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CONTENTS

EDITORIAL

- Placental Transfer of Fluoride 178

ORIGINAL ARTICLES

- Industrial Fluorosis in Wild Mule and Whitetail Deer from
Western Montana - by C. E. Kay, P. C. Tourangeau, and C. C.
Gordon, Missoula, Montana 182
- Effect of Single and Long-Term Sodium Fluoride Administra-
tion on Biosynthesis of the Thyroid Hormone in Rats - by S.
Kahl and Z. Ewy, Kraków, Poland 191
- Inorganic Plasma Fluoride Concentrations and Its Renal Excre-
tion in Certain Physiological and Pathological Conditions in
Man - by H. Hanhijärvi, Kunamylynkatu, Finland 198
- Effect of Sodium Fluoride on the Phosphate Absorption by
Potato Tuber Tissue - Influence of Calcium - by M. Diouris
and M. Penot, Brest, France 208
- An Evaluation of the Potential for Ecological Damage by
Chronic Low-Level Environmental Pollution by Fluoride - by
E. Groth III, Washington, D.C. 224

ABSTRACTS

- Placental Transfer of Fluoride During Methoxyflurane Anaesthesia for Cesarean Section - by V. Weiss and Ch. de Carlini, Genève, Switzerland 241**
- Optimal Fluoridation, The Concept and Its Application to Municipal Water Fluoridation - by J. R. Lee, Mill Valley, California 242**
- The Prevalence and Distribution of Enamel Defects in Four Districts with Different Fluoride Contents in the Drinking Water - by K. Haavikko and A. Helle, Helsinki, Finland 243**
- Excretion of Retained Fluoride in Man - by H. Spencer, L. Kramer, D. Osis, E. Wiatrowski, Hines, Illinois 245**
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EDITORIAL

PLACENTAL TRANSFER OF FLUORIDE

Although considerable research has been carried out on the transfer of fluoride from the mother to the newborn child the available data are not, as yet, conclusive.

In his classical book, Roholm (1) concluded that fluoride does not pass the placental barrier. He found no dental fluorosis upon examining teeth of two children born to mothers who had been exposed industrially to cryolite. In three other children, dental fluorosis was present but was attributed to breast feeding. On the other hand, Kauzal (2) recorded five newborn children who succumbed to bleeding ulcers in the gastrointestinal tract as revealed at autopsy. Their mothers had been working in a fluoride-emitting factory. Kauzal suggested that transplacental transmission of fluoride was responsible for their death.

The question of placental transfer of fluoride was explored by Feltman and Kosel (3). In 20 pregnant women who had been given tablets containing 1 mg of fluoride (2.2 mg NaF) per day throughout their pregnancy, the average fluoride concentrations in the placenta were more than twice as high as in cord blood while in 17 controls the placenta contained more than 5 times as much fluoride as the cord. Also, in six pregnant women who had been drinking artificially fluoridated water throughout pregnancy, fluoride concentrations in the placenta ranged more than twice as high as those in cord blood; both cord and placental fluoride was higher than in women not drinking fluoridated water.

That sizeable amounts of fluoride might be transferred from the mother to the fetus was also demonstrated by the findings of as much as 59.3 ppm in the arteries of an infant who died shortly after birth in fluoridated Ames, Iowa with calcifications throughout his arterial system (4).

In animals as well, placental transfer has been shown to occur (5-8) and seems to correlate with the fluoride content of water and food (9, 10). According to Singer and Armstrong (11) increased feeding of fluoride to animals and humans raises the fluoride concentration in maternal and fetal blood. On the other hand, Bawden et al. (12) administered radiofluoride (^{18}F) to pregnant ewes and found relatively low fetal plasma radiofluoride levels compared to that of the mother. Similar results were obtained by Ericsson and Malmnas (13) in ^{18}F studies on rabbits and on women who submitted to therapeutic abortions. Armstrong, Singer and Makowski (14) found little difference in the fluoride concentrations of maternal and fetal blood in a series of women undergoing Cesarean sections. These findings were confirmed in a more

recent study by Shen and Taves (15).

The situation seems to be somewhat different when high maternal fluoride levels are involved. Gedalia (16) found cord blood in fluoridated areas consistently lower than the levels in their mothers', but this was not the case when the mothers had taken fluoride supplements in water and tablets.

New data has recently accumulated in studies on patients receiving Methoxyflurane anaesthesia during labor. Fry and Taves (17) observed a metabolic breakdown of the anaesthetic into organic and inorganic fluoride and an organic acid-labile fluoride (presumably methoxydifluoracetic acid) which starts within 10 to 25 minutes after the beginning of the anaesthesia (17, 18). They found a 5 to 10 fold rise within 10 to 15 minutes before the delivery but the cord blood showed only less than 25% of that increase. The high concentration of fluoride in blood was presumed to be responsible for the observed nephrotoxicity of the drug. Weiss and de Carlini (19) revealed on 21 women with Cesarean sections a parallel increase of maternal and fetal fluoride in the blood stream but, in the infant, the fluoride concentrations in the cord blood were only one half of that of the arterial blood of the mother. In the urine of the newborn, no rise in the fluoride concentration occurred within 12 hours.

From the above-quoted divergent studies it seems that numerous factors enter into the passage of fluoride through the placenta. In Cesarean sections, the duration of the anesthesia may be an important factor. Since anaesthesia is of relatively short duration, a possible interaction of other medications used simultaneously with the anaesthetic is also conceivable. Undoubtedly the status of the health of the patient is involved, particularly the condition of the mother's kidneys, the major source of fluoride excretion seems to be of major importance. Parathyroid activity might play a significant role particularly since, as has been stated before, calcium deposits in the placenta retain fluoride and prevent it from passing into the newborn. How much the state of nutrition of the mother and the intake of milk or other high calcium and vitamin-containing foods is involved has to be explored. The report of the Iowa infant's death, whose mother had no known fluoride intake other than that contained in fluoridated water, certainly demonstrated how little reliance can be placed upon experimental data and upon studies on a series of women whose health, to all intents and purposes, appears to be "normal".

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The International Society for Fluoride Research will hold its Seventh Conference in Zandvoort, Holland, February 8-10, 1976. The Program Committee is soliciting abstracts (up to 300 words) of papers to be presented dealing with any phase of fluoride research. Kindly mail abstracts to the Society's office, P.O. Box 692, Warren, Michigan 48090. The deadline for the abstracts will be October 15th.

For reservations contact Hotel Zonnewende in Zandvoort, Holland.

INDUSTRIAL FLUOROSIS IN WILD MULE AND WHITETAIL DEER FROM WESTERN MONTANA ¹

by

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Missoula, Montana

SUMMARY: A two year study of bone tissue of white-tail and mule deer in two areas of western Montana revealed that these animals can be used as parameters of fluoride contamination in a given ecosystem. Fluoride in vegetation was markedly higher in the two areas as compared to controls. Because individual deer do not live in precisely the same location every year, the exact consumption of fluoride could not be determined but bone fluoroassays from deer inhabiting the two contaminated areas showed a 5 to 50 fold increase in fluoride concentration as compared to controls. In any particular deer, the level of fluoride was highest in the mandible followed by femur and ribs with the lowest concentration in metacarpals or metatarsals. Cancellous, metabolically active bones accumulated more fluoride than dense compact bones.

Introduction

From 1970 to 1972, the use of mule and whitetail deer (*Odocoileus hemionus* and *O. virginianus*) as indicators of environmental fluoride pollution was evaluated. Previous studies demonstrated that atmospheric fluorides accumulate in vegetation, causing fluorosis when consumed by domestic livestock (1,2); similar reports for indigenous large mammals are rare (3).

From the Environmental Studies Laboratory, Univ. of Montana, Missoula.

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- b. U.S. Dept. of Interior, National Park Service PX 1430-5-A413
- c. Montana Dept. of Natural Resources and Conservation 836-8
- d. Montana Dept. of Health and Environmental Sciences P-703

² Research Associates, Environmental Studies Laboratory

³ Director of Environmental Studies Program

Mule and whitetail deer were collected from Teakettle Mountain which has a history of environmental fluoride contamination due to the operations of an aluminum smelter at Columbia Falls, Montana (4-6). Mule deer were obtained from Douglas Mountain where the ecosystem had been polluted by a phosphate ore concentrator owned by the Cominco American Corporation (7, 8). Hydrogen fluoride gas and particulate fluoride matter released by the Anaconda Aluminum Company contaminated the vegetation near Columbia Falls. Pollution in the Douglas Creek watershed southeast of Hall, Montana, was attributed to fluoroapatite dust. In 1968, industrial processing ceased in the Douglas Creek drainage and fluoride determinations on mule deer from that habitat are a measure of residual contamination. Teakettle and Douglas Mountains are directly adjacent to their respective industrial facilities which are both point sources of fluoride emissions. The degree of pollution was 10 to 20 times greater in the Columbia Falls area (4-8).

Methods

All deer from Teakettle and Douglas Mountains were procured under scientific collection permits by Montana Fish and Game Department biologists. Bones from hunter or highway killed whitetail and mule deer were provided as controls by Fish and Game personnel on a statewide basis (9). Detailed descriptions of techniques for sample preparation and fluoride analyses of bone material (10, 11) and plant matter (5, 8, 12) are presented in the literature. Those techniques were used in this study with the following additions: to obtain an aliquot of entire bones - femur, metacarpus, metatarsus, tibia-fibia, ulna-radius, or humerus - a meat saw was used to bisect each bone perpendicular to the lateral aspect, and sawdust from the entire length was taken as a representative sample (13). A specimen of the mandible was taken by removal of the angular apophysis (11), while the complete 13th rib was utilized for analyses. Rumen and fecal pellet material were preserved with formalin and processed as vegetational matter. Urine and amniotic fluid were combined with TISAB buffer solution on a one-to-one ratio and tested for fluoride. Kidneys were cut into one-quarter inch thick cross sections, air dried, defatted six times with petroleum ether, ground in a Wiley mill to pass a number 40 mesh screen, and assayed as per vegetation. For each material analyzed, fluoride activity was determined with an ORION Fluoride Specific Ion Electrode (10, 11).

Results for bones and kidneys are presented as parts per million (ppm) on a dry, fat free weight while ppm on a dry weight basis is used for vegetation, rumen, and fecal pellet samples with urine and amniotic fluid values expressed as ppm in the original solution. All analyses were performed in duplicate and the single parameter assigned in the data is the average of the two trials.

Results and Discussion

Often the first readily noticeable sign of fluorosis in ungulates

is mottling, pitting, and black discoloration of the teeth, followed by softening and abnormal wear of the dental surfaces as fluoride contamination increases. Since this sequence was well documented for domestic cattle (1, 14, 15) and demonstrated in wild whitetail deer (3), the extent of dental abnormalities prevalent in deer from Teakettle or Douglas Mountains was not evaluated.

Because of tooth softening and anomalous wear, the tooth wear method for estimating the ages of whitetail (16) and mule deer (17) is unreliable and should not be utilized on fluorotic deer. Tooth eruption and replacement tables were used to age mule (18) and whitetail deer (16) until the animals were 1.5 years old; annual layers of growth in the dental cementum were counted for older deer (19). Tooth sections and microscopic slides were prepared by Mr. Gary Matson, Montana Microscopic Inc., Milltown, Montana. This method of aging fluorotic deer appears valid, as the annular rings in the cementum were clearly visible even though there were morphological changes in the cellular configuration of the teeth.

The average fluoride level in control plants was 3.9 ppm (20); thus, the results of fluoride analyses on vegetation from Teakettle and Douglas Mountains (Table 1) clearly demonstrated the intensity of fluoride contamination in those two environments. The floral samples were collected on winter ranges occupied by the deer and represent species that constitute a major portion of their diets (21, 22). However, these determinations are only an approximate measure of the deer's fluoride intake, as assays were not executed on the actual forage consumed by individual deer. Accordingly, direct correlation cannot be calculated between vegetational data and fluoride parameters of rumens (Table 2); in most instances, the fluoride content of rumen material was slightly lower than the fluoride concentration of sampled flora. Fluoride was generally lower in fecal pellets than in rumens of mule deer from Teakettle Mountain and higher in those secured on Douglas Mountain.

Mule and whitetail deer populations in mountainous, western Montana make seasonal migrations and usually inhabit low-elevation ranges only from January to April of each year. Dispersal is varied and individual animals do not necessarily return to the exact same areas in subsequent winters. Because environmental pollution is inversely proportional to distance from an emission point source, deer from Teakettle Mountain summer in a region where the vegetation is less contaminated (4-6). Mule deer wintering on Douglas Mountain frequent summer ranges free of fluoride pollution (7, 8). Therefore, the observed fluoride levels in plant samples and rumen contents are indicators of the maximum fluoride stress that these deer encountered during their lives. This alternating pattern of high and low fluoride ingestion probably magnifies the effects of fluorosis on the deer. Short intervals of high fluoride intake ba-

TABLE 1

Analyses of Vegetation from Teakettle and Douglas Mountains,
PPM Fluoride Dry Weight

Location and species	Sample size	Ave. ppm fluoride
Teakettle Mountain 1970 (from Gordon 1972)		
Douglas Fir (<u>Pseudotsuga menziesii</u>)		
29 months*	3	180.0
17 months	3	89.0
5 months	3	41.0
Lodgepole Pine (<u>Pinus contorta</u>)		
29 months	10	350.0
17 months	10	140.5
5 months	10	22.7
Ponderosa Pine (<u>Pinus ponderosa</u>)		
29 months	12	168.0
17 months	12	77.4
5 months	12	19.5
Grass--various species	6	87.4
Teakettle Mountain 2/2/72 (from Gordon 1973)		
Composite forage	1	430.1
Douglas Fir--5 months	1	158.0
Grass	1	580.0
Serviceberry, stems--5 months (<u>Amelanchier alnifolia</u>)	1	68.6
Red Stem Ceanothus, stems--5 months (<u>Ceanothus sanguineus</u>)	1	186.3
Douglas Mountain 10/71 (from Person 1972)		
Douglas Fir		
29 months	6	39.3
17 months	6	60.8
15 months	6	49.9
Grass	7	63.7

*Exposure time (20).

TABLE 2
Fluoride Analyses (ppm) of Samples Taken From Mule and Whitetail Deer
Collected on Teakettle (TM) and Douglas (DM) Mountains

Sample Number	Location	Date	Sex	Age	Mandible	Femur	Radius	Metacarpus	Metatarsus	Ulna-Radius	Mandible Deposits	Rumen	Fecal Pellets	Kidney	Other
DC-25	DM	3/15/72	♂	8 mo.	1,120	610	560	-	525	-	-	14.4	38.0	19.0	
DC-26	DM	3/15/72	♂	8 mo.	2,175	855	880	-	580	-	-	30.0	35.0	18.5	
DC-28	DM	3/15/72	♂	8 mo.	2,550	1,070	1,090	-	1,075	-	-	37.5	42.0	21.0	
DC-27	DM	3/15/72	♂	8.5 yr.	1,920	3,050	2,400	-	2,450	-	-	17.0	19.2	19.5	Urine 20.0
DC-34	DM	3/15/72	♀	9.5 yr.	2,845	2,550	2,950	-	2,355	-	-	14.2	46.0	24.0	Aminotic Fluid 0.04
DM72-1	TM	2/2/72	♂	1.5 yr.	2,900	2,050	1,845	735	880	-	-	230.0	125.0	-	
DM72-4	TM	2/3/72	♂	1.5 yr.	6,150	3,700	4,150	2,000	2,325	-	-	148.0	116.0	-	
DM72-3	TM	2/2/72	♀	1.5 yr.	6,550	3,300	4,200	1,830	2,450	-	-	188.0	117.0	-	
T-172	TM	3/9/70	♀	3.5 yr.	3,175	-	-	2,440	2,380	-	-	-	-	-	
DM72-2	TM	2/2/72	♀	3.5 yr.	4,785	6,950	5,630	4,075	4,900	-	-	240.0	215.0	-	
DM72-5	TM	2/3/72	♀	3.5 yr.	4,005	5,620	4,625	3,527	4,000	-	-	240.0	240.0	-	
T-184	TM	3/9/70	♀	3.5 yr.	6,950	5,850	-	4,440	-	4,643	11,381	-	-	-	
T-185	TM	4/18/70	♂	8 mo.	9,760	-	-	5,790	4,920	-	-	-	-	-	
T-186	TM	4/18/70	♀	2 yr.	3,435	-	-	1,725	1,855	1,548	-	-	-	-	
DM72-6	TM	2/2/72	♀	2.5 yr.	4,350	-	-	2,550	-	-	-	-	-	-	
DM72-8	TM	3/17/72	♂	2.5 yr.	3,730	3,475	3,295	2,645	2,495	-	-	66.0	-	-	Thia-Fbia 2,195
T-187	TM	4/18/70	♂	3.5 yr.	6,700	5,625	-	4,650	-	-	9,763	-	-	-	Humerus 3,015
T-183	TM	3/9/70	♀	7.5 yr.	3,692	3,845	-	1,825	-	2,700	10,049	-	-	-	

lanced by periods of lower intake were more damaging to domestic cattle than constant exposure to the same average yearly fluoride consumption (15).

In comparing bone tissue fluorassays of mule and whitetail deer inhabiting Douglas and Teakettle Mountains (Table 2) with data from control deer (Table 3), 5- to 50-fold increases in fluoride parameters were noted. The Teakettle Mountain deer were subjected to the highest degree of environmental fluoropollution and their skeletal structures show the greatest amounts of fluoride. In any individual fluorotic deer, as well as the statewide controls, the highest levels of fluoride were usually found in the mandible, followed by the femur or rib, with the metacarpus or metatarsus having the lowest fluoride concentration; insufficient sample size precludes discussion of relative fluoride accumulation in the ulna-radius, tibia-fibia, or humerus. For most fluorotic deer, the amount of fluoride in the mandible was 20 to 100 percent greater than that assayed in the animals' metacarpal or metatarsal bones. This is contrary to what was reported for dairy cattle fed experimental diets containing sodium fluoride (15).

TABLE 3

Average Fluoride Parameters of Mule and Whitetail Deer
from Montana, PPM Dry, Fat Free Weight

Bone	85 Mule Deer		45 Whitetail Deer	
	Sample Size	Mean	Sample Size	Mean
Mandible	50	189.9	23	160.0
Femur	29	152.9	20	114.5
Rib	2	168.0	2	116.0
Metacarpus	19	110.1	8	114.6
Metatarsus	21	137.1	16	78.2
Tibia-Fibia	14	129.7	6	76.1

Cancellous, metabolically active bones accumulate more fluoride than dense compact bones, and the surface layer of a particular bone has a greater fluoride content than the inner tissues of the same bone (15). These factors are probably responsible for the observed differential skeletal fluoride accumulation within individual deer. For example, the concentrations of fluoride in callus type deposits on the exterior of the lower jaw exceeded the bone tissue from which they were removed (11,381/6,950, 9,763/6,700, and 10,049/3,692). The mandible, in general, has a large surface to mass ratio, while the femur or rib have a larger percentage of cancellous matrixes than the metacarpus or metatarsus.

The greatest disparities in skeletal fluoride accumulation rates, as exemplified by the mandibular-metatarsal ratio, were noted in fawns (1, 120/525; 2, 175/580, and 2, 550/1, 075 in mule deer from Douglas Mountain and 9, 760/4, 920 in a whitetail from Teakettle Mountain). These deer were 8 months old when they were collected and bone formation was incomplete (23). The high fluoride content in the metabolically active mandible signifies that maturation of bone structures affects the relative distribution of fluoride in the skeleton.

Significant variation in fluoride parameters was not apparent when mule deer and whitetails from Teakettle Mountain were compared; nor was a sex specific pattern evident within either mule deer population. Fluorosis is cumulative and the fluoride concentration in bone tissues appears to increase with the age of the deer, as would be predicted from research with experimental animals (1). Since these migratory deer experienced considerable latitude in their individual lifetime exposure to fluoride, species, sex, or age specific fluoride accumulations show a high degree of variability and, therefore, these conclusions are tentative.

The one analysis of urine and the several assays of kidneys verify that elevated levels of fluoride were being excreted by mule deer as was demonstrated with other fluorotic animals (1). The single examination of amniotic fluid, 0.04 ppm, showed little fluoride.

Discussion

Fluoride parameters of mule and whitetail deer can be used to delineate the severity and extent of fluoride contamination in an ecosystem. However, the utilization of deer as a biological monitoring system in mountainous regions has inherent variability because the deer's seasonal movements subject them to changing levels of fluoride pollution from an industrial point source. Deer collected from Teakettle Mountain do reflect the greater degree of fluoride pollution resulting from operations of the Anaconda aluminum smelter; the mule deer taken on Douglas Mountain do substantiate that residual fluoride contamination was occurring 4 years after the Cominco American Company ceased production.

For comparative study of fluorosis in deer, the mandibles from all age classes should be examined. Extraction of the lower jaw allows assessment of tooth discoloration and abnormal wear, as well as determination of age. Fluorassay of mandibles is a reliable measure of skeletal fluorosis in deer.

The objectives of this study did not include assessment of the effects of fluorosis on the behavior or population dynamics of fluorotic deer. Nevertheless, lameness, induced by fluorosis, was observed in deer on Teakettle Mountain. It is logical to conclude that such lameness makes

any particular deer more susceptible to predation, snow depth, low temperature, and the like. Additionally, softening of the teeth and anomalous dental wear causes grinding surfaces to erode at an accelerated rate, exposing dental nerves and rendering deer less efficient herbivores. Fluorosis is cumulative and these conditions eliminate the oldest, most fluorotic deer from the population.

During the two collections on Teakettle Mountain, an attempt was made to select the oldest deer, yet only one of the 13 deer secured was older than 3.5 years. Of the five mule deer taken on Douglas Mountain, two were 8.5 and 9.5 years of age and the remaining three were shot specifically as fawns. This apparent shift of the Teakettle Mountain deer populations to a young age structure suggests that fluorosis was so severe that the older, most susceptible deer had been removed from that herd. A similar trend probably was not observed in the mule deer from Douglas Mountain because fluoride pollution was far less severe than in the Columbia Falls area.

In determinations of the amounts of skeletal fluoride harmful to the health and well-being of domestic livestock, the experimental animals were all housed, fed, protected, and cared for by man (1, 2, 14, 15). Therefore, threshold levels of skeletal fluoride concentrations correlated with detrimental effects in dairy cattle are not valid when applied to wild fluorotic deer. The deer are subjected to many environmental variables that were not present in the laboratory experiments. Published data indicate that domestic cattle experience fluoride toxicity when the amount of fluoride found in their metatarsal bones approaches 5,500 ppm (24). This measure should not be applied to deer. Of the 18 animals examined during this study, only one approached 5,500 ppm in its metatarsus; yet, most of the deer were afflicted with various stages of fluorosis that were detrimental to them in their natural ecosystem. Mandibular fluoride levels are a more meaningful indicator of fluorosis in wild deer since individuals with 3,000 ppm exhibited abnormal dental wear.

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EFFECT OF SINGLE AND LONG-TERM SODIUM FLUORIDE ADMINISTRATION ON BIOSYNTHESIS OF THE THYROID HORMONE IN RATS

by

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SUMMARY: In rats given fluoride in daily doses of 2 mg continuously for 65 days no weight gain of the thyroid gland was found but a decrease in the 2-hr uptake of ¹³¹I and in the relative amount of DIT in the thy-

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roid gland was noted. Following a single administration of 2 mg of fluoride by stomach tube, uptake of radioiodine and of DIT decreased but the inorganic iodide and MIT in the thyroid gland increased. The ratio MIT/DIT also increased. The observed changes indicate that long-term fluoride administration to rats depressed the initial uptake of radioiodine exclusively, without reducing the rate of synthesis of the thyroid hormone whereas a single administration of the same dose decreased both the uptake of radioiodine and the rate of the hormone synthesis.

Over 120 years have passed since Maumené (1) first observed the development of goiter in animals following intake of sodium fluoride. Subsequently a number of studies have been reported concerning the effect of fluoride compounds on the size of the thyroid gland, its histological picture, iodine metabolism, basal metabolic rate and the possible relationship between the incidence of endemic goiter and endemic fluorosis (2).

Although the results of early histological studies (3, 4) as well as observations of the therapeutic action of fluoride in the treatment of Graves' disease (5) were suggestive of an inhibition of the thyroid function, numerous papers have appeared, particularly dealing with iodine metabolism, favoring or denying significant goitrogenic effect of fluoride (6-11).

With the exception of the paper of Stolc and Podoba (12) there are no reports describing direct observation of the biosynthesis of thyroid hormone in animals receiving fluoride. We therefore examined the kinetics of radioiodine by the thyroid gland of rats after single and long-term administration of fluoride.

Materials and Methods

Male albino rats of the Wistar strain weighing 200-250 g were used throughout the experiments. They were fed standard laboratory diet containing 0.45 μ g iodine per gram.

In Experiment A, the rats were divided into two groups: the first, as the control group, received distilled water and the second, distilled water containing fluoride as NaF, for 65 days in a concentration of 133 ppm which was equal to a daily ingestion of 2 mg per rat.

In Experiment B, rats were also divided into two groups. The second group was given fluoride as NaF in 1 ml of distilled water by stomach tube in a single dose of 2 mg per rat. The animals of the

first group served as controls and were given 1 ml of distilled water by the same route.

For the study of the composition of iodoamino acids of the thyroid, the animals were injected intraperitoneally with $30\mu\text{Ci } ^{131}\text{I}$. In Experiment B, the ^{131}I -injection was made 5 minutes after the administration of fluoride by stomach tube.

Two hours later, the rats were anaesthetized with ether and exsanguinated by decapitation. The thyroid glands were dissected, weighed and counted per total radioactivity in a well-scintillation counter. Homogenation, digestion and chromatography procedures were carried out according to the method described by Tong and Chaikoff (13) slightly modified in our laboratory. In brief, the glands were homogenized in 0.5 ml of a 0.1% ammonium buffer, pH = 8.5, containing 0.05 M methimazole with an all glass homogenizer. Homogenates were incubated at 37°C with pancreatin, 10 mg/1 ml, for 17 hours. After centrifugation, the aliquots of supernatants were chromatographed on Whatman 3MM paper in two systems: n-butanol-acetic acid-water 200:30:75 (BAW) in Experiment B and n-butanol-ethanol-0.5N NH_4OH 5:1:2 (BEA) in Experiment A. The chromatograms were cut into 0.5 cm strips and the radioactivity of each section was counted in the well. Nonradioactive compounds such as moniodotyrosine (MIT), diiodotyrosine (DIT), thyroxine (T_4), triiodothyronine (T_3) and radioactive iodide were chromatographed under identical conditions to confirm the identity of various peaks. The relative radioactivity of each fraction was expressed as a percent of the total radioactivity on the chromatogram. Incompletely digested iodinated protein remaining at the origin of the chromatographs was almost invariably less than 7% of the total radioactivity.

The statistical comparison of the results was calculated by the Student's t test.

Results

The results obtained from Experiment A are summarized in Table 1. In rats given fluoride for 65 days in the dose equal to 2 mg daily, no increase of thyroid weight was found compared to the control animals. As seen in Table 1, fluoride caused a significant decrease ($P < 0.01$) in 2 hour radioiodine uptake by the thyroid gland and in the absolute amount of injected ^{131}I organically bound ($P < 0.01$).

The relative distribution of ^{131}I between iodide and iodocompounds in the thyroid gland was observed to be nearly identical in experimental animals and controls except that the DIT fraction was slightly decreased ($P < 0.05$) in fluoride-treated rats. No difference in MIT/DIT ratios was found.

TABLE 1

Thyroid Weight After 2-hr ^{131}I Uptake and Intrathyroidal Iodoamino Acid
Distribution in Rats (Mean \pm SE of 6 Rats/Group)
After F- Administration for 65 Days

Group	Thyroid weight mg/100 g body wt	2-hr ^{131}I uptake % of inj. dose	Distribution of ^{131}I in BEA chromatogram % of total chromatogram radioactivity					% of inj. ^{131}I organically bound x	MIT : DIT ratio
			I	MIT	DIT	T_3	T_4		
Control	6.2 \pm 0.4	1.7 \pm 0.1	5.1 \pm 0.6	40.4 \pm 1.5	37.5 \pm 0.4	1.0 \pm 0.2	5.9 \pm 0.8	1.45 \pm 0.12	1.08 \pm 0.04
NaF	6.0 \pm 0.4	1.0 \pm 0.1	5.0 \pm 0.4	40.5 \pm 0.8	32.4 \pm 1.7	0.7 \pm 0.1	3.5 \pm 0.8	0.81 \pm 0.09	1.25 \pm 0.08
p values	NS	<0.01	NS	NS	<0.05	NS	NS	<0.01	NS

NS - non-significant at the 0.05 level.

x - percent of injected ^{131}I present in organic fractions as MIT, DIT, T_4 and T_3
(i.e., rel. % on chromatograms x uptake).

TABLE 2

Thyroid Weight, 2-hr ^{131}I Uptake and Intrathyroidal Iodoamino Acid Distribution
After Single Fluoride Administration 5 Min. Prior to ^{131}I Injection
in Rats (Mean \pm SE of 6 Rats/Group)

Group	Thyroid weight mg/100 g body wt	2-hr ^{131}I uptake % of inj. dose	Distribution of ^{131}I in BAW chromatogram % of total chromatogram radioactivity				% of inj. ^{131}I organically bound x	MIT : DIT ratio
			I	MIT	DIT	T_3+T_4		
Control	5.5 \pm 0.4	2.3 \pm 0.1	7.7 \pm 0.7	39.2 \pm 0.5	32.5 \pm 0.5	6.9 \pm 0.5	1.81 \pm 0.04	1.20 \pm 0.03
NaF	5.5 \pm 0.6	0.8 \pm 0.1	11.8 \pm 1.2	43.7 \pm 1.2	26.4 \pm 0.8	4.8 \pm 0.8	0.58 \pm 0.05	1.65 \pm 0.09
p values	NS	<0.001	<0.05	<0.01	<0.001	NS	<0.001	<0.01

NS - non-significant at 0.05 level.

x - percent of injected ^{131}I present in organic fractions as MIT, DIT, T_4 and T_3
(i.e., rel. % on chromatograms x uptake).

FLUORIDE

The results obtained from Experiment B are summarized in Table 2. As seen, a single administration of 2 mg fluoride induced a significant decrease in the 2 hour ^{131}I uptake ($p < 0.001$) and in the absolute amount of ^{131}I incorporated into organic compounds in the thyroid glands ($P < 0.01$). Furthermore, the distribution of ^{131}I between iodide and iodoamino acids was changed in a manner typical of goitrogenic drugs, i. e., an increase in inorganic iodide ($P < 0.05$) and MIT ($P < 0.01$) as well as decrease in DIT ($P < 0.001$) and iodothyronines, though the decline of the latter values did not reach statistical significance. A marked increase in the MIT/DIT ratios was observed ($P < 0.01$).

Discussion

The data presented here demonstrate that long-term administration of fluoride to rats depressed the 2 hour uptake of radioiodine without reducing the rate of thyroid hormone biosynthesis. A single administration of fluoride in the dose equal to that used in the long-term experiment decreased both the 2 hour uptake of radioiodine and the rate of hormone biosynthesis. The absolute amount of injected ^{131}I incorporated during the 2 hour period into organic fractions was significantly decreased after long-term fluoride ingestion. However, the depression of organic binding was merely due to a decreased ^{131}I uptake but not to a slowdown of hormone synthesis. On the contrary, acute exposure to fluoride in large doses decreased the organic binding of iodide, not only lowering its thyroidal uptake but also lowering the formation rate of iodothyrosines and iodothyronines. It is of interest to point out that, under proper conditions of dosage, fluoride inhibits the formation of diiodotyrosine more than that of iodothyronines.

These experiments did not confirm the earlier observations of Stolc and Podoba (12). Their investigations on rats receiving 0.7 mg of fluoride daily for 2 months revealed a reduced rate of biogenesis of thyroxine and triiodothyronine within 12 hours following the administration of ^{131}I . When a longer period of time elapsed following the test dose, the amount of iodothyronines was the same in fluorized and control rats. Furthermore, the percentage representation of iodinated aminoacids in thyroid assessed by chemical methods did not indicate any alteration in fluoride-treated animals.

Our preliminary unpublished experiments on rats also failed to demonstrate any effect of fluoride intake (2 mg/rat/daily \times 65 days) on the thyroid ^{131}I uptake and distribution between iodocompounds, 24 hours after tracer injection.

The results reported in this paper are in contrast with the

findings of Demole (6), Korrodi et al. (7), and Harris and Hayes (8) that fluoride in large doses did not affect the uptake of radioiodine thyroid. The discrepancy may be due to the fact that, in their experiments, thyroid uptake was measured during the first 10 minutes (8) or more than 12 hours after ^{131}I injection (6, 7).

It has been suggested (14) that the affinity of the thyroid for halides leads to competition by thyroid for fluoride and iodide ions and that fluoride-iodide antagonism depends upon their level in the organism (15). Singer and Armstrong (16) have postulated that there is a homeostatic mechanism in animals which maintains a low level of fluoride in plasma. Although a non-significant increase in that level has been observed after continuous ingestion of fluoride, a significant increase was observed soon after the consumption of a diet containing an elevated concentration of the drug (17). In addition, ^{18}F investigations (8, 18) have demonstrated that the fluoride content of the thyroid is of the same order as that of the blood.

On the basis of the above-mentioned observations and our current experiments we suggest the possibility that, during long-term ingestion, a slightly elevated fluoride level in blood plasma and hence in the thyroid gland, is only sufficient to depress the initial iodine uptake. A significant increase in plasma and thyroid fluoride soon after a single administration decreases both the initial iodine uptake and the rate of hormone formation. This occurs, probably, by inhibition of enzymes (8) involved in iodination of tyrosine and coupling of iodotyrosines as was suggested earlier by Wespi (15) and Stolc and Podoba (12).

In conclusion, this study has shown that even high fluoride intake for 2 months did not affect the synthesis of thyroid hormone in rats. The initial reduction of ^{131}I organic binding appears to have little physiological importance since after long-term administration of the drug no increase in the size of the thyroid gland was observed. The partial inhibition of synthesis following acute fluoride administration does not seem to be a specific reaction for the thyroid gland but rather the result of general enzyme inhibition resulting from an increased fluoride level in the organism.

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INORGANIC PLASMA FLUORIDE CONCENTRATIONS AND ITS RENAL
EXCRETION IN CERTAIN
PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS IN MAN

by

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SUMMARY: In a study involving 2200 patients the inorganic plasma fluoride concentration (IPFC) increased with increasing age. In a fluoridated (1 ppm) community this increase was more pronounced than in a low fluoride (0.2 ppm) community. The mean renal clearance of fluoride and the daily amounts excreted also increased slightly until age fifty, after which a slow decrease was observed. During pregnancy, IPFC decreased significantly until delivery in both fluoridated and non-fluoridated areas. The daily fluoride excretion was also lower during pregnancy than in controls. Patients with renal insufficiency had a mean IPFC of $3.0 \pm 0.45 \mu\text{mol/l}$ in the fluoridated and 2.0 ± 0.14 in the low fluoride community. Their daily fluoride excretion was less than half of that of the control groups. Regularly hemodialyzed patients showed the highest IPFC. In a 6 year-old boy with diabetes insipidus, the IPFC was four times as high as in the corresponding controls. In diabetes mellitus with renal complications, the IPFC was also elevated. Increased water consumption did not cause greater retention of fluoride. In cardiac insufficiency, with normal serum creatinine the IPFC was only slightly elevated.

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Prepared from the transcript of the presentation by H. Hanhijärvi at the Sixth Annual Conference of I.S.F.R., Williamsburg, Va., 11/7-9/74.

During recent years considerable progress has been made in the methodology for measuring the mean free ionized plasma fluoride or inorganic plasma fluoride concentrations in man. The electrometric method first described by Fry and Taves (1) in 1970, slightly modified, has made it possible to make even epidemiological studies on the pharmacokinetics of the fluoride ion in man. The aim of our study was to try to determine whether or not certain physiological or pathological changes in man also affect fluoride metabolism. For this study we collected about 1,600 plasma samples from patients of a hospital situated in an artificially fluoridated (1 ppm) community. We also collected about 900 samples of plasma from a hospital in a non-fluoridated community with up to 0.2 ppm fluoride in the water in order to compare the results from the two areas. Finally, altogether about 200 urinary samples were collected from both communities in order to estimate the excretion of fluoride in the urine.

Both methods have been described elsewhere in a monograph (2).

As shown in Table 1 we found mean, free ionized plasma fluoride

TABLE 1

Mean Age and Mean Free Ionized Plasma Fluoride

Mean age in years		Plasma F ⁻ in the non-fluoridated area $\mu\text{mol/l} \pm \text{S. E. M.}$		Plasma F ⁻ in the fluoridated area $\mu\text{mol/l} \pm \text{S. E. M.}$
2	(4)*	0.72 ± 0.078	(3)	1.0 ± 0.00
7	(29)	0.79 ± 0.029	(78)	1.1 ± 0.033
17	(36)	0.83 ± 0.035	(100)	1.1 ± 0.030
27	(58)	0.87 ± 0.023	(189)	1.2 ± 0.023
37	(77)	0.86 ± 0.021	(148)	1.3 ± 0.042
47	(61)	0.86 ± 0.041	(167)	1.4 ± 0.042
57	(98)	0.89 ± 0.037	(146)	1.5 ± 0.039
67	(84)	0.96 ± 0.031	(125)	1.6 ± 0.039
77	(41)	0.93 ± 0.048	(77)	1.7 ± 0.080
87	(13)	1.0 ± 0.080	(14)	1.8 ± 0.14
TOTAL (501)			(1083)	
MEAN		0.88 ± 0.0093		1.3 ± 0.00048
MEAN SIGNIFICANCE $P < 0.001$				

* Number of patients

concentration in the fluoridated community to be $1.3 \mu\text{M/l}$ (.024 ppm) and $0.88 \mu\text{M/l}$ (.016 ppm) in the non-fluoridated area. This means that

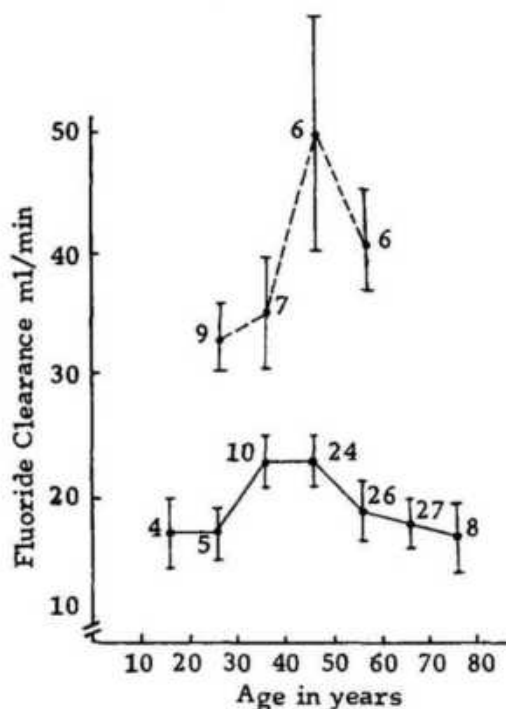
the concentrations of ionized fluoride in the plasma were only about 50% higher in the fluoridated area although the difference in the fluoride concentrations of drinking water is five-fold. This subject has been discussed in detail (2).

Table 1 also shows that the ionized plasma fluoride concentrations increase significantly with advancing age in patients from both areas. The increase in the fluoridated area is 80% in the fluoride community and 40% in the non-fluoridated area, when the mean ages of patients range from 2 to 87 years. Thus it is understandable that the regression coefficient is significantly larger in the curve from the fluoridated area when compared with that from the non-fluoridated community. The reason for this predominance probably is the increasing amount of fluoride in bones with age because under normal conditions there must be a chemical equilibrium between the concentrations of the skeletal fluoride and the blood fluoride. The method with which we selected the patients for the study is outlined in my monograph (2).

The mean renal clearance of fluoride surprisingly enough was not similar in both areas (Fig. 1). In the fluoridated area it was twice as high as in the non-fluoridated community. Thus the clearance of fluoride differs considerably from clearance of creatinine or inulin probably because fluoride accumulates in the bones in direct proportion to the amount ingested. These mean values are significant when compared with normal control values. It must be remembered however that we measured only plasma ionized fluoride concentrations, but did not correlate the clinical signs of toxicity with fluoride clearance. Another interesting finding was the increase of fluoride clearance with age until about age 50 whereafter a slight decline was found. Our explanation for the increases at the earlier age is a possible slow saturation of bones by fluoride with time which leaves more to be excreted. The decrease in fluoride clearance at an older age could be due to diminishing renal function which is characteristic for older people.

Earlier, Gedalia et al. (3) have shown that the urinary excretion of fluorides decreases significantly during pregnancy. We have also found (Table 2) that ionized fluoride concentration decreased rather steadily until delivery of the baby. Comparison of the earlier finding of Gedalia with ours supports the view that both these results are due to fluoride accumulation in the bones of the developing baby. This finding cannot be the result of a dilution of the plasma volume of the mother during pregnancy which would be more likely to increase than decrease the plasma fluoride concentrations in the mother as seen in pre-eclamptic patients.

In the non-fluoridated area also the mean, free ionized plasma

Fig. 1Age and Fluoride Clearance

---- In fluoridated community; — In low fluoride community.

fluoride concentrations decreased significantly when compared to total "non-pregnant" controls. After delivery the plasma levels of fluoride returned to normal within a few weeks. Women with clinical edema or preeclampsia showed slightly elevated values compared to those whose pregnancy was normal. On the basis of these figures we may conclude that, for the baby which is breastfed, the availability of fluorides is better prior to delivery than after it because of the moderate plasma concentrations. In order to confirm this opinion we also measured the ionized fluoride concentrations of maternal milk and found that after delivery of the baby the ionized plasma fluoride concentrations are the same in maternal milk as in plasma. The fluoride concentrations of the maternal milk are only a fraction of those in the drinking water because these values are in $\mu\text{M/l}$ ($1 \mu\text{M}$ equals $1/3$ ppm).

We also studied the effects of certain illnesses on fluoride metabolism, Table 3. In patients with renal insufficiency, we found a correlation between the concentration of serum creatinine and the levels of ionized plasma fluoride, Fig. 2. The mean renal clearance of fluoride was 41 ± 3 ml/min. (S.E.M.) in controls with normal kidney function, which resulted in a mean excretion of $58 \mu\text{mol}/24$ hrs.

TABLE 2

Mean Free Ionized Plasma Fluoride Concentrations, Mean Serum Creatinine Values and Mean Ages of Pregnant Women or Women in Labor in Fluoridated Community

		Plasma F ⁻ μmol/l ± S. E. M.	Serum creatinine μmol/l ± S. E. M.	Mean age in years	Significance
In labor	(149)*	0.93 ± 0.023	63 ± 1	26	p < 0.001
Pregnant					
0-7 months	(18)	1.15 ± 0.070	64 ± 4	30	p < 0.05
8-9 "	(13)	0.87 ± 0.055	67 ± 3	30	p < 0.001
10 "	(16)	0.87 ± 0.051	63 ± 3	26	p < 0.001
All those pregnant	(48)	0.97 ± 0.039	64 ± 2	29	p < 0.01
Edema during pregnancy	(9)				
	(9)	1.1 ± 0.080	66 ± 3	31	p < 0.05
Controls of same age	(67)	1.2 ± 0.036	80 ± 2	30	

* Number of patients

TABLE 3

Mean Fluoride Clearance ± S. E. M., Excretion of Fluoride/24 hrs ± S. E. M. and Mean Age of Patient Groups from the Artificially Fluoridated Drinking Water Community

	Renal clearance of fluoride ml/ min. ± S. E. M.	Daily excretion of fluoride μmol/ 24 hrs ± S. E. M.	Serum creatinine μmol/l ± S. E. M.	Mean age in years	N	P ₁	P ₂
Renal insufficiency	11 ± 3	31 ± 6	649 ± 357	51	5	<0.001	<0.05
Diabetes mellitus	16 ± 2	31 ± 10	200 ± 54	35	2		
Pregnancy	44 ± 5	44 ± 4	72 ± 5	27	11		
Controls	41 ± 3	58 ± 5	74 ± 3	38	28		

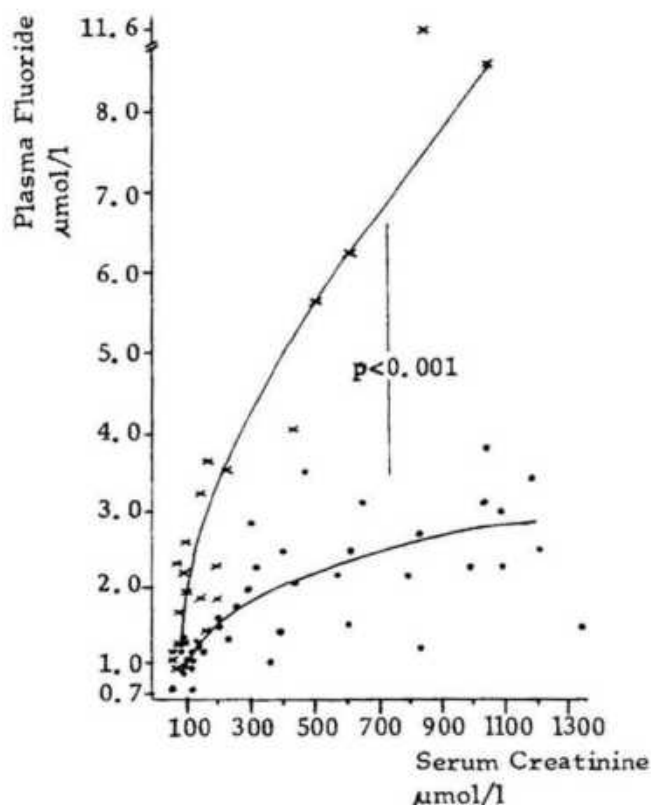
- Renal insufficiency mean fluoride clearance (P₁) and daily excretion (P₂) are significantly lower than in the controls.

FLUORIDE

In patients with renal insufficiency, clearance of endogenous fluoride was significantly lower (11 ± 3 ml/min.). The daily urinary fluoride excretion was also lower than in the control group. In two patients with diabetes mellitus, the same tendency was observed as in the group with renal insufficiency. The mean fluoride clearance was 16 ml/min. and the daily excretion $31 \mu\text{mol}/24$ hrs. The serum creatinine in these two diabetic patients was higher than normal ($105 \mu\text{mol}/\text{l}$ in the woman and $110 \mu\text{mol}/\text{l}$ in the man). An interesting finding was the slightly lower daily excretion of fluoride in pregnant women. The mean decrease was $14 \mu\text{mol}/24$ hrs as compared to the control group. This difference was not significant.

Fig. 2

Correlation Between Serum Creatinine Concentration and Free Ionized Plasma Fluoride in Renal Patients from the Fluoridated Community and Non-Fluoridated Area



(X) represents one patient from the fluoridated community.

(•) represents one patient from the non-fluoridated community.

Table 4 presents data from the artificially fluoridated area. In diabetes mellitus and diabetes insipidus, cardiac insufficiency, liver cirrhosis, LED, cor pulmonale and surprisingly in obstructive icterus we found elevated values. If we excluded patients in whom serum creatinine was elevated the difference from the control group disappeared. Patients with cardiac insufficiency showed elevated values even if serum creatinine was not elevated. Interestingly, patients with hypertension showed significantly lower mean fluoride values than the controls which might be due to the use of diuretic drugs but we did not verify this possibility. Furthermore, sampling in this group was too small for reliable statistical comparisons.

Table 5 shows the corresponding values from the non-fluoridated area with the same disease groups as in Table 3. The only important difference is the fact that here in the cases with hypertension no difference of the free ionized plasma fluoride concentration was noted among those with and without elevated serum creatinine concentration. In patients with LED the mean free ionized fluoride values were significantly higher than in the corresponding control groups.

We also measured the daily excretion of fluoride and the daily renal fluoride clearance in some of the patient groups, (Table 3). The difference between the controls and the patients with renal insufficiency is statistically significant: The mean renal clearance of fluoride was 41 ± 3 in the non-fluoridated controls in subjects with normal kidney function. Endogenous fluoride clearance was significantly lower in patients with renal insufficiency (11 ± 3) and also in diabetes (16 ± 2). However, in both conditions the reduction in fluoride clearance and excretion was more pronounced than in the cases of pregnancy.

In patients with renal insufficiency the mean renal clearance was about one half of that in patients drinking artificially fluoridated water. In addition we found that in patients with cardiac insufficiency the daily excretion of fluoride was somewhat diminished although creatinine values were normal. Therefore it is probable that cardiac disease may also cause at least slight retention of fluoride.

No final conclusions can be made concerning toxicity on the basis of these results because we did not study the clinical phase of fluoride toxicity. However, in patients with severe renal insufficiency the plasma fluoride concentrations were similar to those in patients who received 25 mg of fluoride daily for the treatment of osteoporosis.

TABLE 4

Mean Free Ionized Plasma Fluoride, Mean Serum Creatinine, Mean Ages
in the Artificially Fluoridated Drinking Water Community.
Comparisons with Control Groups of Same Mean Age and from the Same Community.

		Plasma F ⁻ μmol/l ± S. E. M.	Serum creatinine μmol/l ± S. E. M.	Mean age in years	P
<u>Endocrinic diseases</u>					
Diabetes mellitus (all adult patients)	(70)*	1.7 ± 0.088	118 ± 10	54	<0.05
Diabetes with normal serum creatinine	(46)	1.4 ± 0.058	77 ± 3	52	
Diabetes with elevat- ed serum creatinine	(24)	2.3 ± 0.20	182 ± 27	58	<0.01
Diabetes in children	(18)	1.3 ± 0.056	63 ± 3	11	<0.05
Hyperthyroidism	(10)	1.2 ± 0.11	90 ± 7	54	<0.01
Hypothyroidism	(3)	1.5 ± 0.41	128 ± 21	50	
Diabetes insipidus	(1)	4.0	59 ±	6	
<u>Cardiovascular diseases</u>					
Heart insufficiency (all patients)	(49)	1.9 ± 0.12	117 ± 11	63	<0.05
Heart insufficiency with normal serum creatinine level	(33)	1.8 ± 0.14	84 ± 3	60	<0.05
Heart insufficiency with elevated serum creati- nine level	(16)	1.9 ± 0.21	230 ± 66	67	
Heart insufficiency with clinical edema	(8)	2.2 ± 0.33	291 ± 143	62	
Cor pulmonale	(3)	2.6 ± 0.40	121 ± 11	63	
Hypertension (all patients)	(45)	1.2 ± 0.051	91 ± 6	52	<0.001
Hypertension with nor- mal serum creatinine	(34)	1.1 ± 0.045	72 ± 4	45	<0.001
Hypertension with ele- vated serum creatinine	(11)	1.5 ± 0.11	147 ± 8	59	
<u>Liver diseases</u>					
Liver diseases (all patients)	(7)	2.6 ± 0.33	100 ± 10	52	<0.05
Liver cirrhosis	(5)	2.3 ± 0.33	100 ± 10	53	
Obstructive icterus	(2)	3.5 ± 0.40	100 ± 20	52	
<u>Collagen diseases</u>					
Lupus Erythematos. Dissem.	(3)	2.6 ± 0.70	302 ± 56	56	

* Number of patients

TABLE 5

Mean Free Ionized Plasma Fluoride, Mean Serum Creatinine, Mean Ages
in the Non-Fluoridated Drinking Water Community.
Comparisons with Control Groups of the Same Mean Age and from the Same Community.

		Plasma F ⁻ μmol/l ± S. E. M.	Serum creatinine μmol/l ± S. E. M.	Mean age in years	P
<u>Endocrine diseases</u>					
Diabetes mellitus (all adult patients)	(35)*	1.1 ± 0.077	174 ± 8	58	<0.01
Diabetes with normal serum creatinine	(18)	0.98 ± 0.052	95 ± 8	57	
Diabetes with elevat- ed serum creatinine	(17)	1.6 ± 0.36	250 ± 48	59	
Hyperthyroidism	(11)	0.95 ± 0.062	67 ± 3	60	
Hyperthyroidism	(5)	1.0 ± 0.14	89 ± 8	67	
<u>Cardiovascular diseases</u>					
Heart insufficiency (all adult patients)	(56)	1.1 ± 0.045	102 ± 6	67	<0.01
Heart insufficiency with normal serum creatinine value	(49)	1.1 ± 0.049	91 ± 3	64	<0.05
Heart insufficiency with elevated serum creati- nine value	(7)	1.1 ± 0.10	137 ± 19	70	
Heart insufficiency with clinical edema	(12)	1.3 ± 0.091	134 ± 10	65	<0.05
Hypertension (all patients)	(41)	0.99 ± 0.038	117 ± 9	52	
Hypertension with nor- mal serum creatinine value	(30)	0.95 ± 0.039	91 ± 4	50	
Hypertension with ele- vated serum creatinine value	(11)	0.94 ± 0.058	147 ± 12	59	
<u>Liver diseases</u>					
Liver diseases (all patients)	(15)	1.1 ± 0.11	105 ± 8	52	
Liver cirrhosis	(10)	0.95 ± 0.095	101 ± 7	51	
Obstructive icterus	(5)	1.4 ± 0.18	107 ± 15	53	
<u>Collagen diseases</u>					
Lupus Erythematos. Dissem.	(9)	1.7 ± 0.20	254 ± 16	43	<0.01

* Number of patients

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3. Gedalia, I., Brzezinski, A., and Bercovici, B.: Urinary Fluoride Levels in Women During Pregnancy and After Delivery. J. Dent. Res., 38:548-51, 1959.

Discussion

Dr. Burgstahler: In connection with your analytical studies did you ever look at protein-bound iodine levels as some kind of an index of differences?

Dr. Hanhijarvi: No. We researched only fluoride.

Prof. Jolly: We will have more detailed discussion on renal involvement in tomorrow's seminar but I would like to mention that one of the conditions which significantly enhances fluoride toxicity is renal disease of any kind. We have had very few autopsies in our cases but in one case which I remember the patient had originally a polycystic disease of the kidney. He had lived in fluoride areas early in life. In fact, he died of the original disease but the fluoride toxicity was a contributing factor. Similarly the levels of plasma fluoride and fluoride clearances which we have done in our cases along with creatinine clearances are similar to the ones that you have seen in your cases. However, in our cases of fluorosis the levels of plasma fluoride naturally are much higher than yours, namely in the range of 9 to 15 μ M of ionic fluoride as compared to about 1.5 in your group.

Question: How does μ M compare to mg? I think some of us are not familiar with that.

Prof. Burgstahler: 1/10 mg would be 5.3 μ M. .53 would be one hundredth mg.

Dr. Hanhijärvi: Yes we use μ M so that there are not so many zeros in the figures.

* * * *

EFFECT OF SODIUM FLUORIDE ON THE PHOSPHATE
ABSORPTION BY POTATO TUBER TISSUE - INFLUENCE OF CALCIUM

by

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SUMMARY: Phosphate uptake by disks of potato tubers aged for 24 hours in 0.5 mM CaCl_2 was studied after pretreatment for 15 minutes in 5×10^{-2} M NaF. The results indicate that the disks aged in the CaCl_2 solution showed an increase in phosphate uptake only when they were pretreated with NaF. This phenomenon was no longer observed when the calcium was eliminated by the action of EDTA. Absorption of calcium and fluoride by the potato disks is essentially a non-metabolic process. However, disks aged in a calcium solution fixed more fluoride than fresh disks but the subsequent increase in uptake of phosphate did not seem to be directly related to the increased fixation of fluoride.

The rise in phosphate absorption by disks treated with NaF does not appear to be a metabolic process because it occurred when all experiments (aging, fluoride treatment and phosphate uptake) were conducted at 0°C . We conclude that the increased phosphate uptake is largely localized at the physico-chemical level in the membrane.

Fluoride is known as a pollutant for plants. In recent years, the role of fluoride has assumed much importance in plant physiology, primarily because of its toxic properties when absorbed by, or deposited upon plants. The typical symptoms, chlorosis and necrosis of broad-leaved plant species and tip burn of conifer needles, have been extensively studied and have been reviewed in recent papers (1-3).

In considering fluoride as a noxious agent, it is necessary to analyze the symptomatology of plants as well as all other effects of fluoride on plants. Fluoride affects many areas of plant physiology either directly or indirectly. It inhibits growth (4, 5) and many enzymatic processes, as shown by Warburg and Christian (6). Its inhibition of enolase was found to be due to the formation of a magnesium fluorophosphate complex with the enzyme.

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Chang and Thompson (7) reported retardation of growth by slowing cellular division and expansion. Fluoride reduces the amount of free and bound ribosomes (8) and is liable to impair the synthesis of ribosomes by causing an accumulation of ATP (9). Yang and Miller (10) observed that fluoride increases aminoacids and organic acids. Fluoride also affects uptake of oxygen. At high but still sublethal concentrations, fluoride inhibits respiration (11) whereas lower concentrations at a magnitude of 1 to 2 $\mu\text{g}/\text{m}^3$ stimulate respiration (12-13). These changes have not been attributed to any specific enzyme.

Several studies have also underlined the possibility of a relationship of the tissue content of calcium to sensitivity to fluoride. Pack (14) showed, in 1966, that fluoride-induced damage to tomato leaves is more pronounced in a low calcium environment than where calcium levels are adequate. Ramagopal and collaborators (15) concluded that the symptoms caused by fluoride are analogous to those induced by calcium deficiency and suggested that this action is due to an insoluble complex with calcium. Bligny (16) showed, in leaves containing calcium, that the acropetic migration slows down as the retention of fluoride along the lamina increases.

Our present purpose is to define the mode of action of fluoride towards the permeability of phosphates and in particular, the interference of fluoride with the calcium ion. By studying potato tubers we have been able to demonstrate that pretreatment with alkaline fluorides increases absorption of phosphate only after an aging period in a solution containing calcium (17). Nevertheless, aging of potato tuber disks provokes metabolic changes including increases in their absorption capacity (18, 19), their respiratory activity (20, 21) and the synthesis of proteins and nucleic acids (22, 23) and of phospholipids (24, 25).

Under these conditions, the question arises whether or not the increase in absorption of phosphate after treatment with sodium fluoride is directly related to the metabolic changes produced by the aging process. We investigated this problem in the following experiments.

Material and Methods

All experiments were conducted with thin disks of potatoes cut from a large tuber, 1 mm in thickness and of constant diameter (10 mm). The potato tubers (*Solanum tuberosum*, Var. Bintje) were obtained from the station INRA at Ploudaniel (Finistère). They were stored in the dark, in a cold room at 4° C.

Experimental Technique The action of sodium fluoride was studied either on freshly cut tuber disks carefully freed from cell de-

bris by several washings with distilled water or on disks aged for 24 hours. One hundred fifty to 200 disks of the latter group were incubated at 15° C in two liters of 5×10^{-4} M CaCl_2 . In some of the experiments, the disks were incubated in other solutions as indicated later. All solutions were aerated throughout the incubation period by a stream of fine air bubbles and by shaking of the disks in order to insure constant homogeneity of the solution. After the period of aging, the disks were rinsed in distilled water and divided into two lots. One was treated with NaF at 5×10^{-2} M/l, pH 6 for 15 minutes and the other, used as control, with NaCl under the same conditions. Each sample was removed in turn from this first solution, rinsed for 30 seconds in distilled water and then transferred to the absorption solution. The absorption solution contained 1 mM $\text{Na}_2 \text{HPO}_4$ labelled with $\text{H}_3^{32}\text{PO}_4$ 15 to 20 $\mu\text{Ci/liter}$. To discontinue the absorption, each sample was rinsed three times for a total of 30 seconds in distilled water. In some of the experiments, desorption of the ions was effected by placing the disks in 50 ml volumes of distilled water after an absorption period.

Analytical Technique To determine the amount of ^{32}P and ^{45}Ca absorbed, radioactivity in the disks was assayed with a Geiger Muller tube. Calcium uptake was followed either with ^{45}Ca or with ^{40}Ca . ^{40}Ca measurements were made on an atomic absorption spectrophotometer after drying the material at 105° C and digesting it in a (2:1) nitric and perchloric acid mixture. We added lanthane chloride (0.5 mM) to each sample to eliminate the interference.

After employing the stream distillation procedure (26), the fluoride content of the disks was determined spectrophotometrically at 527.5 mm with the use of zirconium cyanine R lake (27).

The amount of mineral content is expressed in micromoles or microequivalents related to dry weight.

Results

I. Pretreatment with NaF and Phosphate Uptake

1) Development of fluoride sensitivity on phosphate uptake by aging the material: Fresh disks do not absorb more phosphate after treatment with NaF. Only after a period of aging in running tap water does absorption of phosphate by the disks increase considerably with aging (Table 1).

This sensitivity to NaF after aging can be attributed either to the composition of the aging solution or to the metabolic changes occurring when a dormant storage organ is sliced and incubated aerobically. In order to decide this question, we experimented with different aging media.

TABLE 1

Effect of Pretreatment with NaF on Phosphate Uptake by Disks of Potato as Related to Aging Period

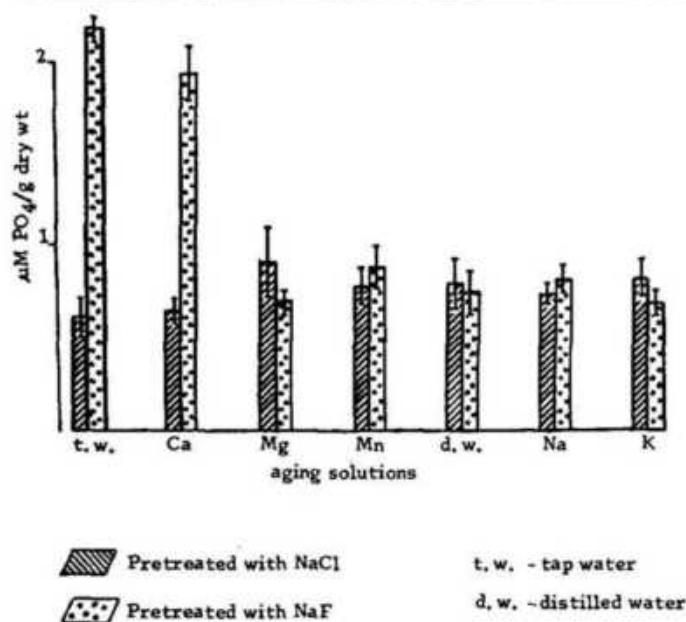
Aging period (hours)	Phosphate uptake lot treated with NaCl (control)	Phosphate uptake lot treated with NaF	% of control
0	0.46*	0.5	105%
2 1/2	0.53	1.70	368%
5	0.57	2.17	370%
10	0.68	2.26	332%
23	0.94	3.70	392%
48	1.51	3.74	248%

* μM per gram dry weight. Uptake period : 1 hour.

2) Relationship of uptake of phosphate after treatment with NaF to the composition of the aging solution: Figure 1 shows the amount of phosphate absorbed in one hour after treatment with NaF (and NaCl as control) as related to the composition of the aging solution. The results

Fig. 1

Uptake of Phosphate After Pretreatment with NaF and NaCl (control) as a Function of the Composition of the Aging Solution



Uptake period : 1 hour - Desorption period : 30 min. at 5°C in Na_2HPO_4 2m M/l

indicate that only the disks, aged in CaCl₂ 0.5 mM/l or in running tap water (calcium concentration:16 mg/l), show an increase in phosphate uptake when pretreated with NaF. In the other aging solutions (MgCl₂, MnCl₂, KCl, NaCl at 0.5 mM/l and distilled water) pretreatment with NaF does not alter the uptake of phosphate.

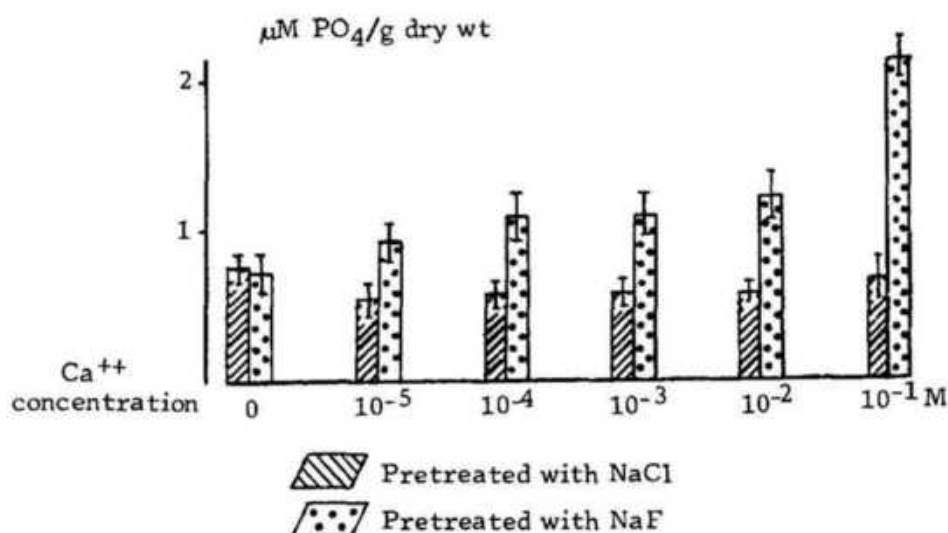
It appears, from this data, that calcium plays an essential role in developing fluoride sensitivity. In order to explore this question further, one may study the influence of the calcium concentration of the aging media as well as the action of EDTA by which calcium can be extracted from the tissue fragments (28). We first determined the mechanism of absorption of calcium and fluoride by the potato disks and demonstrated their interaction.

II. Ca⁺⁺ in the Aging Solution

1) Effect of the Ca⁺⁺ concentration in the aging solution: According to Fig. 2, the uptake of phosphate increases after treatment with NaF in relation to the increase of calcium in the aging solution. Aging the disks in a solution of calcium does not enhance directly subsequent uptake of phosphate. In the controls, there was no statistically significant difference.

Fig. 2

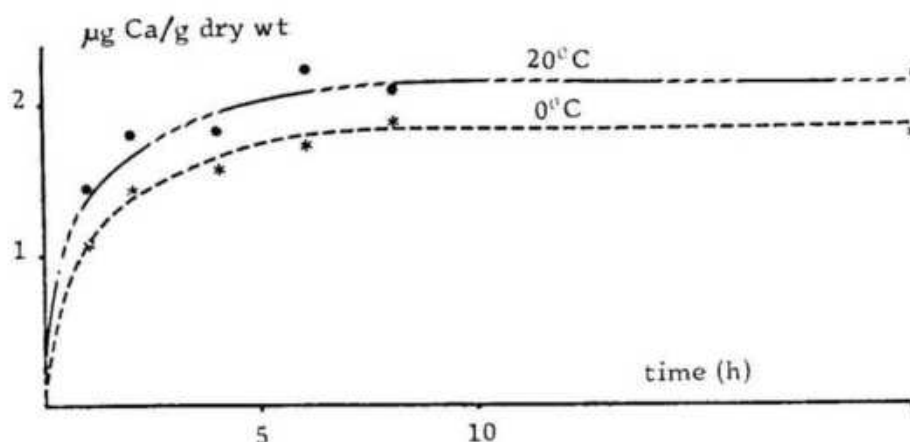
Uptake of Phosphate After Pretreatment with NaF or NaCl (control)
as a Function of Ca⁺⁺ Concentration of the Aging Solution



2) Calcium uptake: Calcium uptake by various plant tissues has been shown to be largely non-metabolic (29, 30). We studied the uptake of calcium as a function of time on freshly cut disks at 0° C and 20° C. The absorption solution was 0.5 mM CaCl₂ labelled with ⁴⁵Ca. The curves showing the absorption time of calcium uptake (Fig. 3) suggest that uptake of calcium is a non-metabolic process because the amount of calcium absorbed reached a maximum at about 5 hours and because there was no marked effect on the temperature.

Fig. 3

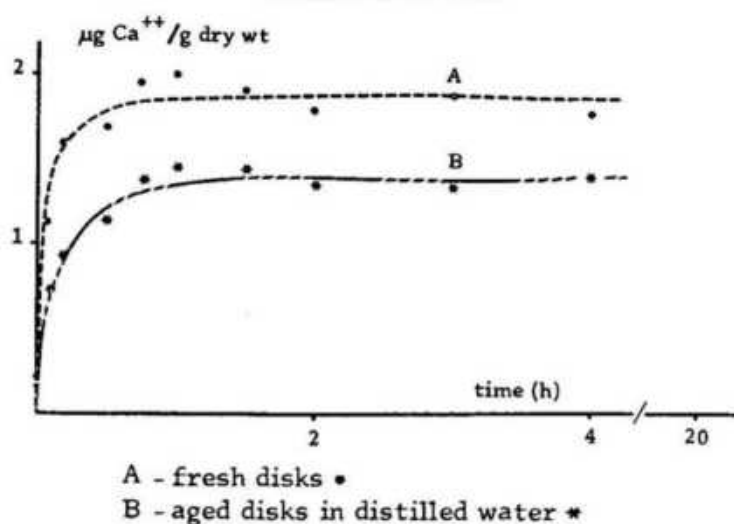
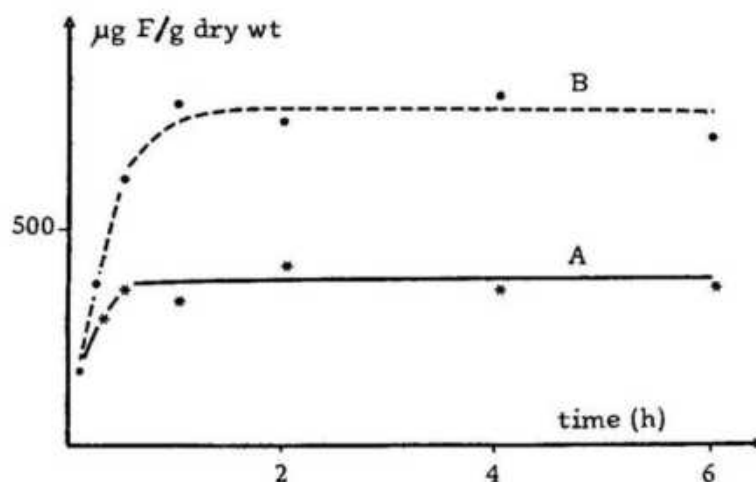
Calcium Uptake by Freshly Cut Disks, at Two Different Temperatures



The evolution in time of phosphate and rubidium uptake differ when one studies the ion uptake by freshly cut disks or aged disks (31). We have followed the ⁴⁵Ca uptake by fresh disks and aged disks (24 hours in aerated distilled water). In both cases, (Fig. 4) calcium uptake is non-metabolic. However disks aged for 24 hours in distilled water absorb more calcium than fresh disks. It is possible that the aged disks lost calcium during the aging period; but in freshly cut disks and in disks aged in distilled water for 24 hours, the amount of calcium, determined by spectrophotometric atomic absorption, was the same. The observed difference must therefore be attributed to a change in absorption capacity and especially to an increase of the adsorption sites.

3) Fluoride uptake:

a) Time of fluoride uptake: The absorption of fluoride as a function of time, either by freshly cut or by aged disks - in this experiment, the disks were aged in running tap water - appeared to be a non-metabolic process (Fig. 5). Fluoride absorption occurred quickly and did

Fig. 4Calcium UptakeFig. 5Fluoride Uptake - Absorption Solution NaF 5×10^{-2} M/l

A : fresh disks, B : aged disks in distilled water

not increase significantly with time. After half an hour, all fluoride which could be absorbed was already present in the disks. These re-

FLUORIDE

sults agree with those of Venkateswarlu and co-workers (32) who found that absorption of fluoride by barley roots from a solution of fluoride labelled with radiofluorine (^{18}F) was a non-metabolic process.

In addition, we found that aged disks absorbed about twice as much fluoride than freshly cut disks. In fresh disks, we never found an increase in phosphate uptake after pretreatment with NaF (Table 2).

TABLE 2

Effect of Pretreatment with NaF on Phosphate Uptake

	min.	min.	min.	hours	hours	hours	hours
Pretreatment time with NaF	5	15	30	1	2	4	6
Fresh disks	105	107	120	113	109	98	95
Aged disks	324	237	227	253	293	295	278

Mean of 2 series of 12 samples

Uptake period - 1 hour. Results expressed as percent of control.

Under these conditions, the question arises whether or not the elevation of phosphate uptake by aged disks may not be explained by, or at least related to, increased absorption of fluoride by the disks.

Nevertheless, for aged disks, a pretreatment of 5 min. was sufficient to bring about a consecutive rise in absorption of phosphate, even though the study of kinetic absorption showed that the absorption of fluoride had not reached its maximum, not even the value obtained with fresh tissues. This indicates that the sensitivity of the aged disks to fluoride was not directly related to their higher fluoride content. In other words the fluoride sensitivity appears to be a manifestation of a metabolic change induced by aging.

In parallel with this study, we have also determined the phosphate absorption after fluoride treatment of fresh and of aged disks (Table 2).

These results confirm that uptake of phosphate by freshly cut disks is insensitive to pretreatment with fluoride even of six hour duration. Even an aging period of 2 hours in running tap water suffices to develop a fluoride sensitivity on phosphate uptake (33). One might have expected that a pretreatment of freshly cut disks with NaF of a few hours duration would bring about sufficient metabolic changes to develop a fluoride sensitivity on phosphate uptake. But this is not the case.

Fluoride sensitivity only appeared after an aging period in a solution containing calcium ions.

Furthermore, these results show that, on aged disks, phosphate uptake was sensitive to pretreatment with NaF regardless of whether it occurred for a few minutes or for a few hours (Table 2).

b) Effect of calcium on fluoride absorption: Fluoride absorption was measured with disks aged either in a solution of CaCl₂ 0.5 mM/l or in distilled water (absorption period is 1 hour). Phosphate uptake was also determined.

According to Table 3 disks aged in a solution of CaCl₂ absorbed about twice as much fluoride than those aged in distilled water. On the

TABLE 3

Effect of Ca⁺⁺ in the Aging Solution on F⁻ Uptake

Aging solution	Distilled water		CaCl ₂ 0.5 mM/liter	
	NaCl (control)	NaF	NaCl (control)	NaF
Pretreatment solution				
Amount of F ⁻ absorbed as µg/g dry weight		547 ± 165		1120 ± 22
Phosphate absorbed for 1 hr : µM/g dry weight	1.15 ± 0.16	1.23 ± 0.15	1.26 ± 0.19	4.35 ± 0.
Stimulation caused by fluoride : % of control	107%		346%	

Mean of 4 measurements ± SE.

other hand, pretreatment with fluoride increased the uptake of phosphate only after an aging period in a solution containing calcium ions. Under these conditions, it is possible that the increase in phosphate uptake, after treatment with NaF, by disks aged in a calcium solution may be attributed to elevation of their fluoride content.

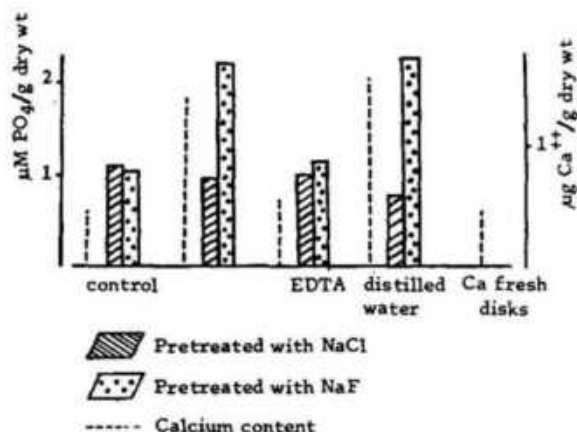
Therefore the uptake of calcium and fluoride by potato tuber disk is essentially a non-metabolic process. These two ions applied successively bring about an increase of phosphate uptake if controlled by replacing fluoride ions with chloride ions. The calcium ion must therefore play an essential role in developing fluoride sensitivity. This could be confirmed, if we could demonstrate the formation of a complex of calcium with EDTA before and after treatment with fluoride.

FLUORIDE

4) Effect of EDTA on phosphate uptake by disks aged in calcium solution and treated with fluoride. After an aging period of 24 hours in a calcium solution, the disks were treated with EDTA (sodium salt pH 6) (Fig. 6). The disks were then treated with NaF or NaCl (as con-

Fig. 6

Effect of EDTA on the Sensitivity to Fluoride of Phosphate Absorption



After aging for 24 hours in CaCl_2 or distilled water (control), the disks are treated with EDTA (10^{-3}M , 1 hour). They are then pretreated with NaF (dotted) or with NaCl (hatching) before proceeding to phosphate absorption - Calcium content (---)

trol) before proceeding to the incorporation of phosphate labelled with ^{32}P . Figure 6 shows that treatment with EDTA 10^{-3}M/l for one hour eliminates fluoride sensitivity, measured by the uptake of phosphate. EDTA also suppresses an important fraction (about 60%) of calcium bound to the tissue.

To verify whether the sensitivity of phosphate absorption is directly linked to the presence of calcium, we conducted a complementary experiment. Disks aged in a calcium solution and treated with EDTA for 2 hours at $5 \times 10^{-4}\text{M/l}$ were returned to a calcium solution for a short time (5 minutes) whereupon the sensitivity of phosphate uptake to F^- reappeared.

From these experiments, it appears that the rise of phosphate absorption after treatment with NaF is directly related to the presence of calcium in the tissues. Calcium is probably fixed to the outer cell walls since a very brief (5 minutes) immersion of the disks in the calcium solution suffices to bring about the reappearance of the phenomenon.

Our next step was to study the absorption kinetics for phosphates, in order to better localize the site of fluoride action, and to define its relation with the metabolism.

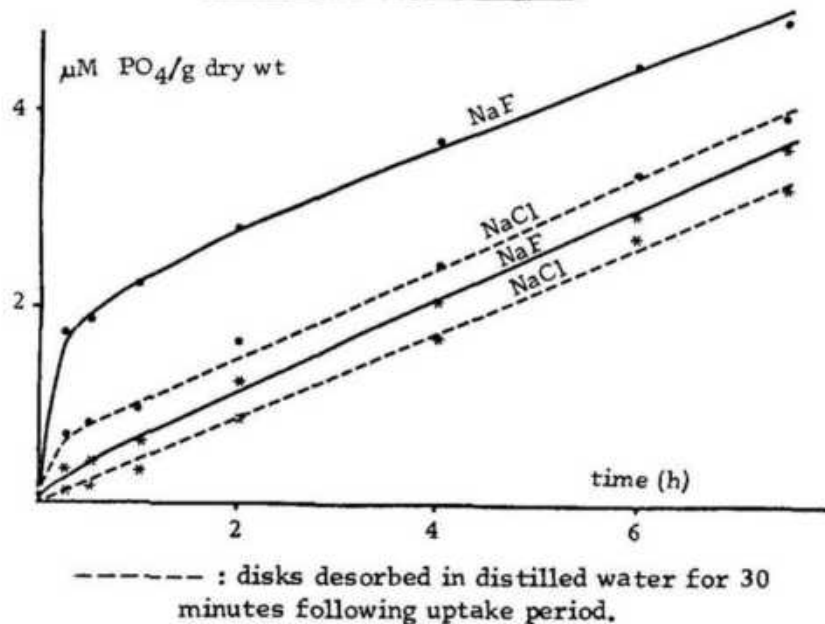
III. Relation with the Metabolism

Absorption of phosphates, from solutions at 20° C or at 0° C, was measured both after rinsing the disks for 30 seconds with distilled water and after a desorption period of 30 minutes at 5° C in distilled water (desorption is complete at this time).

Regardless of whether the kinetics were conducted at 20° C or at 0° C (Figs. 7 and 8) the action of fluoride was noticeable immediately. Ultimately, the curves become parallel. When the kinetics were conducted at 20° C (Fig. 7) fluoride does not appear to modify the metabolic absorptions, at the concentration studied, since the curves are parallel.

Fig. 7

Time Course of Phosphate Uptake at 20° C by Aged Disks Treated with NaF (●) or with NaCl (*)



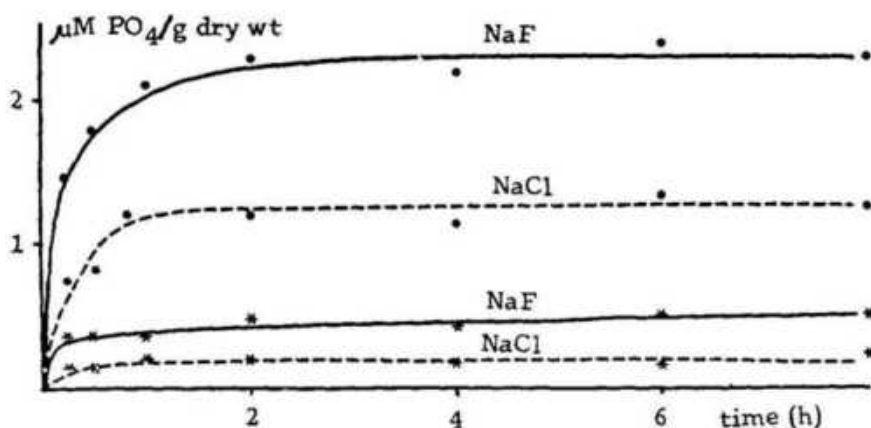
On the other hand, the increased absorption of the lot treated with fluoride persists after a desorption period of 30 minutes regardless of whether the experiments are carried out at 0° C or at 20° C.

Therefore fluoride acts on phosphate absorption by means of an augmentation of the number of adsorption sites (non-metabolic phase of the absorption) which are not entirely diffusable, since they persist after a prolonged desorption phase.

In a parallel study, we placed disks in CaCl_2 for 24 hours at 0°C ,

Fig. 8

Time Course of Phosphate Uptake at 0°C by Aged Disks Treated with NaF (•) or with NaCl (✱)



----- : disks desorbed in distilled water for 30 minutes following uptake period.

and treated them subsequently with NaF or NaCl for 15 minutes at 0°C , before following the incorporation of phosphates (still at 0°C). We reproduced the stimulatory effect of fluoride on the absorption of phosphate.

It therefore appears that the observed effect is largely, if not solely, localized at the level of physico-chemical processes in the membrane and does not constitute a physiological process provoked by aging.

Conclusions

The foregoing experiments show that the increased absorption of phosphates observed many times in storage tissues after pretreatment with alkaline fluorides, appears only after an aging period in a cal-

cium containing solution (CaCl_2 solution or tap water with 16 mg/liter calcium content). We have attempted to define more precisely the nature of this interaction. At first it seems surprising that this appears to be specific to the calcium ion.

The passive character of calcium absorption by different organs and tissues is generally admitted; it appears that for the potato tuber disks, the situation is the same. The absorption of fluoride is also a non-metabolic process according to the curve of Fig. 5. Nevertheless, after an aging period of 24 hours, the quantity of fixed fluoride is doubled. This could be attributed to an increase in the number of absorption sites. Similarly, aging in a calcium solution increases the fixation capacity of the disks for fluoride which appears to be in agreement with the favorable effect of calcium on the absorption of anions (34) but which is difficult to interpret here, because of the possibility of a direct interaction between calcium and fluoride through formation of the low soluble ($17 \mu\text{g/g}$) CaF_2 .

If the calcium is eliminated by the action of EDTA, the increase in phosphate absorption after treatment with NaF is no longer observed; this is further confirmed, by the return of the fluoride sensitivity after reimmersion of the disks for only 5 minutes in a calcium solution. Absorption kinetics for phosphates show that the stimulation is especially marked during the early stages of the absorption, which suggests a passive phase (diffusive) of absorption. The persistence of increased absorption at 0°C (Fig. 9) confirms this hypothesis. On the other hand, fluoride does not appear to influence the active phase of phosphate absorption since, in the stationary regime, the speed of metabolic absorption is not altered.

The elevated absorption of phosphate persists after a desorption phase of 30 minutes, at the end of which desorption is practically completed. This phenomenon appears even when all treatments are carried out at 0°C (aging, pretreatment with NaF, and phosphate uptake). Therefore the sensitivity to fluoride in regard to absorption of phosphate is directly related to the presence of the calcium ion at the level of the membranes and not linked to physiological changes due to aging.

It is concluded that pretreatment with fluoride acts on phosphate absorption by an augmentation of the adsorption sites; calcium appears to be required for the appearance of these sites.

The problem of localization of these sites and that of the interaction of the different ions at the membrane level requires further investigation. Calcium is apparently fixed on the cell walls at the level of the pectates (35) and the plasmalemma (36). Fluoride is perhaps capable of breaking certain pectate-calcium bonds, and forming CaF_2 precipitate.

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AN EVALUATION OF THE POTENTIAL FOR ECOLOGICAL
DAMAGE BY CHRONIC LOW-LEVEL
ENVIRONMENTAL POLLUTION BY FLUORIDE

by

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SUMMARY: The literature dealing with the effects of chronic environmental contamination by fluoride in wild-life plant and animal species has been reviewed, in order to assess the potential for significant impact of fluoride pollution upon natural ecosystems. Research data are far from complete, and many important questions cannot be answered yet; nevertheless, current knowledge supports the tentative conclusion that fluoride has great potential for ecological harm. It is clear that a wide range of organisms can accumulate substantial concentrations of fluoride from the air, water, or soil. Some evidence of toxicity, even at very low environmental levels, is available. These and other characteristics of fluoride pollution should alert biologists to look for likely effects of fluoride stress on natural populations and ecosystems in areas subjected to low-level fluoride pollution.

Introduction

Most research on the environmental effects of fluoride pollution has been directed toward measuring the potential risks to human health and to crops and livestock of major economic significance to humans. While this is perfectly logical, it is necessary also to examine the potential for damage by fluoride pollution to wildlife organisms and natural ecosystems. Environmental science is coming to the realization that the natural world provides many services and amenities which our technology cannot begin to replace, and environmental insults which may damage the balance of nature, even in subtle ways, may threaten human security as much as many a more obvious, direct risk of harm from pollution.

This paper reviews research findings on the impact of fluoride

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pollution on wildlife species in their natural habitat, and laboratory studies which may be models of what occurs in the natural world. Although research on this topic is a relatively new field, and data are very incomplete, the current state of knowledge is more than adequate to mark fluoride as a significant pollutant which appears to have a high potential to cause ecological damage.

A recent publication of the U.S. National Academy of Sciences (1) sets out some guidelines for evaluating the potential for ecological damage inherent in the release of any chemical into the environment. Pollutants which are released by many sources and are widespread in the environment, and substances which are chemically stable and therefore persistent, are of concern. Other properties which suggest increasing potential for harm include toxicity, as known from tests with laboratory animals; the ability to be accumulated in organisms, and magnified in biological food chains; and the potential to be transformed by biological activity into more toxic substances. In addition, long-term, chronic, sub-lethal toxic effects on organisms, which may occur in all members of a population exposed to low-level pollution, may be far more significant, ecologically, than even relatively high mortality due to intense but localized pollution. Even if toxic effects on individuals of a species may not be apparent, effects on characteristics of populations (such as age distribution, reproductive rates, etc.) may lead to significant shifts in ecological balances (1-3).

As will be seen, fluoride meets most if not all of the criteria for a pollutant of major potential ecological impact. Research to measure such potential effects, however, has barely begun, especially in terms of effects at the population or ecosystem level.

Sources and Environmental Levels

Fluoride is released to the air, soil, and water of the United States by a great variety of industrial, agricultural, and municipal activities, in a widespread number of geographical locations. Aluminum, steel, and phosphate production (as well as many other industries) are major sources of fluoride air and water pollution; fluoride is added to community water supplies (for protection against tooth decay), and incorporated into soils (as an impurity in phosphate fertilizers.) Estimates of fluoride air pollution range from 120,000 to 155,000 tons per year for the U.S. (4, 5); fluoride input to soil and water may be roughly of the same order of magnitude (6). Marier and Rose (7) have diagrammed the "Man-made Fluoride Cycle"; quantitative estimates of transfers among the compartments of the environment are very approximate.

Except in the immediate vicinity of a source, fluoride levels in air are generally quite low [below one part per billion (ppb) (8)]. However, concentrations below 1 ppb may still be biologically significant (9,

10), and can occur over widespread areas when pollution is present (10, 11). Surface waters usually contain 0.1 part per million (ppm) or less of naturally-occurring fluoride, and the oceans from 0.7 to 1.4 ppm (7). Near a pollution source (industrial), concentrations of more than 20 ppm have been recorded in rivers and estuaries (12), and levels of 0.3 to 2.1 ppm have been linked to specific sources discharging into other rivers and streams (13-15). There are few systematically gathered water quality monitoring data for fluoride; among the measurements that have been recorded, occasional levels as high as 3 or 4 ppm can be found (16). In soils, natural mineral constituents may lead to fluoride content of several hundred or even thousands of parts per million; and the addition of fertilizers, which may contain 10,000 or more ppm fluoride, can raise the absolute level of the ion. However, much of both the intrinsic and the added fluoride is in insoluble form, or is tightly bound to soil particles, and over 90 percent may be unavailable for uptake by organisms. (See discussion of bioaccumulation, below.)

Uptake and Accumulation

One of the most notable characteristics of fluoride as a pollutant is its tendency to accumulate in organisms, making serious adverse effects possible even at very low levels of exposure, if exposure persists over time. The entry of fluoride into food chains, by accumulation in forage crops, is the chief factor accounting for the extensive damage to grazing livestock that has been caused in the past by fluoride pollution (4, 17). Some plant varieties have been found to build up fluoride concentrations in their tissues to levels one million times that of the surrounding air (18). Fluoride is also stored in animals; in vertebrates, accumulation is primarily in skeletal tissues (4). In recent years, field studies have begun to examine fluoride accumulation in natural vegetation and wildlife in areas subject to fluoride pollution; the information gathered to date suggests strongly that a great many organisms have the capacity to accumulate significant fluoride, even from very low ambient concentrations of the ion.

Table 1 presents, in summary form, some of the data now available on the bioaccumulation of fluoride from the air, water, or soil, by a variety of plant and animal species. Studies by Gordon and co-workers (10), Carlson and Dewey (11, 19), Kay (20), and others, have shown significant buildup of fluoride in vegetation, insects, and herbivorous mammals, in a forest ecosystem area in Montana. Of particular interest are the entomological data of Carlson and Dewey (11, 19); not only is this one of the first broad demonstrations of accumulation of fluoride in insects, but it also shows marked differences in the degree of fluoride buildup among different kinds of insects, with the pollinator group apparently the most prone to accumulate the pollutant. The relatively high fluoride content of some predatory insects indicates at least a

TABLE 1

Summary of Data on Bioaccumulation of Fluoride (a)

Organism and Tissue	Fluoride (ppm)	Parameters of Exposure	Reference (b)
Lichens, (whole plant)	475-990	Air, 1-8 km from source, 4 or 12 months	21
	134-190	Air, 15 km from source, 12 and 4 months	"
	70	Air, 40 km from source, 4 months (control)	"
Mosses, (whole plant)	140-600	Air, 1-4 km from source, 4 or 12 months	"
	78	Air, 15 km from source, 12 months	"
	20	Air, 40 km from source, 4 months (control)	"
Vegetation (all types)	6.1-10.7	Control samples	11
	10-70	Samples 4-14 miles from source (200,000 + acres)	"
	100-1000+	Samples 2 miles or less from source (7040 acres)	"
Insects, (whole body)		(All specimens exposed to F in air & diets)	11, 19
Controls (all types)	3.5-16.5	Controls, captured 50 miles from source	"
Foliage feeders	21.3-255	Captured within 1/2 mile of source	"
Cambial feeders	8.5-52.5	Same	"
Predators	6.1-170	Same; primary source of intake, insects in diet	"
Pollinators	58-585	Same	"
Chipmunk, (femur)	50-303 (108.7)*	Controls	10
"	200-13,333 (1000)	Adults from assorted locations in polluted area	"
Ground Squirrel, (femur)	35-142 (105)	Controls	"
"	17-7168 (775)	From assorted locations in polluted area	"
Deer Mouse, (femur)	(106.5)	Controls	"
"	28-6038 (1300)	From assorted locations in polluted area	"
Deer (2 sp.), (femur)	86-635 (225)	Controls	"
"	124-9400 (1500)	From assorted locations in polluted area	"
Vegetation (all types)	1.6-6.4 (3.9)	Controls	20(c)
"	6-456	From assorted locations in polluted area, 2 years (samples above 35 ppm all 2.5 km or less from source)	"
Deer Mouse, (femur)	(144)	Controls	"
"	288-1995	From assorted locations in polluted area, 2 years	"
Shrew, (femur)	(494)	Controls	"
"	1315-10,500	From assorted locations in polluted area, 2 years	"
Sparrow, (bones)	84-565	Controls	44, 45
"	1013-3527	Captured near factory (aluminum smelter)	"
Frogs, (bones)	392-1067	Controls	"
"	852-7880	Captured near factory	"
Grass	10-30	Controls	"
"	up to 1330	Growing near factory	"
"Freshwater plants"	(40.5)	Exposed in water of unknown F content	46
Water hyacinth, (leaf)	25	Growing naturally in water @ 1 ppm F	47
" (petiole)	60	Same	"
Marine algae, several varieties	2-22	Growing naturally in sea water @ 1.08 ppm F	48

Organism and Tissue	Fluoride (ppm)	Parameters of Exposure	Reference
Eel grass, (leaves)	0.2	Kept in sea water @ 1.05 ppm F, 72 days	49
" "	"trace"	Kept in sea water @ 52 ppm F, 72 days	"
Algae (<i>Cladophora</i>)	3.2	Kept in sea water @ 1.05 ppm F, 72 days	"
" "	2.4	Kept in sea water @ 52 ppm F, 72 days	"
Sand Shrimps (total body)	106	Kept in sea water @ 1.05 ppm F, 72 days	49
" "	3116	Kept in sea water @ 52 ppm F, 72 days	"
Mud crabs (total body)	169.6	Kept in sea water @ 1.05 ppm F, 72 days	"
" "	1414	Kept in sea water @ 52 ppm F, 72 days	"
Prawns (total body)	374	Kept in sea water @ 1.05 ppm F, 72 days	"
" "	3248	Kept in sea water @ 52 ppm F, 72 days	"
Mullets (total body)	141.8	Kept in sea water @ 1.05 ppm F, 72 days	"
" "	7743	Kept in sea water @ 52 ppm F, 72 days	"
Oysters (soft tissues)	4-5	Kept in sea water @ 0.5 ppm F for up to 60 days	50
" "	13-18	Kept in sea water @ 2.0 ppm F for up to 60 days	"
" "	20-30	Kept in sea water @ 8.0 ppm F for up to 60 days	"
" "	50-100	Kept in sea water @ 32 ppm F for up to 35 days	"
Blue crab (exoskeleton)	298	Controls, kept in sea water @ 0.5-1.5 ppm F	12
" (gills)	253	Same	"
" (muscle)	10	Same	"
" (exoskeleton)	800	Kept in sea water @ 8 ppm F for 30 days	"
" (muscle)	21	Same	"
Brown Trout (bone)	400-1700	Living in stream with natural F content 1-14 ppm	23
Trout (fillet)	1.3	Exposure unknown; assumed to be control level	51
Ocean fish (various)			
soft tissues	3, 5-57	Living in sea water (0.7-1.4 ppm F)	52
skeletons	558-6820	Same	"
Clover (leaf & stem)	6-13	Controls	53
" "	8-17	100 ppm F added to soil as NaF, KF, or cryolite	"
" "	17-53	600 ppm F added to soil as NaF, KF, or cryolite	"
" "	62-233	1800 ppm F added to soil as NaF, KF, or cryolite	"
Spinach (leaf)	5.3	Control	54
" "	15.5	280 ppm F as NaF added to soil	"
" "	41.3	560 ppm F as NaF added to soil	"
Winter rye (leaf & stalk)	6.0	Control	"
" "	67.0	500 ppm F as NaF added to soil	"
" "	158	1000 ppm F as NaF added to soil	"
" "	200	1500 ppm F as NaF added to soil	"

^a Data are primarily for wildlife species. See other reviews by Hill (9), Lillie (17), and National Academy of Sciences (4, 55) for extensive additional data on bioaccumulation of fluoride in laboratory and field studies of cultivated plants and domestic animals.

^b Data presented here are selected from much more extensive data contained in many of the references cited. Readers are urged to consult the original works for more detailed information on bioaccumulation of fluoride.

^c See also recent data published by Kay, et al (74) on fluoride accumulation in plants and wildlife from an uncontaminated ecosystem.

* Figures in brackets are average values, when available

FLUORIDE

three-step food chain transfer (plants—herbivorous insects—predatory insects.) Also notable is the report by LeBlanc et al. (21), showing very marked buildup of fluoride in lichens and mosses (plants which are at the base of many food chains) even at substantial distances from a pollution source. The work of Gordon et al. and Carlson and Dewey shows accumulation to potentially toxic levels in vegetation over a very large area, and the former study presents data of skeletal burdens in herbivorous mammals which are quite high. None of these studies include adequate data on predatory species which, because of their positions high in food webs, are often the most heavily affected by cumulative toxins. Accumulation of fluoride in predators is certainly an area where research should be given high priority.

Data from field studies on aquatic organisms are, in contrast, virtually nonexistent. Most of the findings reported in Table 1 for water-dwelling organisms were obtained in the laboratory. Fluoride contamination of rivers and streams is not uncommon; there is both a need, and an opportunity, to sample populations of aquatic plants and animals exposed to low-level fluoride pollution, and examine the extent to which fluoride accumulation may be taking place in these systems. Data on aquatic plants seem to suggest that some plants can accumulate fluoride from waters containing about 1 ppm, though the magnification factors here are nowhere near as large as known for some terrestrial species. Other aquatic plants (e.g., eel grass, and *Cladophora*) are reported not to accumulate the ion. Investigations of the uptake and storage of fluoride in a number of plants which are important elements of aquatic ecosystems need to be conducted. Invertebrates, at least marine and estuarine species, seem to have a general capacity for fluoride accumulation; almost nothing is known yet for similar freshwater varieties. Because of the importance of crustaceans and insects in freshwater food chains, this also is a question of potentially large ecological significance. Fish, like terrestrial vertebrates, show significant skeletal storage of fluoride, though better dose-response data are needed. There are some indications that other environmental factors, particularly calcium hardness of the water, may reduce the availability of fluoride for uptake, at least in fish (22-24). As is the case in terrestrial ecosystems, there are almost no data to evaluate the potential risk to animals at the highest levels of aquatic food chains which may result from biomagnification of fluoride in food chain organisms. Indeed, virtually nothing is known about the potential for fluoride buildup in any species of aquatic birds, mammals, or amphibians.

Some plants may accumulate significant fluoride from soil, at least under certain conditions. Laboratory tests with heavy addition of soluble fluorides to soils showed substantial uptake in a wide range of test plants. However, under field conditions, many factors are involved

in the availability of fluoride for uptake. Calcium content of the soil (25), the presence of other trace elements such as boron (26, 27), pH, temperature, moisture and, in all probability, other variables besides the amount and form of fluoride present, influence uptake. In addition to plants, however, the soil is home to myriad species of microbes, fungi, worms, mites, insects, and other creatures, whose complex interactions play a very large role in the maintenance of a healthy soil system (upon which we rely for food, fiber, and forest products). To date, nothing has been published which examines the potential for accumulation of fluoride by these soil organisms, or the transfer of the pollutant in soil food webs. In view of the substantial amounts of fluoride added to soils in fertilizers, this too should be an area of priority research.

Biological Effects

Extensive damage to both domestic livestock and agricultural and timber crops has long been associated with fluoride pollution; extensive reviews of research on these effects are available (4, 9, 17), and this material need not be re-examined here. It suffices to say that there is a bountiful body of literature in support of the conclusion that fluoride is an element of high biological activity, which can provoke a wide range of toxic effects in a broad spectrum of plants and animals. What is of interest here is knowledge of the potential toxic impact of fluoride on the many plants and animals exposed to the contaminant in their natural environments. The sort of information needed to assess the ecological risk inherent in current or likely levels of fluoride pollution is largely unavailable; research is needed not only on the short-term and long-term toxicity of fluoride to individuals of many species, but also on the long-term impact of any effects which may occur on populations and ecosystems.

Table 2 summarizes current knowledge of potential effects of fluoride pollution on wildlife organisms. Most of the data are from laboratory studies, and many of the reports date back a decade or more. Knowledge of potential toxicity of fluoride to organisms other than domestic and laboratory animals and cultivated plants is essentially in its infancy; recent findings, however, do suggest some areas which need to be pursued. Carlson and Dewey (11), citing the sensitivity of honeybees to fluoride injury and their own data showing that the pollinator group of insects accumulated the highest levels of fluoride, suggest that, should pollinators as a class be susceptible to fluoride injury, the result could be shifts in the types of plants which are pollinated, and eventual major changes in the ecological balance of the polluted region. Other indications of potential fluoride toxicity in organisms which play key ecological roles also should receive high priority research attention; examples are the rather high, and potentially toxic, levels of skeletal fluoride found in herbivorous mammals in Montana (10, 20); the suggested physiological impairment of single-celled algae (28) which are a keystone of

TABLE 2

Summary of Selected^(a) Findings of Effects of Fluoride Pollution on Wildlife Organisms

Organism	Exposure Parameters	Effect	Reference ^(b)
Lichens & mosses (15 species)	Exposed to ambient air 1-15 km from source for 4 or 12 months	Cellular injury, other damage	21
Coniferous trees (several types)	Growing within several miles of a source for a period of years	Foliar injury; mortality; in- creased scale in- sect attack	11
Honeybees	Living and gathering nectar in the vicinity of a source of F air pollution	High level of mortality	17
Silkworm larvae	Feeding on contaminated forage containing: up to 10 ppm F as BaF ₂ , (NH ₄) ₂ SiF ₆ up to 15 ppm F as NaF, KF, other levels below lethal concentrations	Lethal	56
		Lethal	"
		Reduced growth	"
Bacteria (aquatic) (several species)	Kept in water containing 0.5 to 5.0 ppm F	No effect on sewage digestion	57
"	Exposed to water containing up to 360 ppm F	No effect on survival	58
Freshwater protozoa and rotifera	Exposed to up to 450 ppm F for up to 9 days	No effects on survival	59
<u>Chlorella</u> (single- cell green alga)	Exposed to 2 ppm F for 48 hours	37% reduction in growth	28
	Exposed to 2 ppm F for 72 hours	19% reduction	"
	Exposed to 200 ppm F or more for 48 or 72 hours	86-99% reduction in growth	"
	Exposed to 1000 ppm F as NaF	7% decrease in respiration	60
<u>Scenedesmus</u> (green alga, freshwater)	43 ppm F as NaF, 4 days, @ 24°C	Lethal (LC ₅₀)	61
<u>Daphnia</u> (water flea)	225 ppm F as NaF, 48 hours	Cessation of activity	62
"	122 ppm F as NaF, 48 hours	Acute toxicity (TL _m)	63
"	5 ppm F as cryolite, 48 hours	Lethal (LC ₅₀)	64, 65
<u>Simocephalus</u> (water flea)	5 to 10 ppm F as cryolite, 48 hours	Cessation of activity	"
Lobster	0.9, 2.25, and 4.5 ppm F as NaF, 10 days	No effect on survival	66
Oysters	32 or 128 ppm F in sea water, up to 60 days	100% mortality within 40 days	50
	0.5, 2, or 8 ppm F in sea water, 60 days	No effect on survival	"
Blue Crab	20 ppm F in sea water, ambient temp., 6 mo.	Growth reduced 4.5% per molt	12
Prawns	10 or 100 ppm F in sea water, 4 days, 20.5°C	No mortality	49

Organism	Exposure Parameters	Effect	Reference
Fish (3 species)	Same	Same	"
Mussels	1.0 to 1.4 ppm F in sea water @ 20.5°C, up to 20 days (Controls)	Up to 60% mortality	"
"	Higher fluoride concentration, other conditions the same	Increased mortality with increased F level	"
Mud Crab	52 ppm F, 25°C, 72 days, in sea water	71% mortality	49
Sand Shrimp	Same as above	45% mortality	"
" "	Controls (1.05 ppm F)	23% mortality	"
Prawns	52 ppm F, 25°C, 72 days, in sea water	No mortality	"
Mullet	Same	Poor physical condition, lessened activity, infection	"
Rainbow Trout	2.7 to 4.7 ppm F, 48-240 hours, @ 13°C	Acute toxicity (LC ₅₀)	23
Carp	75-91 ppm F, 20-24°C, up to 480 hours, soft water	Same	"
Rainbow Trout	4.0 ppm F, three weeks, soft water	5% Mortality	22
	8.5 ppm F, three weeks, soft water	50% Mortality	"
	75 ppm or less F, three weeks, hard water	No mortality	"
Trout eggs	1.5 ppm F during period of development	7-10 day delay in hatching	30
Frogs	900 ppm F, immersion for 1 week	Lethal (LC ₅₀)	67
"	Kept in water @ 5-300 ppm F for prolonged periods	Decrease in red & white blood cell counts	68
Frog eggs	Kept in well water with 1 ppm F added	Premature hatching, retarded development	29
Frog tadpoles	Kept in water @ 0.5 and 4.5 ppm F	Metamorphosis delayed, abnormal thyroid	31
Toad eggs and tadpoles	Kept in water @ 13-450 ppm F	Retarded development, premature hatching, delayed metamorphosis	69
Fruit trees	Grown in soil fertilized with F and Boron-containing phosphate fertilizers	Leaf damage and fruit injury	26, 27
Winter Wheat	Grown in soil with 1000-1500 ppm F added	40-65% reduction in yield	54
Spiderwort	Grown in soil with 400 ppm F added	28-34% reduction in growth	54
Pea seedlings	Grown in soil with increasing F added	Increasing inhibition of growth	70
Loblolly pine & red maple	Grown in soil with 1.9-190 ppm F added	Reduced growth	71

^a Effects selected are primarily those on wildlife species in their natural habitats, but due to lack of field research, data on many species were obtained in laboratory tests. For a great deal more data on effects of fluoride on cultivated plants and domestic animals, see earlier reviews by Hill (9), Lillie (17), and National Academy of Sciences (55, 72).

^b Readers are encouraged to consult papers cited for additional data and discussion.

most aquatic systems; and the potential interference of low levels of fluoride with the reproductive and developmental cycles in fish and amphibians (29-31).

It is notable that many of the organisms tested for fluoride toxicity (as shown in Table 2) did not experience toxic effects until levels of fluoride far higher than those which might realistically be encountered in the environment were attained. This type of finding can be misleading, first because our techniques of measurement may be inadequate to detect effects, and second because sub-acute effects may have their impact at the population level, rather than in detectable ways in individual organisms (1-3). Many effects are quite subtle; for example, the suggestion in Carlson and Dewey's study that fluoride accumulation might increase susceptibility of some pine species to attack by scale insects (11). Only extensive and careful field research, carried out over a period of years, can bring answers to these and other important questions.

An assessment of the potential toxic impact of a given fluoride exposure is made more difficult by the knowledge that many physical, chemical, or biological parameters can significantly affect toxicity. In fish, for example, temperature, water hardness, pH, chloride concentration, and the strain, age, physiological, and reproductive condition of the fish all are known to influence susceptibility to fluoride intoxication, and other factors may be involved as well (24, 32, 33). Knowledge of this complexity makes drawing of generalizations from existing laboratory data difficult, and accentuates the need for studies of effects under existing field conditions.

Biosynthesis of Organic Fluorides

The ability to synthesize fluoroacetate and fluorocitrate, each far more toxic than inorganic fluorides, has long been recognized in several dozen highly toxic tropical plants (34, 35) and has recently been demonstrated in cell cultures of soybeans (36, 37). Identification of traces of these organic fluoride toxins in tea, oatmeal, and other common plants suggests the possibility that the capacity to synthesize organofluorides may be more widespread in the plant world than was previously recognized (36, 38). If it could be demonstrated that the presence of low-level fluoride pollution in the environment could lead to the widespread synthesis and accumulation of fluoroacetate or similar toxins in natural food chains, this would be a finding of enormous potential ecological significance. Miller and co-workers have published several reports of induced synthesis of fluoroacetate in forage and vegetable plants, grown in the vicinity of a phosphate plant or fumigated with HF in laboratory tests (39-41). Weinstein and associates (42), and Hall (43), among others, have attempted to duplicate these findings by exposing plants to fluoride in the air or in nutrient solutions but have failed, to date, to confirm the synthesis of organic fluorides under these conditions. This

is certainly an area which merits further investigation.

Conclusions

The most obvious conclusion about current knowledge of the potential effects of fluoride at low levels of contamination of natural ecosystems is that what we know is dwarfed by what we need to know. However, it is clear that fluoride fits most of the criteria for potentially important pollutants set down by the National Research Council (1), i. e.:

- (1) Fluoride is a widespread pollutant.
- (2) It is non-biodegradable, and relatively persistent.
- (3) Fluoride is accumulated by a great many organisms; it is nearly certain that the contaminant is building up in wild-life food chains.
- (4) Fluoride is an element of high biological activity, with well-established toxic effects on a great many organisms; and levels of exposure which may occur in the environment appear to be capable of causing some adverse impact in at least some of the relatively small number of wildlife species which have been tested.
- (5) Information on potential effects on populations in the field, and on ecological balances, is virtually non-existent to date.
- (6) It is possible that fluoride may be transformed by some organisms in the natural environment into far more toxic organic fluorides. If this transformation occurs to any significant degree, it could be an effect of profound ecological importance.

These conclusions are not enough to tell us what levels of fluoride in the environment are "safe," but it should be clear that fluoride has most of the earmarks of a pollutant of major ecological impact. Substantial research effort will need to be directed to some of the questions suggested by the findings summarized here, to generate sound factual information for the purpose of setting standards which will protect natural ecosystems against potential damage by fluoride pollution.

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PLACENTAL TRANSFER OF FLUORIDE DURING METHOXYFLURANE ANAESTHESIA FOR CESAREAN SECTION

by

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(Abstracted from *Experientia*, 31:339-41, 1975).

In 21 women who received methoxyfluorane anaesthesia for Cesarean Section the authors measured simultaneously inorganic fluoride in maternal arterial blood, in cord blood and in the urine of the newborn. In addition, the time sequence of the fluoride concentration of the mother's blood plasma was monitored during methoxyfluorane anaesthesia.

Anaesthesia was induced with thiopental 4 mg/kg on the average not exceeding 250 mg. All patients received 100 mg succinylcholin for relaxation as an intravenous drip. The ventilation was carried out with a gas mixture of N_2O-O_2 at a rate of 8 l/min. To this gas flow was added 0.2 volume percent Methoxyfluorane. At the moment of birth, 10 ml arterial blood were drawn from the mother as well as blood between 2 clamps from the umbilical cord vein. The inorganic fluoride was estimated in the maternal and cord blood by means of the Orion fluoride electrode. The first 12-hour urines of the newborn were also collected for fluoride analysis. The anaesthesia lasted from 3 to 23 minutes with an average of 12.25 minutes. The authors recorded a significant correlation between the fluoride concentration in the maternal arterial blood of the patients and the duration of anaesthesia. When the anaesthesia had lasted only 10 minutes the fluoride concentration in the maternal blood was about $4 \mu\text{mol/l}$; it reached $12 \mu\text{mol/l}$ when the anaesthesia lasted 20 minutes or more. A similar correlation was observed for the fluoride concentration of the umbilical vein of the newborn. However, the blood in the newborn was always about one half that of the maternal blood and during the first 12 hours the urines of the newborn showed no increase in fluoride concentration. The authors explained the difference between the levels of fluoride in the mother and the baby on the basis of two mechanisms, the uptake of fluoride by the growing skeleton of the fetus and the uptake by zones of calcification often found in human placentas. The authors found that the mean fluoride values in the mothers blood ($5.6 \mu\text{mol/l}$) were much lower than those capable of impairing renal function.

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G. L. W.

OPTIMAL FLUORIDATION, THE CONCEPT AND ITS APPLICATION TO MUNICIPAL WATER FLUORIDATION

by

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(Abstracted from the Western Journal of Medicine, 431:122-25, 1975).

The author determined the daily average fluoride content of children's diet in a low-fluoride (0.1 ppm) community. Dietary sources of fluoride occur in the food chain through canning, bottling and other modes of preparation of food, through airborne fluoride from industrial operations i. e. absorption by broad-leaved edible plants such as lettuce and cabbage, through fluoride-polluted forage for food animals, and through numerous other direct and indirect ways which inevitably affect humans. Thus, by 1963 food-chain fluoride was approximately 3 1/2 times that found by earlier investigators. According to Marier and Rose (1) and Spencer (2) daily intake of fluoride ranged from 3.6 to 5.4 mg. Children's cereals such as Grape-Nuts and Wheaties, milled in fluoridated Minneapolis, showed values up to 6.2 ppm and 10.1 ppm respectively. In 1971, the United States Army Institute of Dental Research (3) study obtained a "market basket" type analysis of foods collected in 1967 and 1968 by several teenage boys from supermarkets in fluoridated Baltimore. They found 2.1 to 2.4 mg of fluoride per day which included beverages made with fluoridated water.

The author analyzed 17 day-food lists compiled by parents who recorded food, snacks, beverages consumed by an average child in one day in Marin County (California). He obtained exact duplicate sample diets of children in the age groups of 4 to 15 which were submitted to total fluoride determinations. The duplicate meals which included school lunches and snacks were prepared in the quantities consumed. The mothers were cautioned to duplicate only the amount which each child actually had eaten, and amounts equal to what the child left on the plate were disregarded. The individual daily diets were refrigerated overnight and delivered for analysis to the laboratory the next morning. The fluoride determinations on the homogenized, dried diets were made according to current methods by the Association of Official Analytical Chemists (AOAC). Three to four aliquots analyzed for each individual diet resulted in an accuracy of 2.7%.

The local State Board of Health carried out another study of 17 healthy children in the area by examining their daily urine specimens. They found concentrations in the range of 0.2 to 0.7 ppm in the non-

fluoridated (0.1 ppm or less) communities. A subsequent study by the local Health Department on 72 specimens revealed a mean of 0.34 ppm. On the basis of a daily fluid consumption of 1.0 to 1.4 liter the author calculated the daily fluoride intake on the 72 urine tests between 1 to 2.2 mg with the mean being 1.4 mg per day which corresponded remarkably closely to the results of his own food fluoride study.

In his conclusion, the author pointed out that the optimal fluoride intake according to accepted standards occurs when the average child in the formative age group receives 0.4 to 1.5 mg of fluoride per day. The 17 samples indicated an average daily intake of 0.9 to 1.5 mg through the diet alone in the non-fluoridated community.

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G. L. W.

THE PREVALENCE AND DISTRIBUTION OF ENAMEL DEFECTS IN FOUR DISTRICTS WITH DIFFERENT FLUORIDE CONTENTS IN THE DRINKING WATER

by

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(Abstracted from *Proc. Finn. Dent. Soc.*, 70:178-85, 1974).

The authors studied the prevalence and severity of enamel defects in children and tooth populations in districts with different fluoride contents. The causes of enamel defects are ingestion of exces-

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sive amounts of fluoride, exanthematous febrile diseases, rickets, vitamin deficiency, metabolic changes, local infections and mechanical trauma. Mottled enamel has been reported in low fluoride areas (0.06 ppm to 0.3 ppm) varying in prevalence between 21 and 63%.

The authors studied 204 children with an average age of 11.1 years in four communities, namely Lohja (0.1 ppm), Espoo (0.2 and 0.7 ppm), Hanko (0.9 ppm) and the high fluoride community of Elimaki (1.6 ppm). The values of the fluoride contents were provided by the National Board of Health of Finland 1974 and additional checks were made by individual analysis by the ion analyser Orion on samples of 1000 g of water provided by each child. In general the classification of mottling by Dean was used. However, Dean's fifth group "severe" was abandoned and the following two groups were substituted for it: "staining of gingival margin of tooth" and "pitting".

In the three low-fluoride districts the authors observed an average of 0.8% of teeth with mottling classified as "mild" and "moderate". In Elimaki with the highest fluoride level a high prevalence of enamel defects namely 65.4% of the tooth pair population was observed of which "mild" mottling (white spots covering less than 50% of the enamel surfaces) amounted to 4.1% and moderate mottling (whole surface affected) to 11.4%. Pitting of the enamel in Elimaki occurred in 0.4% of the teeth and in Lohja in 2.3%. Brown stains in the gingival margin were recorded most frequently in Elimaki namely in 10.5% of the children. The authors found it difficult to differentiate non-fluoride opacities from dental fluorosis.

In the low-fluoride districts, the percentage of children with enamel defects ranged from 41.2 to 74.1% and in Elimaki the percentage was highest namely 98.2%. The authors stated that these are some of the highest reported; they quoted Dean (1942) who found an incidence of mottling in the range of 2.5 to 43% when the fluoride content was less than 0.5 ppm.

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G. L. W.

EXCRETION OF RETAINED FLUORIDE IN MAN

by

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(Abstracted from the Journal of Applied Physiology, 38:282-87, 1975).

High urinary fluoride excretion has been observed in persons who have moved from a high to a low fluoride area. Therefore, appreciable amounts of previously retained fluoride continue to be released for prolonged periods of time. In this study, the authors investigated the release of stored fluoride from the human body in urine and feces following discontinuation of high fluoride intake. They also related the excess excretions of fluoride to the amount retained during the period of high fluoride intake.

Nine fully ambulatory male patients were kept on a constant diet throughout all the study phases. Complete collections of urine and stool were made and analyzed for fluoride and urinary creatinine was measured daily. In the control cases, fluoride balances were determined in each 6-day metabolic period by analyzing dietary aliquots of 6-day collections of urine and stool during the intake of fluoride supplements and after their discontinuation. In the controls, the fluoride present in food and drinking water ranged from 3.6 to 5.4 mg/day with an average of 4.4.

The fluoride supplements consisted of an average of 9.1 mg fluoride as sodium fluoride given for an average of 32 days and of 7.7 mg of fluoride as fish protein concentrate given for an average of 26 days. The daily fluoride intake was increased to an average of 13.5 mg when the fluoride supplement was sodium fluoride and to 11.2 mg when it was fish protein concentrate. After the fluoride supplements were discontinued, the urinary and fecal fluoride excretions were determined for three weeks in each 6-day metabolic period (with exception of one 10-day study period immediately after the discontinuation of the fish protein supplemented diet). The analysis of fluoride in the diet and drinking water and in urine and stool and in the fluoride supplements was carried out according to a modification of the Singer and Armstrong diffusion method.

Results: In the control study, with an average daily fluoride intake of about 4.4 mg the urinary excretion of fluoride ranged from 1.8 to 3.2 mg/day. An increase to an average of 13.5 mg by addition of the

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fluoride supplement to the constant diet raised the fluoride excretion in the urine promptly in each of four patients and it remained elevated throughout the period of fluoride administration. After discontinuation of the fluoride supplement, the urinary fluoride excretions decreased promptly but remained somewhat higher during the first or second 6-day period than in the controls after which they returned to baseline levels.

The fecal fluoride excretion in the controls was very low (0.2 to 0.3 mg/day) in the different 6-day periods. When the intake of fluoride was increased by addition of the fluoride supplement, to 13.5 the fecal fluoride increased irregularly in the different 6-day periods but remained below 1 mg/day. After discontinuation of the fluoride supplement, the fluoride in the feces promptly decreased but remained slightly higher than in the controls for 6 days after which it was equal to that of the controls.

Discontinuation of the sodium fluoride supplements was followed by greater excretion in urine and feces than in the controls during the first and second 6-day periods but during the third 6-day period excretion returned to control levels. The total excess of fluoride excretion during the 18 days averaged 8.6 mg in the urine and 1.18 mg in the feces.

The amount of fluoride retained during the intake of sodium fluoride averaged 3.6 mg/day or 115 mg for 32 days. Excess fluoride excretion - the release of fluoride that was previously deposited - corresponded to 9.1% of the retained fluoride given as NaF and to 14.1% of the retained fluoride given as fish protein concentrate. Of the excess fluoride elimination, 87% was excreted through the urine and only 13% through stool. There were wide individual differences in the amount of fluoride excreted from person to person.

In a previous study by the authors, after discontinuation of a daily dose (1) of 45 mg fluoride given for three months, excretions of excess fluoride in urine and stool totalled only 16.7 mg in 8 days which represents 2% of the retained amount of fluoride. However, despite such high retention of fluoride no x-ray evidence of skeletal fluorosis nor any other untoward effects were observed. The authors point out that their results here recorded of low excretion of excess fluoride are in contrast to the large excretions of fluoride in urine for prolonged periods of time following the discontinuation of a high fluoride intake, "reported by Largent (2) and by Likins, McClure, and Steere (3).

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* * * *

AUTHOR'S INDEX

- Aasenden, R.: 55-56
 Alary, J.: 25-33
 Armstrong, W. D.: 112-113
 Auermann, E.: 61-85

 Beyer, W.: 176
 Bierenbaum, M. L.: 114-115
 Bourbon, P.: 25-33

 de Carlini, Ch.: 241
 Chan, M. M.: 163-173
 Conover, C. A.: 85-92
 Cordy, P. E.: 118-119

 Diouris, M.: 208-223

 Egyed, M. N.: 134-143
 Ewy, Z.: 191-198

 Farkas, C. S.: 98-105, 105-112
 Fengler, F.: 61-85
 Fleischman, A. I.: 114-115
 Franke, J.: 61-85
 Frazier, P. D.: 112-113

 Gagnon, R.: 118-119
 Gatiyatullina, E. E.: 47-50
 Gileva, E. A.: 47-50
 Gordon, C. C.: 125-133, 182-191
 Greenberg, L. W.: 117-118
 Greenberg, R. A.: 174-175
 Groth III, E.: 224-240

 Haavikko, K.: 243-244
 Hanhijärvi, H.: 198-207
 Hayton, T.: 114-115
 Helle, A.: 243-244

 Jagiello, G.: 52-53
 Jones, W. G.: 56-57

 Kahl, S.: 191-198
 Kay, C. E.: 92-97, 125-133,
 182-191
 Kaye, M.: 118-119

 Kramer, L.: 245-247
 Kramer, ..: 117-118
 Krechniak, J.: 38-40

 Lee, J. R.: 242-243
 Lenart, G.: 61-85
 Lin, J.: 52-53

 Murty, K. J. R.: 12-24, 144-154
 Murty, T. V. S. D.: 12-24, 144-154
 Myers, G. S.: 51

 Nanda, R. S.: 154-163
 Nelsen, C. E.: 117-118

 Osis, D.: 245-247

 Paletta, B.: 176
 Peebles, T. C.: 55-56
 Penot, M.: 208-223
 Plotko, E. G.: 47-50
 Poole, R. T.: 85-92

 Rao, S. R.: 12-24, 144-154
 Rath, F.: 61-85
 Reddy, S. S.: 12-24, 144-154
 Riggins, R. R.: 163-173
 Rucker, R. B.: 163-173
 Runge, H.: 61-85

 Sakla, A. B.: 176
 Saxena, M. K.: 144-154
 Schätzle, W.: 115
 Schlegel, H. H.: 177
 Shen, Y. W.: 54
 Shlosberg, A.: 134-143
 Singer, L.: 112-113
 Snyder, D. S.: 174-175
 Spencer, H.: 245-247
 Stein, R.: 114-115

 Taves, D. R.: 54, 118-119
 Teotia, M.: 34-38
 Teotia, S. P. S.: 34-38
 Tourangeau, P. C.: 125-133, 182-191

Author's Index

Truhaut, R.: 25-33	Wiatrowski, E.: 245-247
	Wörner, H. P.: 116-117
Waldbott, G. L.: 41-47	
Weiss, V.: 241	Zipkin, I.: 112-113
von Westernhagen, B.: 115	

SUBJECT INDEX

- Age and fluoride clearance, 203
- Alkaline phosphatase 17, 112, 119
- Aluminum
 - production, 225
 - workers, 62, 63
- Arthritis, 177
- Atmospheric fluoride, 30, 31, 33, 177
- Blood sugar
 - in Na_2SiF_6 poisoning, 139
- Bone
 - citrate, 113
 - collagen content, 113
 - enzymes, 115
 - strength, 77-84, 163-171
 - x-ray diffraction, 77
- Calcium
 - deprivation, 168
 - effect on
 - bones, 166
 - fluoride sensitivity, 212
 - fluoride uptake, 230
 - in
 - drinking water, 113
 - fluorosis, 17, 21, 35, 57, 112
 - frogs, 232
 - Na_2SiF_6 poisoning, 139
 - placenta, 241
 - urine, 18
 - water, 35
 - leaf necroses, 91
 - uptake in potatoes, 214, 216
- Cardiovascular diseases
 - mortality, 114
 - serum fluoride in, 114
- Cattle, fluorosed, 135
- Cholesterol in serum, 114
- Chromosomal aberrations, 49, 50, 52
- Cordylone terminalis, 85-92
- Creatinine in serum, 201, 205
- Cryolite
 - hydrogen fluoride, 50
- Deer, 93
- Defluoridation, 22, 152
- Dental
 - caries, 55
 - enamel defect, 243, 244
 - fluorosis in deer, 184
- Diabetes, 202
- Diabetes insipidus, 117, 118
- Diammonium phosphate, 28, 29
- Dietary fluoride survey, 159
- Down's Syndrome, 1-11, 120
- Ear
 - fluoride effect on enzymes, 115
- Easton, Pennsylvania, 114
- Enzymes
 - in acute fluoride poisoning, 140
 - in ear bones, 115
- Feces, fluoride in, 19, 21, 184
- Fertilizer, 25, 31
- Fluoridation
 - fluoride clearance, 203-204
 - statistics, 41-47
- Fluoride
 - absorption of, 111, 112
 - analysis, 32
 - ammonia, 33
 - atmospheric, 30, 31, 33, 177
 - balances, 245
 - clearance, 200-202
 - daily intake, 98-105, 242
 - dietary, 245
 - ecological, 224-240
 - effect on
 - palatal development, 51
 - teeth, 55
 - thyroid function, 194
 - tooth hardness, 116
 - emission
 - from fertilizer plants, 31
 - standards, 31
 - environmental, 224-240
 - excretion, 246
 - fetal, 54, 178, 179

Subject Index

Fluoride

fetal clearance, 200, 202
 in
 algae, 228
 birds, 127
 bones, 57, 96, 113, 172, 186, 187
 cereals, 102
 clover, 228
 cord blood, 179
 deer, 96, 182-191
 dental enamel, 55
 diet, 157, 158
 feces, 18, 19, 184
 fir, 195
 food, 161, 242, 243
 food chain, 226
 forage, 185
 gladiolus, 90
 grass, 185
 hair, 38-40
 kidneys (deer), 186
 lichen, 229
 lobsters, 231
 mammals, 127
 mandible, 95
 mice, 129, 130
 milk, 201
 oysters, 231
 placenta, 54, 179
 pine, 195
 predatory animals, 131
 rabbits, 128
 rumen (deer), 186
 serum (domestic animals), 137, 141
 soil, 226
 spinach, 228
 teeth, 55, 56, 116, 117
 thyroid gland, 34, 36, 196
 trout, 233
 umbilical vein, 241
 urine, 18, 19, 137, 141, 162, 176, 242, 245
 vegetation, 133, 185
 water, 158, 159, 226

Fluoride (cont.)

 intake, 98-104, 105-112
 ionized, 179-199
 mutagenic effect, 52, 53, 47-50
 placental transfer, 251
 poisoning
 acute, 135
 in cattle, 134-143
 experimental, 134-143
 in horses, 134-143
 in pigeons, 134-143
 in sheep, 134-143
 symptoms, 138
 retention, 246
 sampling, 31, 32
 silicium, 33
 skeletal, 21
 sources, environmental, 225
 supplements, 55, 56
 tablets, 55
 toxicity, 233
 transmission to fetus, 54
 uptake from soil, 230
 uptake in potatoes, 214, 215
 Fluoroacetate, 233
 Fluoroapatite, 116
 Fluorocarbon sprays, 123, 124
 Fluorocitrate, 243
 Fluorosilicate, poisoning, 134-143
 Fluorosis
 alkaline phosphatase in, 17, 112
 arthritis in, 57
 bone chemistry, 112, 113
 bone resorption, 74
 calcium, 17, 112
 dental, 34, 56
 caries associated with, 156
 in Lucknow, India, 155, 157
 diabetes insipidus and gastric acidity in, 117, 118
 experimental
 bones in, 168, 173
 cyclic AMP, 190
 histology, 168
 in quail, 168
 pyrophosphatase, 179
 histology, of bones, 69, 70, 113

Subject Index

- Fluorosis (cont.)
 - history, 62
 - in cattle, 56, 57
 - in India, 34, 155-157
 - incidence, 35, 36, 56
 - industrial, 61-85
 - kidney stones, 34
 - magnesium, 17
 - manifestations, 12, 62
 - neurological changes, 73
 - osteoporosis, 84
 - phosphorus, 112
 - reversibility, 73, 78
 - serpentine treatment, 144
 - skeletal, 64-66
 - symptoms, 12, 57, 62
 - thyroid gland, 34, 36
 - treatment, 12-24, 78, 144-154
 - urinary fluoride, 84
 - x-ray findings, 62-67
- Freons, 121
- Gladiolus, 85-91
- Glutamate oxalacetate trans-aminase, 140
- Goiter, 34, 36, 191-198
- Growth retardation, 36
- Hair, fluoride content, 38-40
- Hemodialysis, 118, 119
- Hydrogen fluoride, 25
- Hyperparathyroidism, 83, 84
- ¹³¹Iodine uptake, 194, 195
- Lactate dehydrogenase, 140
- Leaf necrosis, 90
- Lucknow, India, 155, 157
- Magnesium
 - in
 - drinking water, 113
 - fluorosis, 17, 21, 35, 57
 - Na₂SiF₆ poisoning, 139
 - soil, 90
 - water, 35
 - metasilicate, 13, 144
 - urinary, 18
- Massachusetts, study on mongolism, 4-6
- Methoxyfluorane, 54, 241
- Mongolism, 1-11
- Monochlorodifluoromethane, 121
- Montana, 93
- Mottled teeth, 244
- Mule deer, 93
- Mutagenic effect of fluoride, 50, 52, 53
- Myopathy in fluorosis, 146, 147
- National Research Council, 98, 99
- Nephritis, 118, 119
- NPK fertilizer, 30
- Oocytes, 52, 53
- Osteitis fibrosa, 119
- Osteosclerosis, 164
- Otosclerosis, 115
- Ozone depletion, 123, 124
- Palatal development, 51
- Phillipsburg, New Jersey, 114
- Phosphatase, alkaline in fluorosis, 17
- Phosphate
 - uptake in potatoes, 211, 217, 218
- Phosphate fertilizers, 224
- Phosphoric Acid manufacture, 26-28, 35
- Phosphorus
 - in fluorspar, 112
 - in Na₂SiF₆ poisoning, 139
 - urinary in fluorosis, 18
- Placenta
 - fluoride concentration, 54
 - transfer of fluoride, 178, 201, 241
- Polydipsia, 118
- Potato tubers
 - calcium uptake, 223
 - phosphate uptake, 208-223
 - sodium fluoride in potato tubers, 211, 212
- Pregnancy, 178, 201, 202
 - fluoride in plasma, 200
- Pyrophosphatase, 169
- Quail, 168
- Radiculomyelopathy, 144-154
- Renal clearance of fluoride, 200, 203

Renal insufficiency, 203, 204	Teeth (cont.)
Serendipity, 58-60	eruption, 157, 162
Serpentine, 12-24	mottling, 56, 157
Sheep, 135	Teleangiectasis, 174
Silicotetrafluoride, 25	Thyroid hormone, 191-197
Sodiumfluosilicate, 134	disease, 192
Steel production, 225	Triamcinolone, 175
Steroids, fluorinated, 174	Trichloromonofluoromethane, 123
Sulfuric Acid manufacture, 25, 26	Triglycerides, serum, 114
	Triple Super phosphates, 28, 29
Teeth	Urine, excretion, 19, 176
calcium content, 116	Water intake, 160
caries in India, 161	
caries prevention, 55	

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