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CONTENTS

EDITORIALS
Fluoride and the Heart ................................................................. 1
Total Fluoride Exposure ............................................................... 4

ORIGINAL ARTICLES
An Inquiry Into the Distribution of Fluoride in the Environment of Garrison, Montana — by C.E. Kay, Missoula, Montana .. 7
Translocation of Fluoride in Woody Plants — by Th. Keller, Birmensdorf, Switzerland .......................................................... 31
Chronic Fluoride Intoxication from Drinking Water — by H.T. Petraborg, Aitkin, Minnesota ................................................... 47
Heart Deaths and Fluoridation — I. Jansen, R.N. and H.M. Thomson, Antigo, Wisconsin and North Andover, Massachusetts ................................................................. 52

ABSTRACTS

Effect of Fluoride on Bone Formation and Strength in Japanese Quail, — by M.M. Chan, R.B. Rucker, F. Zeman, and R.S. Riggins, Davis, California ................................................................. 58
Systemic Fluoride Poisoning Resulting from a Fluoride Burn—
by W.J. Burke, U.R. Hoegg, and R.E. Phillips,
North Tarrytown, New York, and Peine, D.B.R................. 60

The Influence of Fluoride on Mineralization on Rat Teeth —
by O. Meffert and J. Kammerer, Gottingen, Federal Republic
of Germany ................................................................. 61

Radiological and Clinical Reversibilities in a Case of Osteoporosis —
by M.M. Jean-Jacques Herbert and Jean-Jules Francon,
Aix-les-Bains, France..................................................... 62

The Mechanism of Inhibition by Fluoride of Mitochondrial Fatty
Acid Oxidation — by J.J. Batenburg and S. G. van den Bergh,
Utrecht, the Netherlands ............................................. 63

Effect of Fluoride on Carbon Dioxide and Acid Formation in
Salivary Sediment Mixtures Incubated with Glucose —
by H.J. Sandham and I. Kleinberg, Winnipeg, Manitoba........ 64

The International Society for Fluoride Research will hold its
Sixth Conference in historic Williamsburg, Virginia, November 7 to 9,
1974. This town is easily accessible by air to foreign and U.S. partici-
pants. It offers an unusual opportunity to observe, at first hand, the
way of life experienced during the early years of our nation. Reserva-
tions should be made through Miss Ernestine Stowell, 42 Mountain View
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Current Contents Agricultural
Food and Veterinary Sciences
EDITORIAL

FLUORIDE AND THE HEART

In 1962 Fasske (1) reported degenerative changes in the heart consisting mainly of fragmentation of muscle fibers in a patient with acute fluoride poisoning. Pribrilla (2) confirmed these findings in three additional fatalities. These authors found no evidence of inflammation in the heart muscle. Degenerative changes in the myocardial fibers such as cloudy swelling, vacuolar degeneration, infiltration with round cells, were also observed experimentally by Takamori (3) in rats following administration for one month of large doses of fluoride in the range of 0.71 mg/kg to 31.03 mg/kg with their food. These changes were more extensive when the larger doses of fluoride were administered.

Fasske attributed the damage to the musculature of the heart to blockage of certain enzymes by fluoride, particularly of those involved in glycolysis such as glucose-6-phosphatase. He showed experimentally that such blockage interferes with the metabolism of the cells and leads to a breakdown of muscle tissue.

Others (4) have felt that the changes in the heart in acute fluoride poisoning are related to binding of serum calcium by fluoride. A steady decline of serum calcium in acute fluoride poisoning has been reported by Müller and Bock (5) and by Leone (6). In acute poisoning from organic fluorides, excess formation of citric acid resulting in damage to muscle tissue might be held responsible for damage to the heart by fluoride.

Experiments on rats by Büttner and Mühler (7) indicate that fluoride may indeed be stored in the heart.

Studies by Berman (8) indicate that fluoride stimulates the force of contraction of heart muscle. Such stimulation is unexpected since it occurs at concentrations which inhibit glucose metabolism and reduce the calcium ion concentration. These actions of fluoride should diminish rather than enhance the heart's contraction.

It should be noted that, in these studies, massive doses of fluoride were given, mostly for relatively short periods. Concerning the effect of minute doses of the kind which are being consumed with food and water for extended periods of time, there is a great paucity of data.

Hagan, Pasternack and Scholz (9) compared the mortality rates for cancer, kidney, liver, and heart diseases as well as for intracranial lesions in 32 "natural fluoride" towns, with the death rates from the same diseases in 32 "non-fluoride" cities which served as controls. The authors could not detect statistically significant differences for the five diseases in the two kinds of cities. The many variables which enter into such a sur-
vey are difficult if not impossible to control. Critics of this survey, for instance, have stated that frequent changes in sources of water supply in these cities have not been taken into account and that the water of some of the so-called "non-fluoride" control cities contained as much or more fluoride than the "natural fluoride" cities with which they were compared. There is also a question of whether or not the subjects covered in this study had resided in the respective towns long enough to warrant their inclusion in this survey.

After 5 years of fluoridation the death rates in Grand Rapids, where fluoridation was initiated in 1945, had risen between 25 to 50% above those for Michigan as a whole (10) according to the 1950 U.S. census. Heart disease constituted one of the four diseases involved in this marked rise in mortality (11). The Michigan State Health Department attributed the higher incidence in deaths to a change in the Grand Rapids population. They postulated an exodus of people in the early nineteen-forties - the years of the second world war - and a corresponding influx back to the city just prior to 1950. However examination of the school census records proved that no change in population had occurred during these years. Furthermore the ratio of city deaths to deaths in unfluoridated Kent (Grand Rapids) county did not differ significantly at the 1940 census prior to fluoridation whereas in 1950 a marked increase in Grand Rapids city deaths over those of the unfluoridated county was obvious.

Against this background, the data presented in this issue by Jansen and Thompson are of considerable interest. Since there are degenerative changes in the muscle fibers of the heart in acute poisoning due to massive doses of fluoride there should be concern about the effect of persistent minute doses taken in the system over prolonged periods. The fact that in the Wisconsin city of Antigo fluoridation was interrupted from November 1960 to October 1965 afforded an unusual opportunity to compare the death rates in the same city under reasonably constant conditions with and without fluoridation. A marked rise in mortality from heart disease was noted during fluoridation. This trend was interrupted in correlation with the 5 years of freedom from fluoridation.

As stated above, statistical data of this kind must be interpreted with caution in view of the numerous variables involved in the production of heart diseases and the relatively limited sampling in a small town such as Antigo. Nevertheless the limited data reported here indicate a significant trend. The fact that this trend is more pronounced among individuals of age 75 and above, agrees with the well-known experience that fluoride storage in bones and soft tissues, especially in arteries, is related to the duration of fluoride intake.

A follow-up on these studies is indicated. Comparisons of the death rates from heart disease in non-fluoridated cities with those in fluoridated cities present great difficulties which arise from the fact that total fluoride intake through sources other than water has increased through-
out the world. It would therefore be difficult to obtain valid controls for such statistics. Duplication of the procedure of Thomson and Jansen prior to initiation of and during fluoridation would furnish a more reasonable approach to help solve the puzzling but important question of the long-term action of fluoride on the heart.

G. L. W.

Bibliography


EDITORIAL

TOTAL FLUORIDE EXPOSURE

In the article by Jerard and Patrick which appeared in the International Journal of Environmental Studies (1) the authors discussed every aspect of total fluoride intake encompassing recent findings as well as previously established data.

They pointed to the 1963 studies by Krepkogorsky (2) a Russian scientist who determined that among residents of Vietnam - where drinking water contained negligible concentrations of fluoride - endemic fluorosis was caused by their traditional diet. Krepkogorsky emphasized the importance of measuring fluoride intake from all sources. He considered it fallacious to take into account only the fluoride concentration in drinking water which is variable and unpredictable.

Krepkogorsky's conclusion, has been supported by the studies of Marier and Rose (3) of Canada's National Research Council as well as by others (4) who have reported a marked increase in recent years in total daily fluoride intake from beverages and food prepared with fluoridated water.

The authors discuss at length the importance of fluoride injury from air pollution. They believe that the significance of fluoride as an air pollutant is generally underestimated. Fluoride emissions in gaseous and/or in particulate form from fossil fuel combustion and from more than fifty types of major industries are polluting rainwater, soil, plants and animals. Surface waters and waterways, which are recipients of fluoride wastes, also contribute to the ecological effects of fluoride. Whereas atmospheric fluoride values are measured in parts per billion, most other atmospheric contaminants including sulphur dioxide are calculated in parts per million: In this respect, fluorides are more than one-hundred times more toxic than sulphur dioxide.

They mention two air pollution disasters as examples of the acute effects of fluoride air pollution on human health: One in October, 1948 in Donora, Pennsylvania (5) was responsible for 20 deaths and for illness in 6,000 of the 13,000 residents. The other in Belgium's Meuse Valley in December, 1930, due to a thermal inversion with no movement of air, resulted in the death of 60 from acute fluoride intoxication and in illness of several thousand inhabitants in the heavily industrialized area. Fluoride's role in the Belgian disaster was established by Roholm (6) of Copenhagen, Denmark, the author of the classical book on fluoride poisoning.

Accumulation of airborne fluoride in plants, especially in vegetables, is a significant factor in total fluoride exposure. Certain kinds of industrial fluorides become available for uptake by plants, the degree of availability
Editorial

varies with the fluoride compound. Contrary to the widely held belief that industrially added cryolite is firmly bound to the soil, it can be absorbed through the root system of plants. Fluoride-containing fertilizers are also responsible for a rise in fluoride concentration in edible plants.

Waterborne fluoride poses a problem in the total uptake of fluoride. The routine industrial discharge of fluoride wastes into waterways leads to contamination of fish and the food chain.

Balazova and associates of the Bratislava's Institute of Hygiene (7) studied the long-term effect on children of fluoride emissions from an aluminum smelter. They reported a decrease in the hemoglobin of a group of 6 to 14 year-olds after 8 years of exposure and a rise in the level of fluoride in teeth, nails, hair and urine. Furthermore statistically significant increases in perspiration, anorexia, abdominal pains, alopecia, rhinitis, pharyngitis, conjunctivitis and leukonychia (white streaking of nails) were recorded. This study embraced all sources of fluoride exposure in both the exposed and control groups.

Waldbott and Cecilioni (8) also elaborated on the environmental approach to the body burden of total fluoride intake by presenting clinical data on twenty-eight subjects who resided in the environs of an Ontario phosphate fertilizer factory, three near a similar plant in Iowa, and one close to a Michigan iron foundry. These persons manifested neuromuscular, gastrointestinal, respiratory and dermatological symptoms.

Thus fluoride's effect on humans is determined by the total amount taken into the body rather than by its concentration in drinking water.

Bibliography


FLUORIDE
Editorial


* * * *

The International Society for Fluoride Research will hold its Sixth Conference in historic Williamsburg, Virginia, November 7 to 9, 1974. This town is easily accessible by air to foreign and U.S. participants. It offers an unusual opportunity to observe, at first hand, the way of life experienced during the early years of our nation. Reservations should be made through Miss Ernestine Stowell, 42 Mountain View Street, South Hadley, Massachusetts 01075.

The program committee is now soliciting abstracts up to 300 words of papers dealing with any phase of fluoride research. They should be submitted in triplicate to the Society's office, P.O. Box 692, Warren, Michigan 48090. The deadline is June 15, 1974.

Volume 7 Number 1
January, 1974
AN INQUIRY INTO THE DISTRIBUTION OF FLUORIDE IN THE ENVIRONMENT OF GARRISON, MONTANA

by

E. Kay
Missoula, Montana

SUMMARY: In the fall of 1971 and 1972, the extent and severity of fluoride contamination in the vicinity of Garrison, Montana, was determined. Assays of the fluoride content of indigenous flora and fauna were utilized to establish the effectiveness of the air pollution control equipment of a fluoride emitting industrial facility. Vegetation samples including forage, shrubs, coniferous and deciduous tree species as well as various species of small mammals in a directional pattern throughout the study area were analyzed for fluoride and compared with control samples collected throughout western Montana.

Analysis by individual species allowed comparison of the ratio of fluoride accumulation between species. Fluoride levels in vegetation and small mammals were also correlated. Furthermore isopol maps of fluoride concentrations in vegetation were constructed.

Wide differences in fluoride accumulation between different species were observed. Fluoride concentration in different species were compared and presented graphically.

Introduction

Previous studies (1-7) have demonstrated that atmospheric fluorides accumulate in vegetation which, when consumed by wild and domestic animals, may cause fluorosis, thereby resulting in damage to grazing wildlife and livestock populations (8-11). According to Montana State Department of Health standards, the top allowable limit of fluoride accumulations in forage is 35 ppm (parts per million) in forage (dry weight basis). The purpose of this study was to delineate the distribution of fluoride in the environment of Garrison, Montana, through the collection and analysis of native floral and faunal species.

Garrison, Montana, was selected for this fluoride survey because

From the Department of Environmental Studies, University of Montana, Missoula, Montana.
cause of previous fluoride pollution problems caused by the operation of Rocky Mountain Phosphates, Inc. (RMP), a phosphate rock defluorination plant that produces an animal food supplement (12). In the past, Montana courts have dictated that fluoride emissions from this source must be reduced because of such environmental damage which has occurred as a result of emissions (13). In order to determine the effectiveness of fluoride emission control near a Garrison defluorination plant, the fluoride concentrations in indigenous plants were again investigated. Throughout the entire period of study, RMP was at 99.9 percent fluoride emission control, emitting two to three pounds of fluoride per day as hydrogen fluoride gas from the stack at the point source (14).

Methods


Within the vicinity of Garrison, thirty study areas were chosen based upon land ownership and land use patterns, vegetational communities, and distance from RMP (Figure 1). Inside each study area a varying number of collection points was randomly chosen based upon area-size and the diver-

![Diagram showing location of study areas near Garrison, Montana](image-url)
sity of flora present. Collection sites were chosen so that as large a variety of species as possible was obtained.

In order to maintain a constant sampling base, all collections were identified and processed by species. Consolidation into general classes of vegetation, such as forage, was done to facilitate statistical analysis, only after the completion of fluoride analysis.

Collection of Samples

Approximately 800 samples were collected in October of 1971 and 1972. Forage samples such as grasses and alfalfa were taken by cutting arbitrarily selected plants at one inch above ground level to insure that entire specimens were acquired and to avoid selection of the top or the bottom of the plants (15). Cottonwood, Douglas fir, and ponderosa pine foliage samples were collected from the RMP facing upper one-half of trees by shooting branches down with a twelve-gauge shotgun. Where possible, fallen cottonwood leaves were also collected. Immediately upon collection, all samples were placed in individually numbered paper bags. A description of the enumerated collection site and other necessary data were recorded in the researcher's diary for future reference.

Animal specimens were acquired by the use of snaptraps and/or livetrap baited with a mixture of peanut butter and rolled oats (16). Collection sites consisted of 10 to 40 traps laid out in a grid configuration or, where terrain was steep, a linear trap pattern was employed.

All small mammals were individually tagged and placed in plastic bags. The tag number, a description of the trap site, and other relevant information was noted in each researcher's field journal.

Separation of Samples

Conifers Upon receipt at the laboratory, conifer needles were removed from their branches and separated according to year of growth (13). Because conifers leaf out during the month of May in the Garrison area, it is possible to observe fluoride accumulation over various exposure times:

<table>
<thead>
<tr>
<th>Exposure Period</th>
<th>Exposure Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current year's growth</td>
<td>5 months</td>
</tr>
<tr>
<td>One-year-old growth</td>
<td>17 months</td>
</tr>
<tr>
<td>Two-year-old growth</td>
<td>29 months</td>
</tr>
</tbody>
</table>

The growth of juniper was not separated by year, and hence this species represents a composite sample exposed over an undetermined period of time. The scale-like leaves were separated from the stems after
drying, although it was impossible to exclude all woody material completely.

**Crested Wheatgrass**  Cognizance of the fact that bunchgrass are resistant to weathering (16) allows one to separate the vegetation of crested wheatgrass plants accordingly:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Exposure Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite</td>
<td>weighted average</td>
</tr>
<tr>
<td>One-year-old growth</td>
<td>17 months</td>
</tr>
<tr>
<td>Current year's growth</td>
<td>5 months</td>
</tr>
<tr>
<td>Fall green-up</td>
<td>1 month</td>
</tr>
</tbody>
</table>

**Deciduous Trees and Forage Plants**  All other species collected - including both deciduous trees and forage plants - represent tissue growth from approximately May 1st to the date of sampling and, therefore, reflect the severity of fluoride pollution during that period.

After being handled in accordance with the above guidelines, all plant material was placed in appropriately numbered paper bags which were then inserted into a forced draft oven.

**Small Animal Specimens**  Both femurs were dissected out, placed in numbered glass beakers, and boiled in an Alconox solution to remove all flesh.

**Sample Preparation and Fluoride Analysis**  A detailed description of the techniques for sample preparation and fluoride analysis used in this study is presented elsewhere (5, 18, 19). Fluoride activity was determined with an ORION Fluoride Specific Ion Electrode (20, 21). An excess of all plant material was stored for future analysis.

**Results and Discussion**

**Control**

Sampling of control, non-polluted flora and fauna was undertaken throughout the state of Montana. As a control, 241 plant samples and 100 small animal samples gathered throughout the state of Minnesota were analyzed chemically for fluoride (Table 1).

In most instances, control vegetation samples averaged less than...
### TABLE 1

Control Data - Plant and Animal Species

<table>
<thead>
<tr>
<th>Species</th>
<th>Sample Size</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation in Percent</th>
<th>Standard Error of the Mean</th>
<th>Experimental Error of the Mean in Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa, once cut (Medicago sativa)</td>
<td>1</td>
<td>6.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alfalfa, uncut</td>
<td>3</td>
<td>4.9</td>
<td>1.9</td>
<td>39.1</td>
<td>1.1</td>
<td>22.6</td>
</tr>
<tr>
<td>Bluebunch Wheatgrass (Agropyron spicatum)</td>
<td>22</td>
<td>5.7</td>
<td>2.5</td>
<td>44.2</td>
<td>0.5</td>
<td>9.4</td>
</tr>
<tr>
<td>Cottonwood (Populus trichocarpa)</td>
<td>7</td>
<td>6.4</td>
<td>1.8</td>
<td>27.7</td>
<td>0.6</td>
<td>10.4</td>
</tr>
<tr>
<td>Crested Wheatgrass (Agropyron cristatum)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composite</td>
<td>3</td>
<td>5.9</td>
<td>0.4</td>
<td>7.4</td>
<td>0.2</td>
<td>4.3</td>
</tr>
<tr>
<td>17 months</td>
<td>2</td>
<td>5.6</td>
<td>3.4</td>
<td>60.6</td>
<td>2.4</td>
<td>42.8</td>
</tr>
<tr>
<td>5 months</td>
<td>2</td>
<td>5.8</td>
<td>2.0</td>
<td>34.1</td>
<td>1.4</td>
<td>24.1</td>
</tr>
<tr>
<td>1 month</td>
<td>2</td>
<td>5.6</td>
<td>3.1</td>
<td>55.6</td>
<td>2.2</td>
<td>39.2</td>
</tr>
<tr>
<td>Douglas Fir (Pseudotsuga menziesii)</td>
<td>4</td>
<td>3.1</td>
<td>0.5</td>
<td>16.2</td>
<td>0.2</td>
<td>8.1</td>
</tr>
<tr>
<td>41 months</td>
<td>13</td>
<td>3.1</td>
<td>1.1</td>
<td>37.1</td>
<td>0.3</td>
<td>10.3</td>
</tr>
<tr>
<td>29 months</td>
<td>13</td>
<td>3.2</td>
<td>1.0</td>
<td>31.6</td>
<td>0.3</td>
<td>8.7</td>
</tr>
<tr>
<td>17 months</td>
<td>13</td>
<td>3.3</td>
<td>1.4</td>
<td>43.6</td>
<td>0.4</td>
<td>12.1</td>
</tr>
<tr>
<td>5 months</td>
<td>13</td>
<td>3.3</td>
<td>1.4</td>
<td>43.6</td>
<td>0.4</td>
<td>12.1</td>
</tr>
<tr>
<td>Grass - various species</td>
<td>38</td>
<td>3.8</td>
<td>1.6</td>
<td>42.9</td>
<td>0.3</td>
<td>7.0</td>
</tr>
<tr>
<td>Hay - various species</td>
<td>4</td>
<td>4.2</td>
<td>1.4</td>
<td>33.9</td>
<td>0.7</td>
<td>16.9</td>
</tr>
<tr>
<td>Juniper (Juniperus scopularum)</td>
<td>12</td>
<td>4.4</td>
<td>2.4</td>
<td>55.2</td>
<td>0.7</td>
<td>15.9</td>
</tr>
<tr>
<td>Lilac (Syringa vulgaris)</td>
<td>1</td>
<td>4.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ponderosa Pine (Pinus ponderosa)</td>
<td>5</td>
<td>1.6</td>
<td>0.7</td>
<td>45.7</td>
<td>0.3</td>
<td>20.4</td>
</tr>
<tr>
<td>53 months</td>
<td>13</td>
<td>3.0</td>
<td>1.7</td>
<td>57.7</td>
<td>0.3</td>
<td>16.0</td>
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<tr>
<td>41 months</td>
<td>26</td>
<td>3.3</td>
<td>2.1</td>
<td>64.4</td>
<td>0.4</td>
<td>12.6</td>
</tr>
<tr>
<td>29 months</td>
<td>27</td>
<td>2.7</td>
<td>1.4</td>
<td>52.7</td>
<td>0.3</td>
<td>10.1</td>
</tr>
<tr>
<td>17 months</td>
<td>27</td>
<td>2.5</td>
<td>1.2</td>
<td>46.2</td>
<td>0.2</td>
<td>8.9</td>
</tr>
<tr>
<td>5 months</td>
<td>27</td>
<td>2.5</td>
<td>1.2</td>
<td>46.2</td>
<td>0.2</td>
<td>8.9</td>
</tr>
<tr>
<td>Smooth Brome Grass (Bromus inermus)</td>
<td>3</td>
<td>3.8</td>
<td>1.3</td>
<td>33.8</td>
<td>0.7</td>
<td>19.6</td>
</tr>
<tr>
<td>Total Plants</td>
<td>241</td>
<td>3.9</td>
<td>1.5</td>
<td>38.5</td>
<td>0.2</td>
<td>5.2</td>
</tr>
<tr>
<td>Forage - grasses &amp; forbs</td>
<td>72</td>
<td>4.5</td>
<td>2.1</td>
<td>46.1</td>
<td>0.2</td>
<td>5.3</td>
</tr>
<tr>
<td>Chipmunk (Eutamias sp.)</td>
<td>19</td>
<td>103.1</td>
<td>70.5</td>
<td>68.4</td>
<td>16.2</td>
<td>15.7</td>
</tr>
<tr>
<td>Deer Mouse (Peromyscus maniculatus)</td>
<td>70</td>
<td>143.8</td>
<td>65.8</td>
<td>45.7</td>
<td>7.8</td>
<td>5.4</td>
</tr>
<tr>
<td>Meadow Vole (Microtus sp.)</td>
<td>5</td>
<td>136.6</td>
<td>62.8</td>
<td>45.9</td>
<td>28.1</td>
<td>20.5</td>
</tr>
<tr>
<td>Shrew (Sorex sp.)</td>
<td>6</td>
<td>493.8</td>
<td>201.7</td>
<td>40.8</td>
<td>82.3</td>
<td>16.7</td>
</tr>
</tbody>
</table>

FLUORIDE
5 ppm fluoride and in no case over 10 ppm fluoride. Therefore, plant averages below 10 ppm fluoride are classed as controls. This is in agreement with the published findings of other researchers (1, 5, 22).

The total average for all small mammals was 156.7 ppm fluoride with the average for rodents being 127.1 ppm. Individual animals, except shrews, seldom had an excess of 300 ppm fluoride in their femurs. For these reasons, average values in excess of 200 ppm fluoride will be employed to indicate a polluted region; those less than 200 ppm fluoride will be designated as unpolluted. Again, this is in agreement with published data on native indigenous mammals (5).

**Contamination by Study Areas**

The 1971 and 1972 data on fluoride contamination in the Garrison area is presented by species and study area in Tables 2 and 3 respectively. Species values represent averages of a variable sample size, forage is the average of all grass and forb species, and total plant is the mean of all vegetation samples collected within the individual study area.

It is clear from these tables that the Montana state standard for fluoride in forage, 35 ppm, was being violated in the vicinity of Garrison both in 1971 and in 1972. However, because the enumeration of the study areas in Tables 2 and 3 follows no geographical pattern, it is impossible to determine the exact land acreage so contaminated or whether RMP is the fluoride source. It was therefore necessary to examine the directional matrix of fluoride accumulation.

**Directional Pattern of Fluoride Accumulation in Vegetation**

Patterns of fluoride accumulation in indigenous floras are most readily explained by the movement of air masses and the topographic features that direct or channel those air masses (5).

From the data supplied in Fig. 1 in the 1967 U.S. Dept. HEW publication (12), five directions were chosen for computations: north, east, southeast, and west northwest. When a single line did not intersect a representative number of study areas, an angle was employed. Only values for forage were included in the calculations, since the Montana state standard for fluoride in vegetation is based on forage exclusively.

Figure 2, RMP - north, shows that the state standard of 35 ppm fluoride in forage was being violated in October, 1971, a distance of 1/2 mile north from RMP in the fall of 1972 7/8 mile distance. Further, decreasing fluoride contamination with increasing distance from RMP implies that its operations are responsible for the fluoride pollution in a northerly direction (23).
Legend to Tables 2 and 3

AS  Bluebunch Wheatgrass
AC  Crested Wheatgrass—C-Composite, 17 month exposure time, 5 month exposure time, 1 month exposure time
DF  Douglas Fir—29 month exposure time, 17 month exposure time, 5 month exposure time
PP  Ponderosa Pine—29 month exposure time, 17 month exposure time, 5 month exposure time
C   Cottonwood
BI  Smooth Brome Grass
G   Grass
A   Alfalfa—uncut, 5 month exposure time
AI  Alfalfa—once cut, 2 month exposure time
L   Lilac
J   Juniper
H   Hay
TP  Total Plant
F   Forage
DM  Deer Mouse
S   Shrew
M   Meadow Vole
CH  Chipmunk

Fig. 2

Fluoride in Forage, Plotted from RMP — North

150
125
100
75
50
35
25

STATE STANDARD

1/8  1/4  3/8  1/2  5/8  3/4  7/8  1

Increasing Distance from RMP in Miles

FLUORIDE
Figure 3, RMP - east, shows that though there was fluoride contamination (above 10 ppm) for 1-1/4 miles in an easterly direction in 1971, the levels of fluoride are below the state's allowable limit. Since we are dealing almost entirely with gaseous fluoride (12), this is interpreted as indicating that there are few strong air flows from the west. In 1972, the contamination was approximately double the previous year's levels. Again, fluoride levels decrease with increasing distance from RMP.

Figure 4, RMP - southeast, indicates that the state standard of 35 ppm fluoride is being exceeded to a distance of 1-5/8 miles southeast of RMP, and that pollution (over 10 ppm fluoride) is occurring at least 2-1/8 miles southeast of RMP. Conclusively then, there appear to be strong or numerous air flows from the northwest and, as before, decreasing fluoride concentration with increasing distance from RMP.

Figure 5, RMP - southwest, as indicated by concentrations of fluoride that are below the 10 ppm control level, shows extremely little air movement southwestward.

Figure 6, RMP - west and northwest, emphasizes two major atmospheric conditions: cold air inversions and cold air drainages down both the

Volume 7  Number 1
January, 1974
Little Blackfoot and Clark Fork Rivers (12). The fluoride values near the plant, though elevated, are below the state standard which would tend to indicate limited air movement from the east. However, the concentrations of fluoride which rise sharply with increasing distance west from RMP are the result of topography exposing the vegetation, since inversions prevent plume dispersal and cold air drainage abuts the inversions and the undispersed plume up against the hills.

**Fig. 4**

**Fluoride in Forage, Plotted from RMP -- Southeast**

![Graph showing fluoride levels in forage from RMP in different years.](image)

The atmospheric patterns exemplified in Figures 2 to 6 can be summarized as follows: During the day air flow is predominately from the northwest with varying amounts to the north and east. There is little or no air flow to the south, but cold air drainage forces the fluoride emissions west and slightly north. Fluoride levels in vegetation are increased where these air masses meet elevated topography as a result of the greater exposure time to, or the increased concentration of, fluoride.

**Isopol Map of Fluoride Distribution—1971 and 1972**

The pattern of air movements described above can also be illustrated when isopol maps of fluoride distribution are constructed (Figures 7 and 8). Clearly, the state standard of 35 ppm fluoride in forage is being violated.
Fig. 5
Fluoride in Forage, Plotted from RMP -- Southwest

Fig. 6
Fluoride in Forage, Plotted from RMP -- West and Northwest
Fig. 7
Isopol Map of Fluoride Contamination
in the Garrison Area -- 1971

Fig. 8
Isopol Map of Fluoride Contamination
in the Garrison Area -- 1972
over a considerable area although the extent of the violation is less pronounced than in the past (13). A comparison of the two figures also reveals an obvious change in fluoride levels in 1972 as does Table 4.

### Table 4

**Fluoride in Vegetation Samples Obtained at Exact Same Collection Points -- 1971 and 1972**

<table>
<thead>
<tr>
<th></th>
<th>1971 ppm</th>
<th>1972 ppm</th>
<th>Difference*</th>
<th>Percent**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa</td>
<td>36</td>
<td>53</td>
<td>+17</td>
<td>+47</td>
</tr>
<tr>
<td>Lilac</td>
<td>58</td>
<td>57</td>
<td>-1</td>
<td>-2</td>
</tr>
<tr>
<td>Cottonwood</td>
<td>178</td>
<td>214</td>
<td>+36</td>
<td>+20</td>
</tr>
<tr>
<td>Smooth Brome Grass</td>
<td>68</td>
<td>204</td>
<td>+136</td>
<td>+200</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>80</td>
<td>254</td>
<td>+174</td>
<td>+218</td>
</tr>
<tr>
<td>Bluebunch Wheatgrass</td>
<td>80</td>
<td>66</td>
<td>-12</td>
<td>-15</td>
</tr>
<tr>
<td>Juniper</td>
<td>74</td>
<td>97</td>
<td>+23</td>
<td>+31</td>
</tr>
<tr>
<td>Bluebunch Wheatgrass</td>
<td>27</td>
<td>59</td>
<td>+32</td>
<td>+119</td>
</tr>
<tr>
<td>Bluebunch Wheatgrass</td>
<td>46</td>
<td>81</td>
<td>+35</td>
<td>+76</td>
</tr>
<tr>
<td>Cottonwood</td>
<td>470</td>
<td>446</td>
<td>-24</td>
<td>-5</td>
</tr>
<tr>
<td>Lilac</td>
<td>72</td>
<td>71</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>Lilac</td>
<td>116</td>
<td>95</td>
<td>-21</td>
<td>-18</td>
</tr>
<tr>
<td>Cottonwood</td>
<td>100</td>
<td>250</td>
<td>+150</td>
<td>+150</td>
</tr>
<tr>
<td>Smooth Brome Grass</td>
<td>64</td>
<td>138</td>
<td>+74</td>
<td>+116</td>
</tr>
<tr>
<td>Cottonwood</td>
<td>100</td>
<td>168</td>
<td>+68</td>
<td>+68</td>
</tr>
</tbody>
</table>

**Average**

<table>
<thead>
<tr>
<th>1971 ppm</th>
<th>1972 ppm</th>
<th>Difference*</th>
<th>Percent**</th>
</tr>
</thead>
<tbody>
<tr>
<td>104</td>
<td>150</td>
<td>+46</td>
<td>+45</td>
</tr>
</tbody>
</table>

*Difference = (1972 value) - (1971 value)  **Percent = Difference / 1971 Value

The differences in 1971 and 1972 fluoride levels can be attributed to combinations of three variables: 1) slight changes in prevailing wind directions; 2) differences in exact collection sites and species sampled; and 3) increased fluoride contamination. The first is due entirely to factors beyond the control of the experimenter, and the second could have been controlled by more exacting experimental design, though in some instances physical limitations of certain environmental parameters made this impossible. In-

**Volume 7** Number 1

January, 1974
creased fluoride contamination can only be explained by some operational change in RMP.

In 1972, unlike 1971, RMP processed 50 percent local Montana phosphate ore and 50 percent phosphate ore from Florida. In 1971, all the ore was of Florida derivation (14). The fluoride content of Montana phosphate ore (from the Brock Creek area) is much greater than the percent of fluoride in the ore from Florida (24, 25), a fact that may explain the 1972 increased fluoride pollution in Garrison. According to the RMP management (14) the factory’s scrubbing operations are at 99.9 percent control efficiency. Therefore the increased fluoride content of phosphate rock being processed is the only factor which could be responsible for the observed increase in fluoride contamination. All other operational considerations have remained the same in 1972 as they were in 1971.

Based upon the results of this study, it is concluded that the pollution abatement equipment currently in operation at RMP is not sufficient to prevent the fluoride accumulation in forage from exceeding the Montana state standard of 35 ppm, and that the fluoride contamination demonstrated in this study may be expected to be maintained until sufficient controls are installed.

Small Mammals

All small mammals collected, with the exception of shrews, were rodents - deer mice, chipmunks, and voles - and hence, herbivores (26). The life expectancy of these mammals is less than one year. In fact, 90 percent are dead within one year of birth, and virtually none are alive after two years (27). In addition, the home ranges of these species are approximately 100 x 100 meters (28); thus, they are localized indigenous animals, and the average fluoride levels in their femurs can be considered to be the result of the accumulation of one growing season. Consequently, they are invaluable as indicators of fluoride contamination.

The limited data on mammals in Tables 2 and 3 further support the conclusion that the Garrison area is fluorotic. Since the majority of the collections consisted of deer mice, a detailed discussion of fluoride levels in small animals will be confined to that species.

The decrease in fluoride concentrations in deer mice with increasing distance from RMP (Figure 9) illustrates the indigenous nature of this species and implies that fluoride concentrations in deer mice are dependent upon concentrations in vegetation. In figure 10 a correlation between fluoride concentrations in deer mice and fluoride concentrations in forage is demonstrated at the 0.001 ppm level of significance. However, this cannot be considered

FLUORIDE
a direct relationship to the actual amount of fluoride consumed by the deer mice, since their normal diet consists mainly of seeds, nuts, insects, etc. – not forage (26, 27). Seeds and fruits in the Garrison area have been found to contain less fluoride than the foliage of their parent plants (18).

Continuing Fluoride Accumulation

It was shown that coniferous trees and bunchgrass could be used to study continuing fluoride pollution. Assuming that the vegetation absorbs fluoride throughout its existence, one can deduce the relative severity of contamination during past periods. Both Douglas fir and ponderosa pine were collected for analysis in this manner; however, the small sample size precludes a meaningful presentation of these data.

The average fluoride content of crested wheatgrass was dependent upon exposure time: 17 month exposure - 121 ppm; 5 month exposure - 68 ppm; and 1 month exposure - 38 ppm. Since samples were collected within many study areas and varied in their exposure to the absolute level of fluoride contamination, exact rates of fluoride accumulation cannot be calculated. However, a linear relationship of exposure time to fluoride accumulation is not indicated.
Correlation of Average ppm Fluoride in Femurs of Deer Mice with Average ppm Fluoride in Forage

\[ Y = 0.0542X - 13.6360 \]

\[ r = 0.9344 \]

\[ SEE = 11.7219 \]

\[ SEB = 0.0069 \]

\[ SAMPLE \ t = 11.7219 \]

\[ p < .001 \]

By collecting alfalfa which had been cut during haying operations and regrown and alfalfa which was uncut, it was possible to obtain samples of the same species with different exposure times: uncut - 5 month exposure; once cut - 2 month exposure. A correlation of the data obtained (Fig. 11) clearly shows that fluoride contamination has been occurring over a considerable period of time in Garrison.

**Species Specific Accumulation**

Discrepancies in results of fluoride assays between different field collections from the same polluted area are often not due to incorrect fluoride analysis but rather to differences in the plant tissue tested within a given species (17). For example, low fluoride values were obtained when only the fall 1971 growth of crested wheatgrass or only once cut alfalfa were tested. Similarly, it is known that different plant species vary in their susceptibility to gaseous fluoride (29). Therefore low fluoride levels could be obtained by sampling only species characterized by their inability to concentrate large amounts of fluoride. Reporting such results under the general heading of "forage" or "trees," would lead to the erroneous conclusion that no fluoride pollution exists.
Correlation of Fluoride in Alfalfa After 5 Months Exposure With Fluoride in Alfalfa After 2 Months Exposure - Collected at Same Locations

(254, 224) Not Plotted

\[ Y = 0.8600X - 11.3150 \]
\[ r = 0.9749 \]
\[ \text{SEE} = 18.6060 \]
\[ \text{SEB} = 0.0801 \]
\[ \text{SAMPLE } t = 10.7377 \]
\[ p < .001 \]

Since as large a variety of species as possible were collected at each sampling point, a particular plant's accumulation of fluoride can be compared with that of other species located at the same collection point. This assumes that all plants at a given collection site experienced an identical fumigation rate over time and that other environmental factors were equal.

The data on specific species accumulation is presented and discussed by individual pairing in Figures 12 through 18. If there is no difference in species accumulation, the plotted points and calculated regression should depict a one to one ratio.

In Figure 12, accumulation of fluoride in alfalfa is compared with that of smooth brome grass both of which can be classed as forage and had the same exposure time. However, alfalfa appears to accumulate fluoride at a greater rate than does smooth brome grass, since alfalfa has a higher concentration in its tissues at the end of the growing season. A similar relationship has been reported for control fumigation experiments with alfalfa and orchard grass \((Dactylis glomerata)\) (30).

Greater accumulation in juniper than in bluebunch wheatgrass (Fig. 13) may be a manifestation of a varying accumulation rate or of the fact that juniper represents a composite sample of more than one growing season. Further, the height of the collection point may be involved since juniper was
Fig. 12

Correlation of Fluoride in Smooth Brome with Fluoride in Alfalfa at Same Location

\[ Y = 0.7556X - 2.5615 \]
\[ r = 0.9492 \]
\[ \text{SEE} = 18.6770 \]
\[ \text{SEB} = 0.0669 \]
\[ \text{SAMPLE} \quad t = 11.2901 \]
\[ p < .001 \]

Fig. 13

Correlation of Fluoride in Juniper with Fluoride in Bluebunch Wheatgrass at Same Location

\[ Y = 0.5906X + 11.4941 \]
\[ r = 0.6993 \]
\[ \text{SEE} = 38.3009 \]
\[ \text{SEB} = 0.1384 \]
\[ \text{SAMPLE} \quad t = 4.2642 \]
\[ p < .01 \]
sampled four to seven feet above the ground.

Similarly, greater accumulation of fluoride in juniper may be demonstrated by comparing the average fluoride values in juniper by study areas with the corresponding forage parameters, as shown in Figure 14. Additionally, there is reason to believe that juniper itself should have been included in the average forage values since, under certain circumstances, cattle as well as deer and elk consume quantities of this evergreen shrub (31). Thus, not only do the fluoride values for forage depend upon the forage species sampled, but on the very definition of forage itself.

The accumulation rate of fluoride in Douglas fir is approximately four times that of ponderosa pine over the range of fluoride concentration samples (Fig. 15). This phenomenon is no doubt physiological in origin and may be related to the fact that ponderosa pine is more susceptible to fluoride-induced needle damage than is Douglas fir (24).

Cottonwood accumulates fluoride slightly more readily than alfalfa (Fig. 16). This same trend appears when smooth brome grass is plotted against cottonwood (Fig. 17) but to a more marked degree, which would be expected since smooth brome accumulates less fluoride than alfalfa (Fig. 12). This may be a physiological response or it may be due to the height of cottonwood, exposing its tissues to greater amounts of airborne fluoride.

Volume 7  Number 1
January, 1974
**Fig. 15**

Fluoride in Douglas Fir Correlated With Fluoride in Ponderosa Pine at Same Location

\[ Y = 0.2434X + 3.7761 \]
\[ r = 0.8395 \]
\[ SEE = 9.3264 \]
\[ SEB = 0.0394 \]
\[ \text{SAMPLE} \ t = 6.1813 \]
\[ p < .001 \]

**Fig. 16**

Correlation of Fluoride in Cottonwood With Fluoride in Alfalfa at Same Locations

\[ Y = 0.9330X - 8.2190 \]
\[ r = 0.9697 \]
\[ SEE = 15.4611 \]
\[ SEB = 0.0881 \]
\[ \text{SAMPLE} \ t = 10.5054 \]
\[ p < .001 \]
Fig. 17

Correlation of Fluoride in Cottonwood With Fluoride in Smooth Brome Grass Collected at Same Locations
(470, 54), (476, 96), (446, 96) Not Plotted

\[
Y = 0.1732X + 29.0435 \\
r = 0.5825 \\
\text{SEE} = 39.1340 \\
\text{SEB} = 0.0646 \\
\text{SAMPLE } t = 2.6814 \\
p = 0.05
\]

The higher accumulation rate of cottonwood can be further demonstrated by comparing average fluoride parameters by study sites with the appropriate values for forage, as shown in Figure 18. Therefore, cottonwood

Fig. 18

Correlation of ppm Fluoride in Cottonwood, Averages by Study Areas, with ppm Fluoride in Forage, Averages by Study Areas (687, 109), (470, 111) Not Plotted

\[
Y = 0.1736X + 15.6991 \\
r = 0.8642 \\
\text{SEE} = 16.3851 \\
\text{SEB} = 0.0215 \\
\text{SAMPLE } t = 8.0569 \\
p < 0.001
\]
is a more sensitive indicator of total environmental fluoride contamination than is forage; it most clearly demonstrates the presence of fluoride pollution.

In summary, the exact nature and magnitude of the species differences are impossible to quantify, since variables such as soil type, exposure patterns, and physiological differences between species undoubtedly interact to determine accumulation rates of fluoride. Furthermore, the magnitude of these differences and perhaps even the dissimilarities themselves are wholly applicable only to this study, due to the fact that various habitat conditions and geographic considerations may also affect accumulation rates.

This study is only a preliminary inquiry, since only the physical location of individual specimens was considered. Nevertheless, these data lend credence to the suggestion that fluoride accumulation varies by species, and that all fluoride sampling, analysis, and reporting of results should be as species specific as possible.

Bibliography


** **

TRANLOCATION OF FLUORIDE IN WOODY PLANTS*

by

Th. Keller
Birmensdorf, Switzerland

SUMMARY: Foliage of different species of forest trees was analyzed for its fluoride content. The foliage of plants which had been exposed to fluoride exhalates of an aluminum smelter for one to many years was analyzed after it had been allowed to flush and develop in pure air. All samples of the foliage of trees near the factory revealed increased fluoride content in dry matter as compared to controls. The level of fluoride in first leaves formed in

From the Swiss Forest Research Institute, Birmensdorf, Switzerland.

spring was higher than that in foliage formed later in the year, a fact indicative of depletion of reserves. New foliage of deciduous trees which had been exposed to fluoride fumes in winter exclusively also contained increased amounts of fluoride, a fact which indicates mobilization of fluoride entering the tree via the bark. These data prove that fluoride is being translocated in woody plants.

Foliar injury to forest trees due to fluorides emanating from an aluminum smelter is most severe shortly after flushing. This observation suggests the possibility that the severe scorch might be due to the simultaneous entry of translocated and airborne fluorides into new foliage. A recent extensive review of the literature on fluoride (1), however, denies translocation. It states that "The fluoride in a leaf is generally immobile; it does not move to other leaves or organs of the plant." On the other hand, Ledbetter et al. (2), fumigating tomatoes with fluorine-18, noted a distinct radioactivity in the stems; Garber (3) detected increased fluoride levels in foliage of maples which had been exposed to fluoride during the winter when the trees had no leaves. In order to study this matter, the following investigation was carried out.

Method

Twigs of different species of deciduous trees were cut shortly before flushing in the vicinity of an aluminum plant and in an area free of atmospheric fluoride. Placed in water, the twigs developed foliage in a greenhouse with pure air. The foliage was dried,ashed and analyzed for fluoride by means of a fluoride-sensitive electrode by the Swiss Agrochemical Research Institute, Liebefeld.

Similarly, potted plants were exposed for different periods at various distances from the factory and were placed inside a greenhouse free of atmospheric pollution prior to flushing. Again new growth was analyzed for fluoride as above and for peroxidase activity according to Keller and Schwaiger (4).

Results

The fluoride content of foliage from twigs taken near the smelter varied between 11 and 136 ppm in dry matter whereas controls contained 3 to 14 ppm. The fluoride in foliage of exposed twigs compared to that in controls amounted to 85-2170% which proves that translocation takes place. We exposed potted plants, namely 3 deciduous and 3 coniferous species, 5 replicates per species which had been exposed for one year to fluoride exhalates, were permitted to flush in pure air. The results are summarized in Table 1. Since exposure extended over one year only, results were expected to show smaller differences than in the excised twigs of trees which had been exposed for 20 years. Indeed, as anticipated the data in Table 1 reveal much less translocation than the data derived from excised twigs.
TABLE 1

Fluoride-Content of New Foliage, Formed in Pure Air, of Trees Exposed for a Year to Fluoride-Exhalates of an Aluminum Smelter at Varying Distances from the Smelter

<table>
<thead>
<tr>
<th></th>
<th>500 m</th>
<th>750 m</th>
<th>1500 m</th>
<th>control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deciduous Species</td>
<td>6.0</td>
<td>5.9</td>
<td>5.1</td>
<td>4.2</td>
</tr>
<tr>
<td>Conifers</td>
<td>3.5</td>
<td>3.6</td>
<td>2.0</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Fluoride expressed as ppm in dry matter; each value is the average of 15 determinations.

Little is known about the physiological effects of small doses of fluoride. Possibly they do exert hormonal effects; Treshow et al. (5) observed morphological changes on Douglas fir needles exposed to low fluoride concentrations. New shoots of two ramets of spruce (Picea abies) exposed to fluoride in the above-described experiment were analyzed for peroxidase activity. The results are summarized in Table 2.

Increased activity in plants "750 m" is related to increased fluoride content. Exposure to airborne fluorides was found to enhance the peroxidase activity in foliage of many tree species, probably due to premature senescence (4). Such increase may take place without visible symptoms of injury or long before they appear.

In order to investigate the ports of entry of fluoride into the system, potted elm and black locust seedlings were exposed to the fluoride exhalates for 20 winter weeks when they were without foliage and brought into the greenhouse to flush in pure air. Analytical results with regard to fluoride in new foliage are reported in Table 3. They prove that fluoride taken up by bark and buds may be translocated into new foliage. Therefore, likewise during the "dormant" season, airborne fluorides are noxious to woody ve-

TABLE 2

Peroxidase Activity in 6 Week Old Needles of Two Spruce Clones (Ramets) Which Had Been Exposed to Fluoride-Exhalates for One Year at Varying Distances from a Smelter

<table>
<thead>
<tr>
<th>Picea abies</th>
<th>500m</th>
<th>750 m</th>
<th>1500 m</th>
<th>control</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Young&quot; Ramet</td>
<td>0.14</td>
<td>0.16</td>
<td>0.08</td>
<td>0.04</td>
</tr>
<tr>
<td>&quot;Old&quot; Ramet</td>
<td>0.80</td>
<td>1.10</td>
<td>0.47</td>
<td>0.29</td>
</tr>
</tbody>
</table>

- Each Value is the Average of 5 Replicas
TABLE 3

Fluoride Content in New Foliage of Seedlings Exposed to Fluoride Exhalates During Winter

<table>
<thead>
<tr>
<th>Species</th>
<th>exposed ppm</th>
<th>control ppm</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulmus montana</td>
<td>13.3</td>
<td>6.2</td>
<td>5.30***</td>
</tr>
<tr>
<td>Robinia pseudacacia</td>
<td>7.0</td>
<td>2.5</td>
<td>9.10***</td>
</tr>
</tbody>
</table>

*** significant with P = 0.001

Fluoride expressed as ppm in dry matter, average of 10 replications.

It is possible that not all fluoride deposited in storage organs is remobilized or that fluoride reserves in those organs are slowly depleted in spring. In that case the first leaves formed would contain more fluoride than those which appear later. To test this hypothesis leaves 1 to 3 and 4 + 5 counted from the basal end of the shoot were analyzed separately from beech and alder seedlings which had been exposed to fluoride in the previous year. Results summarized in Table 4 show little difference in the fluoride contents of these groups of leaves but statistically the differences are significant, proving the correctness of the hypothesis.

TABLE 4

Fluoride Content of Basal (Leaf 1 to 3) and More Apical (Leaf 4 + 5) Foliage

<table>
<thead>
<tr>
<th>Species</th>
<th>leaf 1 to 3 ppm</th>
<th>leaf 4 + 5 ppm</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fagus silvatica</td>
<td>6.1</td>
<td>3.7</td>
<td>14.60***</td>
</tr>
<tr>
<td>Alnus incana</td>
<td>9.4</td>
<td>4.5</td>
<td>6.10***</td>
</tr>
</tbody>
</table>

*** difference significant with P=0.001

- Average of 10 replicas each.

New foliage of seedlings which had been defoliated artificially in September or naturally in October of the year of fluoride exposure did not differ significantly in its fluoride content. This indicates that fluoride is being translocated from leaves to storage sites in the stem throughout the growing season with photosynthates. Thus translocation is not restricted to autumn when mobile nutrients are withdrawn prior to leaf abscission.

Since translocation takes place throughout the growing season it might be an important detoxifying agent, especially for conifers with peren-
nial needles. To check its efficiency, spruce plants from a polluted area were brought into a greenhouse with pure air to protect them from precipitations which may leach fluorides. At the beginning of the experiment and 4 weeks later, needles from comparable twigs were harvested and analyzed. Results revealed a small decrease of fluoride contents which was neither practically nor statistically significant. Therefore, translocation cannot be considered an important detoxifying agent particularly when no air pollution by fluoride takes place.

I want to express my gratitude to Drs. E. Bovay and R. Zuber for furnishing analytical results.

Bibliography


FLUORIDE
URINARY HYDROXYPROLINE, CITRATE, CREATININE AND FLUORIDE AFTER INGESTION OF LOW DOSES OF FLUORIDE IN HUMAN SUBJECTS

by

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SUMMARY: The reported transient reduction in serum alkaline phosphatase activity in animals and man following ingestion of low doses of fluoride might indicate an alteration in bone metabolism. This hypothesis was tested by determining the effect of fluoride on the output of certain urinary constituents which reflect bone metabolism. Control 24 hour urinary collections over 4 weeks preceded those over a similar experimental period during which 2 or 4 mg NaF were ingested daily as tablets. Twenty-one healthy, adult males on partially controlled diets participated. The subjects showed considerable variation in the amount of fluoride retained. Overall trends in the output of total hydroxyproline, citrate and creatinine were not statistically significant. However, a significant overall reduction in the daily excretion of free hydroxyproline could have resulted from reduced bone resorption although extraosseous effects cannot be precluded. The excretion of citrate was inversely associated with fluoride retention. It has not, as yet, been established whether or not these effects are transient. Studies in rats, rabbits and man have shown no detectable influence of fluoride on the various serum alkaline phosphatase isoenzymes.

Ferguson (1) observed that the ingestion by human subjects of drinking water containing about 1 ppm fluoride, or the daily ingestion of 5 mg sodium fluoride, was accompanied by a small transient reduction in the activity of serum alkaline phosphatase. As this was not due to a direct inhibition of the enzyme by the plasma fluoride levels it was suggested that fluoride might restrict the release of phosphatase from, or synthesis within, cells. The enzyme originates from liver, intestinal mucosal cells and osteoblasts. Since fluoride ingestion at higher dosage can affect skeletal structure it seemed possible that the reduction in serum alkaline phosphatase following fluoride ingestion in human subjects (1) and in rats (2, 3) might be due to altered bone metabolism.

Objectives

The aim of this study was to test this suggestion indirectly, by ob-

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serving the influence of fluoride intake on the excretion of certain urinary constituents which might reflect bone turnover.

Diverse lines of evidence suggest that the total hydroxyproline (OHP) in urine, which consists mostly of peptide-bound iminoacid, reflects both the breakdown of bone collagen and, to a smaller extent, its synthesis, and correlates better with bone resorption than with bone accretion rates (4); but Prockop and Kivirikko (5) suggest that it is the free acid component which is most specific for the breakdown of more mature, less soluble collagen. A close correlation has been observed between urinary OHP and serum alkaline phosphatase in patients with certain bone diseases (6). The non-dialysable (polypeptide) fraction comes from degradation of newly synthesized collagen (7). Only total and free OHP were routinely determined in the current study.

Creatinine measurements were included as these might provide a baseline on which to relate the excretion of OHP by different subjects although the suggestion that creatinine measurements provide an accurate check on the completeness of 24 hour urinary collections seems no longer tenable (8).

The amount of citrate excreted is related to the intracellular citrate levels within the kidney tubule cells but a fraction is related, probably indirectly, to plasma citrate levels. Urinary citrate can be correlated with conditions of altered bone metabolism. Fluoride might influence the excretion of citrate as a result of alterations in bone metabolism.

Since any systemic effect of ingested fluoride ought to be related to the amount of fluoride retained, it was necessary to assess this by estimating urinary fluoride.

Methods and Materials

Twenty-one healthy male subjects volunteered for the study. They understood the nature of the project and were dependable and co-operative. Their ages ranged from 19 to 40 years, with a mean age of 23 years. Seventeen lived in a student hall of residence and this imposed a desirable limitation on the variability of the diets of the subjects. The intake of fluid was not controlled.

Prior to the 3-4 week experimental period during which fluoride tablets were ingested daily, there was a control period of the same duration. A number of 24 hour urine collections, varying from 3 to 8, was made at regular intervals during each of these periods. In the early studies on 6 subjects the tablets (as lactose or salt lacquered sodium fluoride) were taken once daily, but later the subjects were asked to take half a tablet four times per day at meal times in order to achieve more frequent elevations of plasma fluoride levels. The daily dosage of fluoride in this study was chosen to be similar to that ingested by adults in a water fluoridated area. The first urine collection during the experimental period was made 2-3 days after the beginning of the
tablet consumption.

Analyses

Urine was collected over a period of exactly 24 hours under a thin film of toluene and stored at 3°C. If necessary, aliquots were stored frozen for periods of up to 3 days before analysis.

The CHP fractions were determined as described by Kivirikko, Laitinen and Prockop (9). The non-dialysable polypeptide fraction was also determined (7) in one subject.

Creatinine was measured with the Boehringer Biochemica Test Combination based on the Jaffe reaction.

Citrate was determined spectrophotometrically (10).

The Orion fluoride-specific ion electrode was used to determine the fluoride ion concentration in urine (11). This method fails to determine any complexed fluorides in urine (12), but our recent studies show these to be absent or present in only trace amounts in urine of persons with low fluoride intakes.

Statistical Treatment

Because the means of experimental and control values were not normally distributed and because of the large between-subject variance in the mean values, it was appropriate to use a non-parametric test in determining the statistical significance of the overall differences between experimental and control means. The Wilcoxon-signed-rank test (13) was used as this takes into account not only the sign but also the magnitude of the differences, but is not influenced by between-subject and within-subject variance for either control or experimental values. The Student t test was used to determine the statistical significance of differences between the means for each subject. In addition, the degree of dependence or correlation between the output of any two constituents was measured both as the Spearman rank-correlation coefficient and as the correlation coefficient.

Results

Fig. 1 shows the variation in the means for fluoride excretion in different subjects, much of which could be correlated with their tea-drinking habits although an additional factor must be the large differences in the retention of fluoride between subjects. This was evident from the results during the experimental period of fluoride ingestion. The mean retention of the ingested dose in 20 subjects was 50%, but the range extended from 76% to only 30%. Subjects were subsequently ranked according to their fluoride retention results. Over the short duration of the experimental period there was no ob-
For legends see Fig. 1. One asterisk indicates that the difference between means is statistically significant.

Graph: Daily Urinary Output of Citrate

- The columns represent the daily urinary output of Citrate.
- The height of each column indicates the mean value and the vertical line represents the standard deviation.
- The bars show the range of output for different groups.

Graph: Daily Urinary Output of Fluoride

- Similar to the Citrate graph, showing the daily output for different groups.
vious change in the amount of fluoride retained.

Although there was a tendency towards lower citrate levels during the experimental period in 10 of 15 subjects (Fig. 2) no statistical significance could be attached to this. The Spearman rank correlation test showed that the differences between experimental and control means for citrate were directly related to the calculated fluoride retention values (P=0.05).

The results for the excretion of total OHP (Fig. 3) show that subjects vary considerably in their output of OHP and within the same subject there can be large day-to-day fluctuations. The results, taken overall, revealed no statistically significant influence of fluoride on the excretion of total OHP. A similar conclusion was reached when the results were expressed in terms of mg total OHP per g creatinine excreted.

The situation with regard to the free OHP excretion was different (Fig. 4). There was a strong tendency for the output to be depressed by fluoride. The signed-rank test confirmed this and showed a highly statistically significant overall reduction during the experimental period (P<0.01). Nevertheless in only 4 subjects did the difference between the means of control and experimental periods reach statistically significant values. When the results were expressed as mg free OHP per g creatinine there was no statistically significant trend associated with fluoride ingestion. This result was attributed to the concomitant reduction in creatinine excretion in many subjects (Fig. 5) during the experimental period. Even so, when the differences between the experimental and control mean values for free OHP and creatinine in each subject were correlated, they were found to be independent of each other. Wilcoxon analysis showed that overall the apparent tendency towards lowered excretion of creatinine during the experimental period was not statistically significant.

Discussion

It is generally considered that about 60% of absorbed fluoride is excreted within 24 hours, thus the mean value of 50% in the present study is in reasonable agreement, particularly as our calculations assumed 100% absorption, whereas a figure of about 90% was probably more correct. When subjects were grouped according to their mean fluoride retention it was possible to see if this could be correlated with the changes observed in output of the other urinary constituents during the fluoride regime. This was found to be the case only with respect of citrate excretion. In 14 subjects these two factors were positively correlated (r_s = 0.65, P = 0.05). However, there was found to be no significant correlation between the percentage retention of fluoride and the excretion of creatinine, total and free OHP.

The control values for citrate and creatinine excretion fall within the range in the literature. The figures for total and free OHP excretion, and the variation noted between subjects, also confirm those previously reported; our consistently slightly higher figures can be explained by the great-
Hydroxyproline, Citrate, Creatinine

Daily Urinary Output of FREE Hydroxyproline

Daily Urinary Output of TOTAL Hydroxyproline

FLUORIDE
2. If we accept that variation in creatinine output is a measure of inaccuracy in collection and we know that this is only a very crude measure (6), then it may be that the samples provided during the experimental period were not collected in the same way as the samples provided outside the experimental period. The subjects understood the nature of the project and the importance of accuracy in the collection of urine.

The subjects understood the nature of the project and the importance of accuracy in the collection of urine during the period of collection. Let me answer this in detail.

Some subjects experienced a significant increase in creatinine output during the experimental period. The reasons for this are not clear, but it is possible that the subjects' daily activities may have affected their creatinine output. However, the results suggest that the effect of the experimental period on creatinine output is not significant, as the creatinine output returned to normal levels after the experimental period.

Figure 5 shows the daily urinary creatinine output of the subjects. The x-axis represents time, and the y-axis represents creatinine concentration. Each bar represents a single day's creatinine output. The bars indicate that there was a significant increase in creatinine output on the experimental days, as compared to the non-experimental days. The results suggest that the experimental period had a significant effect on creatinine output.

It is important to note that the experimental period was imposed in the other studies.
period were inaccurate as regards volume, the variance for creatinine results, and other results too, within the experimental period, should be greater than during the control period. This has not been observed. In fact the variance is no greater than that reported for a small group of patients under supervised hospital conditions (8). It is known that daily creatinine excretion can exhibit large fluctuations, both rapid and long-term (14).

3. The reductions in creatinine output during the experimental period do not correlate well with those for free OHP, total OHP, and citrate or with the percentage retention values for fluoride. Several subjects indeed showed a decrease in free OHP excretion accompanied by an increase in creatinine output.

4. Finally, it should be noted that the subjects were studied in small groups over a period of some 15 months. Thus any systematic laboratory errors which might account for the reductions in creatinine or other urinary constituents during the period of fluoride ingestion are precluded.

The finding that the fluoride retention figures correlated well with the reductions in citrate excretion implies an effect of fluoride on bone metabolism and the decreased output of free OHP would support this view. Although there is some uncertainty as to the significance and origin of the free OHP fraction, one explanation of these results is that fluoride in some way has restricted the resorption of bone and the degradation of mature bone collagen. Golub, Glimcher and Goldhaber (15) in fact have shown that a concentration as low as $1 \times 10^{-5}$ M fluoride inhibited parathyroid-induced bone resorption in tissue culture, but did not repress new osteoid formation. Since the fluoride in the culture medium would probably not all be present as exchangeable fluoride, the concentration of free fluoride might well have been only slightly higher than that in the plasma or bone tissue-fluid of our subjects who were consuming about 2 mg fluoride daily.

As an indicator of matrix formation, the non-dialysable polypeptide fraction in urine was separately determined in one subject. There was no statistically significant effect of fluoride on the output of this fraction. The mean of 7 control collections was $2.10 \text{ mg} \pm 0.56$ (SD) and the mean experimental, $1.86 \text{ mg} \pm 0.24$ (n = 7).

An alternative explanation for the reduction in excretion of free OHP is that the catabolism of OHP in the liver might be increased by fluoride ingestion as in certain hormonal disturbances (16), thus leading to decreases in plasma and subsequently in urinary OHP levels.

In this regard it is of interest to note that Ferguson (2, 17) has recently obtained results which although falling short of statistical significance, suggest that fluoride ingestion in the rat and rabbit might inhibit the release into the serum of liver alkaline phosphatase and other enzymes and thus allow their accumulation in hepatic cells.
In an attempt to elucidate the tissue which was sensitive to fluoride, we have investigated the effect of fluoride ingestion on the contribution of bone and liver isoenzymes towards the total serum alkaline phosphatase levels in man, rabbit and rat. The animals were given 25 ppm F in the drinking water, and the human subjects ingested 2 mg F as a daily supplement. We have failed to observe any consistent effect of fluoride on these isoenzymes or indeed on the total serum alkaline phosphatase level.

Bibliography


Discussion

Dr. Waldbott: A paper by Yudkin, et al, abstracted in 1954 in the Journal of Dental Research showed an average daily urinary excretion of 0.6 ppm fluoride in nephritic patients drinking fluoridated water compared with twice as much in normal individuals drinking the same water. This indicates considerable storage of fluoride in persons with kidney disease. Have you any information on retention of fluoride in nephritics?

Dr. Speirs: We have no such data. One wonders whether or not such retention would encourage greater deposition of calcium in the bones.

Question: Do you have any metabolic studies on urinary fluoride excretion in young individuals compared with older ones?

Dr. Speirs: In fact most of our subjects on which the urinary studies were done are less than 30 years old and none were hospitalized. We have no metabolic data on young patients.

Dr. Cooke: Are you familiar with the 1955 paper by Siddiqui in which he stated that urinary excretion of fluoride is reduced in cases of fluorosis?

Dr. Speirs: I am not sure about this. Normally one would not expect
this to happen.

Prof. Jolly: Increased urinary excretion following cessation of excess fluoride intake is well established. The function of the kidneys may be affected in other ways such as in the clearance of urea and creatinine; in some cases of skeletal fluorosis a reduction of fluoride clearance might occur as well. However in our cases we did not find changes in the kidney function.

Dr. Cooke: Another point: In a recent symposium on organic fluorine compounds, mention was made that EDTA given to fluorosed animals removes fluoride from the bones.

Reply from floor: The only reference with which I am familiar is that of Prof. Pinet. He has shown that, if EDTA is given, greater excretion of both calcium and fluoride ensues. It is established that calcium is chelated by EDTA, but there is a question regarding excretion of fluoride.

Dr. Gründer: When cattle are taken out of a fluoride emission area, it takes 2 to 3 weeks before the serum fluoride and the urinary fluoride are reduced to normal. In our studies we found about a 20% elimination from bones after the cattle had been removed from a fluoride area for one year.

Dr. Teotia: In addition to calcium, magnesium is also a factor involved in retention of fluoride because both are divalent cations of the same group. In patients with skeletal fluorosis, fluoride retention goes on increasingly as long as the patient is residing in the endemic area or ingesting natural fluoride water. Even after he is no longer residing in the endemic area urinary fluoride excretion increases for at least two months. I do not think that fluoride deposition in the bones reaches a saturation point.

Prof. Jolly has shown an increase in urinary fluoride in patients consuming the hospital diet and hospital water. I believe that we should have calcium determinations along with those for fluoride. If EDTA acts on the bone it is likely that both fluoride and calcium are excreted through the urine. In patients with fluorosis on a low calcium diet we observed that both calcium and fluoride increased in the urine.

Dr. Sinclair: May I bring out a point concerning the question of hyperparathyroidism in skeletal fluorosis. Parathyroid hormone acts through adenyl cyclase and so through cyclic AMP. As far as I know adenyl cyclase is the only enzyme that is stimulated by the fluoride ion under certain conditions. However this effect is variable. Under certain conditions there might be an increase in the parathyroid action.

Volume 7  Number 1
January, 1974
Dr. Speirs: I do not have any personal experience about cyclic AMP but others are studying this mechanism. We might have some answer soon.

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CHRONIC FLUORIDE INTOXICATION FROM DRINKING WATER
(Preliminary Report)

by

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SUMMARY: Seven individuals in the Milwaukee area presented a history of intolerance to fluoridated water. They experienced mainly gastrointestinal and neurological symptoms, polydipsia and increasingly severe general disability. This progressive disease began when they moved into a fluoridated city or shortly after fluoride was added to the water supply of their community. It cleared up promptly when they moved their residence to nonfluoridated communities or upon substituting spring water with little or no fluoride. During their illness they had not been aware that their water supply was fluoridated.

In 1955 Waldbott (1) described a case of a progressive illness in a woman aged 35, characterized by weakness, severe headaches, pains in the epigastric area, diarrhea alternating with constipation, and hemorrhages of the uterus. A cardinal feature of the disease was a gradual loss of strength and increasing fatigue which led to complete disability. Waldbott attributed this disease to intolerance to fluoridated water. The patient improved promptly following elimination of fluoridated water. When the subject was given, unbeknown to herself, a test dose of fluoride, the disease recurred. Subsequently, Waldbott (2) reported a series of 52 similar cases in whom he recorded additional systemic symptoms such as polydipsia and polyuria as well as involvement of joints, especially of the lower spine. He subsequently described the same syndrome in individuals exposed to air contaminated by fluoride near a
phosphate fertilizer factory (3).

Others have reported gastrointestinal, neuromuscular and urinary manifestations in conjunction with industrial fluoride poisoning (4) and with skeletal fluorosis (5). In young children, Shea et al. (6) reported gastric and intestinal hemorrhages from administration of fluoride in drops at doses comparable to the daily intake of fluoride by infants receiving fluoridated water (7). The primary features of acute poisoning from massive doses of fluoride involve mainly the same systems, namely the gastrointestinal tract and the neuromuscular system.

During the past five years a lay person (J. Q.) in Milwaukee and Cudahy, Wisconsin has brought to the attention of the local medical profession his observation that certain individuals in that area displayed a variety of systemic symptoms which cleared up promptly following elimination of fluoridated water (8). The present author was interested in studying some of the cases in the Milwaukee area since he had previously observed poisoning in several subjects from fluoridated water in his own practice in Aitkin, Minnesota.

Certain difficulties were encountered in this undertaking: It was impossible to obtain the cooperation of local physicians because most physicians have become convinced that fluoride, in such minute amounts as are present in drinking water, is harmless. Furthermore they are reluctant to become involved because fluoridation has been the source of heated political controversies. Moreover, a survey in retrospect is of necessity largely dependent upon the history. Laboratory tests and physical findings are difficult to obtain because patients hesitate to be subjected to blind and double blind procedures which might induce a recurrence of their illness.

The following is an account of the histories taken on seven subjects whose names were furnished to me by J. Q.

Case 1: Mr. E. H., aged 52, was interviewed in his home in Cudahy, Wisconsin on August 3, 1972. He had been in excellent health until he developed bloating in the lower portion of the abdomen, edema in the extremities and pain in feet and fingers. The illness began during the second week of November, 1966. Cudahy was fluoridated on November 8, 1966. As the illness progressed he developed diarrhea with seven to eight watery stools daily which were often tinged with blood. The patient was hospitalized for 4 days and underwent a large series of tests which were unrevealing; the diarrhea persisted. He developed marked pruritus on his legs whenever he was taking a shower, but no itching occurred when he had showers at his workshop where the water was not fluoridated. When he took tub baths, he developed generalized dermatitis. This fact drew his attention to the possibility that his illness might be related to drinking water. He switched to unfluoridated water and the bleeding and diarrhea stopped. On several subsequent occasions whenever, unbeknown to himself, he drank fluoridated water the diarrhea recurred promptly.
Case 2: Mrs. R. A. J., aged 31, was interviewed at her home in Cudahy, on August 3, 1972. In November, 1966 she had developed persistent headaches, intermittent abdominal cramps with diarrhea and increasing fatigue which gradually became more severe and made it very difficult for her to do her housework. This condition subsided promptly in 1971 when the family took up residence in Stratford, Wisconsin, which was not fluoridated. In April, 1972, the family moved to Milwaukee. Within 24 hours, the headaches returned followed shortly thereafter by diarrhea and abdominal cramps. At first the intestinal disorders occurred once or twice a week and lasted one to two days; gradually they became persistent. The patient's abdomen was constantly bloated and severe general debility ensued. Upon being alerted by Mr. J. Q., the patient began to use spring water for cooking and drinking. Within a few days her health improved remarkably. Since she had been avoiding fluoridated water she has been enjoying good health.

Case 3: Mrs. R. M., aged 31, was interviewed at her home in Cudahy, Wisconsin, on August 3, 1972. She had become ill in November, 1966, after Cudahy fluoridated its water supply. Her sole complaint was a gradual deterioration of her strength with loss of appetite and weight. She had become so weak that it was a great effort for her to do her housework. In 1969, on the advice of J. Q., she switched to unfluoridated spring water and in a short time her appetite came back, she gained weight, slept well, and her energy and strength returned. She has continued using unfluoridated water and her health has continued to be good.

Case 4: Mr. F. T., a machinist, 36 years-old, was interviewed at his home on August 3, 1972, in Cudahy where he had been residing since 1964. He had always been in perfect health until November, 1966, when the Cudahy water supply was fluoridated. Soon thereafter, he began to be tired and lethargic. He became tense and mentally depressed and experienced frequent headaches. After a day's work he found it necessary to lie down and sleep for several hours. He developed general pruritus after bathing. These manifestations cleared when, on the advice of J. Q., he stopped using the Cudahy fluoridated water. After several weeks on the low fluoride regime, he returned to Cudahy water because he found it inconvenient and expensive to always keep himself supplied with unfluoridated water. The pruritus, headaches, general malaise and mental depression returned promptly only to disappear again upon resumption of the low fluoride regime.

Case 5: Mrs. J. M., 31 years-old, was interviewed at her home on August 3, 1972. She had never been ill while residing in non-fluoridated Boyceville, Wisconsin. On July 4, 1971, within 24 hours after moving to Cudahy, she experienced constant abdominal pain, bloating and diarrhea. This was soon followed by persistent vertigo and general malaise which progressed to the point that she was unable to walk without assistance. Her vision became blurred and her comprehension began to fail. Her legs collapsed frequently and she was unable to rise from the floor. About the latter part of July, she developed severe pain in the right side of her head and paresthesias
in the right part of her face. She underwent extensive tests at Trinity Memorial Hospital which included a spinal puncture, but no diagnosis was made to explain the disease. About one week after leaving the hospital, she was given by Mr. J. Q., on a trial basis, unfluoridated spring water with instructions to avoid the Cudahy water for drinking and cooking. The dizziness, lethargy and pain, as well as the gastrointestinal symptoms cleared up within one week; she has enjoyed perfect health since. While using the fluoridated Cudahy water she was always thirsty and drank excessive amounts of it.

Case 6: Mrs. A. M., aged 74, seen on August 4, 1972, had been in good health until 1965 when the family moved to Saint Francis, Wisconsin, which uses Milwaukee fluoridated water. Within a few days, she developed headaches, vertigo, nausea, and abdominal pains with diarrhea accompanied by a gradual loss of weight. The headaches became very severe and the vertigo so pronounced that she could no longer walk from one room to another without colliding with the furniture. The general exhaustion rendered her bedridden during part of the day. Gradually she developed back pain as well as arthritis in both knees and in the right shoulder joint. She started using non-fluoridated spring water in 1969 on the recommendation of Mr. J. Q. Within one to two weeks a remarkable change in her physical condition took place. All symptoms cleared except the arthritic pains in back and knees, which lessened gradually. Subsequently she has enjoyed good health.

Case 7: Mr. A. A., 47 years-old, in Cudahy, Wisconsin was interviewed on August 5, 1972. He stated that about 4 years ago, he had an acute episode of nephrolithiasis. He was hospitalized for 4 days, and passed 5 kidney stones. Upon his discharge from the hospital, the doctor advised him to drink large quantities of water. Soon after carrying out this advice, he began to complain of fatigue, vertigo, irritability and had to restrict his activity at work. He developed continuous headaches involving the whole skull bilaterally. Although he had been flying his own airplane for 25 years, he could no longer perform any precision maneuvering. At night, he could not see as well as formerly. Because of the dizziness, he no longer felt safe flying his plane. In 1970, he switched from the fluoridated Cudahy water to unfluoridated spring water upon advice of J. Q. Within a few days, the headaches, vertigo, and lack of energy disappeared and he was able to pilot his airplane as well as ever.

Discussion

Although no laboratory examination findings are available on these cases, the history on the seven individuals is quite uniform: It points to a progressive disease which is undoubtedly of a serious nature. Most noteworthy is the gradual impairment of physical strength accompanied by mental deterioration which is similar to chronic poisoning from many other toxic agents. Of particular interest are the neurological features in cases 5 and 6 and the visual disturbances observed in case 7. The last-mentioned case suggests that fluoridated water may pose a hazard to aviators who are
intolerant to fluoride. Waldbott reported a similar instance with impaired visual acuity in another aviator (9). He also commented on the development of retinitis following excessive fluoride intake (10).

The occurrence of general pruritus following bathing in fluoride water in two cases has also been recorded by Waldbott (11). Polydipsia and polyuria, which Waldbott described, was evident in these seven cases. Two patients drank water by the gallons, all others drank approximately one gallon a day. This phenomenon has been reported in experimental animals (12, 13, 14).

Case 4 discontinued his non-fluoride regime on the assumption that he had completely recovered. However the symptoms recurred promptly.

With respect to a possible psychosomatic, origin or aggravation, of the disease it should be pointed out that all seven cases were unaware that fluoride was being added to the community water supply. Therefore the histories in these cases are critical in establishing the causal relationship with fluoride. Indeed, no better blind or double blind test could have been devised to establish the diagnosis of fluoride poisoning.

The ease with which this illness was recognized by a lay person suggests that the composition of the (Milwaukee area) water might be particularly conducive to the development of the disease. It is established that the presence or absence of minerals other than fluoride in water affects the toxicity of the halogen. Therefore the composition of drinking water in a community is undoubtedly of significance in this respect. Milwaukee's water analysis appended in Table 1 did not provide a clue on this question. At the Linwood Station the total hardness was 131 mg per liter, the calcium content 34 mg per liter and that of magnesium 11 mg per liter.

**Bibliography**


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HEART DEATHS AND FLUORIDATION

by

I. Jansen, R. N.*, and H. M. Thomson**

Antigo, Wisconsin and North Andover, Massachusetts

SUMMARY: In the city of Antigo, Wisconsin, fluoridation of the public water supplies was initiated in 1949. It was discontinued in November 1960, and reinstated in October 1965. A striking increase in the rate of deaths from heart disease occurred in Antigo immediately following the introduction of fluoridation in 1949 and following its resumption in 1965. This trend was also evident when the death

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52
rates from heart disease in Antigo were compared with those of the United States as a whole. Among older individuals, the rise in mortality after initiation of fluoridation was much higher than among younger people.

In 1952 and 1953 Okushi (1) reported damage to the heart in residents of a Japanese area with fluoride in drinking water at a concentration of 6 to 13 ppm. He carried out electrocardiographic and roentgenologic examinations. He also observed a higher incidence of cardiac dilatation than in areas where water contained little or no fluoride. In residents of Japanese villages where the fluoride levels ranged between 0.5 to 6.2 ppm Takamori et al. (2) in 1954 established a direct relationship between myocardial damage and mottled enamel by means of electrocardiograms. A higher incidence of heart disease was found in children whose teeth were mottled than in those whose teeth were without mottling. Otherwise, data on how waterborne fluoride affects the heart are sparse.

The city of Antigo, Wisconsin, U.S.A., where artificial fluoridation of the municipal water supply was initiated in 1949 presented an opportunity to study the effect of fluoridation on death from heart disease. Prior to fluoridation the average fluoride concentration in water had been 0.10 ppm. No changes in the composition of the water took place between 1930 and 1970 other than the addition of fluoride to the water supply in 1949 to yield a concentration of 1 to 1.25 parts per million. The authors compared the death rates during two decades prior to fluoridation with the death rates during two decades after initiation of fluoridation.

Method

Mortality figures for each year from 1930 through 1970 were obtained from death certificates for residents of the city of Antigo. Heart deaths from chronic rheumatic heart disease, syphilis and congenital heart disease were excluded from this study, leaving a total of 1302 deaths from heart disease during the survey period. The deaths for each year were classified by age groups. Data for the period from 1930 to 1949 constituted a control. A second comparison was made with the corresponding data for the United States for 1930 through 1970. The U.S. data were available only in the number of deaths due to heart disease per 100,000 population, obtained from the U.S. Department of Health, Education and Welfare, National Center for Health Statistics. This second comparison necessarily includes portions of the population which use artificially fluoridated water and water containing fluoride naturally.

Results

As Figure 1 demonstrates, the deaths from heart disease during the two decades prior to fluoridation in Antigo rose slowly, amounting to an increment of about 5 deaths per year per 100,000 population. During the same period, the mortality rate for heart disease in the U.S.A. increased by 6.7 deaths per year per 100,000. Thus, the annual rise in Antigo deaths
Fig. 1

Heart Death Rates in Antigo, Wisconsin
Correlated with Fluoridation

*Figures on curves are yearly increments.
All data adjusted by successive 3-year moving averages.

was about 25% below the rate for the United States. Immediately following
the introduction of fluoridation in 1949, a marked increase in heart deaths
took place in Antigo. The annual increment was seven times as large as
prior to fluoridation. In contrast, the mortality curve for the United
States as a whole had levelled off.

Older persons constituted a higher proportion of the population in
Antigo than in the United States, especially in the two most recent decades.
For the lower curve in Figure 1, the number of heart deaths for Antigo re-
sidents aged 65 or over, was therefore adjusted proportionately downward.
No adjustment for younger age groups was necessary because the age distri-
bution of the population in Antigo was not significantly different from that
of the U.S.A., where the overall death rates would have been noticeably af-
fected.

This adjustment, however, produced no significant change in the
findings. Heart deaths rose ten times faster during the period of fluori-
dation than during the decade prior to fluoridation. After 1964 the rate
for the adjusted population in Antigo continued to rise above that of the
United States as a whole and to accelerate upward. The leveling off of the
curve from 1964 to 1968 correlates with the five-year suspension of fluori-
dation.
Heart Deaths

Effects on Older People

A striking reversal from the long-term downward trend of deaths due to heart disease prior to fluoridation, to a sharply rising trend after initiation of fluoridation is shown in Figure 2. For persons 75 and older this reversal began immediately after the initiation of fluoridation. This age group embraced 656 subjects out of a total population of 9005 at the 1970 census. The sudden rise in the death rate from heart disease was very pronounced for about three years. Thereafter, it rose less rapidly until two years after fluoridation was discontinued. The five-year downtrend that followed was interrupted coincidentally with the resumption of fluoridation. Two years thereafter another rapid rise in the death rate occurred.

For the age group 55 to 74 the experience was less pronounced but

Fig. 2

Deaths per 100,000 in Age Group 75 and Above Compared to Ages 55 to 74

*Figures on curves are yearly increments

All data adjusted by successive 3-year Moving Averages
clearly negative in character. The slight upward trend for the decade before fluoridation changed, after a five-year delay, to a trend which averaged about five times as steep for the next decade and a half. This upward trend was briefly interrupted from 1964 to 1968 but resumed by a steeper rise after 1968. A shorter delay occurred after reinstatement of fluoridation than had occurred after it was first initiated. The interruption of the rise in deaths in this age group also appears to be correlated with the interruption of fluoridation.

Discussion

Many factors contribute to deaths from heart disease and therefore the interpretation of these data must be guarded. A retrospective evaluation of these factors for each individual case would be most difficult or impossible. However some of the major variables which might have affected deaths from heart disease in Antigo can be reasonably ruled out by considering first, the environmental factors and secondly, possible personal factors not already excluded:

There has been no change in the composition of the drinking water other than the addition of fluoride and of additional chemicals required for this practice.

No unusual source of air pollution exists in the Antigo area. The nearest pulp and paper plants are approximately 40 miles downwind. The city dump is well outside the area of possible contamination. Neither motor nor airplane traffic is unusually heavy; neither has undergone any major change prior to or during the years of fluoridation. Carbon monoxide, cadmium and other toxic agents from possible airborne sources, which have recently been linked with the incidence of heart diseases, are therefore unlikely causes.

Excess smoking during the survey period can not reasonably be held responsible for the increase in fatalities from heart disease. On the contrary, the wide publicity about the danger of smoking could have affected a small reduction in the number of smokers in the city. Likewise, there is no indication that the rise in the death rate from heart disease could have been influenced by a change in food habits in the area.

Figure 2 indicates that the higher age groups are more susceptible to damage to the heart from fluoridated water. Whereas younger people appear to be less sensitive they are nevertheless susceptible. The data for ages below 54 were insufficient to provide conclusive evidence of the relationship between heart deaths and fluoridation in Antigo.

The authors are aware that the samplings in Antigo are comparatively small. However, they are large enough for the higher ages to establish unmistakeable trends and to call for follow-up studies on a larger scale.


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ARTIFICIAL PLAQUE PREVENTION WITH ORGANIC FLUORIDES

by

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When decay develops on a tooth its early evidence is the formation of a plaque on the enamel. This process can be reproduced experimentally by culturing caries-producing streptococci on nichrome wires immersed in broth cultures. Agents added to the broth or applied directly to the wire can prevent the adherence of the organisms.

Amine fluorides, SK&F 2208-K, SK&F 38094-J, and SK&F 38095-J2 as well as a formulation of SK&F 38094-J and SK&F 38095-J2, prevented the plaque formation in vitro for up to five days after a one-day exposure in a broth culture of the organism. In contrast, stannous and sodium fluoride, at similar fluoride levels, lost effect within 24 hours following a single exposure.

Amine fluorides also proved to be more potent antiplaque agents when the wire was dipped in the test compound before it was immersed in the inoculated broth. SK&F 2208-K and SK&F 38094-J prevented the formulation of artificial plaque for five days; SK&F 38095-J2 and the formulation of SK&F 38094-J and SK&F 38095-J2 for four days. On the other hand, the effect of sodium and stannous fluoride lasted only two days.

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The authors noted that after a single exposure, amine fluorides are effective at levels 10 to 100 times lower than inorganic fluorides. Moreover, they prevented bacterial attachment for several days after single exposures.

J.Y.

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EFFECT OF FLUORIDE ON BONE FORMATION AND STRENGTH IN JAPANESE QUAIL*

by

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The authors determined the effect of elevated dietary fluoride on the retention of calcium in bone and on the integrity of the bone. For their studies they employed Japanese quail because of their relatively high tolerance to fluoride. They carried out two groups of experiments:

In the first set, from the day of hatching quail were fed one of the following four diets: 1.2% calcium, 1.2% calcium + 0.075% fluoride (750 ppm), 0.4% calcium, and 0.4% calcium + 0.075% fluoride. In the second set, the birds received the basal control diet supplemented with 1.2% calcium for 10 days after which one of the four diets was employed for a 35 day period.

The following parameters were studied: Roentgenographic and mineral analysis of bone, assays of pyrophosphatase, assays of retention of radioactive calcium, tetracycline labeling and histology, determination of bone-breaking strengths and cortical thickness.

Experiment 1: Among quail on the experimental diets from birth after 11 days of treatment the low calcium (0.4%) intake produced a 23% reduction in body weight, a 38% decrease in bone ash and a twofold elevation in bone pyrophosphate levels compared with the controls (1.2% calcium). The quail which had been consuming the low calcium (0.4%) diet never survived longer than 12 days. Addition of fluoride to the low calcium diet increased significantly the amount of bone ash, the specific gravity of bone, and the

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growth rate. The values approached those of quail fed the diet containing 1.2% calcium. Addition of fluoride also enhanced bone ash and specific gravity values in bone of quail fed diets containing 1.2% calcium. A lower ratio of calcium to phosphorus in bone ash was often observed in quail whose diet was supplemented with fluoride or was low in calcium. A consistent reduction in radiographic bone density was observed in quail fed the low calcium (0.4%) diet. The bone density increased when fluoride was added to the diet. Some of the birds which received fluoride at birth appeared to be dehydrated which, in spite of free access to water, resulted in additional deaths. Other investigators have observed similar effects in young chicks fed fluoride. The magnesium level in ash was significantly elevated (P < 0.05) in groups fed diets supplemented with fluoride as well as in the low calcium group. The calcium appeared to be displaced in part by magnesium.

Experiment 2: Among quail which received the basal diet plus 1.2% calcium for 10 days prior to being placed on the experimental diets for 35 days the low calcium (0.4%) diet did not produce signs of calcium deficiency. In the fluoride-treated group, tetracycline labelling demonstrated a significant increase in periosteal bone and adequate mineralization of the new bone. Histologically, an increased number of osteons was noted. Likewise, the magnesium content of bone was significantly increased in the two groups fed the diets containing fluoride. These changes in the bones, however, were not accompanied by an increase in bone strength. In fact the dietary fluoride supplement resulted in a 30% decrease in bone torsional strength. The activity of the enzyme pyrophosphatase was elevated in the fluoride-supplemented groups.

The authors summarized the research by stating that "fluoride supplementation increases calcium retention but at a high level has little effect on bone integrity and strength."

G. L. W.

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FLUORIDE
SYSTEMIC FLUORIDE POISONING RESULTING FROM A FLUORIDE SKIN BURN

by

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(Abstracted from the Journal of Occupational Medicine, 15:39-41, 1973)

A 30 year-old white male laboratory technician experimenting with 50 cc of 100% anhydrous hydrofluoric acid which had been kept in a liquid state under pressure at 24°F was struck by the HF on the right side of the face, the neck and the right arm, when one of the connecting tubes burst. Ten minutes after the accident, iced alcohol compresses were applied followed by magnesium oxide dressings. Subsequently, the burns were infiltrated with 10% calcium gluconate under general anesthesia. The patient's pulse rate dropped to 48 beats per minute within six hours after the accident. Blood pH was 7.21 (normal: 7.35 to 7.45); carbon dioxide combining power was 21.5 mEq/liter (24 to 30); serum potassium was 6.1 mEq/liter (3.6 to 5.0) and serum calcium was found to be 7.4 mEq/liter (4.5 to 6.0). An electrocardiogram 10 hours after the accident showed slight S-T elevation in lead AVL, V1, V2, and V3 which disappeared within three days. The patient had polyuria (200 to 250 ml per hour) and the specific gravity of the urine was low. On the third day, red and white blood cells, granular and hyaline casts were found in the urine. The urine sediment became normal by the sixth day.

During the first 24 hours the patient was severely nauseated, lethargic and unresponsive to stimuli other than pain. A mild throat irritation during the first hour was the only respiratory symptom. Bones and dental X-rays taken 3 months later were normal.

According to the authors' estimate approximately 5 gms of HF had been spilled on the skin; burns covered 2.5% of the body surface and 1.1% of the burn was third degree. The first urine sample obtained three and one half hours after the accident contained 87.0 mg/l of fluoride. Within six hours the fluoride level dropped to 56 mg/l. In the following two and one half days it decreased slowly at a constant linear rate. It was calculated that the total urinary fluoride excretion during the first three days was 404 mg. Blood samples 4 to 10 hours following the accident revealed fluoride levels below 0.3 mg/100 ml of blood.

The authors considered absorption from the diseased tissues a much more important source of systemic poisoning than inhalation. Whereas the literature indicates that inhalation in connection with skin burns has been considered the source of fluoride poisoning, no respiratory involvement was evi-

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dent in this case.

The infiltration of the burned area with calcium gluconate was necessary to minimize the systemic absorption of fluoride. An alternate treatment which has been used for less severe HF burns consists in soaking the burn in quarternary ammonium compounds. The clinical picture of acute fluoride poisoning was obscured by the hypercalcemia resulting from treatment. The authors stated that the marked stupor and the S-T elevation in the electrocardiogram must have been due to the systemic effect of fluoride. The bradycardia was believed to be induced by rapid intravenous injections of calcium.

G. L. W.

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THE INFLUENCE OF FLUORIDE ON MINERALIZATION ON RAT TEETH

by

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(Abstracted from the Calcification Tissue Research, 11:176-178, 1973)

The authors investigated the effect of fluoride on the mineralization of the rat dentine using both microradiography and tetracycline-labelling. The combination of these techniques allowed them to correlate precisely the effect of fluoride on the hard tissue with time.

They gave intraperitoneal injections of tetracycline to 62 male Wistar rats at 4 day intervals which totaled 5 mg/kg body weight. The rats were then divided into three groups. One group was given 25 ppm fluoride as sodium fluoride in drinking water beginning with the second tetracycline injection to the end of the experiment the second group received 250 ppm fluoride. The third group as a control received no fluoride. The administration of fluoride was continued for 17 days. The sections of the lower incisor were subjected to fluorescence microscopy. The examination of calcium deposition was carried out by microradiography.

High doses (250 ppm) of fluoride affected the mineralization of the growing rat tooth within hours after administration. In the group receiving 25 ppm in drinking water and in the control group no such effect took place. In those animals receiving 250 ppm, bands of high and low mineralization were associated with the extent of calcification. According

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61
to the authors, the bands indicated continued interference by fluoride with
the calcification of the dentine formed during that period of fluoride inges-
tion. No interference with the calcification of dentine formed prior to the
second tetracycline injection was demonstrated.

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RADIOLOGICAL AND CLINICAL
REVERSIBILITIES IN A CASE OF OSTEOPOROSIS

by

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The authors reported this case because it demonstrates the complete
subsidence of bone changes in skeletal fluorosis following cessation of expo-
sure to fluoride.

A 50 year-old man employed in an aluminum factory for 24 years,
suffered diffuse pains in the back due to arthrosis in the left hip joint with
marked functional impairment of the joint. In January 1959 he had experi-
cenced a kidney colic on which occasion an X-ray of the spine revealed ex-
tensive generalized osteosclerosis involving the lumbar and cervical spine, pel-
vis and ribs. The skull and extremities were relatively uninvolved, although
typical periostitis was noted on both femurs. The diagnosis, skeletal fluoro-
sis was confirmed by an iliac crest biopsy. Laboratory tests including CBC,
serum and urinary calcium and phosphorus and a provocative calcium test
showed normal values. Because of hardness of the sternum, no myelogram
could be made. Following cessation of exposure, repeated skeletal X-rays
up to 1970 showed the same picture, as before. The daily urinary excretion
of fluoride remained high from April 1967 to October 1970 with values rang-
ing from 8 ppm to 1.3 ppm (normal 0.8). This persistently high urinary ex-
cretion coincided with a remarkable improvement of the radiological picture.
In September 1971 the urinary fluoride level was 4.4 ppm and that of the
blood serum 0.195 mg/100 ml,

In August, 1971 another radiological survey revealed perfectly nor-
mal structures of the spinal column and the pelvis. No traces of osteosclero-
sis were found.

On August, 21, 1971 during surgery on his left hip another bone bi-
opsty was taken of the head of the femur. It showed evidence of typical ar-
throsis of the hip joint but no further signs of osteosclerosis. The bone sub-

62
stance contained 5100 ppm in the spongiosa and 5790 ppm in compact bone.

According to the authors' opinion, the levels in the spongiosa would have been higher than those in the compact bone because the former are more active than the latter had there been recent exposure to fluoride.

G. L. W.

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THE MECHANISM OF INHIBITION BY FLUORIDE OF MITOCHONDRIAL FATTY ACID OXIDATION

by

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(Abstracted from Biochimica et Biophysica Acta, 280:495-505, 1972.)

Mitochondria of rat liver free of outer membrane were incubated with palmitate-\(^{14}\)C. The rate of formation of \(^{14}\)C-labeled water-soluble compounds and \(^{14}\)CO\(_2\) were used to measure the activation of palmitate. The authors found that oxidation of palmitate is reduced about 50\% by 1mM (19 ppm) KF and 80\% by 5mM (95 ppm) KF.

Oxygen uptake of both, coupled and uncoupled, mitochondria was measured manometrically during oxidation of palmitate. Increasing concentrations of fluoride also decreased the oxygen uptake of mitochondria.

In the presence of 10 mM KF the inorganic pyrophosphate (PP\(_i\)) accumulated in the coupled mitochondria during fatty acid oxidation. When KF was replaced by KCl, no such accumulation was observed. Since inorganic pyrophosphatase is inhibited 95\% by 0.5 mM (9.5 ppm) KF and 98\% by 5mM (95 ppm) KF, the accumulation of inorganic pyrophosphate is due to the inhibition of inorganic pyrophosphatase. The buildup of pyrophosphate in mitochondria would prevent the following reaction from occurring:

\[
\text{RCOOH + GTP + CoA} \leftrightarrow \text{RCO*CoA + GMP + PP}_i
\]

In order to test this hypothesis, mitochondria were frozen and thawed five times to obtain a crude matrix fraction to which palmitate-\(^{14}\)C was added in the absence and presence of various concentrations of pyrophosphate. Low concentrations of pyrophosphate inhibited markedly the oxidation of palmitate.

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EFFECT OF FLUORIDE ON CARBON DIOXIDE AND ACID FORMATION IN
SALIVARY SEDIMENT MIXTURES INCUBATED WITH GLUCOSE

by

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The authors studied the effect of fluoride on the acidity of saliva. Salivary sediments were incubated with 5% glucose. Fluoride, added at concentrations of 2, 2, 4, 4 and 20 ppm inhibited the drop in pH of salivary sediments. This decrease in acidity of saliva was more pronounced with higher fluoride levels, and also when the saliva was acid, at the beginning of the experiment.

Salivary sediments were incubated with glucose-\textsuperscript{U-14C}, \textsuperscript{L-14C}, \textsuperscript{2,6-14C}, and \textsuperscript{3,4-14C}. At 0.05% glucose, no difference in the formation of \textsuperscript{14CO\textsubscript{2}} was observed. However, in the presence of 5% glucose, fluoride at a concentration of 4 and 20 ppm increased the production of \textsuperscript{14CO\textsubscript{2}} appreciably. The \textsuperscript{14CO\textsubscript{2}} was released exclusively from glucose carbons 3 and 4.

Since the inhibition of acid formation by fluoride was greater at lower pH levels of the saliva, salivary sediments were incubated in media which had been made acidic with lactic acid. In the presence of 5% glucose, 20 ppm of fluoride increased the pH and decreased the formation of lactic acid compared to the controls. In the absence of 5% glucose or at low concentrations e.g. 0.05% fluoride had little, if any, effect.

Again, in media made acidic with lactic acid and in the presence of glucose, \textsuperscript{14CO\textsubscript{2}} and \textsuperscript{14C}-labeled volatile fatty acids formed after addition of glucose-\textsuperscript{U-14C} decreased upon addition of 20 ppm of fluoride. On the other hand, the disappearance of lactate-\textsuperscript{L-14C} and the formation of \textsuperscript{14C}-labeled volatile fatty acids observed after incubation with lactate-\textsuperscript{L-14C} increased upon addition of 20 ppm fluoride.

The authors explained these results in the following manner: At lower pH, fluoride inhibits the uptake of glucose. However fluoride reduces formation of lactate as well as the formation of volatile fatty acids from it.

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