may be related to the increased excretion of calcium.

THE EFFECT OF SODIUM FLUORIDE (NaF) ON THE RUMEN DIGESTION IN CATTLE

by

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SUMMARY: 1) Infusoria of the rumen show significant damage in vitro when sodium fluoride in doses of the order of 80 ppm is added to rumen juice for a period of 6 hours. Daily administration of 6 mg of fluoride per kg of body weight to a cow also leads to a marked reduction in the activity of the infusoria. 2) The fermentation of the rumen juice in vitro decreases significantly when fluoride in doses of more than 240 ppm is administered. 3) Long-term administration of fluoride does not cause fluoride to accumulate in the rumen. 4) The pattern of the fatty acid levels (acetic, propionic and butyric acids) in rumen is not affected when doses are less than 4 mg fluoride per kg of body weight. A significant decrease in total production of fatty acids might be related to a reduction of food uptake.

A large body of published data are concerned with fluoride's effect on the skeleton of domestic animals and with production of meat and milk. Studies concerning the influence of fluoride on the rumen, however, are sparse. This is surprising since the rumen is generally considered a most important "chemical factory" where fodder is prepared for subsequent utilization. Therefore we undertook the study of various parameters which are concerned with the digestion of fodder.

We investigated the action of fluoride upon infusoria and upon the production of the three most important volatile fatty acids namely acetic acid, propionic acid and butyric acid, the ability of the rumen juice to ferment, the pH, and the fluoride content of the rumen juice during persistent administration.

Since rumen digestion depends largely upon the composition of the fodder, results cannot be utilized generally but pertain solely to animals subjected to a well-defined method of feeding. In our case, the fodder consisting of cultivated hay available to the animals ad libitum was combined with 2 kilograms of a concentrated mixture with a digestable protein content of 17.9% and of 120 grams of mixed minerals. To the experimental animals, cows from the Simmentaler

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From the Veterinary Medicine Clinic of the University of Zurich, Switzerland

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Presented at the Fourth Annual Conference of I. S. F. R., The Hague, 10/24-27/71.

breed, fluoride in the form of NaF was administered once daily through a fistula in the rumen. In order to study the survival time of the infusoria and the fermentation activity, we added fluoride directly to the freshly obtained rumen juice and studied the changes after six hours.

1. Rumen Infusoria

Freshly gathered rumen juice of butchered cattle was placed immediately after slaughtering into a thermos bottle with a temperature of 38°C and transmitted in the laboratory into a 30 milliliter crucible. The specimens were kept at a constant temperature of 38°C in a water bath which was provided with a shaking device. Fluoride was added prior to the beginning of the experiment which lasted 16 hours. The infusoria were counted by the Usuelli Method (1).

Our experiment showed that upon addition of 40 ppm no visible damage to the infusoria occurred after 16 hours. Doubling the dose to 80 ppm resulted in a distinct reduction of their activity. Higher doses killed all infusoria.

TABLE 1

Viability of Infusoria After Addition of F^- to Rumen Juice

Infusoria Tested	Butschlia	Isotricha	Entodinium	Diplodinium	Ophryoscolex	Miscellaneous	pH
control	very active	very active	very active	very active	very active	very few killed	6.7
40 mg F/liter	very active	very active	very active	very active	very active	somewhat more killed	6.7
80 mg F/liter	active	active	active	slightly active	slightly active	very many killed	6.7
240 mg F/liter	killed	slightly active	killed	killed	killed	many killed	6.7
400 mg F/liter	killed	slightly active	killed	killed	killed	many killed	6.7
600 mg F/liter	killed	killed	killed	killed	killed	all killed	6.7
800 mg F/liter	killed	killed	killed	killed	killed	all killed	6.7

In a second experiment two animals received NaF over an extended period of time and the infusoria per milliliter of rumen juice were studied. No reduction of activity was noted up to a dose of 3 mg/kg body weight per day. Upon increasing the dose to 6 mg/kg body weight per day a distinct reduction of their activity occurred. Higher doses killed all infusoria.

In a second experiment two animals received NaF over an extended period of time and the infusoria per milliliter of rumen juice were studied. No reduction of activity was noted up to a dose of 3 mg/kg body weight per day. Upon increasing the dose to 6 mg/kg body weight per day a distinct decline ensued. Substantial regeneration had occurred within 4 days of discontinuance of fluoride.

2. Fermentation

Further insight into the activity of the rumen was provided through observation of the fermentation. This parameter embraced not only the biological activity of the infusoria but also that of bacteria. We measured the activity of the fermentation of the rumen juice according to the method of Schumacher (2). The volume of gas produced by the fermentation was determined with an open manometer. These assays yielded reproducible values only during the first six hours (3) because with this arrangement neither the addition of fresh rumen juice nor the elimination of the metabolic products is possible. Our studies revealed that a significant decrease of the fermentation took place only following addition of 240 ppm of fluoride. At 800 ppm the decrease amounted to only about 70% of the control values. The limited action of the fluoride ion upon the fermentation can probably be attributed to adsorption of the products of fermentation (4) to organic ingredients or to the mineral salts, especially calcium.

These experiments permit the conclusion that the supplementation of fluoride does not induce an immediate inactivation of ferments as, for instance, magnesium or manganese enolase which, according to Warburg and Christian (5), play an important role as fermentation-inducing enzymes.

3. Volatile Fatty Acids

The determination of the short chain volatile fatty acids, namely acetic acid (C_2) propionic acid (C_3) and butyric acid (C_4) the three most important fatty acids formed during fermentation in the rumen, provide without doubt an evaluation of the production of energy. We used an 8 year-old Simmentaler cow as an experimental animal. The experiment was carried out according to the method of Prabucki (6). The first specimen from the rumen fistula obtained at 7 A.M. was followed by six additional ones at two hour intervals up to 5 P.M. For each specimen we determined the pH at 5 different areas and recorded the mean. Simultaneously we obtained rumen juice in a closed system at four different areas by means of suction equipment daily throughout the whole experiment. The fluoride content of the rumen juice was determined with an ion-specific electrode in acetate buffer.*

Since it was not possible to determine quantitatively the total a-

*Furnished by Möller Brothers, Inc., Zurich.

mounts of the above acid produced, only their concentrations were assayed. According to the available literature, a remarkable parallelism occurs between the concentration of the individual short-chain fatty acids and their quantitative production (6). In general, during the first two hours following feeding the production of fatty acids rises distinctly. During the next four hours production slows down and towards evening it declines further.

Determination of the fatty acids following preliminary experiments, extending over three weeks without administration of fluoride, the following doses were administered:

1st	and	2nd week	2 mg F/kg
3rd	and	4th week	3 mg F/kg
5th	and	6th week	4 mg F/kg
7th	and	8th week	no F
9th	-	11th week	4 mg F/kg

Effects of Fluoride on the Experimental Animal

Up to the end of the 5th experimental week no recognizable disturbances were noted. During the 6th week the animal exhibited a marked loss of appetite. During the feeding period the animal refrained from eating and the content of the rumen at the end of the 6th week was distinctly abnormal. Following two weeks' interruption of the experiment 4 mg F/kg body weight was again administered. After three weeks the experiment had to be discontinued because the animal was visibly ill and distinctly emaciated.

Results

1. Fluoride Concentration of the Rumen: As indicated before, the fluoride determination was carried out with the ion-specific electrode in rumen juice which was acetate buffered. The fluoride concentration increased markedly following the administration of fluoride; it reached its maximum in two hours and then it decreased gradually.

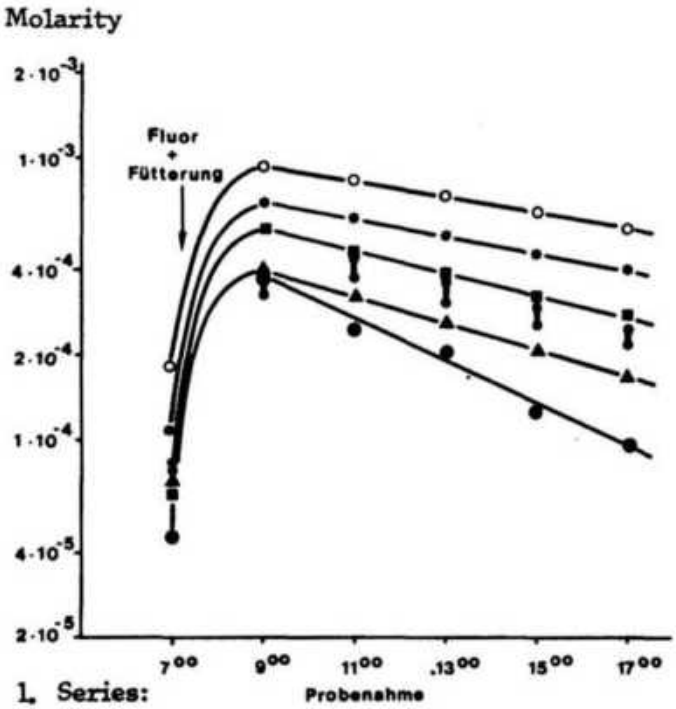
Figure 1 shows that the base-line values become higher in relation to the doses of fluoride; in no case however was there any accumulation of fluoride at the entry of the rumen. This finding indicates that the digestion is little affected if the doses are not too high.

A daily dose of 2 and 3 mg/kg bodyweight caused the fluoride concentration after two hours to average $3.8 \cdot 10^{-4}$ and $4.0 \cdot 10^{-4}$ Mole (i. e. 7.2 and 7.6 ppm F) respectively. At five P. M. only $1 \cdot 10^{-4}$ Mol and $1.65 \cdot 10^{-4}$ Mol (1.9 and 3.1 ppm F) had been retained.

With an intake of 4 mg/kg bodyweight the maximum fluoride concentration in the rumen after two hours in the first week amounted to $5.6 \cdot 10^{-4}$ Mol (10.6 ppm F) and at 5 P. M. $2.8 \cdot 10^{-4}$ Mol (5.3 ppm F).

Fig. 1

Molar Fluoride Concentration in Rumen Juice



1. Series:

● 2 mg F⁻/kg Body Weight
▲ 3 mg " " "
✱ 4 mg " " "
■ 4 mg " " "
○ 4 mg " " "

Mean Value
1. Week
2. Week

2. Series:

4 mg F⁻/kg Body Weight 2. and 3. Week

During the second week of the first series the values increased further with a maximum of $9 \cdot 10^{-4}$ Mol (17.1 ppm F). This distinct rise in the F concentration is probably not caused by actual accumulation but is likely to be due to the fact that, with daily fluoride doses of the same magnitude, the food content of the rumen became smaller. This opinion was supported by the results of the second series of experiments after fluoride administration had been discontinued for two weeks. During this period food uptake rose considerably but, in spite of the renewed administration of 4 mg/kg bodyweight, the values of the first experimental series were not attained. They averaged only between 2 to $4 \cdot 10^{-4}$ Mol.

pH Values of Rumen Content as Related to the Monocarbon Acid Concentration

The relationship between pH and the total concentration of fatty acids was determined by a regression curve. According to Beghelli et al. (7) the pH value of the rumen content is affected by the presence of gases released by fermentation, particularly carbon dioxide. Therefore we carried out the pH determinations immediately following the opening of the fistula. For these assays we employed especially manufactured combined glass electrode* one meter in length. The measurements were carried out at five different points and different depths of the rumen content. The individual values were distributed up to 0.08 pH units around the mean value. The following regression equation resulted from these measurements:

$$pH = 7.094 - 0.0743 \cdot mMol\% \text{ Fatty Acid}$$
$$r = -0.4968; \quad (P \; 0.01)$$

Therefore when the concentration of the volatile fatty acids increased by 1 mMol per 100 ml rumen juice, a decrease of the pH value by 0.0743 units occurred.

Determination of the Volatile Carbonic Acid in Rumen Juice

The following is the procedure carried out for the determination of carbonic, acetic, propionic and butyric acids.

Collection of the specimen with simultaneous pH determination of the rumen.

↓

Rough-filtration followed by centrifuging with 18,000 g for 20 minutes

↓

- - - - → determination of fluoride by means of ion-specific electrode

↓

Addition of 1/5 volume, 25% metaphosphoric acid in 5n H_2SO_4.

↓

After 2 to 3 minutes centrifuging with 5000 Upm

↓

Gas-Chromatography Liquid at 0.5% carbowax, 20 M and 0.5% terephtal acid in Porapak Q 80/100 mesh, 6 ft. 1/8" glass cylinder, T_c=180 C., 60 ml N_2/min. The model: Beckman GC 5 with Double-FID, Electronic Integration: Infotronics CRS 104.

The quantitative titration of the 3 acids monocarbonic acetic (C_2:0), Propionic (C_3:0) and Butyric (C_4:0) resulted under consideration of the widely varying FID- responses with the respective molar titration solutions. Other acids such as valerianic (C_5: 0) and iso-valerianic (C_5:0) were found only in traces.

*Furnished by Möller Brothers, Inc., Zurich.

Fig. 2

Gaschromatographic Separation of Carbonic Acids
C 2:0 to C 5:0

Test Mixture; 12 mMol % C 2:0, 4 mMol % C 3:0, 3 mMol % C:4, 0,5 mMol % C:5 u. 0.5 mMol % C:5 iso. (total 20 mMol %)

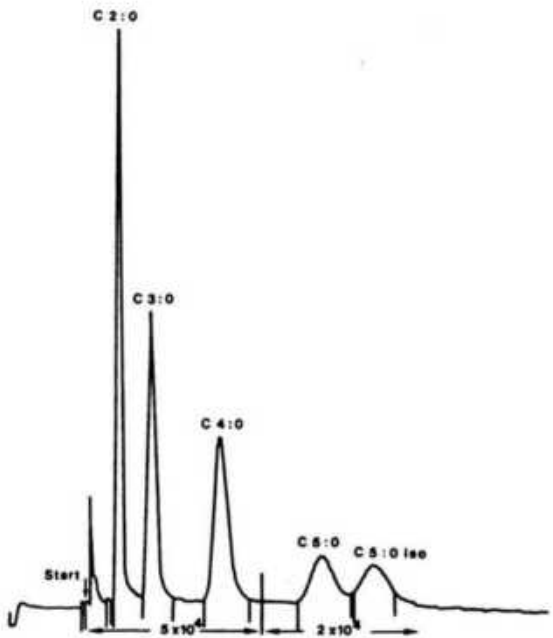


Fig. 3

Gaschromatographic Determination of
Carbonic Acids in Rumen Juice

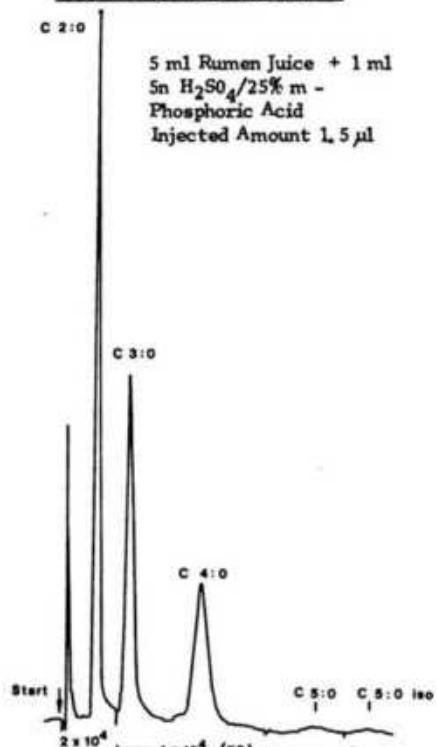


Figure 2 shows the chromatogram of a titration solution, figure 3 the gas chromatographic determination of carbonic acids in the rumen juice.

Total Fatty Acid Concentrations in the Rumen Juice

Without fluoride the average total fatty acid concentrations (the combined amounts of the three fatty acids) amounted to 9.571 mMol%; when doses of fluoride were 2 and 3 mg F/kg bodyweight, to 9.02. With doses of 4 mg F/kg bodyweight the average total fatty concentrations amounted to 8.86 mMol% during the first week; however, only to 2.06 mMol% during the second week. The difference compared with the baseline values was statistically significant.

Following the two weeks' recovery period, the values declined in spite of renewed administration of 4 mg F/kg bodyweight. In the first and second week they were 9.36 mMol% and 9.05 mMol% respectively. A further decline took place during the third week to 7.047 mMol% (statistical probability: 99%).

The Composition of the Three Fatty Acids

No significant change in the percentage distribution of the three individual fatty acids could be established during the term of fluoride administration. Acetic acid constituted the largest portion with 72.98% $\pm$ 2.53 and 72.16% $\pm$ 1.86. This was followed by propionic acid with 18.36% $\pm$ 1.75 and 18.73% $\pm$ 1.08 and the butyric acid with 8.66% $\pm$ 0.95 and 9.09% $\pm$ 1.33 respectively before and during the administration of fluoride.

Discussion

No direct effect upon the fermentation of the rumen juice and upon the reduction of fatty acids occurs before relatively high doses of fluoride are administered. A cow weighing 560 kg with a fluoride supplementation of 4 mg/kg bodyweight would take up 15 kg of hay with a fluoride content of 147 ppm. Our observations therefore confirmed the data of others (1, 8, 3, 9) who have repeatedly pointed out that in chronic fluorosis reduction in productivity occurs only when the animals consume less food because of pains in joints or when their mastication is impeded because of damage to their teeth. Otherwise such changes are observed only in acute fluorosis.

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Discussion

Dr. Bohne: Without reliable controls your studies do not warrant the conclusion that,in fluorosis,gastric upsets and disturbances in mastication resulting from dental fluorosis account for the decrease in milk productivity. You failed to consider that fluoride supplementation is likely to induce serious metabolic changes which account for the decline of the general health of the animals and interfere with milk production.

Dr. Leemann: I have shown that the use of fluoride which induces chronic fluorosis did not interfere with milk production. Our fluoride supplement did not induce acute fluorosis. Only in cattle with acute fluorosis do we see interference with milk production.

Dr. Gründer: The concentration of fluoride in the rumen juice is rather high, is it not? The same experiment was done before and induced acute fluorosis associated with indigestion, diarrhea and general malaise. Your experiments were of short duration. Would your results be the same if they had been pursued for a longer period of time using 5 mg of F per kilogram body weight?

Dr. Leemann: Your question is justified. We intend to continue this experiment for longer periods using 2 mg F per kilo of body weight.

Dr. Waldbott: In 1957 Drs. Murray and Darlow (Gastroenterology, 33:929-936)

at Bedford College, London, found that both the amount of gastric juice and the acidity were reduced materially by administration of large doses of fluoride (0.02 M or 380 ppm) to cats in which the gastric secretory activity had been stimulated by histamine and similar agents. Have you any opinion on this point in connection with your data?

Dr. Leemann: We have only done pH levels on the rumen. Fluoride supplementation in cattle with the doses which we employed produced no changes of the pH level. Blood enzymes, Ca and alkaline phosphatase levels were all normal.

CRIPPLING ARTHRITIS RELATED TO FLUORIDE INTAKE (CASE REPORT)

by

H. A. Cook
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SUMMARY: In a 55 year-old woman with long-standing arthritis, but no obvious signs of fluorosis, X-rays exhibited degeneration of discs and calcification in disc spaces. Her daily fluoride intake, mainly from tea, exceeded 9 mg; her daily urinary excretion was 3 mg. When she discontinued consuming tea, her fluoride intake fell below 1 mg daily; excretion through the urine exceeded intake; the arthritic pains diminished and virtually ceased in 6 months; mobility of her spine was restored and she was able to resume work. These observations suggest that arthritis of the spine of unknown cause might represent subclinical fluorosis which is not demonstrable radiologically.

Rheumatic and joint pains, major manifestations of fluorosis, are not uncommon among people residing in fluoridated areas.

A former public health inspector F. A. I. who resided in Maldon, Essex, a high fluoride area (4-5 ppm), had noted repeatedly that his arthritic pains improved when he moved away from that area, and tended to return whenever he revisited it. He, himself, linked his pains with the fluoride content of the water. Numerous other individuals, who have resided most of their lives in the same small natural fluoride area have had severe arthritis.

In 1968, the author carried out a survey (1) on ingestion of fluoride among British children and adults associated with their fluid intake. From flu-

Presented at the Fourth Annual Conference of I.S.F.R., The Hague, 10/24-27/71.

ids children and adults ingested on the average 2 1/4 mg and 3 1/2 mg fluoride per day respectively in nonfluoridated areas, 3 1/4 and 5 1/2 mg per day respectively in fluoridated areas. These values - far in excess of those previously assumed - did not take into account beer-drinking by adults. Most fluoride was derived from tea: 92.5% of children in Britain over 5 years of age drink tea regularly.

The variations in fluoride intake showed that the maximum fluoride intake of British children from tea alone reaches nearly 6 mg per day in unfluoridated areas and nearly 7 mg per day in fluoridated areas. Maximum intakes for adults were unobtainable.

These figures contrast with the fluoride intake in the U.S.A., where the average tea consumption is 0.6 lbs. per head per year. In Britain 9.2 lbs. per head is consumed annually. Jolly (2) has shown that crippling fluorosis is endemic in the Punjab - another tea-drinking area. Here dental or skeletal fluorosis varied inversely with the hardness of water supplies, i.e. with its calcium and magnesium content.

The actual fluoride intake required to produce skeletal fluorosis is unknown. The disease has been found in areas where the fluoride level in water varies from less than 1 ppm upwards (2, 3). Production of skeletal fluorosis is influenced not only by the fluoride content of the water supplies, but also by that of food and beverages, by industrial gaseous or particulate emissions of fluoride, by the calcium and magnesium intake, and by the efficiency of the kidney function. Siddiqui (4) has shown that individuals with fluorosis retain more fluoride than do normal individuals. In its early stage fluorosis is usually associated with pains in the spine which suggest the diagnosis of rheumatism or arthritis (5, 6). It cannot be diagnosed radiologically until it has reached the advanced stage, at which time_in most cases_it is associated with crippling.

Singh and Jolly (7) noted that a daily intake of 8 mg or more of fluoride is necessary to produce skeletal fluorosis. Those cases in which the disease could not be demonstrated radiologically were excluded. Neuromuscular effects of fluoride ingestion include pains in the muscles and joints (6, 3, 8). In the Hampshire case (3) of skeletal fluorosis with neurological complications, the fluoride content of water consumed by the patient throughout life was low. Apparently tea consumption was this patient's main source of fluoride intake. Therefore the high tea consumption in Britain could have caused the muscle and joint pains diagnosed as "rheumatism" or "arthritis".

Case Report

I encountered the case of a woman M. J. T. age 55, who had the habit of drinking more than 2 pints of tea per day. She had been crippled with arthritis for about 25 years for which she was under constant medication with the analgesic, phenylbutazone. To obtain relief this patient had moved 12 years pre-

viously to a natural fluoride area (0.67 ppm fluoride): she had been told that "fluoride was healthy for teeth and bones."

Her serum calcium, magnesium and inorganic phosphorus were within normal limits. The X-rays of the spine, released from the local hospital showed disc degeneration in the lumbar spine; some discs revealed evidence of excess calcification. There were some exostoses, but no obvious signs of the skeletal changes typical of fluorosis.

Her daily fluoride intake from drinking between 3 and 4 pints of tea was calculated to be 9 mg; the urinary fluoride excretion ranged from 4.5 to 6.5 mg. These figures pertain to fluid intake and urinary excretion exclusively. They do not include the fluoride content of food or feces.

As shown in table 1, the patient stopped drinking tea on 10/7/70 whereupon the urinary fluoride excretion dropped dramatically. Six weeks later she reported relief from pain; she could move easily and her improvement continued progressively. Following elimination of tea, the urinary fluoride excretion equalled or exceeded the intake. The latter had dropped below 1 mg daily.

TABLE 1

Daily Fluoride Intake Through Tea And Water

Date	24-hr F^- intake from tea & water	24-hr urinary F^- excretion
9.2.70	6.90 mg	--
16.2.70	9.34	2.76 mg
4.6.70	6.32	1.56
3.7.70	7.62	3.07
10.7.70	Tea intake ceased.	
24.8.70	0.76	0.75
24.9.70	0.66	1.50
19.10.70	0.68	0.66
25.11.70	0.53	0.58
1.4.71	0.48	0.72

On 11/11/70, X-rays by Dr. J. T. S. at the Kennedy Institute of Rheumatology, confirmed the diagnosis of a long-standing disc degeneration, and the absence of overt signs of fluorosis. There was some calcification in disc spaces. It is noteworthy that the urinary excretion of fluoride before tea-drinking was stopped was in the range of 1.5 to 2.0 ppm, which is indicative of fluoride retention (8).

Little more than 3 months after the patient stopped drinking tea she reported that the pain had lessened to the extent that she was almost able to be without drugs. The mobility of the spine had returned sufficiently to enable her to take a job which involved considerable walking. After 6 months,

she reported that she was virtually free of pain. She no longer required drugs. This improvement was confirmed again in May 1971. In July 1971, one year after she had stopped drinking tea she reported that further improvement had apparently ceased, but there was no deterioration. She was able to do without pain-killing drugs except in emergency. Her physician noted only slight residual restriction in movement of the spine and sensitiveness on palpation which he attributed to the disc degeneration. The condition subsided completely after November 1971 when the patient moved from the Wimborne, Dorset area where water contained 0.67 ppm fluoride to Yeovil where waterborne fluoride is only 0.13 ppm.

This case supports the premise that some forms of arthritis are related to sub-clinical fluorosis, i.e. fluorosis which is not sufficiently advanced to show the characteristic skeletal changes radiologically. It appears that the spinal disc degeneration and sub-clinical fluorosis were co-existent.

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Discussion

Dr. Waldbott: In 1962, I reported a similar case in the International Archives of Allergy and Immunology. Roentgenologists usually interpret the changes in the spine as degenerative osteoarthritis due to "old age."

Retention and excretion studies are of little value when urine is analyzed exclusively and when exact data on fluoride intake are not available. Fluoride content of faeces and sweat is also important.

One of my patients, Mrs. F. O. aged 55 exhibited features indicative of chronic fluorosis, namely arthritic changes in the lower spine, cephalgia, gastritis, ileitis, lower urinary tract disease, paresthesias in arms and legs, ulcers in the mouth. She was in the habit of imbibing 15 to 20 cups of tea daily for 25 years. From this source alone, her daily intake of fluoride amounted to 1.82 to 2.44 mg. She lived in an area where the water supply contained 0.4 ppm; 24-hour urinary fluoride excretion ranged from 1.7 to 6.3 mg (6 determinations).

Dr. Cook: The patient whose case I reported was only 55 years old. Apart from the fluoride intake from tea, that from other fluids and food remained approximately the same. Intake of fluoride through food is highly variable because many foods vary in fluoride content even from day to day.

Dr. Teotia: I consider the data in this case established it as fluorosis from intake of food and tea in Great Britain. We, too, determine fluoride in fluids and food as well as the urinary and fecal fluoride output.

Dr. Franke: Patients with chronic gastritis or gastric hyperacidity should never be treated with NaF for osteoporosis. It would most likely aggravate the gastric symptoms.

RELATION OF MINERAL AND HORMONE METABOLISM TO INTAKE OF WATER WITH A HIGH NATURAL CONTENT OF FLUORIDE

by

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SUMMARY: The metabolic effects of intake of low and high natural fluoride water were studied on 8 subjects over a period of 180 days. In the four subjects drinking high fluoride water (10.35

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* * * * * * *

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ppm) there was a definite increase in the intestinal absorption of calcium, gradual rise in plasma alkaline phosphatase, and increased retention of calcium as revealed by balance studies. Plasma, Ca, P Mg, PBI, plasma-cortisol and urinary steroids showed no significant change. In the four subjects drinking low fluoride water (0.5 ppm) no changes were produced.

Previous studies (1, 2, 3) have demonstrated that subjects with skeletal fluorosis absorb more calcium than normal persons receiving similar intakes of dietary calcium. They indicated that new bone formation and osteosclerosis - the most significant features of the disease - are related to calcium retention. Rich and Ensinck (4) administered sodium fluoride to patients with osteoporosis and reported a transition from a negative calcium balance to a positive one with a drop in the urinary calcium within six to eight weeks of the onset of treatment.

Spencer et al. (5), however, in their study of nine patients noted that, intestinal absorption of calcium and the calcium balances did not improve during an intake of 20.6 mg of sodium fluoride per day for a period of 22 to 42 days. In the majority of their patients, the plasma levels of ^{47}Ca were lower during the intake of sodium fluoride than in the controls, a fact indicative of decreased absorption of ^{47}Ca. Similarly, Ramberg et al. (6) in the performance of calcium kinetics and roentgenologic studies on six Holstein calves reported inhibition of calcium absorption and elevation of the calcium removal rate from bone in fluoride-treated calves.

Because of these conflicting reports we have made further studies of the metabolic effects of the intake of water with a high natural fluoride content (10.35 ppm). We compared them with the effects, on healthy individuals, of water with a low fluoride content (0.5 ppm). Drinking water with a high natural fluoride content was chosen in preference to tablets of sodium fluoride in order to study the effects of fluoride under normal environmental circumstances.

Materials and Methods

In eight individuals subjected to intake of water with a high natural fluoride content we studied the effect on the metabolism of clacium and magnesium and on adrenal and thyroid hormones. All were males and belonged to a poor socio-economic strata. Their ages ranged from 26 to 35 years. The cases were divided into two groups, namely a control group which received water with a natural fluoride level of 0.5 ppm and the experimental group whose water contained 10.35 ppm fluoride.

The eight subjects were admitted to the metabolic wards and the entire studies were carried out on a strictly controlled dietary intake of 800 mg of calcium and 14 mg of magnesium per day. The study extended over a period of 180 days. The intake of fluoride was calculated from the daily intake of water exactly as ingested. Serial determinations of plasma calcium, magnesium, inorganic phosphorus and alkaline phosphatase were done by the use of the esta-

blished procedures. Calcium and magnesium balances were performed in periods of 6 day and at 4 week intervals using carmine as the marker. Protein-bound iodine, plasma cortisol, 24 hour urinary keto and ketogenic steroids were determined in the beginning and at the end of 12 and 24 weeks following intake of water with the high content of fluoride. The 17-ketosteroids were determined by the Zimmerman reaction, urinary ketogenic steroid by modification of Norynberski's method and plasma cortisol by the fluorometric method of Wootton (7).

Results

The results are summarized in Tables I, II, III, IV, V, VI and Fig. I.

Discussion

Our studies have shown that fluoride causes increased absorption of calcium from the intestinal tract (Table 4). The patients who imbibed water with a high fluoride content exhibited progressively positive calcium balances (Fig. 1). The increased absorption of calcium from the bowels and transition of the negative to a positive balance within 4 weeks of the administration of high fluoride water has further supported the observations of Rich and Ensinck (4) as well as our own (3). These findings are in contrast to those of Spencer et al. (5) and Ramberg et al. (6). Such effects were not observed in patients whose drinking water contained low levels of fluoride.

The increased absorption of calcium by fluoride constitutes the basis for the rationale of the use of sodium fluoride in the treatment of osteoporosis and Paget's disease. We therefore believe that fluoride ingestion causes retention of calcium by increasing its intestinal absorption. While during the administration of high fluoride water, the faecal calcium was reduced, there was no significant effect on the excretion of urinary calcium.

In patients with endemic skeletal fluorosis residing in the endemic area since birth we have noted that the retention of calcium, the most important feature of this syndrome results both from the reduction of the fecal calcium and from a decline in urinary calcium excretion. It seems likely that the action of fluoride on the urinary excretion of calcium becomes manifest only after a prolonged period of intake. The progressive fall of the fecal calcium with the duration of fluoride ingestion in the current study suggests the cumulative action of fluoride. Thus it is likely that frequent migration of individuals residing in an endemic area to non-endemic zones would prevent them from the cumulative toxic effects of fluoride, particularly upon the skeleton.

The magnesium balance studies did not show any significant difference from those of the controls. Teotia et al. (3) reported magnesium balance studies in three patients of endemic skeletal fluorosis and analyzed their bone-ash for its magnesium content. The results suggested that the individuals residing in the endemic area for several years develop a tendency to retain magnesium in their body tissues, particularly in the bones.

TABLE 1

Plasma Calcium mg/100 ml*
Before and After the Administration of Fluoride Water

	Case No.	Before	After						Mean Fluoride
		wks.	4	8	12	16	20	24	intake mg/day
Control	1	9.5	9.6	9.4	9.3	9.8	10.5	10.0	1.0
	2	9.2	9.3	9.2	9.2	9.5	9.4	9.6	2.0
	3	10.2	10.8	9.3	10.0	9.5	9.6	10.2	1.5
	4	9.5	9.5	9.2	9.0	9.6	9.6	9.6	1.2
	Mean	9.6	9.5	9.3	9.3	9.6	9.7	9.8	1.4
	Range								
Experimental	1	9.8	9.8	9.6	9.7	9.5	9.7	10.0	16.0
	2	9.4	9.4	9.2	9.3	9.4	9.8	10.2	14.0
	3	10.2	10.2	10.1	10.0	9.8	9.7	9.8	12.0
	4	9.0	9.0	9.2	9.1	9.2	9.2	9.3	15.0
	Mean	9.6	9.6	9.5	9.5	9.5	9.6	9.8	14.3
	Range								

*Plasma Magnesium ranged from 1.8 - 2.6 mg/100 ml (in both groups)

TABLE 2

Plasma Phosphorus mg/100 ml

	Case No.	Before	After					
		wks.	4	8	12	16	20	24
Control	1	3.8	3.6	4.0	4.2	3.8	3.5	3.6
	2	4.2	4.0	9.9	4.1	3.7	4.2	4.0
	3	3.6	3.7	3.6	4.0	4.2	3.9	4.2
	4	3.2	3.2	3.3	3.3	3.2	3.1	3.2
	Mean	3.7	3.6	3.7	3.9	3.7	3.7	3.8
	Range							
Experimental	1	4.1	4.1	4.2	4.3	4.0	3.7	3.9
	2	3.5	3.6	3.5	3.7	3.5	3.6	3.7
	3	3.8	3.9	3.8	4.0	3.8	3.8	4.1
	4	3.5	3.6	3.9	3.5	3.5	3.6	3.7
	Mean	3.7	3.8	3.9	3.6	3.7	3.7	3.9
	Range							

TABLE 3

Plasma Alkaline Phosphatase (K. A. Units)

	Case No.	Before	After					
	weeks:		4	8	12	16	20	24
Control	1	10.0	10.0	9.5	10.0	9.6	9.8	9.5
	2	7.0	6.5	7.2	6.8	7.0	7.0	7.0
	3	5.0	5.0	5.5	5.4	8.0	4.2	4.5
	4	3.0	3.5	4.0	3.6	3.9	3.4	3.5
	Mean	6.2	6.2	6.6	6.5	6.4	6.1	6.1
	Range							
Experimental	1	8.0	8.0	8.5	10.0	12.0	14.0	18.0
	2	6.0	6.0	5.8	7.5	11.2	11.4	11.4
	3	5.0	4.5	7.0	10.0	9.5	12.0	17.0
	4	4.0	4.3	6.0	9.0	9.0	11.0	13.0
	Mean	5.8	5.7	6.8	9.2	10.4	12.1	14.9
	Range							

TABLE 4

Calcium Balance (mg/day)
On a Daily Intake of 800 mg Calcium

	Case No.	Before	After					
	weeks:		4	8	12	16	20	24
Control	1	+5	+5	+10	-6	+8	+12	+10
	2	+15	+12	+20	-16	+28	+20	+12
	3	+18	+22	+20	+31	+28	+30	+24
	4	+2	+5	+10	+26	+5	+7	-16
Experimental	1	-10	+12	+15	+18	+18	+22	+40
	2	+20	+32	+40	+40	+56	+60	+60
	3	+30	+46	+46	+70	+75	+60	+70
	4	+15	+27	+36	+44	+60	+54	+55

TABLE 5

Magnesium Balance (mg/day)
On an Intake on 14 mg/day

		Before	After				
	weeks:		4	8	12	16	20	24
Control	1	+1.6	+1.5	+1	+1.8	+2	+3.2	+1.9
	2	+0.8	+1	+1.2	+1	+1.8	+1.7	+1
	3	+1.5	+1	+1.2	+1.7	+1.8	+2	+1.4
	4	+1	-2	+0.5	+1	+1.4	+0.8	+1
Experimental	1	+0.5	-1.2	-0.6	+0.5	+1	+1.2	+1
	2	+1.2	+2.4	+2	+1.9	+3	+2	+2
	3	+1.8	+0.8	+2.3	+2.2	+1.5	+1.2	+1.6
	4	+0.6	-2	+1.2	+2.1	+1.8	+1.7	+1.5

TABLE 6

Effect of Intake of Water High in Fluoride Naturally
On Thyroid and Adrenal Hormones

	Case No.	P.B.I. (Microgram per 100 ml)			Plasma Cortisol (Microgram per 100 ml) 8 A.M.			Urinary 17-Ketosteroids mg/day			Urinary 17-Keto Genic Steroids mg/day		
		Initial	Weeks		Initial	Weeks		Initial	Weeks		Initial	Weeks	
			12	24		12	24		12	24		12	24
Experimental	1	5	5.5	4.5	9	12	10	11	9	13	6	8	7
	2	6	5.6	7	12	14	10	17	14	21	5	9	7
	3	4.8	5.2	5.6	10	11	9	16	12	18	4.5	6	7
	4	5.2	4.2	4.7	11	21	17	11	14	12	12	11.5	10
Mean		5.2	5.1	5.5	10.5	14.5	11.5	13.8	12.2	16	6.9	8.7	7.8
Range													

Biochemical investigations showed a rise in the plasma alkaline phosphatase as the most distinctive feature seen in all four patients receiving high fluoride water. This probably results from the direct action of fluoride on the osteoblastic cells of the bone. All other parameters showed no significant change from the values obtained in control patients who received drinking water with a low fluoride content.

Equally important are our negative findings on fluoride's effect on thyroid and adrenal hormones. The protein bound iodine, plasma-cortisol, 24-keto and ketogenic steroids determined at 12 and 24 weeks after the ingestion of high fluoride water showed no significant change in the values obtained prior to intake of fluoride water (Table 6).

In the current experimental study the thyroid and adrenal functions were not affected by fluoride water consumed over a period of 24 weeks. Jolly et al., who determined protein bound iodine in plasma of 26 patients of endemic skeletal fluorosis, found normal values ranging from 2.8 to 7.2 µg per cent. They also obtained normal values in all 14 cases tested for adrenal function.

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BIOLOGICAL-BIOCHEMICAL METHOD FOR THE DIAGNOSIS OF FLUOROACETAMIDE POISONING

III. INHIBITION OF ACONITASE

by

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SUMMARY: A biochemical method for the diagnosis of fluoroacetamide (FAA) poisoning is described. The method is based on inhibition of aconitase by an inhibitor extracted from an FAA poisoned animal. The highest concentration of the inhibitor was found in the kidneys. The inhibitor is stable. It is found in the kidneys even 40 hours after death. High levels of the inhibitor are also found in heart and skeletal muscles.

Fluoroacetamide a common rodenticide has caused many poisoning accidents in animals (1). It has been established that the poisoning effect of FAA is due to the enzymatic synthesis of fluorocitrate (2, 3) which inhibits aconitase (4, 5, 6).

Biological assay for the determination of FAA poisoning is based on the intraperitoneal injection of kidney extract from the suspected animal into a sensitive animal such as a guinea pig. After a few hours the guinea pig is sacrificed and citric acid accumulation in the kidney is measured. Accumulation of citrate above normal levels is an indication of FAA poisoning (7). This logical method however is elaborate, prolonged and indirect. In addition the accumulated citrate disappears rapidly after death (8).

The following biochemical method is simple, rapid and direct. It is based on the inhibition of the enzyme aconitase by an extract obtained from the kidneys or heart muscle of the poisoned animal. The aconitase inhibitor is present in the kidneys as long as 40 hours after death.

Method

A dose of 20 mg/kg body weight of FAA was injected intraperitoneally into guinea pigs weighing approximately 300 g. The animals were sacrificed at different intervals following the injection and different organs were re-

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moved and extracted. The extraction of the organs was made by homogenitation of the tissue in distilled water, namely 1 gram of tissue in 2 ml water and by incubation in a boiling water bath for 15 minutes and centrifugation at 15000/ g for 10 minutes. Aliquots of the supernatant were tested for the inhibition of aconitase.

Aconitase activity was measured spectrophotometrically according to a modification of Siebert's method (9). The composition of the reaction mixture is presented in Table 1. Following equilibration, the reaction was star-

TABLE 1

Reaction Mixture

0.1 M Tris-HCl buffer (pH 7.4)	0.3
0.2 M $MnSO_4$	0.7
0.005 M NADP	0.3
Isocitric Dehydrogenase 10 units per ml H_2O (Sigma Type 1)	0.1
Activated Aconitase	0.1
Tested Tissue Extract	0.1
Water	up to 0.95

ted by the addition of 0.05 mg of 0.002 M citrate (pH 7.4). The reaction was followed spectrophotometrically at 340 millimicron in a unicam sp 800-A recording spectrophotometer. The reaction was measured against water or water and tested extract.

Aconitase was activated according to Morrison (10). The existence of the aconitase inhibitor was studied in extracts of liver, kidneys, lungs, brain, muscle, heart, spleen and blood. Also studied was the formation of this inhibitor at varying intervals after the FAA injection until death and at varying intervals following death to check the persistence of the compound in the tissue of the dead animal. To this end the dead animal was kept at room temperature. Its organs were removed 17, 21, 25 and 40 hours after death. Extracts from unpoisoned animals were used on controls.

Results

Extracts of organs from FAA poisoned guinea pigs showed different rates of aconitase inhibition (Table 2). In heart and kidneys, 100% inhibition per gram of organ tissue was obtained. Inhibition took place in the following tissues in a decreasing order: muscle, brain, lungs, spleen, liver and blood. These levels of inhibition were obtained with the equivalent of 30 mg tissue extract.

In a study of the rate of formation of the inhibitor, complete inhibition

TABLE 2

Aconitase Inhibition by Extracts Obtained from Organs of Poisoned Guinea Pig

Extract added		% of aconitase activity
None	Poisoned animal	100
Blood	"	91
Liver	"	90
Spleen	"	77
Lung	"	68
Brain	"	50
Muscle	"	10
Heart	"	0
Kidney	"	0
Liver	Control animal	100
Kidney	"	100

Wherever indicated 0.10 ml tissue extract was added. The reaction was run in a 1.0 ml cuvette, 1.0 on light path at room temperature.

in kidney extract was obtained within two hours whereas heart tissue was completely inhibited within 3 hours after the injection. In muscle tissue the formation of the inhibitor was slower; inhibition reached 90% at death. Inhibitor formation in liver reached a maximum in the third hour and then declined toward death (Table 3).

TABLE 3

Inhibition of Aconitase Related to Time Interval After FAA Injection

Organ	% Inhibition, Hours After Injection					
	0	1	2	3	4	Death
Liver	0	0	9	32	18	10
Kidney	0	77	100	100	100	100
Heart	0	73	77	100	100	100
Muscle	10	23	45	64	78	90

Conditions described in Table 2

Checking the stability of the inhibitor in the tissue after death, it was shown that the kidneys, 40 hours after death, contained high levels of the inhibitor whereas in heart and muscle tissue only a small amount of the inhibitor re-

TABLE 4

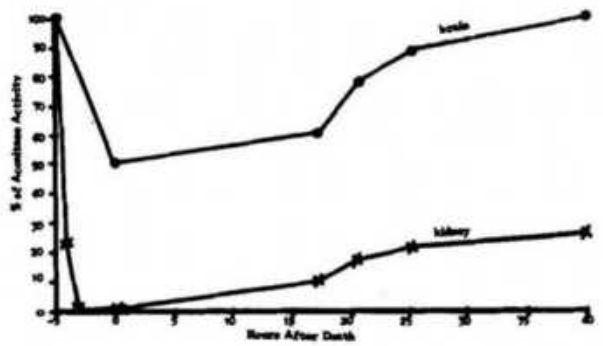
Time After The Animals' Death Related to Concentration of Aconitase Inhibitor

Tissue	% Inhibition, Hours After Death				
	0	17	21	25	40
Kidney	100	90	82	78	74
Muscle	90	22	17	11	11
Brain	50	40	22	1	0
Heart	100	84	79	47	26

Conditions described in Table 2

Fig. 1

Effect of Time on the Inhibitor Concentration in Kidney and Brain from FAA Poisoned Guinea Pig



Conditions of the inhibitor concentration measurements described in Table 2.

mained in the tissue (Table 4; Fig. 1). With regard to brain tissue, all inhibition was lost 40 hours after death.

Discussion

The biochemical method for the determination of FAA inhibition is based on the measurement of aconitase inhibition by tissue extract of an animal poisoned by this compound. The elaborate and prolonged biological method based on measurement of citrate accumulation in tissues, agrees with the values of aconitase inhibition: Tissues which showed high level of aconitase inhibitor also showed high level of citrate accumulation in the poisoned animal (1).

It is presumed that the aconitase inhibitor found in the different tissues is fluorocitrate, which is synthesized by the condensation of fluoroacetate and oxaloacetate. The sites of the synthesis of this inhibitor are not fully known. However, different organ tissues accumulate varying amounts of the inhibitor and the inhibitor is probably synthesized in these tissues. If so, it is possible that the inhibitor is synthesized in those tissues which show high glycolytic and Krebs cycle activities such as muscles; it then leaks into the blood which transports it into the kidneys, where the highest concentration of the inhibitor is found at a time when blood contains no inhibitor at all.

It was necessary to check the different tissues for their inhibitor levels in order to determine which tissue contained the highest level and at the same time retained this compound for the longest period after death. It was established the kidney is the most suitable organ for the test since here the inhibitor was produced fastests (Table 3, Fig. 1) and retained longest after death (Table 4). The use of kidney tissue permits the test to be carried out as long as 40 hours after death. Other tissues which could be employed for the test are heart, and muscle, but they must be extracted within 24 hours following the death of the animal.

It is of interest to establish whether or not the inhibitor of aconitase can be found in the urine of a poisoned animal. If so it would permit early detection of poisoning. Preliminary studies in this laboratory showed that the urine contains an inhibitor of aconitase which interferes with the test. However the injection of urine from a poisoned sheep caused a significant accumulation of citric acid in the guinea pig kidneys which indicates that the toxic principle may be excreted through the kidneys (7).

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INEFFECTIVENESS OF FLUORIDE THERAPY IN MULTIPLE MYELOMA

by

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(Abstracted from the New England Journal of Medicine, 286:1283-1288, 1972.)

This double-blind study, involving 150 patients with multiple myeloma was undertaken by a group of 29 clinicians to determine whether or not sodium fluoride therapy could possibly influence the clincial course of the illness.

METHODS

All patients had a substantiated diagnosis of multiple myeloma. The only additional requirement was that the patient's life expectancy would be increased by at least three months. A total of 150 patients completed at least three months of therapy.

The patients were randomized into three groups: The low dose group received 25 mg sodium fluoride plus 25 mg sodium chloride four times daily; the high dose group, 50 mg sodium fluoride four times daily and a placebo control group was given 50 mg sodium chloride four times daily on a double-blind basis.

Routine myeloma studies were performed at intervals of four months and a specific skeletal survey was performed at similar intervals.

RESULTS

1. SIDE EFFECTS: Side effects, mainly nausea, occurred with almost equal frequency in all three groups. It could not be determined whether or not they were related to the treatment or to myeloma.

2. SKELETAL DISEASE: To evaluate the effect of fluoride, both objective and subjective measurements were used. A loss in height of at least 2 cm was considered a reliable index of vertebral collapse. Several skeletal surveys were performed in order to determine when progressive skeletal disease could first be established roentgenographically or to determine the recalcification of

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* Prepared by Writing Committee: John B. Harley, M. D. (chairman) Albert Schilling, M. D. (chairman), and Oliver Glidewell, M. D. (statistician)

generalized osteoporosis, the recalcification of lytic lesions or the reduction of fluorosis. Also subjective indexes of pain and performance were noted.

The production of increased osteosclerosis was considered a desirable objective in patients with multiple myeloma. A summary of recalcification and fluorosis in each treatment group is given.

3. SERUM CALCIUM: Abnormal calcium levels below 8.5 mg/100 ml and above 12.5 were evenly distributed among the three treatment groups.

4. SURVIVAL: Survival in the three treatment groups was practically identical in the NaF treated patients and the untreated cases. Neither beneficial nor harmful effect could be demonstrated. No beneficial effect, either subjective or objective, on the clinical course nor on survival could be identified during an observation period of 53 to 70 months.

K. J.

EFFECT OF COMBINED THERAPY WITH SODIUM FLUORIDE, VITAMIN D AND CALCIUM IN OSTEOPOROSIS

by

J. Jowsey, B. L. Riggs, P. J. Kelly, and D. L. Hoffman

(Abstracted from the American Journal of Medicine, 53:43-49, 1972)

METHODS

Eleven patients with progressive osteoporosis causing vertebral deformities were studied. None of the patients had diseases other than osteoporosis and only one of them had had previous treatment. Before and 12 to 17 months after treatment the patients underwent metabolic studies. Spinal roentgenograms and specimens of the anterior iliac crest bone were examined for morphologic and microradiographic studies.

Hospitalized in a metabolic ward, serum calcium, phosphorus, alkaline phosphatase and serum protein were determined for each patient. The twen-

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ty-four hour excretion of calcium, phosphorus and creatinine were determined as well as renal clearance studies were made. After treatment a portion of each bone biopsy and a fasting morning sample of serum and urine were analyzed for fluoride content.

The bone biopsy specimens were cut and prepared for quantitative microradiography. By this technique it is possible to identify accurately the appearance of bone formation and resorption. Also, mineralized sections were mounted unstained and the width of unmineralized osteoid tissue was measured.

Fluoride as NaF was taken in divided doses three times daily with meals. The home dietary calcium intake was supplemented with oral calcium and, in addition, each patient took 50,000 units of vitamin D_2, twice weekly.

RESULTS

1. CLINICAL DATA: Most patients continued to have symptoms. In four of the eleven patients spinal roentgenograms following treatment showed additional compression fractures and one showed evidence of skeletal exostoses. Mild arthralgias, stiffness of joints and epigastric dyspepsia were common. Laboratory studies showed no changes attributable to fluoride toxicity.

2. BIOCHEMICAL: Following therapy no significant change in the serum calcium, phosphorus and alkaline phosphatase values, protein electrophoretic pattern or urinary excretion of calcium or creatinine was noted. However, there was a significant decrease in urinary phosphorus excretion. The bone fluoride content could not be correlated with the daily dose but, in all treated patients, bone fluoride content (mean 1.13 μg/mg) was higher than in normals.

3. BONE BIOPSIES: After treatment, bone formation was increased in all but one patient and the group mean was significantly higher than before treatment. Both the increase in bone formation and post-treatment formation levels were related to the total amount of fluoride ingested. No correlation between calcium supplement and the values of bone formation was found.

The post-treatment mean of bone resorption was significantly less for the group than before therapy. This change in bone resorption could not be correlated with the amount of fluoride administered but resorption was significantly correlated with the amount of supplemental calcium administered. Changes in osteoid thickness varied, but thickness tended to increase in the group as a whole.

DISCUSSION

The authors have shown that, when combined with vitamin D and calcium supplements, doses of fluoride can be given which produced an increased

formation of bone and a decrease in bone resorption. Because three therapeutic agents were used simultaneously it was impossible to assess the individual effect of each agent. Moreover, according to the authors' estimate, the optimal pharmacologic doses for fluoride and calcium supplementation are 50 mg fluoride daily, 600 mg or more calcium daily and for vitamin D 50,000 units twice weekly. With these doses they anticipate a modest increase in skeletal mass with only limited undesirable effects on the skeleton.

K. J.

FLUORIDATED WATER, THE SKELETAL STRUCTURE AND CHEMISTY

by

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(Abstracted from the Health Services and Mental Health Administration Health Reports, 86:820-827, 1971)

This study was carried out to provide data on the microscopic structure of and the quantity of fluoride present in skeletal tissues of Grand Rapids residents who had consumed the city's water fluoridated at a level of 1 ppm for a maximum of 20 years. The authors examined the possibility that fluoride present in human bones might have contributed directly to the control of osteoporosis.

Method

Autopsies on individuals between the ages of 21 and 80 who had resided in Grand Rapids for 20 years and less were selected for this study. Most of the subjects in the study had succombed to a short illness such as myocardial infarction, pulmonary embolism, cerebral-vascular disease, pneumonia or trauma. All cases with "chronic diseases known to affect the bone structure" namely cancer, leukemia, severe anemia, parathyroid disturbances and renal disease severe enough to cause uremia, were excluded from the study. On the other hand, subjects with nephrosclerosis of a vascular character if not accompanied by renal failure were included.

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The bone specimens were cleaned of soft tissue, weighed, dried overnight at 100° C, pulverized and ashed at 1030 F. An aliquot was then dissolved in 1 ml of 0.5 M $HClO_4$ and 4 ml of 0.5 M trisodium citrate were added. The fluoride content was determined by means of the fluoride electrode. On another sample calcium was determined by atomic absorption spectrophotometry.

The second part of this study was the histologic examination of specimens of lumbar vertebrae and of the left sixth or seventh rib at its antero-lateral end. Also, in a small number of cases, the abdominal segment of the aorta was examined. Specimens of 62 Grand Rapids residents were matched with 62 controls according to age and sex from New York City and Albany. At the time of the study the water supply in both cities contained 0.1 ppm fluoride; fluoridation had not as yet been initiated in New York City. The thickness of the cortex and of the cortical and medullary trabecular bone was estimated with an ocular micrometer. Qualitative microscopic changes in the bones, the marrow, the intervertebral joint, the parosteal tissue and in the aorta were noted. Forty-one specimens, namely 25 from New York and Albany and 16 from Grand Rapids, could not be matched by age and sex.

Results

1. Fluoride Levels: With the exception of four persons in the age group of 0 to 20 years, fluoride levels in vertebrae and ribs of Grand Rapids residents were consistent, namely 0.095 to 0.307% (950 to 3070 ppm) in the vertebrae and 0.079 to 0.206% (790 to 2060 ppm) in ribs. In the majority of the New York cases, bone fluoride was less than 0.10% (1000 ppm). The data suggest an increase of bone fluoride in older age groups, both in Grand Rapids and in New York.

The ash and calcium content of the vertebrae and ribs of the New York residents did not differ significantly from that in Grand Rapids subjects.

With respect to the fluoride analysis of the aorta, the number of the specimens from New York was limited to 19. Within the age group of 41 to 60 years, fluoride in the Grand Rapids aortas averaged 0.15% (1500 ppm) with a range of 0.021 to 0.246% (210 to 2460 ppm). However in the New York state group 0.234% (2340 ppm) was found in the aorta of an individual less than 20 years old; in two other individuals above 80 years of age, 0.155% (1550 ppm).

2. Histological Findings: The 62 matched pairs of subjects from Grand Rapids and New York state showed no consistent differences in quantitative bone structure in the comparison microscope. The cortex of the ribs of the Grand Rapids women aged 61 to 80 seemed to be thicker than those of the controls. However this impression was not supported in any other female or male age group. There was no correlation between the thickness of the bones and their high and low fluoride content nor between qualitative changes in bone structure and fluoride content of bones.

In bones of 14* Grand Rapids subjects and in 5 New York state control bone specimens an increase in focal osteoclasia was noted. Subacute periostitis was observed in one Grand Rapids and in four New York state cases. "Spurs" and "lips" of the vertebral bones indicative of osteoarthritis of the spine were noted in 89 matched pairs.

Atherosclerosis of Abdominal Aorta

	Grand Rapids	New York
Mild	8 (11.6%)	5 (25%)
Moderate	22 (32%)	6 (30%)
Severe	39 (56.5%)	9 (45%)
	69	20

Atherosclerosis of the abdominal aorta was studied in 20 New York controls and in 69 Grand Rapids residents. The results presented in the above table suggest a slightly higher trend in Grand Rapids but were not statistically evaluated by the authors.

EDITOR'S NOTE

The following are some of the major deficiencies of the above survey:

1. The specimens from New York City and Albany residents are not suitable as a control for those in Grand Rapids: In spite of the low (0.1 ppm) fluoride content of water in New York City and Albany, fluoride intake into the system of New York City residents prior to fluoridation must have been unusually high through sources other than water, probably through air pollution. This was demonstrated in 1958 by Herman et al. (1) who recorded exceptionally high levels of fluoride in soft tissues of individuals with kidney stones all of whom had resided in unfluoridated New York City. Excess fluoride uptake is also evident from the authors' data which show as high or higher levels in bones from the control cities than previously reported in skeletal fluorosis. Singh (2) for instance reported fluoride levels in bones in skeletal fluorosis starting as low as 600 and Soriano (3) as low as 905 ppm.

2. No data on minerals other than fluoride in drinking water have been furnished by the authors in any of the three cities, particularly levels of calcium and magnesium in water, and of phosphorus and other elements in the diet which affect fluoride uptake.

3. Exclusion of cases of parathyroid disease and a selected group of cases with kidney disease renders comparison of the skeletal structure and chemistry of the Grand Rapids cases with the New York cases difficult to assess. Both diseases are known to be related to fluoride intake. Comparison of fluoride levels and microscopic studies on bones and tissues of such cases are of

*The number of subjects is given as 14 on page 825, as 19 on page 826.

paramount importance.

4. The exceptionally high fluoride levels in the aortas of both groups - particularly in one New York individual less than 20 years of age - is signifi - cant. The value of 2340 ppm constitutes the highest level of fluoride ever recorded in the literature in any soft tissue organ. This high fluoride accumulation in the aorta cannot be discounted by pointing to "the propensity of aortic and other soft tissue to calcify." Similarly the calcium levels of aortas from Grand Rapids are much higher than any others recorded in the literature. Calcification of aortas has been established frequently in the literature as a manifestation of skeletal fluorosis (4). Whether or not consumption of fluoridated water damages blood vessels is also of utmost importance.

5. The exact duration of the residence of each individual under study in the three cities was not presented nor were parameters other than sex and age investigated; no clinical data were made available on individual cases.

The study reveals the following facts pertaining to the evaluation of fluoridation:

a. Fluoride levels of the abdominal aorta in low fluoride (0.1 ppm) cities are much higher than those previously recorded anywhere in the literature. How such storage occurs and how much concomitant fluoride storage occurs in other soft tissues requires further investigation.

b. The fluoride content of bones in Grand Rapids after 20 years of fluoridation is within the range of values reported in skeletal fluorosis (2, 3).

c. Storage of fluoride in bones following prolonged use of fluoridated drinking water does not benefit patients with bone disease such as osteoporosis.

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