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EDITORIAL

FLUORIDE AND KIDNEY STONES

In 1936 Volkmann, a German surgeon, examined 14 kidney stones for their fluoride content. He found 0.4 mg to 180 mg per stone, but did not specify their weight (1). In 1954, Dillon, a Scottish dentist, reported 2,000 ppm fluoride in a urinary calculus using the Willard-Winter method of analysis (2). About that time Taylor suggested that fluoride might be related to the formation of at least some kind of urinary calculi (3). During experiments pertaining to the carcinogenicity of fluoride, four out of 645 mice which received 1 ppm fluoridated water for their life span, developed bladder stones "a condition never before observed in our mouse colony."

Two years later, Spira obtained kidney stones from 10 patients aged 4 to 80 and found fluoride in the range of 0-1790 ppm (4). All his patients were residents of New York City where the natural fluoride content of the municipal water was only 0.1 ppm. Spira suggested that fluoride in food and drinks may play a role in the formation of calculi. A subsequent assay of the same calculi by another analyst recorded by Herman (5) yielded somewhat different results with a high of 1560 ppm. Herman pointed to the difficulties encountered in obtaining accurate, quantitative data. He suggested that fluoride levels may differ in different portions of a calculus. The fluoride and the calcium content of the stones were directly related. He presented data from the literature which shows that fluoride "values found in calculi greatly exceed those hitherto reported in biological materials including those obtained in experimental poisoning" (5). In a subsequent study (6) Herman and Papadakis gave 150 rats up to 500 ppm in drinking water for two months; he failed to find any kidney stones.

Zipkin et al. (7) compared the fluoride content of kidney stones with that of bones from individuals residing in low (0.0 - 0.6 ppm in water) and high (2.6 ppm) fluoride areas. His values were distinctly higher than those of previous investigations. He found a mean concentration of 2600 ppm in the low and 3700 ppm in the high fluoride communities.

No hard data on this subject have emerged until more recently when two groups of investigators shed new light on the subject. On page of this issue, Jolly, et al. recorded in kidney stones obtained in an endemic fluorosis area of India, a mean of 2.2 mg/100 mg (2200 ppm) and 0.02/100 mg (200 ppm) in nonendemic areas. Urinary and phosphorous levels were within normal limits, but greater amounts of fluoride appeared in the urine of persons residing in the endemic area than in the nonendemic one. Jolly et al. found no correlation between the calcium and the fluoride content of the stones. No data

however on the kind of kidney stones were presented.

This feature was explored by two teams of investigators from East Germany. In 56 urinary calculi from adults residing in Karl-Marx-Stadt DDR (0.25 ppm in drinking water), Auermann and Kuhn (8) reported a range of 210 to 10,650 ppm fluoride with a mean of 1130. Twenty-eight stones were calcium oxalate stones, 11 mixed calcium oxalate and phosphate, 13 uric acid stones; the remaining 4 consisted of a mixture of the above ingredients. The uric acid stones contained the lowest amounts of fluoride, namely 70 to 430 ppm with a mean of 200 ppm.

Hesse et al. (41)classified 150 kidney stones according to their chemical composition. The Whewellite and Weddellite stones, i.e. those containing calcium, showed the greatest amounts of fluoride, whereas cystin and uric acid stones contained considerably less.

All the above findings leave no doubt that in calcium-containing kidney stones, the magnitudes of fluoride under certain conditions exceed the fluoride content of bones. The question arises: Can fluoride in water, food and air cause urinary calculi?

Among theories concerned with this problem, Auermann proposed that the halogen encourages and accelerates the formation of calculi. Fluoride enters the apatite lattice from uromucoid and renders the cyrstal more dense and less soluble. Substantiation of this theory would require further studies on the fluoride content of the body fluids surrounding the stones.

That fluoride levels in the blood and other body fluids of patients with kidney stones might be elevated is suggested by a 1958 study by Herman (9). In organs of 38 persons afflicted with urolithiasis, he found unusually high fluoride levels, e.g. 290 ppm in the skin, 181 ppm in the kidneys, 185 ppm in the bladder. Jolly has found a significant correlation of fluoride in the urine with that in kidney stones. (10-16)

Whether or not a correlation exists between the calcium and the fluoride content of the stone is of paramount importance since such a correlation would indicate that calcium might be the primary factor and that its presence would account for deposition of fluoride because of its strong affinity to fluoride. In calcified aortas, Waldbott (10) found no such correlation in assaying the two electrolytes, which suggests that the primary source of such calcifications might be the presence of fluoride. With respect to kidney stones, as indicated above, the findings are contradictory. Neither Jolly nor Zipkin found a correlation between fluoride and calcium.

It is clear that additional research is needed in order to establish whether or not and to what extend fluoride contributes to the production of kidney stones.

Bibliography

1. Volkmann, J.: Versuche mit Fluornatriumbehandlung bei Knochenbrüchen verzögerter Knochenbruchheilung und ähnlichem. Bruns' Beiträge Zur Klinischen Chirurgie, 164:487-491, 1936.
2. Dillon, C.: Fluorine Content of Renal Calculus. Dental Practitioner, 4:181, Feb. 1954.
3. Taylor, A.: Fluoride and Cancer. Saturday Review, 48:73, 1965.
4. Spira, L.: Urinary Calculi and Fluorine. Exp. Med. and Surg., 14:72-78, 1956.
5. Herman, J.R.: Fluorine in Urinary Tract Calculi. Proc. Soc. Exp. Biol. Med., 91:189-191, 1956.
6. Herman, J.R. and Papadakis, L.: The Relationship of Sodium Fluoride to Nephrolithiasis in Rats. Journ. Urol. 83:799-800, 1960.
7. Zipkin, I., Lee, W.A. and Leone, N.C.: Fluoride Content of Urinary and Biliary Tract Calculi.In McClure, F.J. Ed. Fluoride Drinking Waters. P.H.S. Publ. 825, 1962, p. 435-437.
8. Auermann, E., and Kuhn, H.: Untersuchungen über den Fluorgehalt von Harnsteinen. Deutches Gesundheitswesen, 24:565-569, 1969.
9. Herman, J.R., Mason, B. and Light, I.: Fluorine in Urinary Tract Calculi. Journ. of Urol. 80:263-268, Oct. 1958.
10. Waldbott, G.L.: Fluoride and Calcium Levels in the Aorta. Experientia, 22:835-841, 1966.

G.L.W.

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ADRENAL FUNCTION AND CHANGES OF SODIUM AND POTASSIUM IN SERUM AND URINE IN FLUORIDE-INTOXICATED RATS

by

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SUMMARY: Administration of fluoride to intact and adrenalectomized rats reduced the serum sodium and elevated the serum potassium level. The response of serum sodium and potassium to angiotensin became more pronounced in intact rats which received fluoride than in the controls. The increased sensitivity of serum sodium to angiotensin by fluoride was associated with greater excretion of sodium. Interestingly, the elevation of serum potassium by adrenalectomy could be suppressed by angiotensin and the decreased urinary excretion of potassium induced by adrenalectomy was markedly suppressed by angiotensin. Fluoride did not alter the suppression effect of angiotensin.

Introduction

Many of the biological effects of fluoride have been extensively studied, but damage to the kidneys and adrenal glands induced by relatively large doses of fluoride has received little attention.

Schwalb et al. (1) found no pathological changes in the kidneys of a dog sacrificed a few hours after receiving a single large intravenous injection of 20-64 mg/kg fluoride (as NaF), although the volume of urine increased and excretion of urea decreased. Taylor et al. (2) reported that after administration of 20 and 30 mg sodium fluoride, the major changes in renal function were an increased volume of urine and decreased specific gravity.

Changes in physiological ion mobilization such as sodium, potassium, magnesium and calcium ions, and their related enzyme activities in the blood and kidneys were produced by a single oral dose (50 mg/kg) sodium fluoride to rats (3-6).

The following experiment was designed to study the relationship between adrenal function and changes in sodium and potassium in serum and urine during fluoride intoxication. Changes in adrenal and renal function due to fluorosis were evaluated by adrenalectomy and by the response of sodium and potassium in urine and serum to aldosterone and angiotensin.

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Materials and Methods

Treatments: Male Wistar albino rats weighing about 100g were used in this experiment. The rats were housed in an airconditioned room at 22° C. After being maintained on basal diet MF (purchased from Oriental Yeast Ind., Japan) and water ad libitum for five days, the rats were adrenalectomized. The intact rats, which served as controls, as well as the adrenalectomized rats received the same diet and water ad libitum for two additional days. All animals were fasted for 24 hours before the start of the experiment in order to minimize the effect of fluoride absorption from the bowels and to stabilize the urinary excretion on ions. Urine was collected in metabolic cages each of which housed one pair of rats.

Aldosterone (30μg/kg or angiotensin (angiotensin II) (12 μg/kg) or 0.9% saline (2 ml/kg), respectively, were administered subcutaneously twice at four-hour intervals, four hours after a single oral dose of fluoride (NaF, 50 mg/kg) or of chloride (NaCl, 69.8 mg/kg) in the control rats. The rats were sacrificed four hours after having received the last dose. Aldosterone 30 μg or angiotensin 12 μg, respectively, were contained in 2 ml. of 0.9% saline.

Analyses: The determinations of sodium and potassium in serum and urine were carried out by the method of Wills (7) by means of an Hitachi Model 518 Digital Atomic Absorption Spectrophotometer.

Materials: Angiotensin II was obtained from Protein Research Foundation (Osaka, Japan). Aldosterone was purchased from Sigma Chemical Company, (St. Louis, Missouri, U.S.A.).

Results

Adrenalectomy caused the serum sodium levels of rats to decrease significantly ($p < 0.02$). In intact rats, fluoride lowered the serum sodium to 0.9 of the control. In the adrenalectomized rats, this decrease was slight as shown in Table 1. In intact rats, treatment with aldosterone and angiotensin elevated the fluoride-induced serum sodium levels. In the adrenalectomized rats, the serum sodium was elevated by aldosterone ($p < 0.02$), in both the control and fluoride-intoxicated rats, but was not significantly elevated by angiotensin ($p > 0.05$) as shown in Table 1.

Adrenalectomy increased the urinary excretion of sodium by the rats 3.36 times above the control level in intact rats. This increase due to adrenalectomy was further enhanced to 1.81 times by fluoride. Treatment with aldosterone inhibited the increase in urinary sodium excretion when fluoride was given both in the intact and adrenalec-

tomized rats. On the other hand, angiotensin inhibited the increase of urinary sodium in intact rats which received fluoride, but failed to do so in the adrenalectomized rats given fluoride (Table 2).

Table 1

Response of Serum Sodium to Aldosterone and Angiotensin in Rats F-Intoxicated After Adrenalectomy[a]

		Intact			Adrenalectomized		
		μEq/ml	T/C	T/F	μEq/ml	T/C	T/F
Control	None	146.3±8.4	1	-	128.7±7.5	1	-
	Aldosterone	181.2±5.5	1.24[b]	-	142.5±4.7	1.11[b]	-
	Angiotensin	159.7±6.5	1.09[c]	-	125.2±5.3	1.05	-
Fluoride	None	131.3±1.9	0.90[b]	1	118.0±6.5	0.92	1
	Aldosterone	172.7±0.9	1.18[b]	1.32[d]	128.4±4.8	1.00	1.09[e]
	Angiotensin	171.5±7.5	1.17[b]	1.31[d]	123.5±0.8	0.96	1.04

Table 2

Response of Urinary Sodium to Aldosterone and Angiotensin in F-Intoxicated Rats After Adrenalectomy

		Intact			Adrenalectomized		
		μEq/12 h	T/C	T/F	μEq/12 h	T/C	T/F
Control	None	465±57	1	-	1560±152	1	-
	Aldosterone	234±21	0.50[b]	-	1027±113	0.66[b]	-
	Angiotensin	232±23	0.50[b]	-	1083±115	0.69[b]	-
Fluoride	None	1553±158	3.34[b]	1	2821±301	1.81[b]	1
	Aldosterone	1033±110	2.22[b]	0.67[d]	1728±182	1.11	0.61[d]
	Angiotensin	715±74	1.54[b]	0.46[d]	2727±294	1.75[b]	0.97

A slight elevation of serum potassium levels of adrenalectomized rats was not statistically significant ($p > 0.05$). When fluoride was given, serum potassium in adrenalectomized rats increased significantly as shown in Table 3. Fluoride induced elevation of the serum potassium in intact rats, but this elevation was suppressed in the animals treated with aldosterone and angiotensin. The angiotensin-induced suppression of serum potassium was more pronounced in the fluoride-intoxicated rats than in the controls. In adrenalectomized rats, serum

potassium levels in control and fluoride-intoxicated rats were suppressed by aldosterone as shown in Table 3.

The urinary excretion of potassium in intact rats was increased by fluoride 1.93 times above that of the control. Urinary potassium excretion decreased 0.22 times of the control level in intact rats by adrenalectomy as shown in Table 4.

In adrenalectomized rats, the urinary excretion of potassium increased 3.38 times of the control when fluoride was given. In the intact rats, the suppressive effect of aldosterone and angiotensin on urinary potassium excretion was similar in the fluoride-intoxicated rats compared to the control. In adrenalectomized rats, treatment with angiotensin caused a twofold increase in urinary potassium excretion both in the control and in the fluoride-intoxicated rats, but aldosterone failed to alter urinary potassium excretion in control and fluoride-intoxicated rats (Table 4).

Table 3.

Response of Serum Potassium to Aldosterone and Angiotensin in Rats Fluoride-Intoxicated after Adrenalectomy[a]

		Intact			Adrenalectomized		
		μEq/ml	T/C	T/F	μEq/ml	T/C	T/F
Control	None	4.96±0.06	1	-	5.42±0.43	1	-
	Aldosterone	4.46±0.06	0.90[b]	-	4.87±0.40	0.90	-
	Angiotensin	4.48±0.15	0.90[b]	-	4.58±0.40	0.85[c]	-
Fluoride	None	5.74±0.23	1.16[b]	1	6.19±0.12	1.14[b]	1
	Aldosterone	4.74±0.30	0.96	0.83[d]	5.53±0.41	1.02	0.89[e]
	Angiotensin	3.96±0.37	0.80[b]	0.69[d]	4.58±0.04	0.85[b]	0.74[d]

Discussion

The characteristic symptoms of experimental fluorosis are mottled teeth and osteosclerosis; they represent a disorder of bone metabolism (8). Polyuria relates to renal lesions (2). In our previous papers we observed changes in ion mobilization in fluorosis (3,4).

In the present study, we were able to demonstrate a relationship between adrenal function and ion mobilization of sodium and potassium in fluoride intoxication by adrenalectomy and by treatments with aldosterone or angiotensin. Response of the serum sodium to angiotensin became much more marked by administration of fluoride in the intact

rats than in the controls, whereas fluoride did not alter the response in adrenalectomized rats. In intact rats sodium and potassium in urine did not respond to angiotensin and aldosterone after administration of fluoride. In adrenalectomized rats the response of urinary sodium to angiotensin was more noticeable when fluoride was given than in the control, but the response of urinary sodium excretion to aldosterone was not altered by fluoride. However, in the adrenalectomized rats, the response of urinary excretion of potassium to angiotensin and aldosterone was unaltered by fluoride.

Table 4.

Effect of Aldosterone and Angiotensin on Urinary Potassium Excretion in Fluoride-Intoxicated Rats after Adrenalectomy[a]

		Intact			Adrenalectomized		
		μEq/12 hr	T/C	T/F	μEq/12 hr	T/C	T/F
Control	None	457±41	1	-	101±11	1	-
	Aldosterone	325±35	0.71[b]	-	108±10	1.07	-
	Angiotensin	232±20	0.51[b]	-	198±15	1.96[b]	-
Fluoride	None	883±89	1.93[b]	1	341±35	3.38[b]	1
	Aldosterone	679±71	1.49[b]	0.77[d]	348±32	3.45[b]	1.02
	Angiotensin	685±70	1.50[b]	0.78[d]	714±72	7.06[b]	2.09[d]

[a]Rats sacrificed 12 hrs. after oral dose of 50 mg/kg NaF.
Values are averages from three pair of rats.
T/C: Values related to intact control.
T/F: Ratio of hormone treated to number of F-intoxicated rats.
[b,c]Significantly different from control (none): [b], $p<0.02$, [c], $p<0.05$.
[d,e]Significantly different from fluoride-intoxicated rats: [d], $p<0.02$, [e], $p<0.05$.

Luetscher and Axelrod (9) observed that low sodium intake augments the secretion of aldosterone. The principal adrenal steroids which affect the mineral balance (known to be aldosterone and angiotensin) stimulate aldosterone secretion from the adrenal gland (10).

From our results in this experiment and the findings of other workers described above, it is suggested that adrenal function in fluorosis plays an important role by maintaining a strong homeostasis on ion mobilization of sodium and potassium.

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Bibliography

1. Schwalb, H., Bauernfeind, A., and Hesel, H.: Die Wirkung von Natriumfluorid auf die renale Ausscheidung von p-Aminohippur säure, Kreatinin, Chlorid und Harnstoff beim Hund. Arch. Exp. Pathol. Parmakol., 224:285-294, 1955.
2. Taylor, J.M., Scott, J.K., Maynard, E.A., Smith, F.A., and Hodge, H.C.: Toxic Effects of Fluoride on the Rat Kidney. I. Acute Injury from Single Large Doses. Toxicol. Appl. Pharm., 3:278-289, 1961.
3. Suketa, Y., Mikami, E., Sato, Y., Hayashi, M., and Yamamoto, T.: Changes of Ion Mobilizations and Their Related Enzume Activities in the Blood of Fluoride-treated Rats. J. Toxicol. Environ. Health, 2:301-309, 1977.
4. Suketa, Y., and Mikami, E.: Changes in Urinary Ion Excretion and Related Renal Enzyme Activities in Fluoride-treated Rats. Toxicol. Appl. Pharm., 40:551-559, 1977.
5. Suketa, Y., Mikami, E., and Hayashi, M.: Changes in Calcium and Magnesium in the Kidneys of Rats Intoxicated with a Single Large Dose of Fluoride, Toxicol. Appl. Pharm., 39:313-319, 1977.
6. Suketa, Y., Sogo, T., and Yamamoto, T.: Effect of Fluoride on Ca^{2+}-and Mg^{2+}- stimulated ATPase in Mitochondria of Rat Lung. Chem. Pharm. Bulletin, 25:1094-1097, 1977.
7. Willis, J.B.: The Determination of Metals in Blood Serum by Atomic Absorption Spectroscopy. III. Sodium and Potassium. Spectrochim. Acta, 15:551-555, 1960.
8. Shupe, J.L.: Clinical and Pathological Effects of Fluoride Toxicity in Animals. In Carbon-Fluorine Compound; Chemistry, Biochemistry & Biological Activities. A Ciba Foundation Symposium (13-15 September, 1971). Elsevier/Excerpta Medica/North-Holland, Amsterdam, pp 357-388.
9. Leutscher, J.A., Jr., and Axelrod, B.J.: Increased Aldosterone Output During Sodium Deprivation in Normal Men. Pro. Soc. Exp. Med., 87:650-653, 1954.
10. Levine, R.: Some Mechanisms for Hormonal Effects on Substrate Transport. In Metabolic Pathways (L.E. Hokin, ed.,) 6:627-641 (3rd Edition). Academic Press, New York, 1972.

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KIDNEY CHANGES AND KIDNEY STONES IN ENDEMIC FLUOROSIS

by

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SUMMARY: Urinary tract calculi obtained from 25 patients residing in an endemic fluorotic area revealed a significantly higher fluoride content compared to calculi from 25 persons from a nonendemic area. However the difference in their calcium and phosphate content was not significant. Twenty-four hour urinary fluoride excretion was significantly higher in 10 fluorotic patients than in 10 controls. The incidence of urinary tract calculi was elevated in most of the fluorotic districts of Punjab.

Chronic ingestion of high levels of fluoride is known to produce harmful effects on hard tissues of the body leading to widely recognized clinical manifestations in teeth and bones. The kidneys are the chief organ of the body responsible for excretion of a large portion of ingested fluoride. Patients with endemic fluorosis must excrete excessive amounts of fluoride from the blood into urine. The renal involvement by high doses of fluoride has been described in experimental animals at various concentrations, but no precise studies on humans of the structure and function of the kidneys are available. The present study was undertaken for the purpose of determining the effect of chronic ingestion of high levels of fluoride on the kidneys in humans.

I. Renal Changes

Complete urine examinations including urea, creatinine and fluoride clearances were carried out on 25 cases of endemic fluorosis. The specific gravity of the urine was measured in all cases after water loading and water deprivation. Successful renal biopsies were possible in 18 fluorotic individuals. In 10 healthy nonfluorotic subjects urea, creatinine and fluoride clearances were measured simultaneously as a control. The following results were obtained:

1. The mean values for maximum urea clearance and standard urea clearance were low compared to mean control values. The decline in creatinine and fluoride clearances compared to the controls was statistically significant, an indication that chronic fluoride intoxication leads to a distinct impairment of glomerular function in human beings.

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2. The renal tubular functions, as determined by water loading and deprivation tests, were found to be normal. Therefore the tubular functions remain unaffected.

3. On histological examination, the sections from the needle biopsy specimens of the kidneys did not reveal any structural change in glomeruli as seen under the light microscope. The only abnormality was the presence of vacuolation in the cells lining the distal convoluted tubules. Thus, a subtle functional impairment of the kidneys is present in chronic fluoride intoxication which requires further investigation.

II. Urinary Tract Calculi

Fluoride has been demonstrated to be present in almost all body tissues. It is eliminated mainly in the urine (1,2). Its excretion is said to closely parallel its intake from drinking water(3-5). Hence fluoride concentrations are elevated in the urine of subjects residing in endemic areas. Fluoride has been reported to be present in renal tract calculi (6,7).

The presence of fluoride in such calculi combined with excess fluoride in the urine induced us to assess the role of fluoride in the causation of renal calculi. The problem was studied from three different aspects:

1. We estimated the concentration of fluoride, calcium and phosphate in the urinary tract calculi obtained from people residing in endemic fluorotic areas in Punjab compared with calculi obtained in nonendemic areas.

2. Levels of fluoride, calcium and phosphate in 24-hour urinary specimens of fluorotic patients were compared with those of nonfluorotic persons.

3. The records for the past five years of the Rajendra Hospital, Patiala were reviewed to determine the incidence of urinary tract calculi in endemic and nonendemic areas.

Material and Methods

The kidney stones of 50 patients admitted to the hospital were obtained at surgery. One half of them came from endemic-fluorotic areas, the other half from nonfluorotic areas. The urinary calculi thus obtained were dried overnight at 105° C, weighed and pulverized. They were then extracted with alcohol for 8 hours and with ether for 4 hours, ashed at 550° C for 3 hours and analyzed for calcium, phosphorous and fluoride. The calcium levels in the stone ash were determined according to the method recommended by "Official Methods of Analysis of the Association of Official Agricultural Chemists" (8).

Inorganic phosphorous was estimated according to the method of King and Wooten. Fluoride assays were made by the thorium nitrate titration method.

Ten patients of radiologically proven skeletal fluorosis in the age group of 40 to 70 years were hospitalized. Their 24-hour urine was collected in glass receptacles containing 50 ml of 10% hydrochloric acid. The volume of this urine was measured and analyzed for calcium and phosphorous. Another 24-hour urine specimen was collected in glass containers with a buffer preservative and analyzed for fluoride. The same procedure was carried out in 10 normal subjects from nonendemic areas as controls.

The 1961 to 1965 admission records of Rajendra Hospital, Patiala were examined to ascertain the number of cases with urinary calculi. By charting these records districtwise, the incidence of urinary calculi from the endemic area as outlined by Singh et al. in 1961 (9) was compared with that from the nonendemic areas in the Punjab. In addition, the records of approximately 5000 hospitalized cases during four months in 1963 were reviewed in order to obtain the total number admitted from each district of Punjab.

1. Analysis of Urinary Tract Calculi: The districtwise distribution of the 25 cases from the endemic area is presented in Table 1 and their distribution in terms of age and sex is given in Table 2.

Table 1

Case Distribution from Endemic Area

Districts	No. of Cases
Bhatinda	14
Sangrur	5
Ferozepur	3
Hissar	3
Total	25

Table 2

Age and Sex of Endemic Group

Age Group In Years	Sex	
	Male	Female
0 - 1	5	1
11 - 20	2	-
21 - 30	2	1
31 - 40	5	-
41 - 50	2	-
51 - 60	3	-
61 - 70	3	-
71 - 80	1	-
Total	23	2

Of the 25 calculi, 3 were renal stones, 4 ureteric stones and 18 were bladder stones. In two cases multiple calculi were present. The weight of the calculi varied from 0.5 to 13.7 gms.

In the nonendemic districts of Punjab, we had 25 patients, 22 males and 3 females. Their age range and sex ratio was similar to those from fluorotic areas. Nineteen of the 25 calculi were present in the bladder, 4 in the kidneys and 2 in the ureters. The weight varied from 0.5 to 9.2 gms.

Table 3 shows the fluoride, calcium and phosphorous contents of the urinary tract calculi obtained from endemic and nonendemic groups.

Table 3
Analysis of Urinary Tract Calculi

		Fluoride mg	Calcium mg	Phosphate mg
Endemic Group	Range	0.84-4.15	10.1-31.2	1.01-18.2
	Mean	2.2	23.0	4.8
	S.D.±	0.99	6.5	4.2
Nonendemic Group	Range	0.03-0.91	4.9-30.5	1.15-18.2
	Mean	0.62	21.2	6.03
	S.D.±	0.243	6.2	5.5
Endemic & Nonendemic Group	S.E. +	0.20	1.80	1.38
	't' value	7.09	1.0	0.89
	Remarks	Significant	Insignificant	Insignificant

Significance P = 0.01

The mean fluoride content of stones formed in endemic fluorotic areas was 2.2 mg/100 mg (22,000 ppm) compared to 0.62 mg/100 mg (6200 ppm) stone ash obtained from nonendemic areas. The rise in fluoride levels was statistically significant in the urinary calculi derived from the endemic areas. However no significant change was observed in the calcium and phosphorous content of stones formed in endemic and nonendemic areas.

The calcium/fluoride ratio was 2.8/37.5 in fluorotic calculi and 17.7/840 in control stones. There was no definite correlation between the calcium and the fluoride content of stones. Similarly the calcium/phosphate ratio was 0.9/24.9 in fluorotic calculi and 0.7/22 in control stones. Thus the phosphorous content was also independent of the calcium and fluoride concentrations of the calculi.

2. Analysis of Urine for Fluoride, Calcium and Phosphates:

(a) Fluorotic Group: Ten patients varying in age from 27 to 70 years were hospitalized at Rajendra Hospital, Patiala. Nine of them were males, one was female. Six cases came from District Bhatinda, two from Ferozepur and 2 from Sangrur. All 10 had been residing in the high fluoride areas since birth and calcification of their interosseous membrane was considered proof of skeletal fluorosis.

(b) Control Group: The 10 cases, 9 males and 1 female, of this group came from a nonendemic area. Their ages varied from 30 to 70 years similar to those of the fluorotic group. Eight were residing in the District Patiala, one in Ambala and one in Ludhiana.

The daily excretion of fluoride, calcium and phosphate in the 24-hour urine of both groups is shown in Table 4.

Table 4
Daily Urinary Excretion of Fluoride, Calcium and Phosphate

		Fluoride mgs	Calcium mgs	Phosphates mgs
Fluorotic	Range	2.4-12.7	70.5-271.5	321-853
	Mean	6.1	117.4	606.9
	S.D.±	3.13	57.7	178.8
Control	Range	0.21-0.83	70.2-249	390.1-861
	Mean	0.54	122.9	592.3
	S.D.±	0.20	54.8	161.9
Fluorotic & Control	S.E.±	0.99	25.16	76.28
	't' value	5.62	0.22	0.19
	Remarks	Significant	Insignificant	Insignificant

Significance P = 0.01

According to Table 4 the daily excretion of fluoride in the urine of fluorotic patients is significantly higher than in the control group. However the daily excretion of calcium and phosphates in urine is almost the same in both groups.

3. Incidence of Urinary Tract Calculi in Endemic and Nonendemic Areas:

The districtwise records of cases with urinary calculi admitted at the Rajendra Hospital, from 1961-1965 and the total number of admissions to this hospital during four consecutive months of the year 1963 are shown in Table 5.

Table 5
Incidence of Calculi in Hospital Admissions

	District	Total cases with calculi admitted 1961-65	Total admissions in 4 consecutive months of 1963	% calculi in 5 yrs. to total no. of admissions in 4 mos.
Endemic	Sangrur	97	591	16.8
	Hissar	79	228	35.1
	Bhatinda	72	301	23.7
	Ferozepur	88	212	41.1
Nonendemic	Patiala	284	2714	10.3
	Karnal	61	249	23.0
	Ambala	61	332	18.3

Table 5 shows a higher percentage of urinary calculi in fluorotic districts than in the nonfluorotic ones.

Discussion

Fluoride has a pronounced affinity to calcium and combines with it to form insoluble calcium fluoride. It is conceivable that with high concentrations of fluoride in the urine, calcium fluoride may be precipitated and the precipitated particles may form the nucleus around which other salts are laid down to produce the calculi. In areas where the fluoride content of water is high, it is reasonable to assume that it might be responsible for the formation of urinary tract calculi or, at least, that it might accelerate their formation.

Zipkin, Lee and Leone (7) found that the fluoride concentration of calculi was considerably higher than that of bones. They also observed a slightly higher concentration of fluoride in calculi obtained from patients residing in endemic fluorotic areas (0.06 to 1.11%) although the increase was not statistically significant.

In the present series, the mean fluoride content of calculi obtained from patients in endemic fluorotic areas was 2.2 mg% compared to 0.62 mg% in those from nonendemic areas, a statistically significant difference. The discrepancy between our figures and those obtained by Zipkin et al. (7) can be explained on the basis that most of our patients were farmers who work in the scorching heat of summer and drink an average of 8 to 12 liters of water daily compared to people in cooler damp climates where the average intake of water is only 2 to 3 liters daily. Consequently they ingest large amounts of fluoride. Furthermore, the average fluoride concentration of water in the endemic fluorotic areas of India is 16 ppm (10) whereas in the area analyzed by Zipkin et al. (7), it was 2.6 ppm.

No statistically significant difference was observed between the calcium content of the calculi obtained from the endemic fluorotic areas and that from the control group. The calcium content of the calculi in the endemic group was unrelated to the fluoride content of the calculi. The calcium/fluoride ratio varied from 2.8 to 37.5. Zipkin et al. (7) made a similar observation.

The mean phosphate content of calculi from the endemic group (4.84 mg%) was slightly lower than that of the controls (6.03 mg%) although the difference was not statistically significant.

In the ten fluorotic patients in whom fluoride assays of the 24-hour urine were made, the mean value was 6.1 mg as contrasted to 0.54 mg in the controls, a significant difference. Similar observations were made by Largent and Heyroth (11). However, calcium and

phosphorous excretion was similar in the control and fluorotic groups. The difference was not statistically significant.

With respect to the incidence of urinary tract calculi in the endemic and nonendemic areas of Punjab, we observed a higher percentage of urinary calculi in the fluorotic than in the nonfluorotic districts. This technique of calculating the incidence of urinary calculi, however, is approximate and cannot be considered accurate. Nevertheless the data comparing the incidence of urinary calculi in the fluorotic and the nonfluorotic districts as well as our other findings, strongly support our hypothesis that kidney stones are influenced by the level of fluoride in drinking water.

Bibliography

1. Machle, W., Scott, E.W. and Largent, E.J.: Absorption and Excretion of Fluorides, Part 1 The Normal Balance. J. Ind. Hyg. Toxicol. 24:99-204, 1942.
2. Majumdar, D.B. and Ray, S.N.: Fluorine Intoxication of Cattle in India II. Effect of Fluorosis on Mineral Metabolism. Indian J. Vet. Sci. 16:107-112, 1946.
3. McClure, F.J., and Kinser, C.A.: Fluoride Domestic Waters and Systemic Effects II, Fluorine Content of Urine in Relation to Fluorine in Drinking Water. Publ. Hlth. Rep. 59:1575-1591, 1944.
4. Smith, F.A., Gardner, D.E. and Hodge, C.H.: Investigations on the Metabolism of Fluoride II. Fluoride Content of Blood and Urine as a Function of the Fluorine in Drinking Water. J. Dent. Res. 29:596-600, 1950.
5. Machle, W. and Largent, E.J.: The Absorption and Excretion of Fluoride II. The Metabolism at High Levels of Intake. J. Ind. Hyg. and Toxicol. 25:112-123, 1943.
6. Herman, J.R.: Fluorine in Urinary Tract Calculi. Proc. Soc. Exp. Biol. Med. 91:189-191, 1956.
7. Zipkin, I., Lee, W.A. and Leone, N.C.: Fluoride Content of Urinary and Biliary Tract Calculi. Proc. Soc. of Exp. Biol. Med. 97:650-653, 1958.
8. Official Methods of Analysis of the Association of Official Agricultural Chemists. 8th Ed. 1955, Washington, D.C.
9. Singh, A. and Jolly, S.S.: Endemic Fluorosis with Particular Reference to Fluorotic Radiculo-Myelopathy. Quart. J. Med. 30:357-721, 1961.
10. Singh, A., Dass, R., Hayreh, S.S. and Jolly, S.S.: Skeletal Changes in Endemic Fluorosis. J.Bone Joint Surg. 448:806-815, 1962.
11. Largent, E.J., Bovard, P.G. and Heyroth, F.F.: Am. J. Roentogenol and Radium Therapy. 65:42, 1951.

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THE PARATHYROID IN HUMAN FLUOROTIC SYNDROME

by

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SUMMARY: The present report deals with autopsies in two endemic fluorotic subjects. In one (S 721), there were three parathyroid glands with a total weight of .795 gm In the other (S 723), a single large parathyroid weighed 5.715 gm. Histologically the parathyroid glands showed hyperplasia. The nuclei of the chief cells were larger and more vesicular than in normal controls. In case S 721, a trend toward acinous formation was noted. Oxyphil cells were seen occasionally. In case S 723, the chief cells showed acinous formation with large vesicular nuclei. No oxyphil cells were seen and the parathyroid showed degenerative changes similar to those reported by us in experiments on rabbits.

Introduction

The literature on the role of the parathyroid gland in human endemic fluorosis is sparse. Singh et al. (1) found, in radiologically proven fluorotic patients, the parathyroid functions, e.g. serum calcium, inorganic phosphorus, phosphate clearance and calcium deprivation tests within normal limits. However, they noted significantly elevated levels of serum alkaline phosphatase.

Similarly Srikantia and Siddiqui (2) observed elevated serum alkaline phosphatase activity up to 98 K.A. units in juvenile fluorosis. In five patients reported by Teotia and Teotia (4), the immunoreactive parathyroid levels were increased. They concluded that the hyperactivity of the parathyroid gland was due to the body's attempt to maintain a normal extracellular ionized calcium equilibrium.

We are presenting this report because in the past no histological findings of the parathyroid glands in human fluorosis have been available. Whether parathyroid hyperplasia is primary or secondary can be determined solely by histological findings early in the disease.

Methods

Autopsies were carried out on two fluorotic patients S 721, age forty-four and S 723, age fifty-five. Both exhibited neurological manifestations of endemic fluorosis. Two nonfluorotic (S 722, S 729)

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cases served as controls. In addition, we attempted to locate the normal parathyroids in ten cadavers.

The parathyroids were identified, dissected and removed. Gross and histological studies were made. X-rays of various comparable bones were studied.

Results

Microscopic Findings: The parathyroid glands of the two fluorotic cases showed a marked increase in weight as compared to those of the nonfluorotic controls. In case S 721, three parathyroids were found A, B, and C weighing 312 mg, 278 mg and 205 mg respectively with a total weight of 795 mg. In case S 723, only a single nodular mass weighing 5.715 mg was identified and removed. In one of the nonfluorotic controls S 722, the three parathyroids weighed a total of 100 mg, in another S 729 the total weight of the four parathyroids was 175 mg. The hypertrophy in the fluorotic cadavers resulted from an increase in the size and number of parenchymal cells.

Radiological Findings: Radiologically there was ossification of the interosseus membrane, ligaments of the spine, osteosclerosis of the spine, but the distal ends of the radius and ulna were osteoporotic with cystic cavities.

Histological Changes, etc.: Histologically, the parathyroids in case S 721 showed hyperplasia. The nuclei of some of the chief cells were larger and more vesicular than in the normal controls. Occasional oxyphil cells were seen and there was a trend to acinous formation. In case S 723, we found acinous formation of chief cells with larger than normal vesicular nuclei. No oxyphil cells were seen. Degenerative changes were observed occasionally.

Discussion

Faccini and Care (5) found, by direct immunological assay, elevated levels of circulating parathyroid hormones in young sheep maintained on drinking water containing 100 ppm fluoride. Under similar circumstances Faccini (6) reported changes indicative of parathyroid hyperactivity. However, in other experimental animals, marked species variations were observed. According to Hac et al. (7) rats given 125 ppm fluoride in drinking water for nine weeks responded normally to parathormone administration.

Nicholas et al. (8) and Yates et al. (9) postulated that fluoride might depress bone mineral solubility and tend to reduce serum calcium levels sufficiently to stimulate the parathyroid gland to secrete parathyroid hormone and reestablish the normal serum calcium level. Nicholas et al. (8) added a further hypothesis based on evidence that the

inhibition of proline incorporation into the collagen of rat bone produced by parathormone extract is followed by a stimulation of collagen biosynthesis. They further suggested that the fluoride induced a special kind of secondary hyperparathyroidism in which increased bone resorption is blocked and only the stimulation of new bone formation is manifest.

The histological changes seen in the human parathyroid in the present report are similar to those presented by Makhni et al. (10).

Bibliography

1. Singh, A., Singh, B.M., Singh, I.D., Jolly, S.S. Malhotra, K.C.: Indian J. Med. Res., 54:591-596, 1966.
2. Srikanta, S.G., Siddiqui, A.H.: Calcium Metabolic Studies in Fluorosis. Clin. Sci., 28:477-485, 1965.
3. Teotia, M., Teotia, S.P.S. and Kanwar, K.B.: Endemic Skeletal Fluorosis. Arch. Dis. Child., 45:686, 1971.
4. Teotia, S.P.S. and Teotia, M.: Secondary Hyperparathyroidism in Patients with Skeletal Fluorosis. Brit. Med. J., 1:637 1973.
5. Faccini, J.M. and Care, A.D.: The Effect of Sodium Fluoride on the Ultrastructure of Parathyroid Gland on the Sheep. Nature, London. 207:1399-1401, 1965.
6. Faccini, J.M.: Inhibition of Bone Resorption in the Rabbit by Fluoride. Nature, London. 214: 1269-1271, 1967.
7. Hac, L.R., Freeman, S., Nock, W.B.: Effects of Fluoride and Parathyroid Extract on Citrate and Bone Metabolism. Am. J. Physiol. 212:213-216, 1967.
8. Nicholas, G.Jr., Flanagan, B. and Woods, J.F.: Influences on Bone Biosynthetic Mechanisms. 19:243-260, in The Parathyroid Glands, Ultrastructure Secretion and Function. Ed. Gaillard, P.J., Talmage, R.V., Budy, A.M. Univ. Chicago Press, Chicago, 1965.
9. Yates, C., Doty, S. and Talmye, R.V.: Effects of Sodium Fluoride on Calcium Homeostasis. Proc. Soc. Exp. Biol. Med. 115:1103-1108, 1964.
10. Mahni, S.S., Sidhu, S.S., Singh, P., and Grover, A.S.: Long-term Effects of Fluoride Administration in Rabbits -- An Experimental Study: Histological Changes in the Parathyroid Gland and Correlation in the Changes in the Bone. Fluoride. 11:124-128, 1979.

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ELECTROPHORESIS OF SERUM PROTEINS IN GROWING CHICKS FED A DIET SUPPLEMENTED WITH NaF

by

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SUMMARY: The electrophoretic patterns of serum samples from growing chicks fed a diet supplemented with 150 ppm fluoride as NaF showed, after 5 and 8 weeks, variations in the mobility and the concentrations of protein components.

Introduction

Previous studies from our laboratory showed changes in the weight of various tissues and their vitamin C level in young chicks fed a diet supplemented with 150 ppm fluoride as NaF (1). Subsequently, the serum albumin, glucose, and cholesterol level, and the alkaline phosphatase activity were found to be altered in the experimental birds (2). These data strongly suggest that marked biologic changes occurred in growing chicks exposed to fluoride at a level generally considered safe.

The electrophoretic constituents of plasma and serum proteins in chickens at various growth stages have been reported (3-6). Ferguson (7) studied the influence of low doses of fluoride on serum proteins and serum enzyme in human subjects and found that the protein patterns did not differ significantly. Kaur et al. (8), on the other hand, reported significant changes occurring in the different protein patterns in fluoride-injected rabbits. In a continued effort to investigate the biologic effect of low-level fluoride intake on growing chickens, we have conducted an electrophoretic analysis of serum proteins in these animals.

Materials and Methods

Ten one-day old White Leghorn cockerels (Gallus domesticus) were obtained locally and were subjected to habituation for one week on a basal diet. They were then divided equally into two groups: the controls and the experimentals. The chicks in the control group continued to receive the basal diet, whereas those in the experimental group were fed the basal diet supplemented with 150 ppm fluoride, as NaF. Other growing conditions were the same as reported previously (1).

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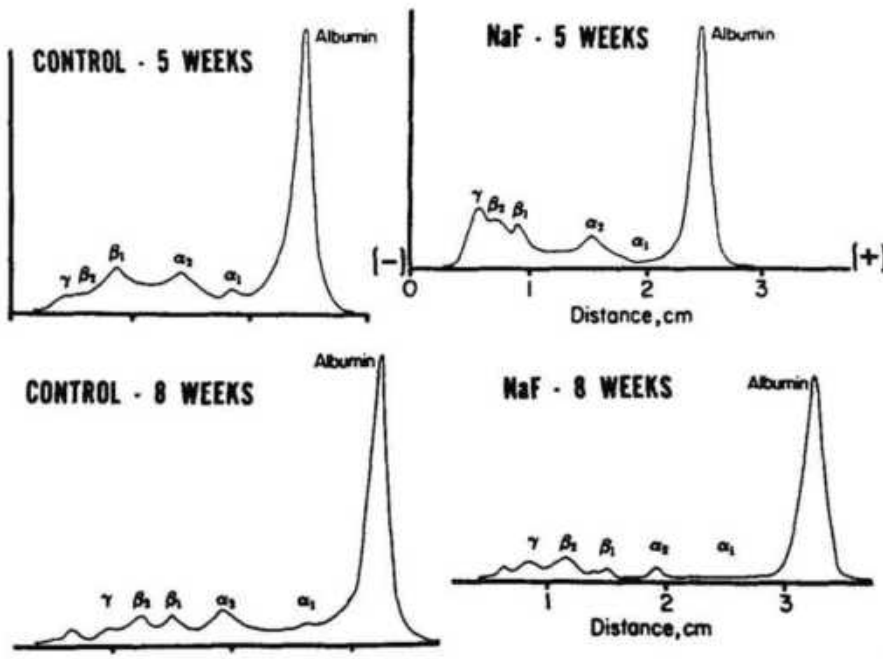
At the end of 5 weeks and 8 weeks, respectively, blood samples were taken from the wing veins and the sera prepared by the method of Weiser et al. (9). Electrophoresis was carried out in a Gelman Sepratek apparatus with Gelman Sepraphore III cellulose acetate strips, using 0.05 M Tris buffer at pH 8.6. Running times were 60 and 75 minutes for the 5 week and 8 week samples, respectively. The strips were stained with a solution of Gelman Panceau S in 5% trichloroacetic acid, destained and cleared by the method described by the manufacturer. They were then scanned in a densitometer (Quick Scan Jr., Helena Laboratories Corp., Texas, U.S.A.) at 525 nm. Identification of each protein fraction was done by comparing the data with those reported by other investigators (5-6). To obtain representative values for the percent composition of the individual protein constituents, each strip was scanned three times, each covering different portions of the individual bands. Calculation of the percentages was based on the areas under the curves.

Results

Under the experimental conditions, six to seven distinct bands were obtained and identified. These include albumin, alpha-1-, alpha-2-, beta-1-, beta-2-, and gamma-globulins, according to their decreasing mobility towards the anode. Figures 1 and 2 show the electrophoretic patterns of serum samples obtained from chicks treated with fluoride for 5 weeks and 8 weeks, respectively. The average mobility and the percent composition of various components are given in Tables 1 and 2, respectively. Prealbumin has been included in the fraction of albumin, as separation of these proteins was unsuccessful.

Figs. 1 & 2 Electrophoretic pattern of serum proteins



Fluoride at the level used in this study did not have appreciable effect on the mobility of albumin, but it slightly enhanced the mobility of the globulin fractions (Table 1).

Marked differences in the percent composition of serum proteins were observed between the control and the experimental birds. At the end of 5 weeks, the percentage of gamma-globulin showed about a twofold increase over the controls (Table 2). This increase was statistically significant ($p<0.01$). Albumin, on the other hand, showed a 12% decrease. At the end of 8 weeks, however, all the globulins showed significant decreases. The percentage declines of α_1- and β_1-globulins were particularly marked, each giving a value of 45 and 56%, respectively (Table 2). The percentage composition of albumin was consequently enhanced (17%). These changes were found to be significant at greater than the 99% level.

Table 1

Electrophoretic Mobility[a] of Serum Proteins of Chickens

Time (week)	Treatment	Albumin	Globulin α_1	α_2	β_1	β_2	γ
5	Control	7.2±0.1	5.5±0.1	4.0±0.3	2.3±0.2	1.5±0.2	1.0±0.1
	NaF	7.1±0.1		4.1±0.1	2.5±0.4	2.0±0.3	1.6±0.4
8	Control	7.3±0	5.3±0.6	3.6±0.4	2.4±0.6	2.0±0.4	1.6±0.4
	NaF	7.3±0		3.8±0.4	2.6±0.4	2.1±0.3	1.9±0.5

[a] $\times 10^{-5}$ cm^2 per volt per second

Table 2

Percent Composition of Serum Proteins of Growing Chicks Fed a Diet Containing Supplemental Fluoride

Time (week)	Treatment	Albumin	Globulin α_1	α_2	β_1	β_2	γ
5	Control	62.6±9.9	4.6±1.4	11.6±3.0	4.8±1.0	9.8±2.3	5.8±5.2
	NaF	54.8±4.6	4.6±1.9	13.1±2.4	5.5±1.5	10.7±1.7	11.1±3.6
	% Difference	-12.4	0	+12.9	+14.6	+9.2	+91.4
8	Control	64.6±3.6	5.1±1.6	9.8±2.2	1.6±0.5	7.2±1.9	12.9±2.4
	NaF	75.9±6.4	2.8±1.4	6.8±1.6	0.7±0.4	4.9±1.2	8.8±3.2
	% Difference	+17.5	-45.1	-30.6	-56.2	-31.9	-31.8

Discussion

The electrophoretic patterns obtained in this study are comparable to those reported by other investigators using samples from normal chickens (5-6). The data on the mobility and, in particular, on the percent composition of serum proteins suggest that significant changes occurred in these constituents of growing chicks exposed to 150 ppm fluoride. They suggest a

phenomenon that may occur in chickens in response to fluoride-induced stresses. Since the blood proteins are essential to the maintenance of blood pH and blood volume, it is possible that the observed decrease and increase in the albumin fraction at 5 and 8 weeks, respectively, may be necessary in order to preserve proper colloidal osmotic pressure.

Of particular interest is the markedly elevated gamma-globulin level in the experimental birds at 5 weeks' treatment (Table 2). This increase was also reported by Kaur et al. (8) in their work on fluoride-injected rabbits. As the gamma-globulins include antitoxins, antibacterial agglutinins as well as antibodies, such increase may be important in fluoride toxicosis in chickens. On the other hand, the marked elevation of these components may reflect hepatic abnormalities. Kaur et al. (8) suggest that the increased serum globulins and the concomitant albumin/globulins ratio inversion found in the sera of fluoride-injected rabbits point to impaired liver function as a result of fluoride intoxication. A significant decrease in the liver glycogen content of acutely and chronically injured rabbits has been shown by Tadashi (10). Zebrowski and Suttie (11) observed a depressed glycogen turnover rate in the liver of fluoride-fed rats. Since the liver is the site of gamma-globulin catabolism, it may be surmised that damage to the hepatic tissue by fluoride would result in increased levels of the various protein components of the sera.

It is striking that at 8 weeks' treatment the increases in gamma-globulins not only diminished, but the percentages showed a decline, as did the other globulins (Table 2). This is in contrast to the findings of Kaur et al. (8) with rabbits. The importance of these changes is unknown. It has been suggested (7, 11-12) that the changes in serum proteins and enzyme concentrations resulting from fluoride intake may be due to fluoride-ion induced changes in membrane permeability. A diminishing effect with time of the fluoride-induced changes had also been reported, thus raising the question of how a membrane system could adapt within a short period of time (7, 13-14). Furthermore, Bogin et al. (14) indicated that the lowered levels of serum proteins and enzymes may be due to lowered levels of these components in the tissues and organs of origin.

The experimental data obtained in this study support those reported by Kaur et al. (8), and Riekstneice et al. (12) in their work on rabbits and rats, respectively, indicating that fluoride treatment caused changes in serum proteins. These changes may include charges and molecular sizes, as would be reflected in the mobility patterns (Table 1). Such changes could alter their permeability and/or their binding capacity for certain important blood electrolytes. Many of the globulins are known to function in the transport of such ions as copper, calcium, and iron. It is possible that fluoride, through binding to blood proteins, may compete with different electrolytes for the binding sites or may alter the configuration of the proteins, thus influencing their functions.

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Bibliography

1. Yu, M.H. and Driver, C.J.: The Effects of Fluoride on the Growth and L-Ascorbic Acid Levels of Tissues from the Domestic Chicken (Gallus domesticus). Fluoride, 11:60-67, 1978.
2. Yu, M.H. and Stoehr, M.P.: Serum Compositional Changes in Chicks Caused by Supplementary NaF in Diet. Abstracts of Papers of the 144th AAAS Meeting, 12-17 February, 1798.
3. Suttie, J.W., Phillips, P.H., and Faltin, E.C.: Serum Fluoride in the Chick. Proc. Soc. Exp. Biol. Med., 115:575-577, 1964.
4. Carlson, J.R.: Studies on the Metabolism of Sodium Fluoride I. Plasma Fluoride in Relation to Dietary Fluoride in Dairy Cattle. Ph.D. Dissertation, University of Wisconsin, 1967.
5. Deutsch, H.F. and Goodloe, M.B.: An Electrophoretic Survey of Various Animal Plasmas. J. Biol. Chem., 161:1-20, 1945.
6. Heim, W. and Schechtman, A.M.: Electrophoretic Analysis of the Serum of the Chicken During Development. J. Biol. Chem., 209:241-247, 1954.
7. Ferguson, D.B.: Effects of Low Doses of Fluoride on Serum Proteins and a Serum Enzyme in Man. Nature. New Biology, 231:159-160, 1971.
8. Kaur, R., Singh. P. and Makhni, S.S.: Long-term Effects of Fluoride Administration-An Experimental Study: II Effect on Serum Proteins. Fluoride, 11:25-28, 1978.
9. Weiser, R.S., Myrvik, Q.N. and Pearscelli, N.N. In: Fundamentals of Immunology. Henry Rimpton, London, England, 1969, p. 350.
10. Tadashi, I.: Studies on the Glycogen and Phosphorylase Variations in Myocardium, Skeletal Muscle and Liver in Experimental Fluorosis. Part I. Influence of Fluorine on Glycogen. Shikoku. Acta Medica, 12:616-623, 1958.
11. Zebrowski, E.J. and Suttie, J.W.: Glucose Oxidation and Glycogen Metabolism in Fluoride-fed Rats. J. of Nutr. . 88:267-271, 1966.
12. Riekstneice, E., Meyers, H.M. and Glass, L.E.: In Vivo Effects of Sodium Fluoride on Serum Proteins and Enzymes as Studied with Starch Gel Electrophoresis. Arch. Oral Biol., 10:107, 1965.
13. Doberenz, A.R., Kurnick, A.A., Kutz, E.B., Kemmerer, A.R. and Reid, B. L.: Effect of a Minimal Fluoride Diet on Rats. Proc. Soc. Exp. Biol., 117:689-693, 1964.
14. Bogin, E., Abrams, M., Avidar, Y. and Israeli, B.: Effect of Fluoride on Enzymes from Serum, Liver, Kidney, Skeletal and Heart Muscles of Mice. Fluoride, 9:42-46, 1976.

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A FOLLOW UP STUDY OF FLUOROSIS A DECADE AFTER A CHANGE IN THE SOURCE OF DRINKING WATER

PRELIMINARY REPORT

by

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SUMMARY: An intensive survey of Bindapur Village, a decade after the inhabitants were first provided with piped drinking water (0.6 ppm) revealed that the decline in the prevalence of dental fluorosis was statistically insignificant. The study was designed to determine the incidence of skeletal fluorosis and of dental mottling in children born after 1970.

Foods high in fluoride, excessive placental transfer and its deposition in the fetus during pregnancy appears to be the source of fluoride toxicity. Fluoride intake through water and food, the hot climate, and malnutrition induced increased deposition. Evidence of dental fluorosis was observed in all twelve villages surveyed near Delhi (India) in 1963. The highest incidence (75.4%) occurred in Bindapur. Male residents suffered more than females and adults more than children.

Material and Method

A house to house survey of Bindapur Village (population 850) was conducted by means of an especially prepared questionnaire. All residents were given a thorough clinical investigation. Evidence of dental mottling, caries, and skeletal fluorosis as well as complaint of easy fatigueability, gastrointestinal disturbances, vague pains, backache, bony exostosis and stiffness of cervical or dorsolumbar joints were recorded. Patients suspected of skeletal fluorosis were subjected to radiological investigation. Dean's (2) criteria were used for recording the severity of dental mottling and Roholm's (3) classification for the radiological changes.

The fluoride content of piped water, as well as of other sources of water, milk, other food, soil, and forage was estimated by means of the Orion fluoride electrode (4) coupled to an ionometer. Incineration was done wherever necessary. Three repeat estimations were made on each sample.

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The results of the analysis are presented in Tables 1-3.

Table 1

Fluoride Content of Bindapur Water Supply

Source	Range	Average
Pipe water	0.59-0.66 ppm	0.63 ppm
Well water	1.60-2.09 ppm	1.83 ± 0.32 ppm
Hand pumps	1.40-2.10 ppm	1.80 ± 0.17 ppm
Panghat	1.20-2.50 ppm	1.85 ± 0.65 ppm

Table 2

Soluble Fluoride Content in Soil of Bindapur Village

Agri. Soil (Superficial)	14.4. µg/gm
Agri. Soil (1 ft. deep) with standing crop	12.0 µg/gm
Soil (from path between the field)	8.0 µg/gm

Table 3

F^- Content of Various Foods and Fodder at Bindapur

	Type of Food	Fluoride ppm
I Milk	Human Milk	8.45-16.86
	Buffalo Milk	12.30-18.50
	Boiled Milk	27.30 (boiled 8 hrs.)
II Cereals	Wheat Flour	0.90
	Whole Wheat	0.70-0.87
	Rice	0.96
	Pulses	0.4-0.60
	Gram	1.80
	Beans	1.00
III Vegetables	Green Leafy Mustard (spinach, etc.)	0.30, 3.2
	Onion	0.21-29
	Pudine (Mint)	12.50
	Potato	2.80-6.30
	Garlic	2.31-10.0
	Tomato	0.13-2.30
	Fodder	8.00-18.20

A preliminary survey indicated that the prevalence of dental mottling had not declined (Table 4) although a decade had elapsed since abandonment of the high fluoride source of drinking water.

Table 4

Distribution of Dental Fluorosis in General Population

	Total Population	850	
A.	Total number of cases with dental fluorosis varying between mild and severe	526	61.88%
	Adults	229	26.94%
	Children	297	34.94%
i)	Children above 8 yrs. and below 17 yrs.	169	19.88%
ii)	Children below 8 yrs.	128	15.05%

Results

For the final evaluation, the population was divided into two groups, one 17 years and above, the other below 17 years of age. The group below 17 years was further subdivided (Table 5) to evaluate the effect of the change in drinking water on teeth.

Table 5

Distribution of Severity of Dental Fluorosis By Age

	0-2 yrs.	3-4 yrs.	5-6 yrs.	7-8 yrs.	9-17 yrs.	17 & above yrs.	Total
Nil	41	36	36	24	12	43	192
	(66.13)	(46.75	(34.29)	(25.43)	(5.94)	(13.87)	(22.59)
Quest.	16	23	16	18	21	38	132
	(22.81)	(29.37)	(15.24	(19.15)	(10.4)	(12.25)	(15.53)
Mild	5	18	51	30	86	161	351
	(8.07)	(23.38)	(48.57)	(31.91)	(42.57)	(51.94)	(41.29)
Moderate	0	0	2	2]	38	48	109
			(1.91)	(22.34)	(18.81)	(15.48)	(12.82)
Severe	0	0	0	1	45	21	66
				(1.06)	(22.28)	(6.45)	(7.76)
TOTAL	62	77	105	94	202	310	850
	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)

* Figures in parenthesis indicate percentage.

Analysis of the data revealed that dental mottling was more severe in children than in adults. Even among children, those 7-8 years old suffered more than those 3-4 years of age. No difference was observed between males and females. After eliminating questionable cases, the results were compared to data on fluoride toxicity from Dabir (5), an adjacent village. Our failure to observe a statistically significant difference, suggested that the change in water supply had not affected the prevalence of dental fluorosis, especially in children born after mid 1970.

The prevalence of caries and dental mottling was also high. Children below the age group of 5-6 years (12.38%)had both caries and dental fluorosis. As the children grew older, the prevalence of both caries and fluorosis increased, so that among adults the incidence was 29.35% (Table 6).

Table 6

Distribution of Dental Caries & Fluorosis According to Age

Age in Years	No. of Persons	With Fluorosis	With Caries & Fluorosis
0-2	62	5 (8.06)	4 (6.45)
3-4	77	18 (23.37)	6 (7.79)
5-6	105	53 (50.48)	13 (12.38)
7-8	94	52 (55.32)	18 (19.14)
9-17	202	169 (83.66)	53 (26.23)
7-17	310	229 (73.87)	91 (29.35)
Total	850	526	185

Skeletal Fluorosis

Skeletal fluorosis was suspected in 12 cases, five of which were radiologically confirmed. All radiologically positive cases had osteosclerotic changes except one who had osteoporosis in some bones and osteosclerosis in others associated with multiple fractures. The biochemical investigations suggested hyperparathyroid activity.

Discussion

A decade earlier , in mid 1969, Bindapur Village, a zone of proven fluoride endemicity near Delhi (India), was first to receive piped water containing 0.6 ppm. Within a few months all villagers discontinued the use of water from other sources for drinking purposes. The anticipated marked reduction in prevalence of fluorosis in children born after mid 1970 did not materialize. In 1963, Anand et al. (1) noted dental fluorosis in 65.4% of the population of Bindapur and Bagga et al. (5) reported it in 62.7% in Dabir, a nearby village which shares subsoil water with Bindapur. In the present study, the

prevalence was 61.9%. Children below 4-5 years of age suffered less than others; in children 7-8 years old mottling was most severe (Table 5).

The presence of fluorosis in young children and animals suggests sources of fluoride other than the water supply. The analysis of milk, food stuff, and forage revealed that milk, yogurt, green vegetables, and forage were the richest sources of fluoride in this area. Daily intake of fluoride by a child ranged between 6.5-12 mg, a high dose capable of producing dental changes especially in the presence of malnutrition and poor dental hygiene. Moreover breast feeding for two years or more, which is not uncommon in this area, is one of the important factors responsible for nutritional and other deficiencies.

Ericsson et al., by using F^{18} (6), demonstrated placental transfer of fluoride ions. Zeigler (7), Armstrong (8) and others (9) reported that high intake of fluoride during pregnancy results in increased accumulation in placental tissue. Brzezinski et al. (10) and Gedalia et al. (9) observed increased deposition of fluoride in fetal femora regardless of the mother's fluoride intake.

Like calcium, we believe that there is a mechanism which influences fluoride deposition in bones, and that it is modified in favor of increased deposition by the pH of water, soil, and climate, by the total amount of fluoride ingested and by the nutritional status of the individual.

Acknowledgements

The authors are grateful to Dr. S. Chawla, Principal and Medical Superintendent, and to the staff of the Departments of Biochemistry and Physiology, Lady Hardinge Medical College and Sucheta Kripalani Hospital, New Delhi for encouragement and for providing facilities for this work.

Bibliography

1. Anand, D., Bagga, O.P. and Mullick, V.D.: Endemic Fluorosis and Dental Decay. Ind. J. Med. Res. 52:117-123, 1964.
2. Dean, H.T., Arnold, F.A. Jr., Jay, P., and Knutson, J.W.: Studies on Mass Control of Dental Caries Through Fluoridation of Public Water Supply, Public Health Report, 65:1403-1408, 1950.
3. Roholm, K.: Fluoride Intoxication, a Clinical Hygienic Study. H.K. Lewis, London, 1937.
4. Taves, D.R.: Serum Fluoride Analysis with the Fluoride Electrode. J. Lab. Clin. Med. 75:1020-1025, 1970.
5. Bagga, O.P., Mehta, S.P., Berry, K., Sarada, L. and Parkash, V.: Dental Caries and Fluoride Toxicity. Fluoride, 12:38-45, 1979.

6. Ericsson, Y. and Malmas, C.L.: Placental Transfer of Fluorine Investigated with F^{18} in Man and Rabbit. Acta Obst. and Gyn. Scand. 41:142-158, 1962.
7. Ziegler, E.: Mitt. Naturw. Ges. Winterthur 28:1-63, 1956. Quoted in W.H.O. Monograph, No. 59, 1970.
8. Armstrong, W.D., Singer, L. and Makoski, E.: Placental Transfer of Fluoride and Calcium, Am. J. Obstet. and Gyn. 197:432-434, 1970.
9. Gedalia, E., Brzezinski, A., Zukerman, H. and Myersdorf , A.: Placental Transfer of Fluoride in the Human Fetus at Low and High Fluoride Intake. J. Dent. Res. 43:669-671, 1964 B.
10. Brzezinski, A., Bercov, C.B. and Gedalia, E.: Fluorine in the Human Fetus. Obstetrics and Gynecology. 15:329-331, 1960.

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BORON AS AN ANTIDOTE IN ACUTE FLUORIDE INTOXICATION IN RABBITS: ITS ACTION ON THE FLUORIDE AND CALCIUM-PHOSPHOROUS METABOLISM

by

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K. Khelfat and M. Tabet Aoul
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SUMMARY: The kinetics of calcium, phosphorous and fluoride were studie after daily administration of 60 mg/kg fluoride to rabbits in drinking water for two months and subsequent addition of boron following a lapse of 11 and 45 days during which fluoride was discontinued.

Boron tends to increase the elimination of fluoride from the body. Fluoremia returned to normal levels in all groups on day 11. At that time, the fluoride balance was negative compared with normal levels. When boron was added the negative balance was more pronounced because of relative hyperfluoria and because of a decrease in the digestive utilization coefficient. In bones the fluoride content decreased more slowly. It remained very high on day 45 in the fluoride group, but returned to normal when boron was given.

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Calcium and phosphorous balances were normal in all cases on days 11 and 45. This was due to normalization of calcium and phosphorous digestive utilization coefficient, in the 45-day fluoride group after addition of boron.

Introduction

In 1977 Baer et al. (1) studied the use of boron as a possible antidote for fluoride. Elsair et al. (2) also observed that the simultaneous administration of boron and 60 mg/kg fluoride daily in drinking water during a two month period tends to normalize the toxicological action of fluoride upon the calcium and phosphorous balance. In subacute fluoride intoxication of 7 months' duration, boron reduces the toxic effects of fluoride upon hemostasis (2), upon the calcium-phosphorous metabolism and upon the skeletal changes . In this study, we shall investigate the effect upon fluoride, calcium and phosphorous metabolism of boron administered for 45 days following interruption of fluoride intoxication previously induced by daily doses of 60 mg/kg/day fluoride for two months.

Materials and Methods

Forty-five albino female adult rabbits with a mean weight of 2.5 kg were used in this study. Ten of the 45 served as controls (Group C). They were given tap water containing 0.46 ppm fluoride and a synthetic ration containing 2.09 ppm F. Tnirty-five animals received for two months, water containing 3.45 g/l NaF. Considering a daily water intake of about 100 ml, a rabbit weighing 2.5 kg given 60 mg fluoride a day consumed about 150 mg fluoride per day. Seven rabbits were studied during the last 4 days of intoxication (F Group), after which they were sacrificed for blood and bone assays. After fluoride was discontinued, the rabbits were separated into two groups of 14 animals each. In order to evaluate the effect of spontaneous detoxification, the first group received tap water for the following 45 days, whereupon balance studies were performed during two 4-day periods, namely between day 7 and 11 in the Fll group, and day 41 and 45 in the F 45 group. The second group (termed B group) received tap water containing borax 5.03 g/l or 23.1 mg/kg. boron a day in order to determine a possible detoxifying action of boron. The fluoride/boron ratio was thus equal to that used by Baer et al. (1). In the boron group, balance studies were performed at the same time as in groups Fll and F45, namely on day 11 and 45 (Bll and B45). In order to evaluate any possible benefit of boron, we had to compare its action in the B groups simultaneously with that of the F groups (spontaneous detoxication) at the same time. In the F group, the data presented are those prevailing during the last days of fluoride intoxication, compared to the controls (C).

In the five groups (C, Fll, Bll, F45, B45), the balance studies comprised four day periods during which food ration, feces and urine

were analyzed. Immediately after the animals were killed, blood samples were taken from the carotid arteries and tibias were dissected for fluoride analysis. Plasma and urinary fluoride were measured by means of the specific fluoride electrode after mineralization (3, 4, 5). Calcemia, phosphatemia, alkaline phosphatasemia, calciuria and phosphaturia were measured. We also determined the 24 hour urinary excretion of fluoride, calcium and phosphorous as related to the creatininuria and the phosphorous renal reabsorption coefficient (P.R.C.). Food and feces were analyzed for calcium and phosphorous after acidification and for fluoride after alkalinization. The mean daily fluoride, calcium and phosphorous retentions or losses were calculated by the formula:

$$mg/day = feed - (feces + urine)$$

The fluoride digestive utilization coefficient (D.U.C.) for fluoride, calcium and phosphorous was calculated by the formula:

$$\frac{Food - Feces}{Food} = (\%)$$

The bone fluoride content was analyzed after breaking, drying, delipidation, and complete alkalinization. The results were statistically evaluated by the STUDENT t method.

Radiograms were taken of the tibia at the termination of our experiments, after 60 days' intoxication and after 45 days' detoxification. The same technique was employed and all studies were carried out at the same time in the controls, the fluoride and the boron groups.

Results

1. Fluoride Metabolism: In the fluoride groups and those receiving boron, fluoremia was markedly elevated at the termination of the intoxication, (Fig. 1), but returned rapidly to normal. The fluoride balances (Fig. 2) were strongly positive in the fluoride group ($p<0.01$), but became negative between day 7 and day 11. This effect was more pronounced in the rabbits receiving boron (B11) than in those without (F11), ($p<0.10$). The negative fluoride balance in the B group was related to a decrease of the digestive utilization coefficient and to hyperfluoruria ($p<0.01$). These two parameters involving intestinal absorption and urinary excretion were about normal in the F group on day 11. After day 41-45 following the interruption of fluoride intake, the fluoride balances were normal in both fluoride groups F11 and F45. However, it required more time for reduction of fluoride in bone (Fig. 2) which was considerably increased in the F group ($p<0.01$). It remained high ($p<0.01$) in the two fluoride groups on day 11 and on day 45, whereas it decreased significantly in the boron group (B45) ($p<0.01$), gradually returning to normal levels.

2. Calcium and Phosphorous Metabolism: The calcium (Fig. 3) and

Figure 1
Blood Levels of Fluoride, Calcium, Phosphorous, Alkaline Phosphatase

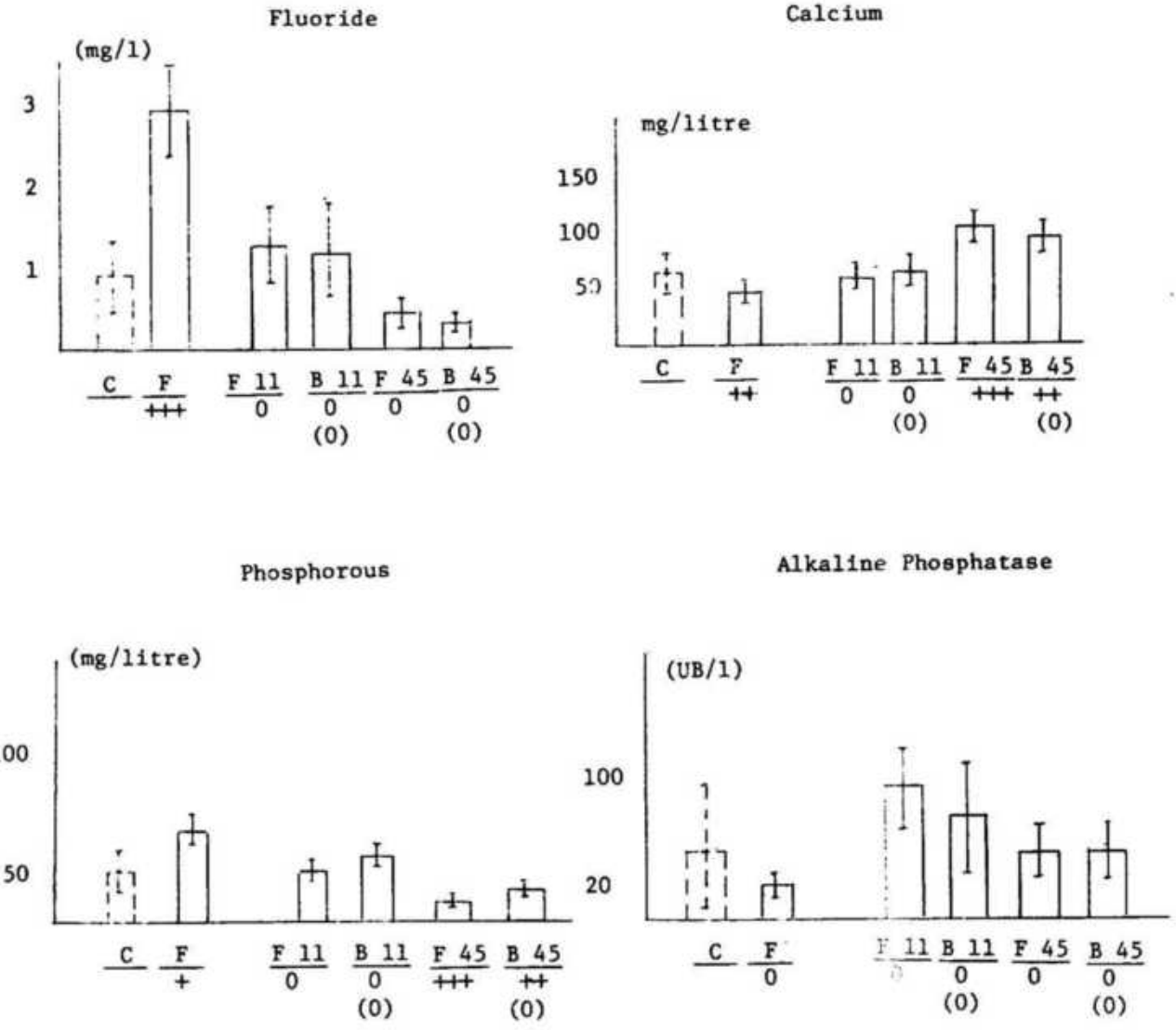
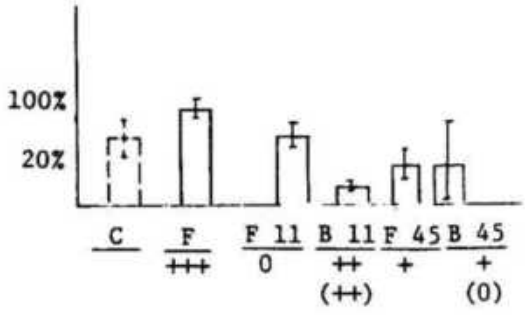


Figure 2A

Fluoride Balances
(during and 11 and 45 days after intoxication)

F Digestive Utilization Coefficient



Creatininuria

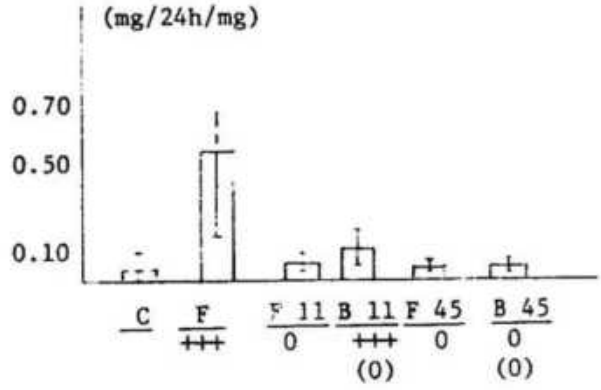


Figure 2A

Fluoride Balance
Food-(Feces & Urine)

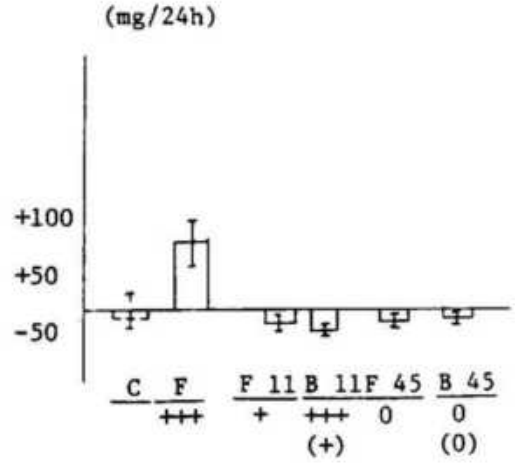
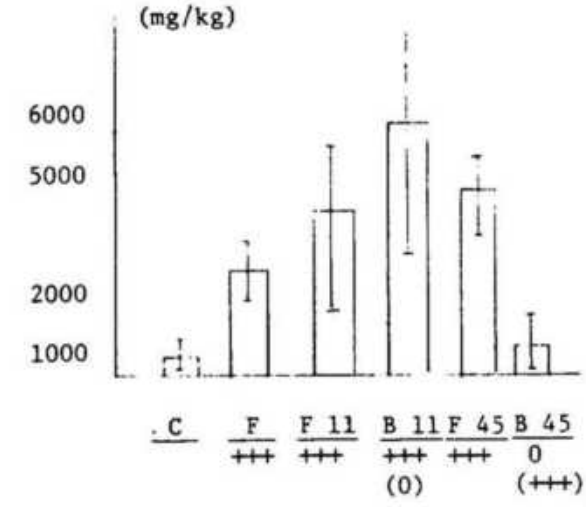


Figure 2B

F in Bone (dry)



phosphorous balances (Fig. 4) were much depressed during the fluoride intoxication (p 0.05), because of reduced absorption of calcium and phosphorous in the intestinal tract (p 0.05), whereas calciuria remained normal. The renal reabsorption coefficient for phosphorous decreased only slightly (p 0.10). After interruption of fluoride, however, the calcium and phosphorous balances returned to normal in all groups in the two time periods of 7-11 days, and of 41-45 days. But the normalization was more complete in the boron groups. The calcium balance decreased slightly in the fluoride groups (p 0.10) because of a relative hypercalciuria (p 0.05 or p 0.10), but remained normal in the B groups with normocalciuria. The phosphorous balances were normal in all groups, in spite of a reduction of the phosphorous renal reabsorption coefficient in the two fluoride groups (p 0.10 or p 0.05) corrected in the boron groups.

During fluoride intoxication the blood calcium levels decreased (p 0.05), but returned to normal before day 11 after fluoride was discontinued in groups F and B (Fig. 1). At this time, calcemia, phosphatemia and alkaline phosphatasemia were within normal limits. However, after 45 days, (group F45) a highly significant hypercalcemia and hypophosphatemia were noted (p0.01), which was partly corrected by boron in group B45 (p 0.05).

3. Radiography of Tibia: Among the animals of group F45 and B45 radiological changes were present as compared to the appearance of normal tibiae. We noted increased thickness of the cortex in the F45 group, which was somewhat less pronounced following the administration of boron (Fig. 5).

Discussion

Baer et al. (1) obtained a reduction of fluoride toxicity as demonstrated by skeletal radiography, histology of bones and by some of the plasma enzymes when they administered boron (2.7 mg/kg) together with fluoride (7 mg/kg) twice a week for one year. We carried out the same experiments with a larger dose, but the same F/B ratio, administered simultaneously daily in drinking water for seven months, (40 mg/kg/day F + 15.4 mg/kg/day B). This combination reduces the toxic effect of fluoride upon hemostasis (2), the calcium and phosphorous metabolism and skeletal radiography. Under the same experimental conditions, biological parameters, histology, and tissue respiration of liver remain unchanged in the fluoride and fluoride + boron groups, but a large dose of fluoride + boron "in vitro" reduces the toxic effect of a large dose of fluoride upon tissue oxygen consumption (6). Baer et al. believe that the beneficial effect of boron may be due to the formation of an anionic complex BF_4 in the body which is less toxic than fluoride.

In order to evaluate this hypothesis, we studied fluoride, calcium and phosphorous balances during acute fluoride intoxication with

Figure 3
Calcium Balances

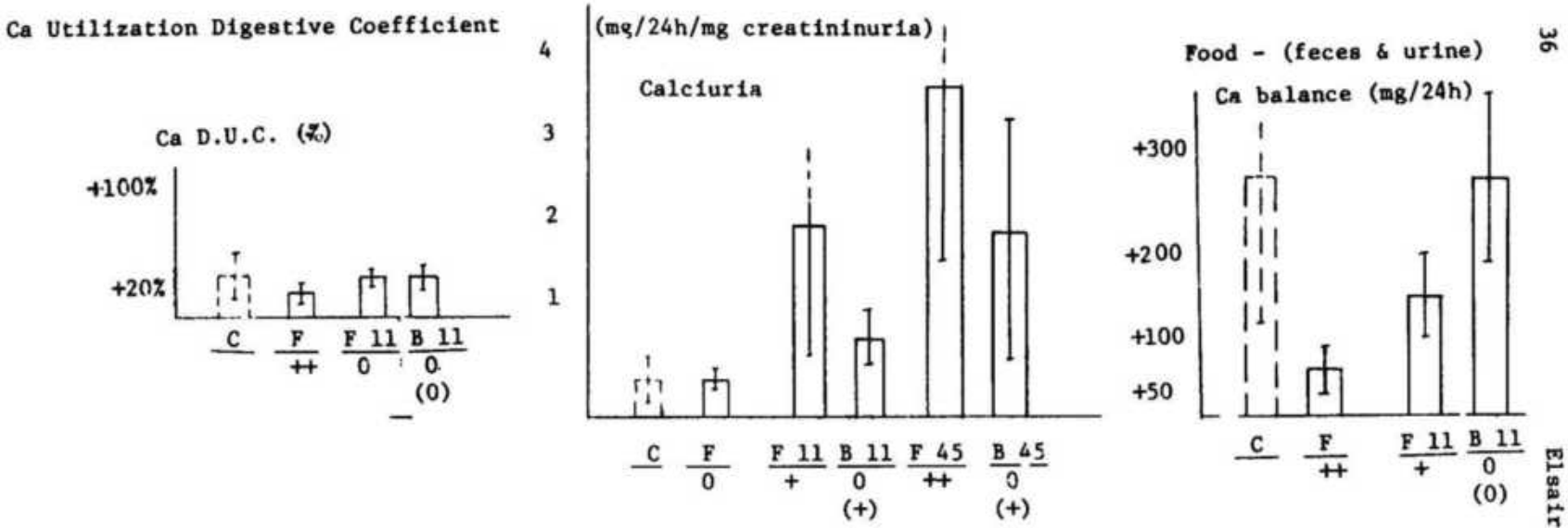
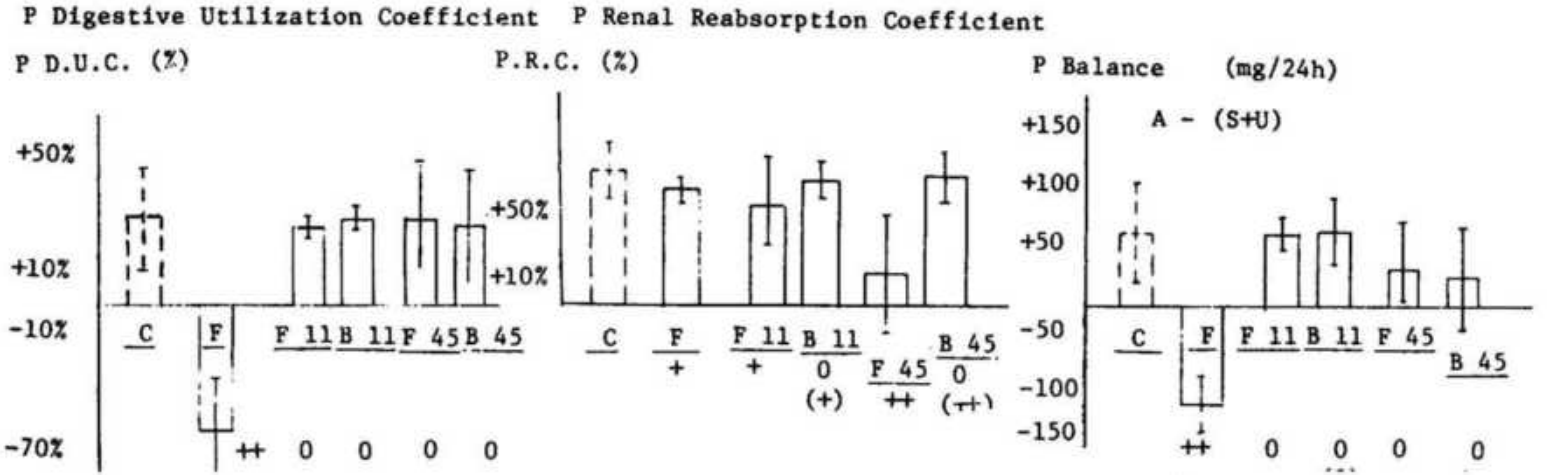


Figure 4
Phosphorous Balance



60 mg/kg per day fluoride for two months. We showed that boron, administered simultaneously with fluoride at the same fluoride / boron ratio as used by the German investigators did not alter the fluoride balance (intestinal absorption, urinary excretion, retention and bone content), as compared to fluoride alone. But the fluoride-induced negative calcium and phosphorous balances, due to decreased intestinal absorption of calcium and phosphorous and due to renal reabsorption of phosphorous was counteracted by the presence of boron. This fact is in agreement with the concept that a fluoride-boron complex is formed in the body, which follows the same metabolic pathways as fluoride alone, but shows less toxic effects.

We therefore studied fluoride, calcium and phosphorous balances under the same experimental conditions giving 60 mg fluoride/kg/day for two months. But boron in this case was administered after interruption of fluoride intoxication at the same F/B ratio. We thus investigated the possibility of a curative effect of boron. We observed, during fluoride intoxication (F group), a marked retention of fluoride due to extensive fluoride absorption in the intestinal tract, associated with hyperfluoruria, increased fluoride content of the skeleton, and hyperfluoremia. The fluoride retention subsided after fluoride was discontinued with and without boron. According to literature (8) fluoremia promptly returns to normal prior to day 11.

Fluoride balances were negative at first, namely on days 7-11,but with boron, the negative fluoride balance was more pronounced (Fig. 2) because of decreased intestinal absorption and of reduced reabsorption of fluoride and because of hyperfluoruria. On days 7-11, boron seems to increase the fluoride excretion through the intestinal tract and through the kidney. Later, on day 45, fluoride elimination from bones returned to normal levels in the rabbits receiving boron, but remained high without boron (Fig. 2). It is therefore likely that a fluoride-boron complex is more readily eliminated from the body than fluoride by itself.

Boron seems to correct the effect of fluoride on the calcium-phosphorous metabolism. Calcium and phosphorous balances declined during fluoride intoxication (F group), because of a marked reduction in calcium and phosphorous absorption in the intestinal tract and because of a slight decrease of renal reabsorption of phosphorous (Figs. 3 & 4). Hypocalcemia, present during fluoride uptake, returns to normal after its discontinuance (Fig. 1). The calcium and phosphorous balances tend to return to normal levels without boron, in spite of hypercalciuria and reduced renal reabsorption of phosphorous. All these values are corrected by boron (Figs. 3 & 4).

In the fluoride group, there is some evidence of a possible secondary hyperparathyroidism, as related by some authors (8, 9 ,10) which contributes further to the toxicity of fluoride (11), with hypercalcemia, hypophosphatemia and a reduction of phosphorous renal reabsorption. These values are partially corrected by boron(Fig.1 and Fig.

4). During detoxification, boron tends to induce mobilization of fluoride from bones. By means of greater intestinal absorption of calcium, boron reduces the effect on the parathyroid glands with greater economy of the calcium-phosphorous output. The skeletal radiography, which is due to fluoride-induced osteosclerosis and hyperparathyroidism - two antagonistic mechanisms - improves and becomes partially corrected by boron (Fig. 5).

It is concluded that boron, in drinking water, should be considered a possible antidote when administered at the proper F/B ratio following termination of acute fluoride intoxication. Boron enhances the sequestration of fluoride from bones, its excretion through the kidneys and, possibly, from the intestinal tract. Boron also corrects the radiographic changes of the skeleton and the seconday effect of fluoride on the calcium-phosphorous metabolism. Additional experimental studies concerned with the preventive and curative effect of boron in chronic fluoride intoxication are desirable, particularly its effect as a possible antidote upon different organ systems.

Bibliography

1. Baer, H.P., Bech, R., Franke,J., Grunewald, A., Kochmann, W., Melson, F., Runge, H. and Wiedner, W.: Ergebnisse tierexperimenteller Untersuchungen an Kanienchen mit Natriumfluorid unter Einwirking von Gegenmitteln. Ztschr. Ges. Hyg. Grenzg. 23:14-20, 1977.
2. Elsair, J., Merad, R., Denine, R., Reggabi, M., Benali, S. and Alamir, B.: Effects of a Subacute Fluorine Intoxication of Several Months on Hemostasis in Rabbits in the Presence and Absence of an Antidote (Boron). Fluoride, 12:136-143, 1979.
3. Baker, H.L.: Determination of Fluoride in Vegetation Using the Specific Ion Electrode, Analyt. Chemistr. 44:1366-1377, 1972.
4. Frant, M.S. and Ross, J.W.: Use of a Total Ionic Strength Adjustment Buffer for Electrode Determination of the Fluoride in Water Supplies. Analyt. Chemistr. 40:1169-1171, 1968.
5. Collembel, C., Bureau, T. and Cotte, J.: Dosage des fluorures dans les eaux et les urines au moyen d'une électrode spécifique. Ann. Pharma. Franç. 29:541-552, 1971.
6. Elsair, J., Merad, R., Denine, R., Reggabi, M., Alamir, B., Benali, S., and Khelfat, K.: Effect of Fluorine and an Antidote (Boron)on Respiration of Liver Tissue in Rabbits. Fluoride, 12: 172-176, 1979.
7. Heraud, G. and Roux, E.: Metabolisme du fluor, Med. et Nutr. 9: 17-31, 1973.
8. Poey, J., Elsair, J., Morgan, P., Reggabi, M. and Hattab, F.: Evolution du bilan biologique en fonction du stade radiologique chez une population vivant dans une zone d'endémie fluorée du sud Algérien. Europ. J. Toxicol. 9:179-186, 1976.
9. Elsair, J., Poey, J., Reggabi, M., Hattab, F., Benouniche, N. and Spinner, C.: Effects de l'intoxication fluorée subaigue du lapin sur les metabolismes fluoré et phosphocalcique et sur la radiographie du squelette. Europ. J. Toxicol. 9:429-437, 1976.
10. Teotia, S.P.S., Teotia, M., Singh, R.K., Teotia, N.P.S., Taves, D.R., Heels, S. and D'Mello, V.P.: Plasma Fluoride, 25 Hydroxicholecalciferol , Immunoreactive Parathyroid Hormone and Calcitonin in Patients with Endemic Skeletal Fluorosis. Fluoride. 11:115-119, 1978.
11. Waldbott, G.L., Burgstahler, A.W. and McKinney, H.L.: Fluoridation, The Great Dilemma. Coronado Press Inc., Lawrence, Kansas, 1978.

METABOLISM OF FLUORINE: REPORT OF A MEETING

by

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Szczecin, Poland

On May 25 - 26, 1979, the Polish Biochemical Society held a symposium on Metabolism of Fluorine at Szczecin, Poland. It was attended by some 150 scientists from various scientific centers in Poland and abroad. Nine papers and fifty-three poster presentations made up the program. The inaugural lecture was delivered by Z. Machoy, Ph.D., Szczecin, entitled "The Influence of Fluorine Compounds on the respiratory Chain."

The first session dealt with analysis and biochemistry of fluorine.

A.Marut of Jelenia Góra discussed the fluoride content of body fluids and of other biological material as determined by the photocolorimetric method. A paper by E. Kun, San Francisco, presented data on the application of erythrofluorocitrate as a probe of enzymatic and transport processes in mitochondria, and a paper by S. Kahl, Cracow, dealt with the influence of fluoride on the physiology of the thyroid gland.

The poster presentations in this section chaired by M. Gumińska, Cracow, pertained to methods of fluoride determination in biological material, to the action of fluorides on electrolyte components in the organism, the influence of fluorides on non-electrolyte but non-enzymatic substances and the influence of fluorides on the enzyme systems.

The methods of fluoride assays in common use were discussed as well as those in the experimental stage. Evidence was presented that there are different pools of fluoride in the organism which require separation and individual studies. It was concluded that in certain areas fluoride is present in both soft and hard tissue and in cellular elements from which fluorides can be released into the surrounding biological fluids. Special attention was given to the effect of fluoride on cations, particularly on iron.

In vitro studies have usually been performed with doses of fluoride which exceed many times those encountered under physiological conditions. Some participants asserted that fluoride has a favorable effect upon the circulation in atherosclerosis and on the state of dentition, whereas others warned about evidence of a possible carcinogenic effect of fluoride in the human organism.

With respect to the biochemistry of fluoride, the magnitude of fluoride in breeding animals appears to be related to their water re-

quirements. Under certain conditions, levels up to 200 mg per 24 hrs. have been eliminated in the urine without permanent ill-effect. Airborne fluoride compounds affect the mucous membranes of the upper respiratory tract both in humans and in domestic animals. It was suggested that minor complaints of persons exposed to airborne fluoride should be heeded and that guidelines for appropriate biochemical investigations be established.

The possibility of individual susceptibility to adverse effects of fluoride was emphasized. Many people residing in fluoridated areas are exposed both to atmospheric fluoride at work, and to fluoride in drinking water.

The second section "Prophylaxis and Therapy with Fluorine Compounds" included four papers, the first two by Z. Janczuk et al. of Szczecin and by N. Wigdorowicz of Makowerowa, Wroclaw. They related their experience with an 8-year trial of fluoridation.

Dr. T. Zuk of Szczecin discussed the therapeutic application of fluoride compounds in diseases of the locomotor system, Dr.K.Zielinski et Z. Machoy, Szczecin, the application of fluororganic compounds as a blood substitute.

The poster presentations in this section covered data by dentists who felt that a daily dose of 1 mg of sodium fluoride would not lead to adverse effects. However this view was not shared by other participants. There was unanimous agreement concerning the fact that water fluoridation delays dentition in children. Some felt that fluoridation may affect the natural environment. Others expressed the view that our knowledge of the metabolism of fluoride is still very limited and recommended caution in its therapeutic application. The absence of pathological findings does not justify the widespread administration of fluoride.

The solubility of fluoride is largely dependent on the pH of the medium. It was emphasized that the effects of so-called physiological levels of fluoride in serum and in the blood cell should be determined. The Szczecin Medical Center is the sole area in Poland where the effect of fluoride for treatment of otosclerosis is being investigated. Favorable results are reported. The fluoride dose of enteric-coated tablets manufactured by Polfa Pharmaceutical Company is 40 mg a day. It was found that fluoride affects lysine oxidase which contains copper and takes part in the formation of cross-bindings of collagen.

The third section dealt with the toxicology of fluoride. Two papers and twenty-two poster presentations dealing with the toxicology of fluoride made up this section. It was introduced by Dr. K. Jacyszyn

of Wroclaw with an article entitled "Fluororganic Compounds of Fluoride: Their Biological Role and Toxicity" and by T. Dziubek of Poznan on the toxicity of fluorine compounds for living organisms.

These presentations covered the effects of fluoride in industry on the natural environment of man, particularly with respect to fluoride emission from factories. Data on large doses of fluoride were presented with respect to their action on bones in animals and humans. The possibility of adverse effects in children of parents exposed to atmospheric fluoride were discussed.

There are wide differences in the degree of tolerances from person to person. Convulsions, paresis and paralysis occur in chronic fluoride poisoning. The action of inorganic fluorides is different from that of organic fluorides. Antimetabolites block the metabolic cycles. As analogues they display a positive pharmacological effect.

Enzymatic inhibition induced by fluorides is largely dependent on pH of the medium which changes the biochemical properties of this element. The synergism and antagonism of fluoride with other elements requires further studies.

The transactions of this meeting will be published by the Szczecin Scientific Society. Several commercial products such as fluoride preparations, toothpastes, and reagents for analysis were on display at the meeting.

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AN ANALYTICAL STUDY CONCERNING THE SIGNIFICANCE OF THE FLUORIDE CONTENT OF KIDNEY STONES

by

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Karl-Marx-Stadt DDR

(Abstracted from Urologe 17: 207-210, 1978)

The authors determined the fluoride content of 50 urinary calculi obtained in Karl-Marx-Stadt (KMS), a community artificially fluoridated for 17 years, and in the nonfluoridated city of Jena. The fluoride content of the municipal water in KMS was 1.0 ppm and that of the city of Jena 0.25 ppm. The calculi were classified according to their composition into 5 groups: Whewellite (calcium oxalate-monohydrate), Weddellite (calcium oxalate dehydrate), carbonate apatite, struvit and

uric acid stones. In addition 10 cystin stones were assayed for fluoride. From each community, thirty-five 24-hour urine specimens were analyzed. The degree of crystallization and the crystal lattice was related to the fluoride content.

Results:

The 24 hour urinary fluoride concentrations in the 35 specimens from the two cities averaged 0.794 ± 0.155 mg/liter and 0.470 ± 0.31 mg/liter respectively. The average fluoride content of 70 kidney stones in fluoridated KMS was 1.1 mg/gm, as contrasted to 0.644 mg/gm of the stone substance in the nonfluoridated town. The difference in the fluoride content of the urine between the two areas was 41.4%, that of the fluoride content in the kidney stones was 45%.

Accumulation of fluoride in the kidney stones depended on their composition. The two calcium oxalate stones showed distinct increases in fluoride: Whewellite contained 807.8 µg/g fluoride in nonfluoridated Jena and 2005.0 µg/g fluoride in fluoridated KMS. The difference in the fluoride content of Weddellite was 959.3 mg/g versus 2103.0. The degree of crystallization was also dependent on the fluoride content of the stone.

Increased fluoride content of the stones was associated with a characteristic contraction of the crystal lattice.

Comparison of the fluoride content of Whewellite stones with that in other large cities in East Germany revealed 62.6 in fluoridated KMS, whereas 50.3 was the average found in fifteen other nonfluoridated cities.

*****

EFFECTS OF A SINGLE DOSE OF FLUORIDE ON CALCIUM METABOLISM

by

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Aarhus, Denmark

(Abstracted from the Calcif. Tiss. Res. 26:199-202, 1978)

The authors administered 60 mg sodium fluoride (27 mg fluoride) to 14 subjects and determined the change in the levels of fluoride, calcium, phosphates and immunoreactive parathyroid hormones.

To 5 normal subjects 23-38 years old, fluoride was administered

in the morning after 8 hours of fasting. Blood samples were taken before and 1, 2, and 24 hours after the fluoride intake. The same procedure was followed in 9 nonfasting individuals 22-28 years old, after their usual lunch one hour before beginning the investigation.

The findings did not differ in the two groups with the exception that a greater increase in serum fluoride concentration occurred in the fasting group. The fluoride concentration in serum increased considerably within the first hour after which it decreased. Three hours later the fluoride level was still significantly above normal and the serum calcium had decreased; after twenty-four hours, both had returned to the normal range.

A nonsignificant decrease in the concentration of serum phosphate paralleled that of calcium. The parathyroid hormone concentration increased during the first three hours but after 24 hours it likewise had returned to normal.

The authors concluded that since fluoride, calcium and phosphate are removed from the liquid phase of the living body, under intensified mineral formation fluoride is likely to induce mineralization. The low serum calcium phase increased the secretion of the parathyroid hormone and induced mobilization of minerals from the bones, which may "obscure or reverse an impression of bone mineralization."

Three hours after the administration of fluoride, the parathyroid hormone was at its highest measured value and the serum calcium at its lowest. The authors stress the significance of the lapse of time between fluoride administration and collection of blood when studying the effect of fluoride therapy.

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TOTAL FLUORIDE INTAKE OF INFANTS

by

L. Singer and R. Ophaug

(Abstracted from Pediatrics 63:460-466, March, 1979)

In view of the dissenting views in the literature on the maximum safe daily dosage of fluoride in infants up to six months of age, the authors determined the fluoride content of commercially prepared infant foods processed with fluoridated and nonfluoridated water. They obtained samples of the major infant foods and milk formulations and calculated the minimum and maximum total daily fluoride intake by infants six months old and less.

The fluoride content of these foods was not related consistently to the levels in the water used for processing. Meat products averaged 0.14 to 0.43 ppm fluoride except for chicken, the mean fluoride content of which was as high as 5.29 ppm. In vegetables the fluoride levels were between 0.02 to 0.67 ppm. However, in a given vegetable processed at different plants fluoride levels varied up to twentyfold; they were not related to the level of fluoride present in the water used for processing. The average fluoride content of fruits was approximately 0.05 ppm, considerably less than that in vegetables and in meats.

However, the level of fluoride in the water used in processing dry cereals, fruit juices and milk definitely influenced the product's fluoride levels. Cereals processed in plants which were using fluoridated water contained approximately 4 to 6 ppm as contrasted with less than one half this amount in cereals prepared in plants using nonfluoridated water. In rice cereal, the highest values were obtained, namely 6.35 ppm, in plants where fluoridated water was used and 2.11 ppm where the water was nonfluoridated. Similarly, when fluoridated water is used in processing fruit juices their fluoride content is increased up to twentyfold.

With respect to milk, commercially prepared formulas prepared with fluoridated water contributed materially to the total daily fluoride intake of infants. Four ready-to-feed milk formulations prepared with fluoridated water contained an average of 0.67 ppm in contrast to an average of only 0.15 ppm in milk processed with nonfluoridated water.

On the basis of these data the authors calculated the total daily fluoride intake by an infant consuming processed food, milk and juices prepared in a fluoridated plant. The total fluoride intake was estimated to be 0.633 mg per day at two months of age and 0.763 at six months or, 0.127 mg/kg at two months and 0.094 mg/kg at six months expressed in terms of mgF/kg of body weight. The higher values for milk formulas may be offset by lower values for vegetables and fruit.

With cereal, milk and fruit juices not prepared with fluoridated water the comparative figures are 0.01 mg/kg at two months 0.015 mg/kg and 0.019 mg/kg at four and six months of age. If ready-to-feed milk formulas processed in a nonfluoridated plant are substituted for human or cows milk the calculated fluoride intake would be approximately 0.025 mg/kg at two, four and six months of age. The authors concluded that use of fluoridated water in processing may significantly increase the total fluoride intake of infants up to six months of age.

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URINARY FLUORIDE CONCENTRATION IN 6-9 YEAR OLD CHILDREN DRINKING WATER WITH A VERY LOW FLUORIDE LEVEL

by

I.L. Shannon and D.M. Sanders
Houston, Texas

(Abstracted from Caries Res. 13:18-22, 1979)

The authors studied the relationship of fluoride intake from drinking water to urinary excretion of fluoride. They collected 623 mid-morning urine specimens from children 6-9 years of age, in a residential area the drinking water of which showed a mean of 0.024 ppm (55 samples).

The creatinine levels of each urine sample was also measured as a reference measurement on which to base the levels of other constituents. No differences were found in the urine concentrations of creatinine due to sex, age, or years of residence. Since the creatinine levels agreed with those of the urinary fluoride, only the data for unadjusted fluoride were presented.

The urinary fluoride concentration ranged from 0.37 to 0.53 ppm with a mean of 0.42 ppm (S.D. = 0.22). No significant differences in the urinary fluoride were found with respect to sex and age. However a significant effect was attributed to the duration of residence of children in the community: those who had resided in the area for less than one year showed a significantly higher urinary fluoride output.

In the discussion, the authors referred to conflicting observations in the literature on the water fluoride - urinary fluoride relationship. Gedalia reported in 1958 that the younger the child the lower the urinary fluoride and the greater the fluoride storage in the system. In the present study, the water fluoride mean (0.024 ppm) was less than 6% of the urinary fluoride mean of 0.42 ppm. It was therefore concluded that much of the excreted fluoride is derived from sources other than drinking water.

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Correction: The English version of the article The Effect of Fluorine and Lead Ions on the Chromosomes of Human Leukocytes in Vitro by Jachimczak, Danuta and Bogumila Skotarczak(abstracted 12:212, 1979) appeared in Genetica Polonica, 19:353-358, 1978.

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