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The Tenth Conference of the International Society for Fluoride Research will take place at Magdalen College, University of Oxford, Oxford, England, September 16-19, 1979. The Program Committee is soliciting abstracts (up to 300 words) of papers to be presented at the conference dealing with any phase of fluoride research. Abstracts should be submitted as soon as possible to the Society's office, P.O. Box 692, Warren, Michigan 48090.

For details regarding program and accomodations kindly contact Dr. Gene W. Miller, Department of Biology, UMC 53, Utah State University, Logan, Utah 84322.

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MANUSCRIPTS for publication should be submitted in English, double-spaced with generous margins. References should be arranged according to the order in which they are cited in the text, and written as follows: Author, title, journal, volume, pages and year. Each paper must contain a summary of not more than 12 lines.

FLUORIDE is listed in
Current Contents Agricultural
Food and Veterinary Sciences

EDITORIAL

ANOTHER FLUORIDE FATALITY: A PHYSICIAN'S DILEMMA

In the October 1978 issue of Fluoride (1) two fatalities due to acute fluoride poisoning were reported which resulted from a widely held, but mistaken, opinion of physicians and dentists concerning the toxicity of fluoride. Relying on outdated textbook data, the physicians involved in these cases underestimated the magnitude of fluoride's toxic action.

In response to the editorial, the editor received a communication from the parents of another fatal case, that of a 27-month-old boy (A.J.B.) who expired under similar circumstances, i.e. due to a faulty estimate of the toxicity of fluoride.

After the child had swallowed an unknown number of fluoride tablets he was promptly taken, in an unconscious state, to a physician's office where gastric lavage yielded 4 tablets. The physician advised the parents to take the still unconscious child home with the assurance that he needed no further treatment and that "he would be okay". Three and a half hours later, when respiratory failure began to develop, the child was admitted to the Mater Misericordiae Children's Hospital, South Brisbane, Australia, where he expired 5 days later (May 15, 1973). The death certificate #41182 of the Brisbane District, State of Queensland, carried the diagnosis "Fluoride Poisoning". At the hospital the physicians and nurses also assured the parents that it would take "200 to 500 tablets to make him so sick". Actually the bottle had contained less than 100 tablets.

Two factors may have contributed to this fatality: The mother, on the advice of the hospital, had been taking fluoride tablets during her pregnancy and the child, on the advice of the Welfare Clinic, had been given fluoride tablets (0.5 mg) daily for 15 months prior to his death. Both measures, combined, undoubtedly contributed to an excessive fluoride load in the child's body and therefore to a lowered tolerance to additional doses.

Why do physicians fail to correctly evaluate the toxicity of fluoride? Most textbooks rely on the now outdated views of Smith and Hodge who 25 years ago designated 5 to 10 g of fluoride the fatal toxic dose (2).

Only recently (3) the Journal of the American Dental Association warned editorially against the use of fluoride supplements in infancy and early childhood presumably because of dental mottling. Unfortunately this editorial did not spell out clearly that doses of 0.5 mg or less can cause hemorrhages in the stomach (4), and bowels (5), atopic dermatitis (6), and other serious disabilities (7).

Similarly an editorial in the Journal of the American Medical Association (8) warned against the use of massive doses of fluoride in the treatment of osteoporosis but failed to indicate the potential harm of this medication. Therefore, neither editorial presents sufficient data to convince physicians and dentists that this drug is hazardous in doses formerly considered safe.

Administration of fluoride to pregnant women for prevention of tooth decay in the newborn has also been abandoned ostensibly because of its ineffectiveness (9). Unfortunately the danger of this treatment to the fetus and newborn is rarely mentioned in the available literature.

Moreover, many clinicians still adhere to the theory that the placenta forms an effective barrier which protects the fetus and newborn from damage by fluoride consumed by the mother. Teotia in this issue, page 58, shows that fluoride naturally in drinking water of pregnant women (21 and 1.5 mg/day) penetrates the placental barrier. Waldbott (10) showed that a newborn infant who expired shortly after birth with calcifications of arteries had stored 59.3 ppm fluoride in arteries, 5.85 in lungs, 2.86 in the thymus, 0.85 in kidneys, 0.81 in the heart. The mother's main known source of fluoride intake had been artificially fluoridated water. Newborn calves exhibit evidence of dental fluorosis in an endemic area (this issue page 100). Thus there cannot be any doubt that toxic amounts of fluoride pass through the placenta.

All these facts point to the urgent need for a thorough reevaluation of all available data on the toxicity of fluoride. Life can be saved in poisoning from even larger doses than those generally considered toxic provided that prompt and efficient therapy is instituted (11). However, this can only be accomplished if physicians are made aware that fluoride must be used with extreme caution.

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G.L.W.

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METABOLISM OF FLUORIDE IN PREGNANT WOMEN RESIDING IN ENDEMIC FLUOROSIS AREAS

by

M. Teotia, S.P.S. Teotia and R.K. Singh
Meerut, India

SUMMARY: Free ionic fluoride concentrations were measured in the maternal blood plasma, cord blood plasma and the urine of pregnant and age matched nonpregnant women in two groups of subjects. Group 1 included females who had been living in endemic fluorosis areas with the mean intake of 21 mg/day of fluoride from drinking water and Group 2 consisted of women from nonendemic areas with the mean daily intake of 1.5 mg of fluoride from drinking water. The ionized fluoride concentrations in the maternal plasma and the urine decreased during the course of pregnancy; they were at their lowest at 36 weeks of gestation. In the nonpregnant controls these values remained largely unchanged. In the maternal and cord blood plasma obtained at the time of cesarean section the fluoride concentrations were similar and did not support the concept of a placental fluoride barrier. The higher fluoride content in the plasma and urine of the women in the endemic group (10 ppm F^- in drinking water) indicated a direct relationship of these values to the amount of fluoride ingested. The fall in the maternal plasma and urine fluoride concentrations during pregnancy is believed to be due to increasing accumulation of fluoride in the rapidly mineralizing fetal skeleton.

There have been several studies on placental transfer of fluoride in humans. Roholm (1) concluded that fluoride does not pass the placental barrier. Feltman and Kosel (2) found the fluoride concentrations in the placenta and cord blood higher in pregnant women who had been drinking artificially fluoridated water than in controls. Gedalia et al. (3) reported significantly lower urinary fluoride levels in pregnant women than in nonpregnant controls. Increased feeding of fluoride to animals and humans raises the fluoride concentrations in maternal and fetal blood (4). The mean fluoride content of blood in pregnant women shortly before delivery did not differ significantly from that of the umbilical cord obtained after delivery of the placenta. In four women subjected to therapeutic abortion (6), fetal blood concentrations of radiofluoride never exceeded 25% of that of maternal blood sampled simultaneously within 10 minutes after intravenous injections of the

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From the Department of Human Metabolism and Endocrinology, L.L.R.M. Medical College, Meerut, India.

isotope.

Armstrong et al. (7) noted insignificant differences in the fluoride concentrations of maternal and fetal blood in 16 women at the time of cesarean sections. In a study on fluoride concentrations in maternal and cord blood plasma of 16 mothers who had not been subjected to methoxyflurane anesthesia during delivery, Shen and Taves (8) observed a direct linear relationship between the concentration of maternal and cord serum with a correlation coefficient of 0.86. The fluoride concentration of the cord serum was about 75% of the maternal value in contrast to 25% reported by Ericsson and Malmnas (6). This discrepancy could be attributed to the rapid decrease of radioactive fluoride in the maternal blood. Hanhijarvi (9) reported that the plasma ionized fluoride concentration decreases steadily and significantly during pregnancy. A review of the literature by Waldbott (10) indicates that the available data on placental transfer of fluoride are not, as yet, conclusive.

There are no reports on fluoride metabolism during pregnancy in women exposed to drinking water naturally high in fluoride. The purpose of the present work is to study in detail the metabolism of fluoride during pregnancy in women residing in endemic and nonendemic natural fluoride areas.

Material and Methods

This study of ten years' duration (1968 - 1978) was conducted on two groups of women. Group I included 33 females residing in an endemic fluorosis area (10 ppm fluoride in drinking water); the women of Group II were living in an area with 1 ppm fluoride in drinking water. Each group contained pregnant females and age-matched nonpregnant controls (Table 1). All women had been residing in their natural endemic and nonendemic areas for 10 to 20 years, and were of child-bearing age (25-36 years). The women of the endemic fluorosis areas had been exhibiting skeletal changes of the disease for 1 to 10 years, those in the nonendemic areas showed no clinical evidence of fluorosis. All women remained in the same place for the duration of the study.

The blood samples for ionized plasma fluoride were obtained from pregnant mothers prior to their pregnancy and at 12, 24 and 36 weeks of the pregnancy. Blood samples were also obtained from the control females at similar intervals after the initial investigations. During the same periods 24 hour urine samples were likewise collected from each subject in polythene jars without any preservative (Table 2). The fluoride content was measured in specimens of maternal and cord blood plasma, at the time of cesarean sections, in 8 females from the endemic areas and 10 females from nonendemic areas in their ninth month of pregnancy (Table 3). Blood was collected in the fasting state in polypropylene heparinized tubes using disposable gamma radiated plastic syringes to avoid any interaction of fluoride and glass.

Table 1
Ionized Plasma Fluoride in Various Groups of Women Studied

Groups	No. of Cases	Mean F^- Content in Drinking Water (ppm)	Mean F^- Intake per day (mg ± S.D.)	Plasma F^- (μM/l) (Mean ± S.D.) Initial	At 12 weeks	At 24 weeks	At 36 weeks
I. Endemic		10	21 ± 15.52				
Pregnant	15			10.5 ± 5.05	10 ± 4.07	9 ± 2.05	5 ± 1.08
Nonpregnant	18			10.5 ± 5.05	10 ± 4.07	10 ± 3.09	10.8 ± 3.08
II. Nonendemic		1.0	1.52 ± 0.51				
Pregnant	10			1.52 ± 0.38	1.47 ± 0.35	0.9 ± 0.07	0.7 ± 0.05
Nonpregnant	10			1.52 ± 0.38	1.47 ± 0.35	1.7 ± 0.05	1.75 ± 0.05

Table 2
Urine Fluoride in Various Groups of Women Studied

Groups	No. of Cases	Urine F^- (μM/day) (Mean ± S.D.) Initial	At 12 weeks	At 24 weeks	At 36 weeks
I. Endemic					
Pregnant	15	862 ± 489.3	845.5 ± 483.9	645 ± 133.6	385 ± 87.5
Nonpregnant	18	860 ± 480.4	855.5 ± 483.9	850 ± 492.7	857 ± 483.5
II. Nonendemic					
Pregnant	10	34 ± 2.7	32.5 ± 2.9	23 ± 2.7	12 ± 3.5
Nonpregnant	10	34 ± 2.7	33.5 ± 2.9	31 ± 3.2	33 ± 3.6

Table 3
Fluoride in Maternal and Cord Blood Plasma
(Obtained at Cesarean Section)

Patients	No. of Cases	Mean Intake of F^- per day (mg)	Plasma F^- (μM/1) (Mean ± S.D.) Maternal	Cord Blood
* Endemic	8	20.5	14.1 ± 3.8	13.6 ± 3.8
** Nonendemic	10	1.7	1.70 ± 0.61	1.45 ± 0.35

*Nutritional osteomalacia associated with skeletal fluorosis.
**Nutritional osteomalacia.

The method of Fry and Taves (11) was used for the determination of fluoride ion concentrations in the plasma and urine.

Results

The results are summarized in Tables 1-3 and Figures 1 and 2. Laboratory investigations did not reveal any evidence of parathyroid hyperfunction or renal insufficiency in any of the patients. All mothers, who were subjected to cesarean section , had pelvic deformities due to

Figure 1
Ionized Plasma F^- Concentrations in Pregnant Women and in Their Age-Matched Nonpregnant Controls Residing in Endemic and Nonendemic Fluorosis Areas

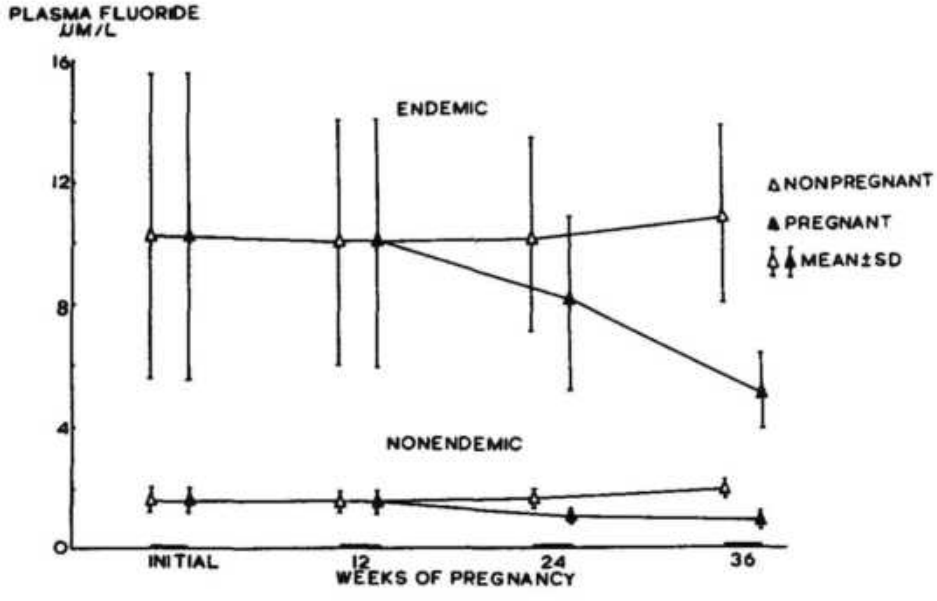
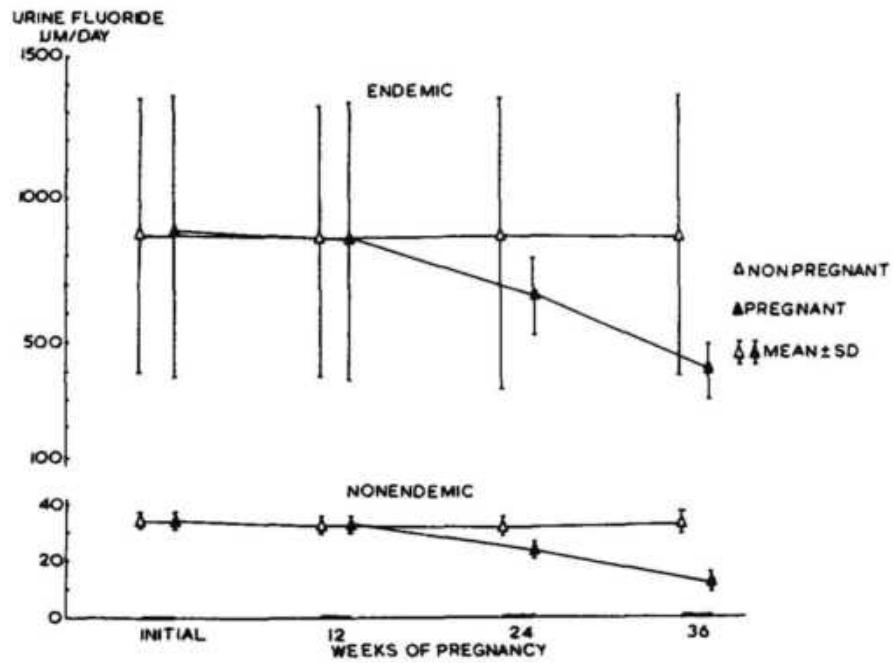


Figure 2
Ionized Urinary F^- Concentration in Pregnant Women and in Their Age-Matched Nonpregnant Controls Residing in Endemic and Nonendemic Fluorosis Areas



nutritional osteomalacia. Their dietary intakes of calcium and vitamin D were extremely low (calcium < 200 mg, vitamin D < 20 I.U. per day). Women from endemic areas in this group had nutritional osteomalacia combined with skeletal fluorosis.

Discussion

Our results clearly established that during the course of pregnancy a gradual and significant decline in the maternal plasma and urine ionized fluoride concentrations occurred and that they were at their lowest at 36 weeks of gestation (Tables 1 and 2). In the nonpregnant controls the fluoride values remained largely unchanged during the follow up. Gedalia et al. (3) reported that urinary fluoride levels in pregnant women were significantly lower than in nonpregnant women. According to Hanhijarvi (9) the free ionized plasma fluoride concentrations decreased steadily and significantly during pregnancy in women residing in an artificially fluoridated community (1 ppm fluoride in drinking water). These reports as well as our own data suggest that the decrease in fluoride in maternal plasma and urine during pregnancy is due to the fact that the developing fetus retains

increasing amounts of fluoride in the bone. This explanation is further supported by the fact that the mother's plasma fluoride concentration would inevitably have increased had she been accumulating fluoride.

The fluoride concentrations in the maternal blood and cord blood plasma collected at the time of cesarean section showed similar values (Table 3) indicating that the placenta does not constitute a barrier to the transport of fluoride ion between maternal and cord blood plasma. This also indicated that placental transfer of fluoride to the fetus during pregnancy is closely related to the metabolism of fluoride in the mother. In 16 samples each of maternal and fetal blood reported by Held (12) fluoride concentrations were similar. According to Armstrong (7) fluoride concentrations in the maternal arterial, maternal venous, fetal arterial and fetal venous blood plasma specimens were quite similar and showed evidence that the placenta does not act as a barrier to the rate of fluoride transport from the maternal to the fetal circulation. Gedalia et al. (13) reported placental transfer of fluoride to the human fetus at low and high fluoride intakes and found fluoride in cord blood in fluoridated areas consistently lower than the levels in their mothers. However, this was not the case when the mother had taken fluoride supplements in water and tablets.

Our results clearly indicate that the concentrations of fluoride in the maternal plasma and urine, that passage of fluoride through the placenta and that its accumulation in the fetal bones were in direct proportion to the amount of fluoride ingested by the mother. According to Singer and Armstrong (4) the increased feeding of fluoride to animals and humans raised the fluoride concentrations in maternal and fetal blood. The higher ionic plasma fluoride concentrations in the nutritionally deficient mothers (Table 3) suggest that calcium and vitamin D deficiency increase intestinal absorption of fluoride and that such mothers and their developing fetuses are more susceptible to the toxic effects of fluoride.

For the exact rate and distribution of the blood fluoride on either side of the placenta as well as differences in concentration between fetal and maternal blood (concentration gradient) further studies are in progress during the course of gestation for possible toxic effects on the critical phase of fetal and placental development.

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EFFECT OF SODIUM FLUORIDE ON ADRENAL GLAND OF RABBIT
I. STUDIES ON ASCORBIC ACID AND DELTA 5-3BETA HYDROXYSTEROID DEHYDROGENASE ACTIVITY

by

K. Rao* and A.K. Susheela
New Delhi and Hyderabad, India

SUMMARY: Rabbits were given 50 mg sodium fluoride/kg body weight through the intragastric route every 24 hours for a total period of 200 days. The left adrenal gland was removed and its total weight recorded. Adrenal glands from rabbits sacrificed at varying intervals for other investigative purposes were also collected and their weights recorded. The data indicate a significant rise in the total weight of the gland.

Both ascorbic acid and steroid dehydrogenase (Delta 5-3Beta hydroxysteroid dehydrogenase) were localized in the adrenal gland by histochemical methods. The results indicate that, in rabbits exposed to NaF, a reduction in ascorbic acid content as well as a depletion of steroid dehydrogenase activity occurs especially at the zona glomerulosa.

The significance of the increase in the weight of the gland to the reduction of the ascorbic acid content and steroid dehydrogenase activity is discussed.

The fluoride content of drinking water is considered to be the cause of hydrofluorosis, but the nutritional status of the patient plays an important role. In cattle malnutrition enhances the ill-effects of excessive fluoride ingestion (1). In this report, the effect of fluoride ions on the ascorbic acid (vitamin C) content and on steroid dehydrogenase activity was investigated.

Rabbits were chosen for the experiments which, unlike humans, synthesize ascorbic acid, thus making it feasible to follow up the changes to the cellular level. It should also be noted that ascorbic acid is essential for the hydroxylation of proline in the synthesis of collagen (2,3). Its role in the biosynthesis of steroid hormones is equally important; it is intimately associated with the enzyme Delta 5-3Beta hydroxysteroid dehydrogenase (4) which converts Delta 5-3

From the Fluorosis Research Laboratory, Dept. of Anatomy, All India Institute of Medical Sciences, New Delhi-110016, *National Institute of Nutrition, Hyderabad, India.

Beta hydroxysteroids to Delta 4-3ketosteroids (5). Ascorbic acid acts as a co-enzyme in one of the reactions leading to steroid synthesis namely, the conversion of deoxycorticosterone to hydroxycorticosteroid (6). The adrenal gland was chosen because it synthesizes ascorbic acid and because the steroid dehydrogenase activity is significantly high in this endocrine gland (7).

Materials and Methods

Adult, healthy rabbits whose body weight ranged from 1 to 1.6 kg were used as the experimental animal. Sodium fluoride, in a dose of 50 mg/kg body weight, was administered every 24 hours through the intragastric route. Control animals were pair fed but deprived of sodium fluoride. The rabbits were sacrificed on day 200. The left adrenal gland was removed and its weight recorded. The tissue thus obtained was used for the localization of 1) ascorbic acid and 2) Delta 5-3Beta hydroxysteroid dehydrogenase activity.

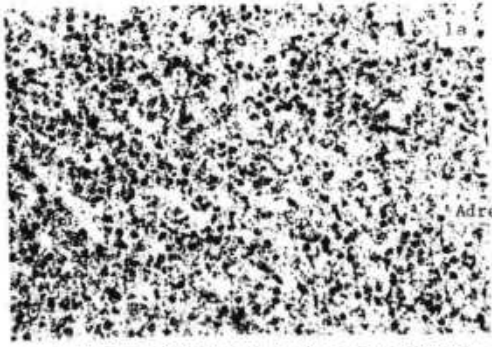
Histochemical Localization of Ascorbic Acid: Ascorbic acid was localized by the silver nitrate reduction method (8). The adrenal gland was cut into halves in a transverse plane; one half was used as sample, the other half as control. The sample tissue was incubated in a medium consisting of silver nitrate at pH 2 to 2.5 for 24 hours at 0 - 4°C. The control tissue was treated with 10% formalin for 3 to 4 hours in order to destroy the vitamin content following which it received the same treatment as the sample tissue. At the end of the incubation period, the tissues were dehydrated and blocked in paraffin wax (M.P. 58 - 60°C). Sections of 5 µm thickness were obtained, deparaffinized and rinsed with 50% alcoholic ammonia to remove the unreacted silver nitrate. The sections were toned with 0.2% gold chloride, stained with Mayer's haemalum and mounted in canada balsam. The control and sample sections of both the normal and fluoride-treated rabbits were examined under a light microscope and photomicrographs were taken. The ascorbic acid, while reducing the silver nitrate to silver oxidizes to dehydroascorbic acid. The black silver precipitates seen in the tissue sections indicate the cellular location of ascorbic acid (Figs. 1a,1b,2a,2b).

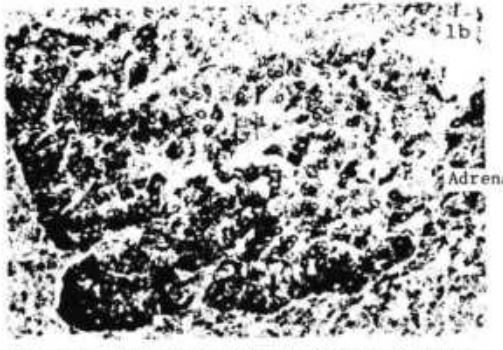
Histochemical Localization of Delta 5-3Beta Hydroxysteroid Dehydrogenase: The cellular localization of steroid dehydrogenase activity in the adrenal gland was carried out by the Tetrazolium reduction method (9). The adrenal gland was dissected out and chilled using isopentane at -60°C. Sections of 12 µm thickness were cut by the Ames Lab Teck Cryostat. The sections were rinsed with acetone to remove the lipid content. The slides were then ringed using Teflon rings of one inch diameter. We added to the wells the following incubation medium:

1.	Dehydroisoandrosterone	5 mg/ml DMF*	0.1 ml
2.	Nicotinamide adenine dinucleotide	6.6 mg/ml	0.1 "
3.	Nitro-BT	2 mg/ml in DMF	0.1 "

*DMF = Dimethylformamide

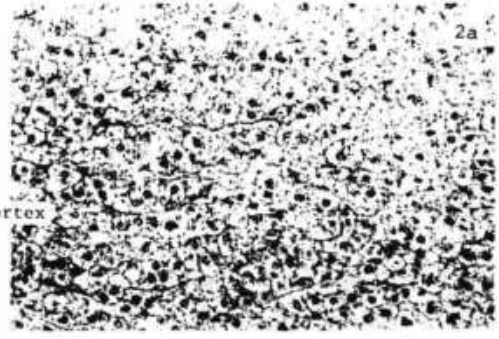
Figures 1a & 1b
Ascorbic Acid Localization in
Normal Adrenal Gland of Rabbit

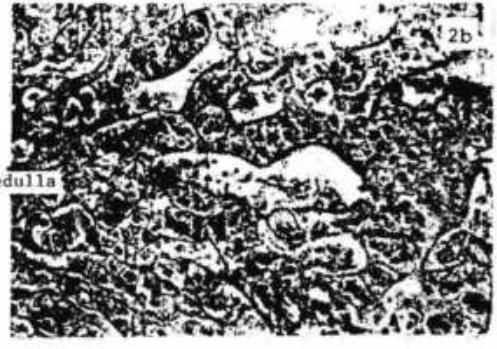




The fine black precipitates of silver indicate site of ascorbic acid X 250.

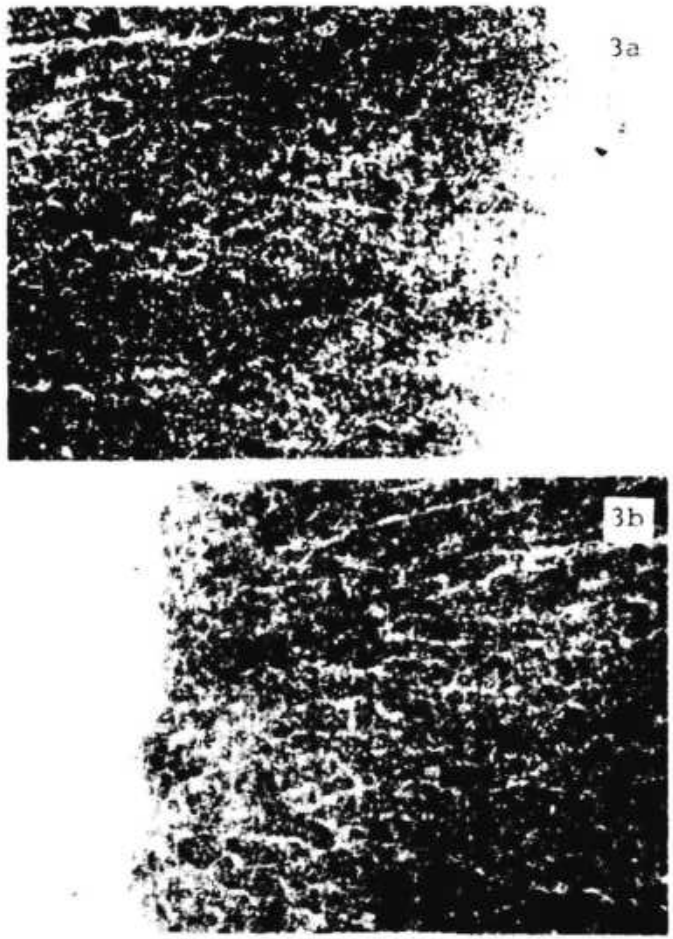
Figures 2a & 2b
Ascorbic Acid Localization in Adrenal Gland
Exposed to NaF for a Period of 200 Days





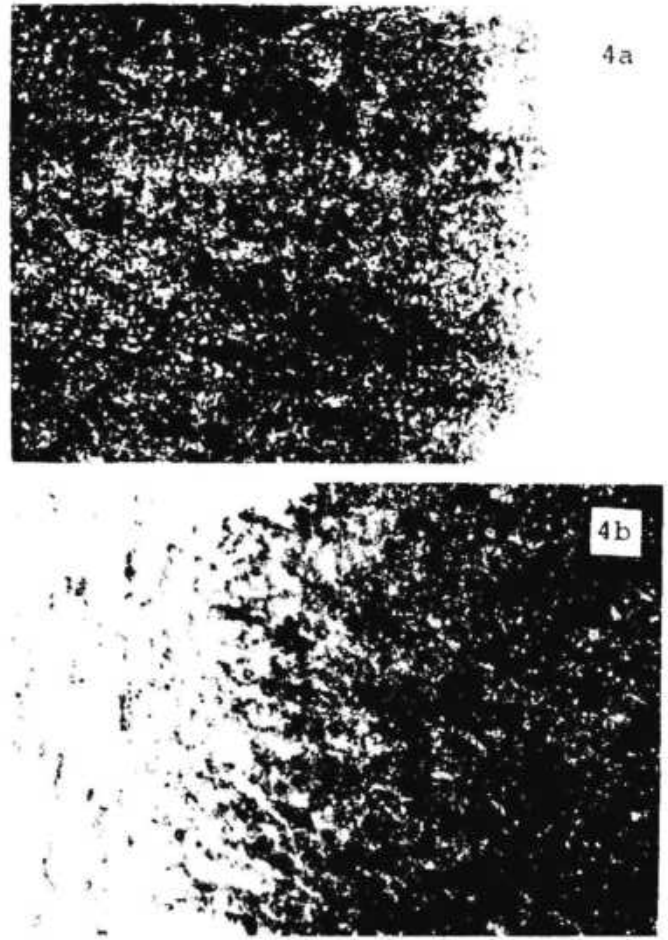
Cortical and medullary regions are shown in Figs. 2a & 2b respectively. Note the reduction in the amount of silver precipitates. It is also evident that cells, both in the adrenal cortex and medulla are hypertrophied.

Figures 3a & 3b
Delta 5-3Beta Hydroxysteroid Dehydrogenase Activity in the Cortical (3a) and Medullary (3b) Regions of the Normal Rabbit Adrenal Gland



Note the zona glomerulosa of the cortex revealing negligible activity X 100.

Figures 4a & 4b
Steroid Dehydrogenase Activity in the Adrenal Gland Exposed to NaF for 200 Days



Note the depletion of the enzyme activity and hypertrophy of the zona glomerulosa X 100. Figs. 4a & 4b represent the cortical and medullary regions respectively.

4.	Magnesium chloride	4.8 mg/ml	0.1 mg
5.	Tris buffer pH 8.3, 0.2M		0.6 "

The sections were incubated at 37°C for 50 to 60 minutes. Control slides were prepared by denaturing the protein (boil the sections for 30 minutes) and incubating the sections with the sample slides. Substrate-free incubation medium was also used for preparing control slides.

At the end of the incubation period, the sections were rinsed with distilled water and fixed for 30 minutes in formol saline (pH 7) in the cold. They were then rinsed with distilled water and mounted in glycerine jelly. The sample and control slides were examined under a light microscope and photomicrographs taken (Figs. 3a,3b,4a,4b).

Results

On gross examination it was seen that the adrenal gland of those animals which had received sodium fluoride, were enlarged as compared to those of the control animals. The weight of the gland increased after administration of fluoride (Table 1). It is evident from the table that the increase in weight of the adrenal gland after exposure to sodium fluoride is statistically significant.

Table 1
Effect of NaF on Adrenal Gland Weight
(Left Side Only) in Rabbits

CONTROL		NaF TREATED		
BODY WEIGHT(Kg) & SEX	ADRENAL WEIGHT (mg)	BODY WEIGHT(Kg) & SEX	EXPOSURE TO NaF (IN DAYS)	ADRENAL WEIGHT (mg)
1·0 (M)*	39	1·3 (F)*	158	94
1·2 (M)	80	1·6 (M)	165	62
1·3 (M)	45	2·0 (M)	200	228
1·4 (M)	110	2·0 (?)	225	178
1·6 (F)	64	2·6 (M)	265	200
MEAN ± 6·0	67·6 ± 28·69	P VALUE = P < 0·05		152·4 ± 31·79

M*, F* INDICATE MALE AND FEMALE RABBITS.

The cyto-architecture of the adrenal gland of the animals which had received large doses of sodium fluoride did not reveal any specific localized lesion, but a generalized hypertrophy of both cortex and medulla cells was noted.

From the histochemical localization of ascorbic acid in the

normal adrenal gland, it is evident that the vitamin is synthesized in all zones of the cortex, namely the zona glomerulosa, zona fasciculata and zona reticularis as well as in the cells of the medulla. The results of the cellular localization revealed in the animals, exposed to large doses of sodium fluoride, a considerable reduction in the ascorbic acid content of the adrenal cortex and medulla cells.

The localization of steroid dehydrogenase activity in the control animals revealed that there was abundant deposition of diformazan granules in the zona fasciculata and reticularis. The zona glomerulosa showed comparatively less activity and the cells of the medulla the least. In the rabbits exposed to sodium fluoride, the adrenal glands revealed reduced activity of steroid dehydrogenase both in the zona fasciculata and reticularis. In the zona glomerulosa the activity was depleted to an even larger extent. The activity in the medulla was unaltered compared to control animals.

Discussion

The results of the present investigation reveal a reduction in the ascorbic acid content in the adrenal gland indicating that fluoride ions have interfered with the vitamin synthesizing pathway of the gland or alternatively with the utilization of the vitamin. It is known that ascorbic acid is essential for the hydroxylation of proline in collagen biosynthesis. When fluorosed bone was viewed under polarized light, abnormality of the organic collagen matrix of the entire osteon as well as an absence of the birefringence of normal osteons was seen (10). Could it be, then, that the deficiency of ascorbic acid observed in fluoride toxicity is the cause of the abnormal collagen synthesis?

The second observation that emerges from the present investigation is the reduction of steroid dehydrogenase activity implying that in fluoride toxicity steroid production is impaired. Since steroids inhibit collagen synthesis (11-14), does this investigation provide evidence that impaired production of steroids leads to enhanced collagen content? Quantitative studies carried out in our laboratory on the effect of NaF on total protein content, indicate a definite increase in the protein content of adrenal gland by day 200 (15). This was the reason for sacrificing the animal on day 200. If the total protein content is increased, it is likely that collagen protein is also increased, since 25 to 30% of the total protein is known to be collagen protein.

The increase in the weight of the gland and the generalized hypertrophy of the cells observed in the present investigation may possibly be due to the increased content of protein and collagen.

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URINARY FLUORIDE EXCRETION IN ENDEMIC FLUOROSIS

by

O.P. Bagga, S.P. Mehta, V. Parkash, A. Raizada,
K.N. Singh, K.A. Sankhyan, R. Gupta and B. Sood
New Delhi, India

SUMMARY: Fifty control volunteers and fifty cases of established fluorosis from Bindapur as well as Dabri village were investigated for the fluoride level of their serum and urine. Mobilization of fluoride from skeletal reserves continues for a prolonged period. A direct correlation between the fluoride intake and urinary excretion was observed.

Homeostasis of plasma fluoride is effected by skeletal sequestration and urinary excretion (1). However, this regulatory mechanism may be overwhelmed by very high fluoride intake (2). According to Hodge and Smith (3) rapid urinary excretion is one of the two major means by which the body prevents the accumulation of fluoride ions to toxic levels. Largent and Heyroth (4), from their fluoride balance studies, reported that excretion of fluoride continues at progressively decreasing rates for as long a period as two years after ingestion of a large amount of fluoride. Likins et al. (5) observed that after defluoridation of drinking water from 8 ppm to 1 ppm, urinary excretion of fluoride took 27 months to decrease to 2 ppm, suggesting the mobilization of stored skeletal fluoride.

Anand et al. (6) had reported in 1964 endemicity of fluorosis in Bindapur village of Delhi. Later, in 1970, this village was provided filtered water containing 0.6 ppm fluoride. The present study was designed to evaluate the excretion of fluoride in the urine of established cases of fluorosis.

Material and Method

Fifty subjects with established severe dental fluorosis with or without skeletal improvement were selected from Bindapur village where the fluoride content of the water supply had been reduced to 0.6 ppm. Since we had no previous urinary fluoride excretion data on these cases, a group of fifty patients from Dabri village, falling in the same age group and having approximately the same degree of fluoride toxicity were also included in the investigation. The fluoride content of the drinking water supply of Dabri village ranged between 1.6 ppm and 3.9 ppm.

From the Lady Hardinge Medical College, New Delhi-110001, India.

A control series of fifty healthy volunteers with no clinical evidence of fluorosis were also analyzed from Janakpuri, a colony situated near Dabri village. A thorough clinical check-up was made of each volunteer who was then advised to collect the first urine in the morning in the container provided. Later, the samples were transported to the laboratory for analysis. The majority of the inhabitants of these villages were illiterate and somewhat uncooperative. They refused to give blood samples and hence the investigation had to be confined to urine analysis. However, wherever possible, blood samples were collected from willing volunteers after an overnight fast.

Serum and urine samples were analyzed for their fluoride content by the fluoride electrode method (7); however, in some urine samples fluoride was estimated by the ashing technique.

Results

Fifty serum samples obtained after an overnight fast in the control group of Janakpuri and their urine samples collected in the early hours of the morning were analyzed for fluoride (Table 1). Urine obtained from fluorotic patients of Dabri and Bindapur villages, who had ad lib access to their water supply,was also analyzed. The results have been statistically evaluated and tabulated in Table 1.

Table 1
Serum and Urinary Fluoride Excretion

	Controls*		Dabri**	Bindapur***
	Serum F⁻	Urinary F⁻	Urinary F⁻	Urinary F⁻
Range (ppm)	0.05-0.078	0.83-3.12	3.8-28.3	21-14.7
Average (ppm)	0.0623	1.627	10.486	6.89
S.D.	± 0.011	± 0.546	± 7.99	± 5.29
S.E.	0.003	0.146	2.06	0.74

*Control=50 cases using centralized water supply; **Dabri= 47 cases of severe dental fluorosis and 3 cases of skeletal fluorosis; ***Bindapur=45 cases of severe dental fluorosis and 5 cases of skeletal fluorosis

Discussion

McClure and Mitchell (8) stated that soluble fluoride, regardless of concentration, is almost completely absorbed and quickly distributed throughout the body fluids. Hodge (9) suggested that one third of the fluoride absorbed is incorporated in the hydroxyapatite crystals of bone, whereas the remaining two thirds is excreted in the urine. From experimental studies on rats with and without previous exposure to high concentrations of fluoride, Yeh et al. (10) concluded that animals with previous low fluoride exposures had an efficient mechanism of skeletal fluoride deposition, faster and quantitatively

larger than excretion of fluoride by the kidney.

Largent (11) also had noted a progressively increased urinary excretion under conditions of excess intake in subjects on defluoridated water. The excretion continued for a period of two years or more, even after cessation of high fluoride intake.

In the present investigation, fluoride excretion in the urine was high in both Dabri and Bindapur villages. Excretion of fluoride in fluorotic patients of Bindapur village was, in some cases, about 10 - 15 times higher than that of the control group. The data supports the findings of Siddiqui (12), Singh et al. (13), Largent (14), Jolly et al. (15) but differs from those of Teotia et al. (16) (Table 2). A correlation appeared to exist between intake and excretion.

Table 2
Urinary Fluoride Excretion in Humans

Author	Urinary Fluoride ppm Range and Mean	Intake Water Fluoride ppm - Range
Siddiqui (1955)	1.2 - 5.8 (2.75)	
Singh et al. (1961)	1.7 - 25.0 (5.46)	1.2 - 14.0
Largent (1961)	1.98 - 15.1	2.0 - 20.0
Jolly et al. (1969)	1.0 - 18.0 (4.29)	1.0 - 18.0
Teotia et al. (1971)	2.2 - 3.2 (2.8)	10.35 - 13.5
Present Study	3.8 - 28.3 (10.49) (Dabri) 14.7 - 21.0 (6.89) (Bindapur)	1.5 - 3.93 (Dabri) 0.6 (Bindapur)(since 1970)

An analysis of the unexpected results of the Bindapur data was obtained since the urinary excretion was significantly higher when compared to that in Dabri village and several times higher when compared to the control group. The results contradicted the observations by Likins et al. that after 27 months or a little more the urinary fluoride excretion returns to normal levels. However, they support the thesis that mobilization of stored fluoride continues over a prolonged period. Why excretion of urinary fluoride was high in the Bindapur population where the water supply contained only 0.55 to 0.6 ppm of fluoride is unexplained.

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MASS POISONING IN DOGS DUE TO MEAT CONTAMINATED BY SODIUM FLUOROACETATE OR FLUOROACETAMIDE (SPECIAL REFERENCE TO THE DIFFERENTIAL DIAGNOSIS)

by

M.N. Egyed
Beit Dagan, Israel

SUMMARY: The results of laboratory investigations are reported in a mass poisoning at which time about 800 dogs died shortly after consuming purchased poultry meat. Clinical and pathological findings were suggestive of poisoning by either strychnine, sodium fluoroacetate (FAC) or fluoroacetamide (FAA). Toxicological examinations implicated organofluorides. At the convulsive stage, the clinical symptoms of FAC (or FAA) and strychnine poisoning are similar and the pathological lesions may be identical. Therefore, toxicological analysis and bio-assays are required for an accurate diagnosis. The toxicological and public health implications of mass poisoning are discussed.

Over a period of 2 weeks, numerous cases of suspected poisoning were reported in dogs that had consumed frozen minced poultry meat marketed in 1 kg lots. About 80 dogs died with acute signs characterized by diarrhea and intermittent tetanic convulsions. Circumstantial evidence indicated that the meat was the source of the poison and, based on the clinical evidence, poisoning by strychnine, sodium fluoroacetate (FAC) or fluoroacetamide (FAA) was the tentative diagnosis.

These chemicals are used in bait for rodent control and, in addition, strychnine is also used for extermination of stray dogs. Often, unleashed pets or guard dogs are accidental victims of poisoning after consumption of poisoned bait or poisoned rodents.

Although the mode of action and course of poisoning of these toxicants differ, the clinical signs at the convulsive stage can be confusingly similar (1,2). Thus the diagnosis in the absence of confirmatory laboratory tests can be erroneous. In considering these problems and their possible legal implications, as well as the relatively large number of cases and materials submitted for diagnostic purposes, it seemed appropriate to summarize recent findings and eval-

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From the Kimron Veterinary Institute, Beit Dagan, affiliated with Tel Aviv University, Israel.

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uate the diagnostic procedures employed in suspected cases of FAC and strychnine poisoning. Some comparative toxicological data of these toxicants which might be useful for the clinician are included (Table 1).

Table 1
Comparative Toxicological and Differential Diagnostic Data in Dogs

	Sodium Fluoroacetate (FAC)	Strychnine Poisoning
Oral toxicity (mg/kg)	0.06-0.2	0.75
Pathogenesis	Blocking of aconitase.	Blocking of spinal cord reflex arch.
Metabolism	"Lethal synthesis" of fluorocitrate.	Possible detoxification (oxidation) in liver.
Latency	Relatively long (most often over 4 hrs., sometimes 24 hrs.	Relatively short (between minutes to 2 hrs.)
Secondary poisoning	Possible.	Possible.
Clinical signs	Restlessness; wild running; barking; vomiting; defaecation; urination; intermittent tetanic seizures with opisthotonus.	Nervousness; restlessness; hypersensitivity to external stimuli; spasms; opisthotonus; retraction of lips; auricular stiffness; cyanosis.
Post-mortem Findings	General cyanosis; hepatic congestion; often empty stomach; colon and urinary bladder; pulmonary edema; frothy mucus in the trachea and bronchi; diffuse gastric mucosal congestion; hemorrhages in the thymus and pancreas.	Venous congestion; usually full stomach; hemorrhages in the thymus and pancreas.
Treatment	Futile. - Specific antidotes (acetate donors) may prevent poisoning only.	Maintenance of relaxation and prevention of asphyxia. For relaxation, pentobarbital or methocarbamol.

Materials and Methods

Pathological and toxicological examinations were carried out on the carcasses of 8 dogs and 1 cat. In addition, 10 samples of the suspect meat were examined toxicologically and 3 dogs were experimentally fed suspect meat and subsequently autopsied and examined.

The laboratory procedures consisted of pathological examinations to determine lesions specific for the two poisons and toxicological examinations.

a. Rapid Test for Strychnine in Mice: The test was performed only when sufficient stomach content was available. The stomach content was mixed with distilled water to obtain a thick slurry. The material was agitated for 1 minute and 1 ml supernatant was injected intraperitoneally into three 21-day-old white mice. Tetanic convulsions and opisthotonus occurring within 10 minutes of inoculation were regarded as strongly suggestive of strychnine. Other symptoms, or those that appeared after 10 minutes but less than 1 hour following the injection were regarded as suspicious and additional chemical examinations for strychnine or FAC by other methods were performed.

b. Thin Layer Chromatographic Method (TLC) for Strychnine: A few drops of extracted and concentrated stomach content or liver were placed on a TLC aluminum sheet precoated with silica gel. The sheet was placed in a tank containing a mixture of ethanol and chloroform (2:8) and sprayed with an iodoplatinate reagent after drying. Strychnine was visualized as a dark violet spot with an Rf value of 0.35 (i.e. 3.5 cm from the base line).

c. A Combined Biological-Biochemical Method for FAC Poisoning (3,4): The method is based on the presence of fluorocitrate, a toxic metabolite of FAC. Tissue extracts of cadavers (most often prepared from kidneys and heart) were injected intraperitoneally into guinea pigs. A significant increase of citrate concentration in their kidneys is indicative of FAC poisoning. In control guinea pig kidneys, citrate concentration is less than 70 µg/g, whereas in positive cases it is above 100 µg/g and may reach several hundred µg/g (4).

Results and Discussion

On the basis of toxicological findings, the meat was found to be contaminated with either FAC or FAA and the cause of death in the dogs was poisoning by one of these compounds. The results are summarized in Tables 2-4.

The lesions found in FAC and strychnine poisoning in nearly all cases (Tables 5 and 6) were petechial and ecchymotic hemorrhages in two of the endocrine glands, the thymus and pancreas. Hemorrhages in the thymus were found in every one of the 15 cases of strychnine poisoning and hemorrhages in the pancreas in 8. In the cases of FAC poisoning, the thymus revealed hemorrhages in 20 out of 24 cases, and the pancreas in 15. One might postulate that the presence of these hemorrhages in FAC and strychnine poisoning was due to the violent nature of the terminal stages of the poisoning. This, however, does not explain why these lesions were located principally in these glands.

Since such lesions in FAC and strychnine poisoning can be identical, they cannot be used for differential diagnosis. Therefore, performance of confirmatory toxicological tests is essential.

The literature concerning pathological lesions in poisoning from the above agents is incomplete. In a description of the pathology of strychnine poisoning in dogs, Kamel and Ahlamy noted few characteristic findings (5). They reported generalized congestion diffusely distributed throughout all the splanchnic organs, a sign of asphyxia. However, they did not mention congestion, ecchymosis or petechiation of the thymus and pancreas. In one textbook on toxicology, FAC is listed as a poison that causes gastroenteritis (6). This finding, however, was not characteristic of our series, although diffuse congestion of the gastric mucosa was found in experimental poisoning of dogs that had ingested FAC from poisoned sheep carcasses (9). We also noted that, in strychnine poisoning, the dog's stomach is not empty

Table 2
Laboratory and Post-Mortem Findings in Fatal FAC Poisoning (Dogs)

Case No.	Hemorrhages in Thymus	Hemorrhages in Pancreas	Other Findings	a) Inoculation test in mice* (with stomach content)	b) TLC examination for strychnine in liver	c) Citric acid** (µg/g in guinea pig kidney after injection of extract of heart & kidney from dogs)
1	+	+	Pulmonary edema, empty stomach			188
2	+	-	Hemorrhages in epicardium	Tonic-clonic convulsions	* * *	600
3	+	+	None	(1 died)		188
4	+	-	Empty stomach			221
5	+	-	Empty stomach			176
6	+	+	Pulmonary edema, empty stomach			188
7	+	-	Hemorrhages in myocardium	No effect	* * * All Negative * * *	341
8	+	+	Empty stomach			255
9†	+	-	None	No effect		212

† cat; * Evaluated up to 15 minutes after injection; ** Citric acid concentration in kidneys of normal guinea-pigs is less than 70 µg/g (4).

Table 3
Results of Toxicological Tests of Suspect Meat Samples

Sample	Citric Acid (µg/g) in Guinea Pig Kidneys After Injection with Meat Extracts*	Effects of Injection of Extract of Meat in 3 Mice	Origin of Suspected Meat
1	875	Not done	
2	3858	Tonic-clonic convulsions in 1 mouse; died after 20 min.	Avian
3	400	Ibid.	
4	275	Not done	
5	1529	Tonic-clonic convulsions in 1 mouse; died after 40 min.	Avian
6	370	Not done	
7	275	Not done	
8	337	Convulsions in hind legs in 2 mice, death in one after 30 min.	Avian
9	2325	Convulsions, later depression in 1 mouse	
10	537	Not done	

* Citric acid concentration in kidneys of normal guinea pigs is less than 70 µg/g (4).

Table 4
Dogs Fed Samples of Suspect Meat

Dog No.	Amount of Meat Fed	Result	Hemorrhages in Thymus	Hemorrhages in Pancreas	Citric Acid (g/g) in Guinea Pig Kidneys* after Injection of Extracts (dogs heart-kidney)
1	1 kg	Died within 12 hrs.	-	+	188
2	1 kg	Died within 16 hrs.	+	-	305
3	700 g	Died within 24 hrs.	+	+	231

* Citric acid concentration in kidneys of normal guinea pigs is less than 70 µg/g (4). Comparative laboratory findings in strychnine and FAC poisoning (not related to mass poisoning) are summarized in Tables 5 and 6.

Table 5
Laboratory Findings in Strychnine Poisoning in Dogs

	Post-Mortem Examination			Toxicological Tests		
Case No.	Hemorrhages in Thymus	Hemorrhages in Pancreas	Other Pathology	Mice Inoculation with Stomach Contents		Chemical Test (TLC) with Stomach Contents of Liver
1	+	-	-	+	Opisthotonus	Not done
2	+	+	-	+	"	+
3	+	+	-	+	"	+
4	+	+	Organs congested	+	"	+
5	+	-	-	+	"	+
6	+	+	Pulmonary edema	+	"	Not done
7	+	-	-	+	"	+
8	+	-	-	+	"	+
9	+	+	Pulmonary edema	+	"	Not done
10	+	+	-	+	"	+
11	+	+	-	+	"	+
12	+	-	-	Mild	Convulsions	+ (liver)
13	+	-	-	+	Opisthotonus	+ "
14	+	-	-	Mild	Convulsions	+ "
15	+	-	-	Mild	Convulsions	+ "

as it is in FAC poisoning. This enabled us to perform a rapid biological test for strychnine from the stomach content. The presence of digesta in the stomach cannot be regarded as pathognomonic, because the quantity of the ingested bait and previous repletion of the stomach may influence this finding. Petechiae or ecchymoses, without exact localization as evidence of struggling, and a hypoxic state are mentioned in strychnine poisoning in dogs (7) as well as hemorrhages in the pancreas and thymus (8).

If the results of toxicological analysis are unequivocally negative for strychnine, while the autopsy lesions, e.g. hemorrhages in the thymus and/or pancreas, are indicative of poisoning from one of these substances, then the biological-biochemical method described for diagnosis of FAC should be carried out. In the positive cases of FAC poisoning listed here, the citrate content of poisoned guinea pig kidneys averaged 217 µg/g tissue, i.e. 3 times higher than in normal kidneys.

The clinical diagnosis of FAC and strychnine poisoning in dogs is fraught with difficulties. On the basis of a relatively long de-

Table 6
Laboratory Findings in Sodium Fluoroacetate (FAC) Poisoning in Dogs

	Post-Mortem Examination			Toxicological Tests
Case No.	Hemorrhages in Thymus	Hemorrhages in Pancreas	Other Pathology	Citrate Concentration in Guinea Pig Kidneys (µg/g)
1	+	+	-	106
2	-	-	Splenomegaly	153
3	+	-	-	305
4	+	+	-	121
5	-	+	Empty stomach	221
6	+	-	Hemorrhages on myocardium	112
7	+	+	-	225
8	+	+	-	113
9	+	+	-	217
10	+	-	-	1000
11	-	+	-	166
12	+	+	-	110
13	+	+	-	200
14	+	+	Pulmonary edema	308
15	+	+	Pulmonary edema	140
16	+	+	-	160
17	+	+	Pulmonary edema	120
18	-	-	Pulmonary edema, petechiae on pleura and epicardium	283
19	+	+	-	237
20	+	-	-	153
21	+	-	-	123
22	+	+	-	122
23	+	-	-	122
24	+	-	-	111

lay between the ingestion of the poison and the appearance of the clinical symptoms as well as the other signs listed in Table 1, only a provisional diagnosis can be established.

In the outbreak presented here, purchased meat was incriminated as the cause of mass poisoning with numerous fatalities in dogs. After having eliminated the possibility of strychnine poisoning, detailed toxicological analyses revealed the presence of an organofluoride compound (FAC or FAA) in the meat. The diagnosis was based on the results of a biological-biochemical method (4), in which secondary poisoning

is induced in guinea pigs by i.p. injection of extracts of tissue taken from the poisoned cadaver (heart, kidney or muscles). A significant increase in citrate concentration in the guinea pig kidneys, as a consequence of inhibition of the enzyme aconitase, is indicative of FAC or FAA poisoning. This was demonstrated in all cases summarized in Tables 2-4. The highest values of citrate in the kidney were 278 - 3858 µg/g (normal < 70 µg/g); they occurred after the injection of extracts from the suspect meat.

FAA is metabolized to FAC in the body and therefore the original toxicant cannot be determined solely on a chemical basis. We do not know whether the poultry was incorporated into the dog food after emergency slaughter consequent to FAC or FAA poisoning or whether the fowl had subclinical poisoning. The latter is a possibility because of the relatively high LD50 of FAA in chickens—4.25 mg/kg (9)—whereas in dogs it is 0.06-0.2 mg/kg (7). Therefore meat of poisoned but clinically normal chickens might be fatal to dogs. These investigations indicate that the diagnosis of strychnine or FAC/FAA poisoning cannot be determined solely on the basis of clinical and pathological findings, and confirmatory toxicological analysis is mandatory in order to establish an accurate diagnosis.

Relatively few reports of FAC or FAA poisoning in dogs have appeared. The most publicized case occurred in England in 1963, when the meat of a pony found dead was subsequently sold for animal consumption. Within the next two days about 100 dogs and cats died after eating the meat (10). It was concluded that the contaminant was either FAC or FAA. This incident led the British to restrict usage of FAA as a rodenticide solely to ships and sewers.

The public health aspects of organofluoride—poisoned meat should also be stressed. Since boiling of such meat does not completely destroy the toxicant, consumption by man could be extremely hazardous.

The toxic dose of FAC or FAA for humans is higher than for dogs (1); dogs can therefore be used in feeding trials in suspected cases of FAC/FAA poisoning.

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CHANGES IN GLUCOSE-6-PHOSPHATASE ACTIVITIES IN KIDNEY AND LIVER OF RATS AFTER ADMINISTRATION OF A SINGLE DOSE OF FLUORIDE

by

Y. Suketa, K. Ohtani and T. Yamamoto
Shizuoka, Japan

SUMMARY: Experimentally, glucose-6-phosphatase activities in the kidney and liver of rats were found to be maximally decreased to 73% and 68% of the respective control levels 6 hours after a single oral dose of 50 mg/kg sodium fluoride.

The decrease of renal glucose-6-phosphatase activity in fluoride-intoxicated rats was markedly stimulated to recover and/or increase by parathyroid hormone, but not by glucagon. On the other hand, the de-

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From the Shizuoka College of Pharmacy, Dept. of Environmental Health, 2-2-1 Oshika, Shizuoka, Shizuoka 422, Japan.

crease in hepatic glucose-6-phosphatase activity in fluoride-intoxicated rats recovered significantly with glucagon, but not with parathyroid hormone.

Introduction

In studies on endemic fluorosis in man and animals, mottled teeth and osteosclerosis are established, but little attention has been given to enzymatic changes in the kidney and liver caused by fluoride.

Taylor et al. (1) reported an increase in urinary excretion of glucose in rats after intravenous administration of fluoride (20-30 mg/kg). Shearer (2) found increases in kidney glucose in rats receiving 25 mg/kg sodium fluoride intraperitoneally. Net glucose liberation from gluconeogenic tissues of liver and kidney has been believed to be controlled through opposing actions of glucose phosphorylation via hexokinase and/or release of glucokinase and glucose through glucose-6-phosphatase (3). In general, glucose-6-phosphatases from various sources are known to be inhibited by fluoride in vitro (4), but evidence on the in vivo effect of fluoride on the enzymes is sparse.

The present study was designed to examine the effect of administration of fluoride on the hepatic glucose-6-phosphatase activity compared to the renal glucose-6-phosphatase activity in rats administered a single oral dose of 50 mg/kg sodium fluoride. In addition the responses of parathyroid hormone and glucagon to the renal and hepatic glucose-6-phosphatase activities in fluoride-intoxicated rats were studied.

Materials and Methods

Treatments: Male Wistar albino rats weighing about 100 g were maintained on basal diet MF (purchased from Oriental Yeast Ind., Japan) and water ad libitum. The animals were maintained at a 22° temperature for a minimum of one week. All animals were fasted 24 hours before the experiments in order to minimize the effects of glucose absorption from the bowels and to stabilize the urinary excretion of glucose. The rats were sacrificed at various intervals after a single oral administration of 50 mg/kg sodium fluoride. 69.8 mg/kg of sodium chloride was given to control rats.

Parathyroid hormone (PTH) and glucagon were injected subcutaneously in doses described in the text 4 hours after administering a single oral dose of fluoride. The control solution, isotonic saline to pH 3.5 with hydrochloric acid, injected in the same volume, was used to dilute the hormones.

Preparation of Microsomes: Microsomes were prepared according to the method of Jørgensen (5). At the time of sacrifice, the rats

were anesthetized with ether and killed by cardiac puncture. The kidneys and livers were removed and the tissues (1 g) were immediately homogenized in a Potter-Elvehjem teflon-glass homogenizer with 5 ml of ice-cold 0.25M sucrose-0.03M histidine buffer (pH 7.2).

The reproducible preparations of the heavy microsomal fraction were obtained by centrifugation (25300 x g, 30 min) of supernatant after sedimentation of the mitochondria at 10800 x g for 30 min. The preparations of the heavy microsomal fraction (1 mg of protein/ml of 0.25M sucrose-0.03M histidine buffer, pH 7.2) were stored in a refrigerator (-20°).

Assays: Glucose-6-phosphatase activity was determined according to the method of Swanson (6). Protein was determined by the method of Lowry et al. (7).

Materials: Glucose-6-phosphate (disodium salt) was obtained from Wako Pure Chemical Ind. Ltd. (Osaka, Japan). Parathyroid hormone (280 U/mg) and glucagon (crystalline) were purchased from Sigma Chemical Co. (St. Louis, U.S.A.).

Results

Responses of Renal and Hepatic Glucose-6-Phosphatase Activities in Fluoride-Intoxicated Rats to Parathyroid Hormone and Glucagon: Kotake et al. (8) observed that following an injection of PTH, glucose-6-phosphatase activity increased in kidney but not in liver microsomes. The hepatic glucose-6-phosphatase activity also increases when glucagon is injected into fetal rat in utero (9), but not when injected after birth or when applied to fetal rat liver tissue culture (10). On the other hand, Ashmore and Weber (11) reported that the enzyme activity in rat liver was increased by glucagon injection.

Then, the responses of hepatic and renal glucose-6-phosphatase activities in fluoride-intoxicated rats to PTH and glucagon were examined as shown in Table 1. The response of renal glucose-6-phosphatase activity in fluoride-intoxicated rats to PTH was higher than in control rats, whereas the hepatic glucose-6-phosphatase activity in fluoride-intoxicated rats was not changed as much as that in the control rats by the treatment of PTH ($P < 0.05$). By the glucagon treatment, the hepatic glucose-6-phosphatase activity in fluoride-intoxicated rats was increased in direct relation to the increase in dosage but 500 μg/kg in the control rats induced no change. On the other hand, the renal glucose-6-phosphatase activity in fluoride-intoxicated rats was slightly elevated by treatment with glucagon, but it induced no change in control rats as shown in Table 1.

Renal Glucose-6-Phosphatase Activity in Fluoride Intoxication: The changes in renal glucose-6-phosphatase activity following a single

Table 1

Response of Renal and Hepatic Glucose-6-Phosphatase Activities in Fluoride-Intoxicated Rats to Parathyroid Hormone and Glucagon

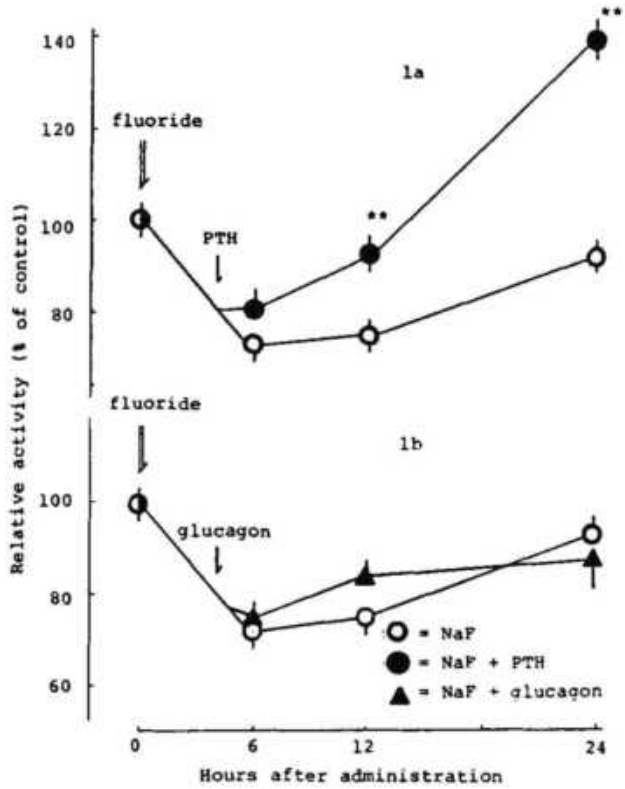
		Renal G-6-Pase Activity		Hepatic G-6-Pase Activity	
		n moles Pi/mg protein/min	F/C	n moles Pi/mg protein/min	F/C
Control	None[1, 2]	525.4 ± 18.4	1	666.0 ± 18.2	1
	PTH (200 U)[2]	709.3 ± 21.0	1.35	673.0 ± 23.1	1.01
	Glucagon (500 µg)[1]	530.3 ± 13.5	1.01	670.2 ± 15.3	1.01
Fluoride	None[2]	483.4 ± 15.8	0.92 (1)	599.4 ± 23.5	0.90 (1)
	PTH (200 U)[2]	735.6 ± 36.8 [3]	1.40 (1.52)	617.4 ± 58.1	0.92 (1.03)
	None[1]	382.8 ± 6.6	0.73 (1)	454.2 ± 14.2	0.68 (1)
	Glucagon (20 µg)[1]	413.6 ± 12.8	0.79 (1.08)	479.5 ± 14.4	0.72 (1.06)
	Glucagon (100 µg)[1]	382.2 ± 4.2	0.73 (1.00)	572.8 ± 20.0 [3]	0.86 (1.26)
	Glucagon (500 µg)[1]	417.4 ± 1.9	0.79 (1.08)	596.2 ± 10.1 [3]	0.90 (1.31)

The rats were killed [1] 6 hrs. or [2] 24 hrs. after a single dose of 50 mg/kg NaF was administered orally or 69.8 mg/kg NaCl in control rats. Glucagon or PTH were administered subcutaneously once 4 hrs. after an oral administration of fluoride or chloride. Values, expressed as average data of five to eight rats and standard error, differed [3] significantly from values of fluoride-intoxicated rats ($P < 0.02$). The number in parenthesis shows relative value of fluoride-intoxicated rats.

50 mg/kg dose of sodium fluoride are presented in Figs. 1a & 1b. The enzyme activity reached a maximum decrease 6-12 hours following the dose, after which it gradually increased to near the control value. With PTH (200 U/kg), the enzyme activity in the kidney increased progressively for the duration of the experiment as shown in Figure 1a. The enzyme activity in the kidney of the fluoride-intoxicated rats reached 1.45-times of the control level 20 hours after the PTH dose.

On the other hand, the pattern of the enzyme activity in the kidney of the fluoride-intoxicated rats was not changed by glucagon as shown in Figure 1b.

Figures 1a & 1b
Effects of PTH and Glucagon on Renal Glucose-6-Phosphatase Activity in Fluoride-Intoxicated Rats

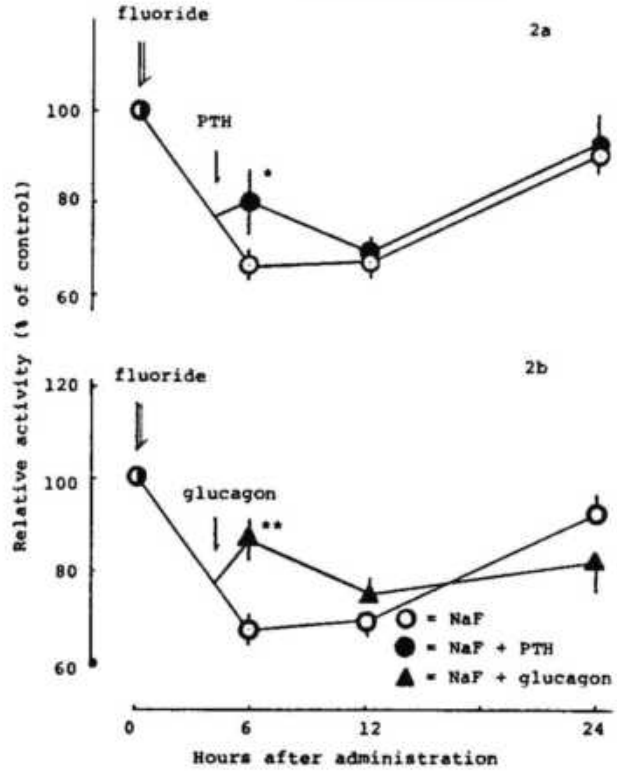


50 mg/kg NaF was administered orally at zero time. 200 U/kg PTH or 500 µg/kg glucagon in saline solution, were injected subcutaneously at the time indicated by the arrow. Values are expressed as average data of five rats and SE are indicated by the bars. Significant difference from fluoride-intoxicated rats, *P < 0.02; **P < 0.01.

Hepatic Glucose-6-Phosphatase Activity in Fluoride Intoxication: In animal fluorosis, lesions in the liver are less severe than those in the kidney. Large doses of sodium fluoride produced an elevation of blood glucose (12) and an increased excretion of glucose (1). Taylor et al. (1) reported that the increased excretion of glucose might be due to tooth tubular injury and elevated blood glucose.

The effect of fluoride on glucose-6-phosphatase activity in the liver as a large part of glucose metabolism in various organs was examined using rats intoxicated by 50 mg/kg NaF given orally. The level of the enzyme activity decreased 68% of the control level 6 hours after administration of the dose and returned to near the control level in the same pattern as the kidney of fluoride-intoxicated rats (Figs. 2a & 2b).

Figures 2a & 2b
Effects of PTH and Glucagon on Hepatic Glucose-6-Phosphatase Activity in Fluoride-Intoxicated Rats



50 mg/kg NaF was administered orally at zero time. 500 µg/kg glucagon or 200 U/kg PTH in saline solution, were injected subcutaneously at the time indicated by the arrow. Values are expressed as average data of five rats and SE are indicated by the bars. Significant difference from fluoride-intoxicated rats, *P < 0.02; **P < 0.01.

The maximum effect of glucagon on the enzyme activity was observed within the first 2 hours following administration as shown in Figure 2b. On the other hand, a significant effect of PTH administration on the hepatic glucose-6-phosphatase activity in fluoride-intoxicated rats was observed the first 2 hours after administration of the hormone as shown in Figure 2a.

Discussion

In this experiment, glucose-6-phosphatase activities in kidney and liver were maximally decreased to 73% and 68% of the respective control levels 6 hours after a single oral dose of 50 mg/kg sodium fluoride.

On the other hand, Stetten (4) reported that glucose-6-phosphatase activity in vitro in rat liver was inhibited about 90% by addition of 20mM sodium fluoride to the incubation media. Recently, we reported that renal alkaline phosphatase and Mg^{++}-ATPase activities decreased by a single oral dose of 50 mg/kg NaF (13).

Moreover, the suppression of the renal and hepatic glucose-6-phosphatase activities by fluoride in this experiment was not changed by washing of 0.25M sucrose-0.03M histidine buffer (pH 7.2) indicating that the suppression was irreversible. The suppression of the renal and hepatic glucose-6-phosphatase activities by fluoride intoxication was markedly stimulated to recovery by PTH ($P < 0.02$), but not by glucacon ($P < 0.05$). The suppression of hepatic glucose-6-phosphatase activity by fluoride was significantly alleviated by glucagon ($P < 0.02$), but not by PTH ($P < 0.05$).

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MEASURING OF CORTICAL THICKNESS, A MEANS FOR CONTROLLED DIAGNOSIS OF FLUORIDE-EXPOSED PEOPLE

by

H. Runge, J. Franke and A. Theuring
Halle, G.D.R.

SUMMARY: In 230 aluminum workers, 5662 measurements were made on the radiographs of the thorax and the extremities. The cortical index of the clavicle, 4th rib, tibia, and fibula were determined by means of a measuring magnifier and a pair of compasses. The determination of the cortical index of the 4th rib is recommended

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From the Orthopedic Hospital of the Martin-Luther-University, 402 Halle/Saale, G.D.R.

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for the early diagnosis of skeletal fluorosis, because this bone appears to be least affected by outside influences and because it showed the most distinct increases of the cortical index.

It is well known that formation of exostoses and ossification of muscle attachments, as well as a gradual narrowing of the medullary cavity, occur in chronic fluoride intoxication (1-4). For diagnostic purposes, measurement of the cortical thickness and of the width of diaphysis has gained importance (5-24). This approach has been particularly useful in the diagnosis of the initial stage of fluoride-induced bone diseases (4,5,25,26) when early diagnosis is important for prophylaxis.

Iliac crest biopsy (27) and the use of a bone mineral analyzer (28) permit evaluation of fluoride-induced bone changes in the clinic. They are not available, however, to the practicing physician. We therefore devised a simplified method of examination that can be used as a standard in making a diagnosis.

Since 1967, we X-rayed the thorax, spine, lower legs and, recently, also the left forearm in 230 aluminum smelter workers. With the aid of X-ray pictures, it is possible for an experienced team of examiners to determine whether or not skeletal fluorosis exists.

According to regulations in the G.D.R. all persons exposed to fluoride are required to undergo X-ray examinations every three years. Special attention is given to the ossification of muscle attachments, osteosclerosis, exostoses and fluoride-induced structural changes of bones. Measurement of the width of diaphysis and of the cortical thickness was possible at the clavicle, ribs, tibia and fibula because only these bones had been X-rayed for diagnostic purposes. The most difficult problem in this study was the search for the bone most suitable to serve as a diagnostic control in bone fluorosis. Whereas some authors find the metacarpal bones, ulna, radius or tibia most suitable for estimating bone mineral content (4-6,9,10,29), we agree with Helelae (16), who stresses that the weight-bearing bones (such as the femur and the tibia) are less suited for measurements of bone mineral content. Bones serving as insertions for big muscles, also, are less suitable for measurements of cortical bones. Fischer and Hausser (13) recommended the 4th or 5th rib and the clavicle for the determination of bone mineral content.

Material and Methods

X-rays of the bony thorax and the lower leg were analyzed in 230 aluminum smelter workers at intervals during their employment in the aluminum factory. With the help of a magnifier and a pair of compasses, 5662 measurements were made of the clavicle, ribs, tibiae and fibulae.

Figures 1 and 2 illustrate the measuring technique. The medial and lateral cortical thickness as well as the width of diaphysis, 10 and 15 cm distal from the knee joint, were determined in the tibia and fibula. We measured the cranial and caudal corticalis and the width of

Figure 1
Measurement Points for Determination of
Cortical Thickness in Tibia

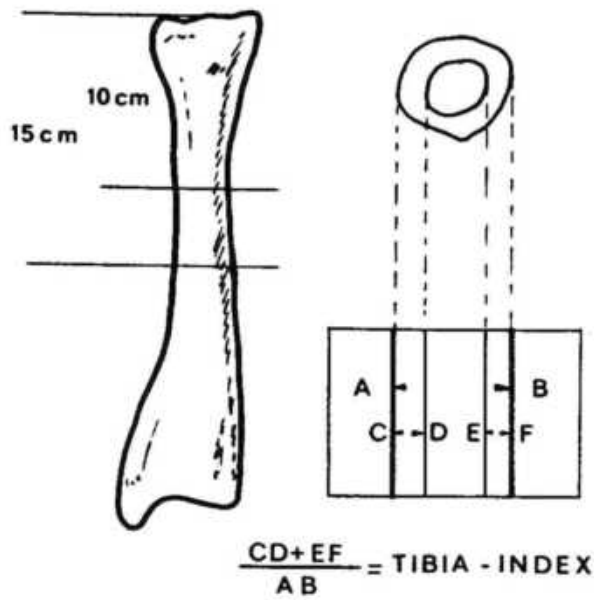
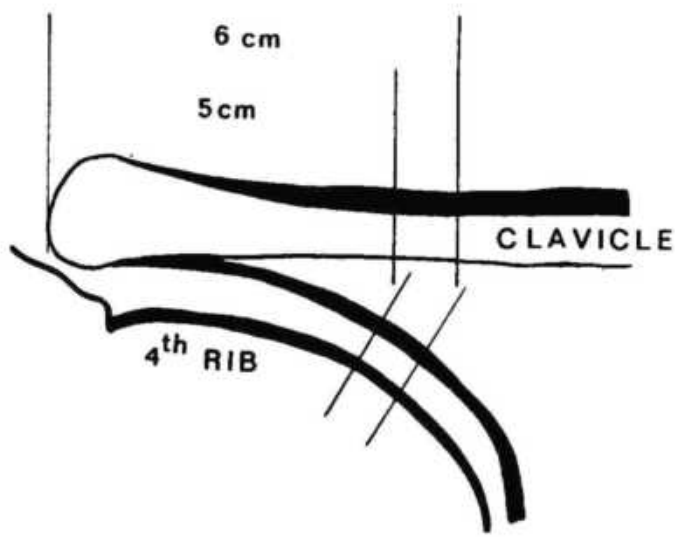
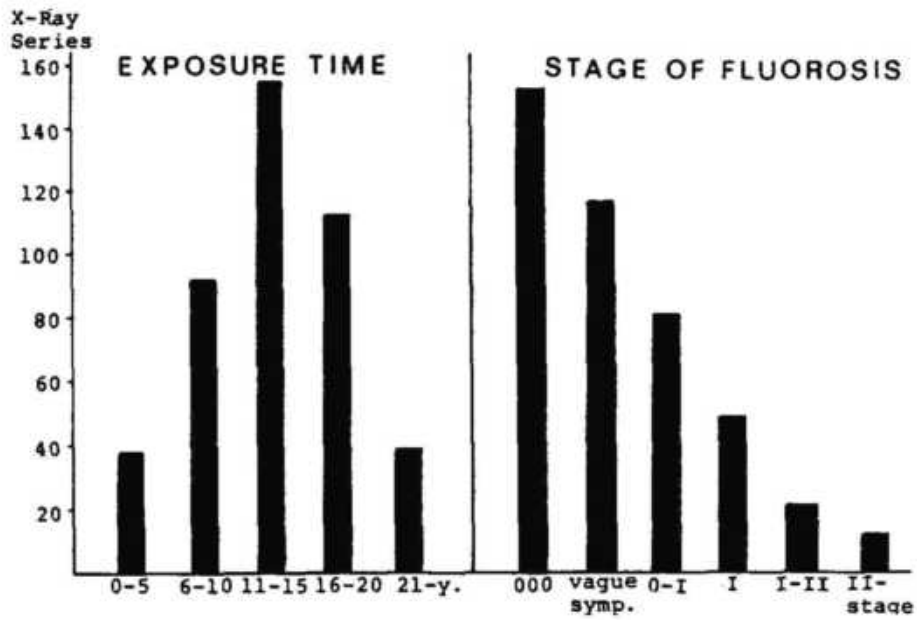


Figure 2
Measurement Points for Determination of
Cortical Thickness in Clavicle and 4th Rib



diaphysis 5 and 6 cm lateral from the sternum in the 4th rib right and left as well as the cranial corticalis and the width of the diaphysis 5 and 6 cm lateral from the sternoclavicular joint in the clavicle. It is not always possible to demonstrate clearly the caudal corticalis of the clavicle (30). That is why the caudal corticalis was not included in the analysis. Figure 3 shows the distribution of the X-ray findings (single exposure) with respect to exposure to fluoride and stages of fluorosis (as classified by Roholm).

Figure 3
Distribution of the X-ray Findings, Times of Exposure and Stages of Fluorosis (Classified According to Roholm)

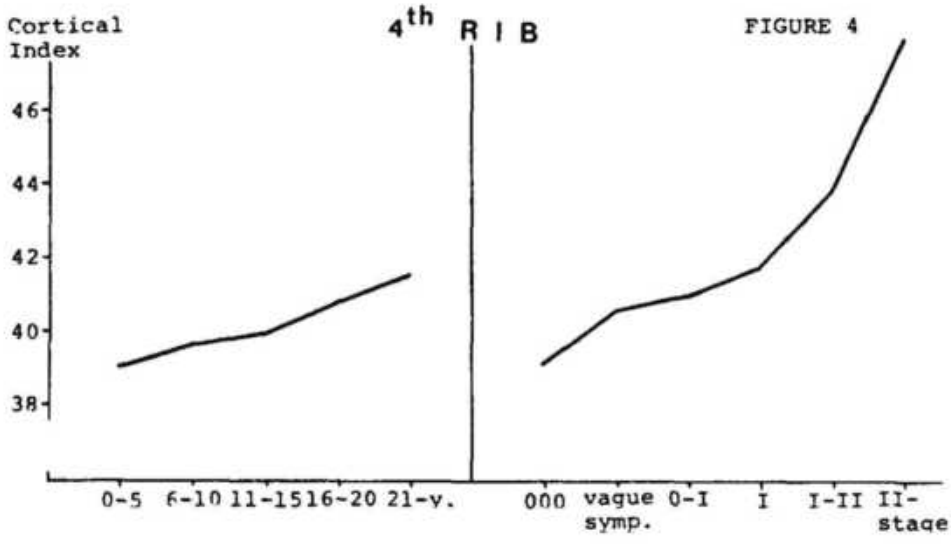


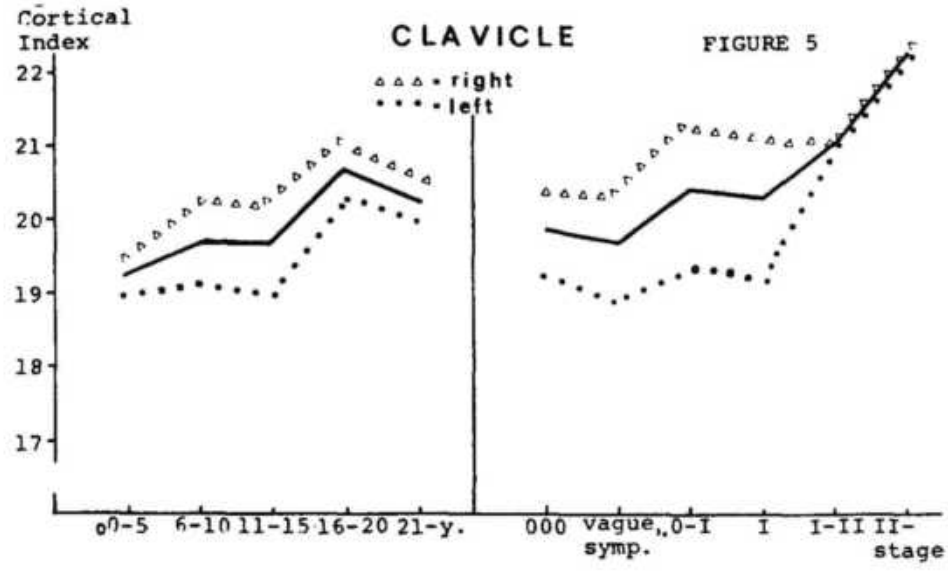
The majority of workers (155) had been employed in the aluminum factory for 11 to 15 years. The distribution to the right in the direction towards the higher fluorosis stages shows a continuous decrease because workers are not permitted to continue working under fluoride exposure and must start another occupation. It should be emphasized that long-term work in an aluminum factory will not always result in skeletal fluorosis. For example, 20 aluminum smelter workers had been employed for 20 years, and 10 for more than 25 years under fluoride exposure without signs of bone fluorosis. Therefore, it is necessary to distinguish between fluoride-sensitive and fluoride-resistant workers.

Results

Figures 4 and 5 show the results of our investigations. A relatively constant increase of the cortical index is related to the duration of exposure and especially to the stage of fluorosis in the clavi-

Figures 4 & 5
Cortical Index Related to Time
of Exposure and on Stage of Fluorosis





cle and rib. A distinct difference between the right and the left clavicle as shown in Figure 5 is undoubtedly due to the predominance of the physical activity of the right upper extremity. On the ribs no such differences exist. The increase in the cortical index at the rib and clavicle becomes more significant when one considers that the min-

eral content of bones decreases with advancing age. Therefore, fluoride exposure results in a reversal of the usual process of aging and the cortical thickness increases. The curves of fibula and tibia in Figures 6 and 7 show no correlation.

The width of the diaphysis of the examined bones did not show essential changes in comparison with the single groups. Figure 8 provides no clue concerning the sensitivity of the investigative method. The clavicle, for instance, demonstrates that even slight changes of

Figures 6, 7 & 8
Cortical Index Related to Time
of Exposure and on Stage of Fluorosis

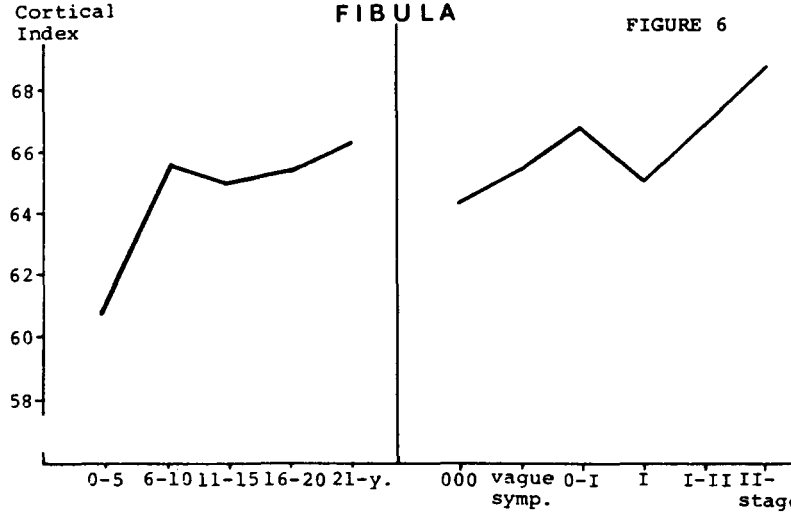


FIGURE 6

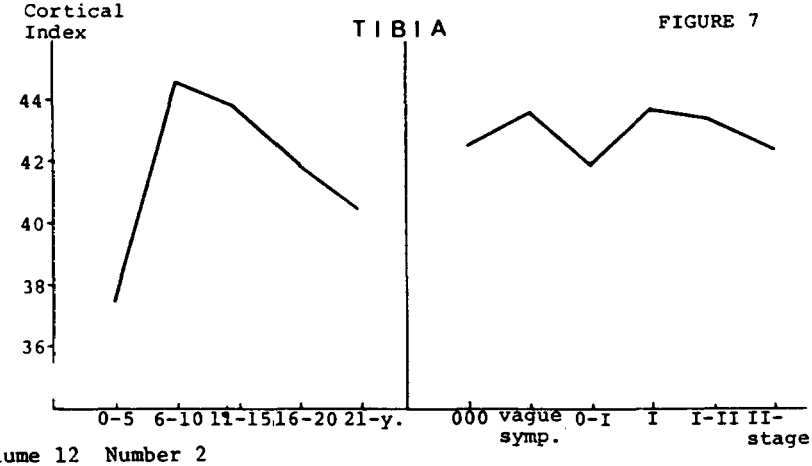


FIGURE 7

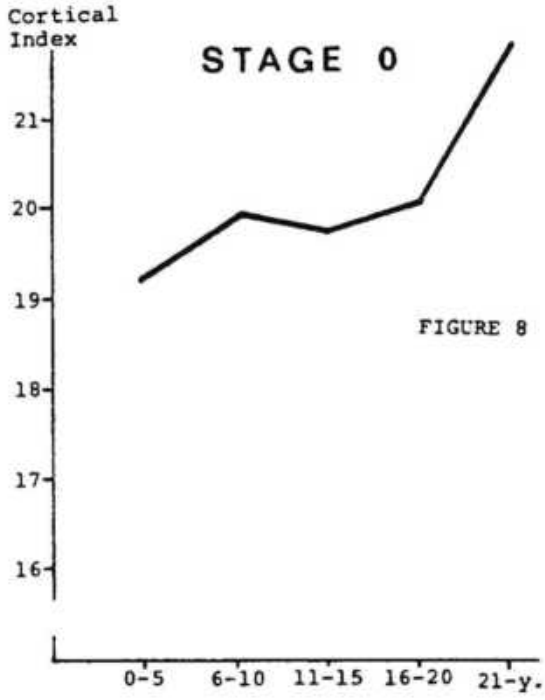


FIGURE 8

cortical thickness can develop during one stage (here stage 0) at a time when fluorosis cannot as yet be diagnosed by other means. By the computer Robotron 300 we calculated the average, the variance, and the standard deviation for the single exposure groups and the stages of fluorosis.

Discussion

Czerwinski (4,5) observed an increase of the cortical index of the ulna, radius, and tibia in aluminum workers after prolonged exposure. He recommended the use of this method in support of the diagnosis of skeletal fluorosis. We, too, found an increase of cortical thickness in the bones which were examined. But, in accordance with the recommendations of Helelae (16) and Fischer and Hausser (13), we studied non-weight-bearing bones, to which no large muscles are attached. Upon examining the clavicles, we found a distinct difference between the right and left half of the body, due to increased strain at the right upper extremity.

Since aluminum workers are subject to great physical strain, the clavicle is not suitable for the diagnosis of fluoride-induced bone changes as indicated by our results.

Following the recommendation of Fischer and Hausser (13), Barer and Jowsey (31) and Takahashi and Frost (23) we selected the rib as suitable for the determination of bone mineral content. We found that

the ribs of one side did not differ from the ribs at the other side and in almost all cases the measuring was easy to perform. Therefore, we recommend this approach in support of the early diagnosis of fluoride-induced bone changes.

It will be the task of the polyclinic physician at a factory with fluoride-exposed workers to determine every third year the cortical indices of the 4th rib according to routine X-rays of the thorax. Should the increase of this value be more than 2%, a more thorough examination is warranted.

This method is easy, reproducable, and independent of outside influences, especially if it is carried out by the same examiner using the same technique. The rib reflects the fluoride-induced bone changes independent of the effect of hard physical work. This method permits early diagnosis of fluoride-induced bone changes. Further investigations, especially a follow-up of individuals workers, are now in progress.

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FLUOROSIS FROM PHOSPHATE MINERAL SUPPLEMENTS IN MICHIGAN DAIRY CATTLE

by

D. Hillman, D. Bolenbaugh and E.M. Convey
East Lansing, Michigan

(Abstracted from Research Report 365, Michigan State University Agricultural Experiment Station, East Lansing, November, 1978)

During 1975 and 1976 more than 75 Michigan dairymen reported in their cows subnormal milk production, loss of weight after calving, failure to exhibit estrus or to re-breed as expected as well as a high incidence of uterine infections and lameness. Death from undetermined causes ranged between 10 and 15 percent among adult cows and among calves up to 1 year or more in age many failed to grow normally or died from undetermined causes. Analysis of milk and tissue fat for PBB (polybrominated biphenyls) which had been responsible for a similar epidemic in Michigan revealed no detectable traces. Severe dental fluorosis and exostoses of metatarsal bones led to the discovery that mineral supplements containing up to 6300 ppm of fluoride and protein supplements containing up to 1088 ppm fluoride consumed by the cows were responsible for this epidemic.

Twelve lactating cows in each of 4 typical problem herds were selected for a study in which the daily amount of milk produced, blood and urine tests were recorded. In each herd, 2 cows had been lactating less than 30 days, 2 between 31 and 120 days, 4 between 120 and 200 days and 4 between 200 and 300 days. Blood serum was analyzed for calcium, phosphorus, glucose, cholesterol, bilirubin, albumin, total protein, uric acid, blood urea nitrogen, alkaline phosphatase and SGOT activity by SMA_{12} autoanalyzer. Blood serum thyroxine (T_4) was determined in 6 herds by radioimmunoassay and in 3 problem herds by competitive protein binding. In addition, fluoride determinations were made on bones, teeth and drinking water.

Results: Post-calving, lactating cows although offered an adequate diet lost excessive body weight. Before calving most cows appeared to be healthy; they were producing a normal amount of milk which subsequently dropped dramatically; they exhibited a ketosis-like syndrome unresponsive to treatment. The average milk production declined 1,000 to 2,000 kg per lactation during a 1 to 2 year period. The cows increasingly failed to conceive; visual evidence of fluorosis increased. The adult cow mortality averaged 15% in four of the problem herds mainly due to post-calving uterine infections, mastitis and general infections which ordinarily respond to therapy. Several cows manifested "atypical pneumonia"; temperature was only slightly elevated.

The discoloration of the dental enamel varied from mild yellowish to mottled brown and black and could not be removed by scraping. In several cows, the surface of teeth showed enamel hypoplasia, in others the teeth were completely eroded to the gum-line. In one cow the 4th molar (ash) contained 4510 ppm fluoride. In cattle less than 2 years of age, the fluoride content of deciduous incisors averaged twice as high as that of the permanent incisors (963 ppm and 444 ppm respectively). Calves less than 6 weeks old exhibited black teeth, which is indicative of placental transfer of fluoride.

Between 2% to 34% of the cows showed lameness due to an "arthritis-like" condition of hip and shoulder joints. In all herds, stiffness and lameness were associated with exostosis of bones and with enlarged, reddened, and swollen joints. In 20 to 58% of the cows, hoofs were curled and sprawling. The fluoride concentration in 22 ashed samples of bones ranged from 885 to 6918 ppm with an average 2406 ppm. This contrasts with 350 to 1000 ppm in cattle which received no fluoride other than that contained in natural feedstuffs. Ribs and coccygeal vertebrae contained twice as much fluoride as the metatarsal and metacarpal bones. The fluoride content of various skeletal parts of the same animal varied widely. In milk it varied from 0.072 to 0.64 ppm. The drinking water of the animals which contained 0.1 to 0.8 ppm of fluoride, contributed only 10 to 100 mg to the daily fluoride intake compared to 1143 mg from the mineral and protein supplements.

The urinary fluoride excretion in 72 cows of the six herds studied averaged 5.31 ± 2.84 ppm fluoride with a range of 1.04 to 15.7 ppm.

The thyroids of calves were enlarged 2 to 5 times their normal weight and the cows afflicted with fluorosis showed evidence of hypothyroidism. The depression of the serum thyroxine (T_4) and triiodothyronine (T_3) correlated with the increase in urinary fluoride, with the number of red blood cells, with the hemoglobin, serum cholesterol, calcium, glucose and albumin. Fluoride also decreased the serum cholesterol at a lower level of significance ($P < .06$).

With respect to the hematological findings eosinophilia increased in correlation with urinary fluoride ($P < .004$) and should be considered an early manifestation of fluoride toxicity. Moreover fluorotic animals manifested anemia. The herd with the lowest average urinary fluoride showed the highest hemoglobin and calcium concentrations in the blood.

Administration of thyroprotein (iodinated casein) to the hypothyroid-afflicted cows resulted in a dramatic increase in thyroxine, milk production, hematopoiesis, serum albumin and calcium whereas the eosinophils tended to become normal. Some animals had nephrosis and fibrosis of the kidneys.

The authors suggested that the National Research Council recommendation of a level of 30 ppm maximum dietary fluoride "may be too high for high producing dairy cattle fed phosphate sources of fluoride".

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PRESKELETAL FLUOROSIS NEAR AN OHIO ENAMEL FACTORY: A PRELIMINARY REPORT

by

G.L. Waldbott
Warren, Michigan

(Abstracted from Veterinary and Human Toxicol., 21:4-8, 1979)

The author describes what appears to be an epidemic of neighborhood fluorosis in 23 individuals residing within 1/4 to 3 miles of an Ohio frit producing factory, in operation since 1966. On the side facing the factory vegetation showed characteristic fluoride changes at tips and margins of foliage. The tooth and bone of a dog which had died in convulsions contained 1480 and 2050 ppm fluoride respectively. In pine needles and leaves, fluoride levels ranged up to 53.6 ppm.

Of the 23 individuals, 7 were males and 16 were females aged 9 to 76 with a mean of 40.7. Nine of the 23 patients were examined; four of them were hospitalized at Hutzel Hospital, Detroit. Only 1 of the 23 was a smoker and none were habitual tea drinkers. In a family of four, every member and, in another family, four out of five members were adversely affected.

In every case, generalized progressive fatigue was an outstanding feature. Other typical characteristics were a distinct decline in mental acuity, forgetfulness, inability to coordinate thoughts, and even reduced ability to write and to form words.

All individuals complained of pains in muscles and joints. Eleven exhibited muscular fibrillation, 8 retrosternal pain, and 3 bursitis. All, but one, complained of symptoms referrable to the central nervous system, i.e. paresthesias in arms and legs, headaches and vertigo; in 7, visual changes occurred.

Respiratory symptoms in 20 of the 23 individuals involved mainly the upper respiratory tract, i.e. nasal and sinus disease; 3 complained of episodes of laryngeal edema.

In 18 individuals gastrointestinal manifestations, mainly gastric pains, nausea, abdominal distention, diarrhea, were present presumably due to consumption of food grown in the area contaminated by airborne fluoride. The same explanation applies to the occurrence of acute abdominal episodes of which 7 of the 18 patients complained. These episodes simulated intestinal obstruction as previously described by the author in fluoride-contaminated areas. Less frequent manifestations were referrable to the lower urinary tract (8 patients). Dermatological symptoms such as hives, dermatitis, and stomatitis were sporadic. Four patients had "Chizzola" maculae.

The severity of the disease correlated with the distance of the patients' residence from the factory. Persons absent from their home during the day were less affected than those confined to their homes. All patients were well balanced individuals who did not exaggerate their complaints.

Physical and laboratory findings were sparse and inconsistent. X-ray examinations failed to exhibit the changes usually associated with skeletal fluorosis. The 24-hour urinary fluoride excretion in 21 patients ranged from 0.35 to 2.4 mg/day and was directly related to the distance of their residence from the factory.

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HISTOLOGICAL STUDY OF BONE TISSUE IN INDUSTRIAL FLUOROSIS

by

C.A. Baud, G. Boivin and R. Lagier
Geneva, Switzerland

(Abstract)

This study was a part of a broader one including clinical, radiological and histological observations on iliac crest biopsies of 43 men exposed to fluoride in aluminum factories.

The bone fluoride content, measured on calcinated compact bone with a specific-ion electrode, shows a significant difference ($p < 0.00005$) in the values obtained for fluorotic subjects (5617 $\pm$ 2143 ppm) and controls (1036 $\pm$ 627 ppm). No correlation was found between bone fluoride content and duration of exposure. However there is a correlation between bone fluoride content and the duration of the period between the end of fluoride exposure and the biopsy; the fluoride content decreases linearly by about one half in 20 years.

The topographical distribution of fluoride and the fluoride content of bone sections were determined by means of an electron probe X-ray microanalyzer. The fluoride was seen to be unevenly distributed in fluorotic bone tissue. The histograms, showing the distribution of fluoride content in percentage volume of compact fluorotic bone tissue, clearly demonstrate the presence of small zones of high fluoride content (> 4400 ppm) which were not observed in control bone tissue.

The examination of the microradiographs of bone tissue reveals that the most striking histological modifications of bone tissue associated with industrial fluorosis are: (a) more marked bone remodeling activity, (b) important cortical porosity, (c) hypervascularization, (d) increased number of enlarged periosteocytic lacunae, (e) presence of mottled periosteocytic lacunae, (f) existence of linear defects of bone matrix formation and, occasionally, (g) presence of newly formed and hypermineralized periosteal bone. These histological changes appear to be more frequent when the bone fluoride content is high. As for the two different changes in the periosteocytic lacunar walls simultaneously observed in fluorotic bone tissue, it is important to note that the mottled lacunae are formation defects with alteration of perilacunar organic matrix and hypomineralization, but the enlarged lacunae are the result of the periosteocytic osteolysis. In the present study, the abundance of enlarged lacunae can not be attributed to hyperparathyroidism.

The morphometric analysis (TAS, Leitz) of these microradiographs allowed a quantitative evaluation of trabecular bone volume, trabecular thickness, cortical porosity, periosteocytic lacunar surface and osteocytic population. If the values obtained in the fluorotic subjects are compared with those of the controls of the same age group, significant increases are found for trabecular bone volume, cortical porosity and periosteocytic lacunar surface, in the fluorotic subjects. On the other hand, there are no significant differences between the two groups, with respect to trabecular thickness and the osteocytic population. These results confirm the qualitative modifications observed on the microradiographs.

The degree of mineralization of the bone tissue, measured by a microradiographic-microdensitometric method, was found to be significantly higher in fluorotic bone tissue than in the control samples.

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ALLEVIATION OF FLUORINE TOXICITY IN STARTING TURKEYS AND CHICKS WITH ALUMINUM

by

A. Cakir, T.W. Sullivan and F.B. Mather
Lincoln, Nebraska

(Abstracted from Poultry Sci., 57:498-505, 1978)

In laboratory animals and ruminants, calcium and aluminum compounds have effectively countered the toxicity of fluoride. Since data on alleviation of fluorosis in poultry is lacking, the effect of aluminum sulfate and aluminum oxide at different levels in the diets of newborn turkeys and broiler chicks with respect to their action on fluorine toxicosis was investigated.

In the first experiment newborn turkeys and broiler chicks were given all combinations of five levels of fluoride (0, 200, 400, 600 and 800 ppm as NaF) and four levels of $Al_2(SO_4)_3 \cdot 18H_2O$ (0, .2, .4, and .6%) in a 5 x 4 factorial design for a period of 28 days. In a second experiment all combinations of four levels of fluoride (400, 600, 800, and 1000 ppm), two aluminum compounds (aluminum trioxide and aluminum sulfate) and four aluminum fluoride ratios (0, .4, .6 and .8 mg Al/mg F) were given to 30-day-old poults for 28 days. In the third experiment, colostomized turkeys received one of the following dietary treatments: a) a control containing 26 ppm fluoride; b) control + 1000 ppm fluoride; c) control + 1000 ppm fluoride + 800 ppm aluminum (as aluminum sulfate). After a five-day adjustment period for all groups, samples of urine and feces were analyzed for fluoride.

Results: Decline in body weight and feed consumption which began at the 600 to 800 ppm level of fluoride given alone was without statistical significance ($P < 0.05$). Above this level severe fluoride toxicity started in young turkeys, which were less tolerant to fluoride than broiler chicks. Up to the 800 ppm fluoride level, body weight and feed consumption were not affected by addition of aluminum sulfate. However, at 800 ppm and 1000 ppm fluoride the aluminum sulfate completely prevented the toxic effect in contrast to aluminum trioxide which failed to prevent fluoride toxicosis in poults, relative to weight gain, food efficiency and feed consumption.

In the third experiment, designed to explain the mode of action of aluminum, it significantly reduced the absorption of fluoride in turkeys. The urinary fluoride levels were 2.4 ppm with the control diet and 17.8 ppm in the high fluoride group. Aluminum sulfate reduced this level to 6.7 ppm and increased the fecal fluoride by 63 percent. Aluminum sulfate did not totally prevent but greatly reduced the absorption of fluoride. Poultry seem to have a more effective protective system

than ruminants.

****

NORMAL VALUES OF FLUORIDE FROM A DEFINED REGION OF THE HUMAN ILIAC CREST

by

B. Schellmann and A. Zober
Erlangen, D.B.R.

(Abstracted from Arch. Occup. Environ. Hlth., 35:233-244, 1975)

In order to establish standards for fluoride determinations in the human iliac crest, the authors analyzed bone samples from 100 cadavers of both sexes varying in age from 6 months to 97 years, 25% of which were accident or suicide cases. They excluded cases with renal or primary osteopathies and malignant tumors with skeletal metastases. Two parallel 5 mm wide bone specimens, sawn 3 cm dorsal from the right anterior superior iliac spine were analyzed for fluoride.

The fluoride values ranged from 69 to 1750 ppm in the ash specimens and from 14 to 264 ppm in fresh weight specimens. A significant correlation was found between the fluoride concentration and age but none with sex nor with the basic illness of the subjects. Histologically the specimens showed no abnormalities.

The authors recommend that the fluoride assays always be made from bone ash since removal of fat from bone may carry the risk of including impurities. They noted a discrepancy between their fluoride values and those of Smith and Hodge who in 1953 had recorded, in ribs and vertebrae, almost double the levels found in the present study. The authors also point out that high bone fluoride concentrations may occur without the radiological changes of fluorosis.

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RETENTION OF FLUORIDE FROM DIETS CONTAINING MATERIALS PRODUCED DURING ALUMINUM SMELTING

by

D.A. Wright and A. Thompson
Newcastle upon Tyne, England

(Abstracted from Br. J. Nutr., 40:139-147, 1978)

Of the total atmospheric load from plants manufacturing phosphate fertilizers, smelting aluminum and steel, and firing brick and ceramic materials, half is particulate fluoride and the other half the gaseous fraction, mainly hydrogen fluoride (HF).

Five kinds of dust found in and around an aluminum smelter were analyzed for the availability of fluoride to experimental rats by means of fluoride balances. They were:

1) Cryolite (Na_3AlF_6) which is used in the electrolytic reduction of aluminum.
2) Aluminum fluoride ($AlF_3 \cdot H_2O$) which is added to the cell during the electrolytic process.
3) Sodium fluoride (NaF) which serves as a reference standard for comparison with other materials.
4) Reclaimed alumina with absorbed fluoride which is recycled to the cells for reduction.
5) Mist eliminator grid solids (MEG) an ill-defined combination of agents passing through the wet-scrubbing system which uses sea water.

Forty-two male albino rats, 50 days old, from six litters (seven litter mates), were randomly allocated to seven diets. Each rat was given 9 g/day of one of these experimental diets for a seven day preliminary and seven day experimental period. The seven diets consisted of: 1) the basal diet containing 2 g/kg fluoride; 2) containing 71 g/kg NaF; 3) 138 g/kg NaF; 4) $AlF_3 \cdot H_2O$ 182 g/kg; 5) mist eliminator grid solids 401 g/kg; 6) Na_2AlF_6 153 g/kg; and 7) reclaimed alumina 203 g/kg. After a preliminary control period the animals were given 9 g diet per day which the majority of the animals consumed completely; only one animal receiving diet 3 (NaF) refused about 30% of it and all animals receiving diet 5 refused approximately 14% of it.

The feed, urine, feces, kidney and left femur were analyzed for their fluoride content.

One of the animals on diet 2 died early in the preliminary period due to food blocking the trachea which apparently made the animal choke to death. The basal diet group (#1) had a very low fluoride

intake (0.113 mg/7 days). The availability of fluoride in NaF at both levels of ingestion (diet 2 and 3) was virtually 100% and of the absorbed fluoride approximately 45% - 50% was retained in the body. In the MEG group (diet 5), the availability was as high as 83.4% but only about one third of the absorbed fluoride was retained. Surprisingly the availability for cryolite (diet 6) was as high as 85% and its retention by 64% showed that its overall yield of fluoride was even higher than that of NaF.

The authors stressed "the weakness of using urine fluoride levels alone as a measure of comparative fluoride potential". Whereas "urinary fluoride levels give a reasonable prediction of fluoride availability, differences in retention by bone tissue may be missed."

There was a very significant correlation between the amount of fluoride retained and that absorbed. The fluoride content of the kidneys and femurs showed a significant correlation with the amounts of fluoride absorbed or retained.

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BOOK REVIEW

EFFECTS OF A CASE OF AIRBORNE FLUORIDE POLLUTION ON THE HEALTH OF THE SURROUNDING POPULATION

by

G. Thiers
Institute for Hygiene and Epidemiology, Juliette Wytsmanstraat 14, 1050 Brussels, Belgium, November 1978

The book written in Dutch with an English summary, is a compilation of extensive environmental and health data concerning airborne fluoride pollution near the Belgian town of Bruges. The study was prompted by illness and sudden death in 1974 of cattle grazing near the Bayer-Rickmann enamel factory situated in the northern part of the city in close proximity to three residential areas and concerns, primarily, the health of the surrounding population.

In the first chapter a survey is given of the available environmental data on the case. The factory began production of enamel in 1964. Fluoride emissions which,in 1975, exceeded 2000 gms/hour were reduced by 1977 to less than 100 gms/hour. Some 20,000 gms fluoride/hour are believed to have been emitted during 1973 and 1974. The mean atmospheric values during 1975 to 1977 ranged from 0.13 to 6.08 micrograms fluoride/m^3. The highest fluoride burden was recorded within 600 to 700 meters from the emission source.

Whereas the water supply of the district contains less than 0.30 ppm fluoride, values of > 1 ppm have been found in surface, ground and rainwater close to the factory. At a distance of less than 1000 meters from the factory, budding and flowering of gladioli was retarded. In ornamental plants, fluoride concentrations sometimes exceeded 400 ppm and in 1976 the mean fluoride values in grass ranged between 4.5 - 132.9 ppm, depending on the sampling site, but in 1977 they had declined to between 8 and 60.2 ppm. In vegetables, measured concentrations were much higher than the usual values recorded in the literature. In 1975, red and green cabbage contained 65 to 85 times more fluoride than is considered normal. In 1977, 2.75 - 4.0 ppm fluoride was found in apples, compared to a normal range from 0.13 to 0.43 ppm. Fluoride levels in rhubarb ranged from 3.25 to 14.0 ppm.

Twenty-four hour urine examinations for fluoride revealed concentrations above 2 ppm in 1974 and early 1975 but, subsequently, there was "no noticeable increase in fluoride excretion".

Etching of window glass was observed during 1973-1975.

Questionnaires covering the complaints of individuals were

circulated early in 1977 among two population groups, one close to the factory and the other (a control group) in the south of Bruges.

In people residing close to the factory who were convinced that some of their complaints were related to the fluoride, additional health investigations were made at the end of 1977. A subsequent questionnaire failed to provide conclusive information since "the interpretation [of such data] is very difficult and it is concluded that only strict clinical double-blind observations can prove a relation between certain complaints and environmental factors". Acute gastrointestinal symptoms seem to have occurred during the years 1973 through 1975 in many persons who consumed home-grown vegetables, fruit and chickens. Chizzola maculae were not reported.

In addition, clinical and radiological investigations and a follow-up on urinary fluoride excretion were carried out. An 11-year-old girl had diffuse unexplained osteoporosis and two adult men, aged 34 and 40, had beginning periostitis deformans in the 5th metacarpal bones. In other cases, calcifications in muscular tendons were observed.

In 1978, the urinary fluoride values were in a range lower than previously, namely between 0.2 and 1.0 ppm.

Extensive studies were carried out in the Bruges schools within and outside the area considered polluted. The prevalence of mottled enamel was consistently higher in children living in the polluted area but the difference was statistically significant only for the age group 9 - 11 years. The "mottled enamel community index" for the age group 15 - 17 almost reached the borderline of what is termed "objectionable" fluorosis. All 7 children,with mottled enamel of degree 5 or more, lived within the polluted area except one whose residence was about 1900 meters distant from the factory. Fifteen children with the highest dental fluorosis score excreted only little fluoride in the urine (mean values of 0.22 to 0.46 ppm). On X-ray examination, these children had no indication of fluoride related abnormalities.

In the polluted area total fluoride concentration in teeth ranged between 66 and 892 micrograms/gram (ppm) with a mean of 185.35. At a depth of 20 micron fluoride concentrations in teeth ranged between 186 and 716 ppm with a mean of 445.85.

The authors point to the difficulties encountered in establishing a relationship of fluoride to the complaints of persons residing in the area. They obtained definite evidence of a high prevalence of mottled enamel in children above age 9; some of them were called serious "unaesthetic dental anomalies with possible dysfunction", especially in the presence of dental hypoplasia. (Editor's note: There is a question whether the southern part of the city was an adequate control since the atmosphere in this area was undoubtedly also polluted).

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INSTRUCTIONS TO AUTHORS

"Fluoride", the official journal of the International Society for Fluoride Research (ISFR) is published Quarterly (Jan., Apr., July, Oct.). Its scope is the publication of papers and reports on the biological, chemical, ecological, industrial, toxicological and clinical impacts of inorganic and organic fluoride compounds. Papers presented at the annual conferences of the ISFR are, in general, also published in "Fluoride." Submission of a paper implies that it presents the results of original investigations and relevant bio-medical observations Review papers are also accepted for publication.

Preparation of Papers

1. General - No precise limit is given on the length of a paper; however, it should be written concisely in English and submitted in two copies, doublespaced with generous margins. This facilitates editorial corrections. Words or letters in the text which are to be printed in italics should be underlined. Measures are given in metric system.

2. Title - Papers should be headed by a concise but informative title. This should be followed by the name of author(s) and the location and state (country) where the work was carried out. At the bottom of the first page (below line) the name and address of the institution should be given, where the work was carried out.

3. Summary - The paper should begin with the Summary. This should be brief (not more than 10 lines) and factual. The summary should be intelligible in itself without reference to the paper.

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5. Material and Methods - This should be condensed; however, if the methodology is new or developed by the author(s) it might be more detailed.

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1. Fiske, C.H. and Subba Row, Y.: The Colorimetric Determination of Phosphorus. J. Biol. Chem., 66:375-400, 1925.

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