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EDITORIAL REVIEW

PHENOMENAL CANCER MORTALITY RATES AMONG DANISH CRYOLITE FACTORY WORKERS

Grandjean, Juel, and Jensen have recently reported (1) that, compared with standardized national mortality rates for the population of Denmark, or even for Copenhagen, a cohort of 425 male workers in a Copenhagen cryolite factory showed marked excess death rates for respiratory cancers, violent deaths (suicide and accidental), pulmonary and other known causes of death, but relatively few or none for cardiovascular diseases (Table 1, Col. 1-4). Their results are summarized in Col. 4 in terms of conventional standardized mortality ratios (SMR = [observed/expected] deaths), where an SMR greater than unity indicates a positive excess relative to the standard population employed, within a chosen confidence level of 95% based on a Poisson distribution.

Table 1

Mortality 1941 - 1981 in 425 male cryolite workers employed in Copenhagen six months or more between 1924 and 1961

	No. of Deaths		Standardized Mortality		
cause of death	Observed	Expected*	Ratio (SMR) Obs/Exp#	Difference (SMD) Obs-Exp	SMD per 100,000 workers per year +
Cancer	54	37.9	1.43	16.1	94.7
Respiratory	27	11.4	2.37	15.6	91.8
Urogenital	7	5.9	1.19	1.1	6.5
Other	20	20.6	0.97	-0.6	-3.5
Violent	27	12.5	2.15	14.5	85.3
Suicide	13	5.6	2.34	7.4	43.5
Accidents	14	6.9	2.03	7.1	41.8
Pulmonary	15	8.8	1.70	6.2	36.5
Cardio-vascular	72	69.5	1.04	2.5	14.7
Other known	31	20.6	1.50	10.4	61.2
Unknown	7				
TOTAL	206	149.3	1.38	56.7	334

*Expected numbers computed from national rates for Denmark as standardizing population.
#Given for 95% confidence limits.
+Calculated for 1941 - 1981 (40 years), e.g., (SMD X 100,000)/(425 X 40).
Data in Col. 5 and 6 re-express data in Col. 2 to 4 and thus have equivalent confidence limits.

A fundamental defect in the widely used SMR is that it does not, of itself, give a direct indication of the absolute numbers of excess deaths involved -e.g., whether 10, 1000, 100,000 etc., from which usual mortality rates in terms of excess deaths per unit of population per unit time would be directly evident.

A better perspective is given, however, by the standardized mortality differences (SMD = [observed - expected] deaths), which state directly the absolute number of excess deaths in terms of excess deaths per unit of population per unit time, as here provided in Table 1, Col. 5-6. Since these SMD values are, like the SMR values in Col. 4, based on the identical observed and expected values in Col. 2-3, they are of equivalent statistical merit. Due consideration of these SMD values, which was not undertaken in the paper cited (1), greatly alters some of the important conclusions and emphases therein.

The excess deaths among the Copenhagen cryolite workers can fairly be described as overwhelmingly devastating. Thus, the total excess deaths given in Col. 6 - 334 per 100,000 cryolite factory workers per year - is about one half of the observed total deaths from all causes per 100,000 population per year in Denmark, averaged over the 40-year period 1941-1981. Not all of these 334 excess deaths were statistically significant as such, but the excess cancer and violent deaths were regarded in the paper (1) as clearly statistically significant. The sum of these, as indicated in Col. 6 to be 180 per 100,000 cryolite factory workers per year over the 40-year average, are thus, by themselves, about equal to a quarter of the total mortality rate for Denmark; and, for cancer by itself, 94.7 (Col. 6), about one eighth. Such an average increase in cancer death rate - about 12% per year - can certainly be described as catastrophic when linked with one known cause, namely working in a cryolite factory. Table 2 re-expresses the data in Ref. 1, Table 2 in terms of excess SMD.

Table 2

Mortality 1941-1981

Class	(A)	(B)	(C)#
No. of workers	184	241	425
Cause of death			
Cancer	119.4	76.8	94.7
Respiratory	122.3	68.5	91.8
Urogenital	10.9	3.1	6.5
Other	-14.9	5.2	-3.5
Violent	157.6	30.1	85.3
Suicide	99.2	1.0	43.5
Accidents	58.4	29.0	41.8
Pulmonary	69.3	11.4	36.5
Cardiovascular	25.8	6.2	14.7
Other known	31.3	84.0	61.2
Unknown			7
TOTAL	486	217	334

Recapitulated from Col.6, Table 1.

(A) 184 cryolite workers between 1924-1939 alive in 1941.

(B) 241 workers employed between 1945-1960 followed up from first day of employment (Ref. 1, Table 2) re-expressed here in terms of excess deaths (SMD) per 100,000 cryolite workers per year over an average of forty years.

The enormity of the excess cancer death rate (C.D.R.) of 94.7 C.D. per 100,000 workers per year can be still better appreciated when compared with American studies (2) showing an approximately equivalent SMD one-hundredth as great (0.75 excess C.D. per 100,000 population per year, see Table 3, next to last line), for the ten largest fluoridated cities (population ca. 11,000,000) compared to the ten largest comparable nonfluoridated cities (population ca. 7,000,000), reported for the 20-year period 1950-1970, with high statistical significance. Table 3 cannot be analyzed in further detail here, beyond its immediate derivation (Tables 4-6). (The value of SMD/year of 1.09 instead of 0.95 results from additional consideration of the prefluoridation period, which was not involved in the Danish studies).

Although the Danish workers did not regard the excess deaths found for pulmonary and other known diseases as "statistically significant" it is to be noted that the reported SMR values for both these assigned causes of death

Table 3

Age-Sex-Race standardizations of time trends of human cancer death rates in the ten largest fluoridated (F) and ten largest comparable nonfluoridated (NF) American central cities, by indirect method of simultaneous adjustment, with U.S. 1940 population as standard.*

Period:	Prefluoridation (PF)			Fluoridation (F)			Net difference, (F - PF)		
Year:	1940	1950	Difference per year = Slope per unit time	1950	1970	Difference per year = Slope per unit time	Per Year	Per 20 years	% of CDRo
Fluoridated (F)									
CDRo (Observed)	154.2	181.8	2.76	186.3	222.6	1.815	-0.945	-18.9	
CDRo (Expected)	123.2	141.2	1.80	141.2	165.9	1.235	-0.565	-11.3	
CDRo - CDRe (=SMD)	31.0	40.6	0.96	45.1	56.7	0.580	-0.380	- 7.6	
CDRo/CDRe (=SMR)	1.252	1.288	0.0036	1.319	1.342	0.00115	-0.00245	- 0.049	
Nonfluoridated (NF)									
CDRo (Observed)	153.5	181.3	2.78	183.6	188.8	0.26	-2.52	-50.4	
CDRe (Expected)	135.7	150.5	1.48	150.5	159.1	0.43	-1.05	-21.0	
CDRo - CDRe (=SMD)	17.8	30.8	1.30	33.1	29.7	-0.17	-1.47	-29.4	
CDRo/CDRe (=SMR)	1.131	1.205	0.0074	1.220	1.187	-0.00165	-0.00905	- 0.181	
F - NF									
CDRo (Observed)			-0.02			1.555	1.575	31.5	100.0
CDRe (Expected			0.32			0.805	0.485	9.7	30.8
CDRo - CDRe (=SMD)			-0.34			0.750	1.090	21.8	69.2
CDRo/CDRe (=SMR)			-0.0038			+0.00280	0.0066	0.132	

*Divided into the conventional ten age groups, each for white male, white female, nonwhite male, nonwhite female, a total of forty groups, based on rates measured during a base-line prefluoridation period of 1940 to 1950, and a fluoridation period of 1953 to 1968, with linear least square regression analysis to calculate trends over the indicated periods and with extrapolations therefrom to 1950 and 1970, to permit direct comparison with reports by others choosing these dates corresponding to censal years.

(SMR = 1.70 and 1.50, resp.) were indeed greater than for cancer (SMR = 1.43, Col. 4) where the excess was assigned statistical significance; we have here another illustration of the inadequacy of the SMR per se, without due consideration of absolute as well as relative numbers.

Which aspect or aspects of working in a cryolite factory resulted in the high excess cancer rates is still uncertain, and a question to which Grandjean, Juel and Jensen devoted considerable attention. Cryolite, Na_3AlF_6, contains 54.3% fluorine and 12.9% aluminum, both of which are potential and indeed demonstrated agents of chemical carcinogenesis. However, virtually all of the carcinogenesis, reported as mortality, took place in the respiratory tract (Table 1, first and second data lines), so that solid carcinogenesis from inhaled dust may be the primary agent, similar to solid carcinogenesis from asbestos. Inhaled cryolite dust, whether soluble or insoluble in pulmonary tissue, could certainly in principle act as a carcinogenic irritant. Grandjean (3) had earlier indicated that "occupational fluoride exposures result in much higher intake rates than does ingestion of fluoride in drinking water," possibly a daily uptake of several milligrams of fluoride; and that, in the cohort here studied (1) there had been "at least 74 cases of skeletal fluorosis."

With regard to "the safety of fluoridation of drinking water" Grandjean, Juel, and Jensen naively relied mainly on the "WHO-IARC Monograph 27"(4) and stated, "During the last decade, many studies have compared cancer mortality in countries and cities with fluoridation with that in comparable areas without fluoridation. These studies have not identified any risk for all cancer

Table 4

Average cancer death rates (CDR = cancer deaths per 100,00 population per year, weighted per person) in the ten largest fluoridated (F) and ten largest comparable nonfluoridated (NF) American central cities for the prefluoridated epoch 1940-1950 and the fluoridated epoch 1953-1968.

Year Prefluoridation epoch	Fluoridated cities (F)*	Nonfluoridated cities (NF)+	Difference
1940	155.6	159.9	-4.3
1941	156.3	154.5	1.8
1942	158.3	154.7	3.6
1943	162.4	159.8	2.6
1944	164.2	163.2	1.0
1945	168.9	167.0	1.9
1946	171.8	169.9	1.9
1947	173.9	175.0	-1.1
1948	174.3	177.8	-3.5
1949	181.1	180.4	0.7
1950	180.8	179.0	1.8
Linear least square regression analysis:			
Mean/year	168.0	167.3	0.7
Slope/year	2.76	2.78	-0.02
r, correl. coeff.	0.99	0.952	0.0155
P(from r-P tab.)	<0.001	<0.001	>>0.05
1940	154.2	153.5	0.7
1950	181.8	181.3	0.5

Year Fluoridation epoch	Fluoridated cities (F)*	Nonfluoridated cities (NF)+	Difference N - NF
1953	190.2	185.9	4.3
1954	192.3	182.6	9.7
1955	193.9	186.1	7.8
1956	201.6	187.6	14.0
1957	204.5	185.2	19.3
1958	199.7	184.3	15.4
1959	201.0	188.8	12.2
1960	205.8	185.0	20.8
1961	206.0	185.7	20.3
1962	204.6	183.8	20.8
1963	208.6	184.8	23.8
1964	208.7	184.8	23.9
1965	212.5	187.0	25.5
1966	218.5	188.2	30.3
1967	218.4	190.1	28.3
1968	219.7	191.1	28.6
Linear least square regression analysis			
Mean/year	205.4	186.3	19.1
Slope/year	1.81	0.26	1.55
r, correl. coeff.	0.959	0.535	0.947
P(from r-P tab.)	<0.0001	0.04	<0.0001
1950‡	186.3	183.6	2.7
1970‡	222.6	188.8	33.8

*Washington (1952), Baltimore (1952), San Francisco (1952), Pittsburgh (1952-1953), Milwaukee (1953), Philadelphia (1954), Buffalo (1955), St. Louis (1955), Cleveland (1956) and Chicago (1956); dates refer to year fluoridation initiated.

+Atlanta, Boston, Cincinnati, Columbus, Kansas City (Mo), Los Angeles, Newark, New Orleans, Portland, Seattle

‡These extrapolations of regression values to 1950 and 1970 are given to provide direct comparison with census--year values reported by other investigators.

Table 5

Standard cancer death rates in U.S.A. in 1940, per 100,000 population, with respect to age, sex, and race.

Age (years)	White males	White females	Nonwhite males	Nonwhite females
0–4	5.2	4.6	2.1	3.1
5–14	3.2	3.1	1.9	1.6
15–24	6.3	4.5	4.3	6.3
25–34	11.9	20.5	11.6	39.4
35–44	38.3	78.3	47.0	122.4
45–54	133.3	198.3	156.4	267.2
55–64	357.1	385.4	291.9	376.6
65–74	759.5	677.1	445.0	486.1
75–84	1320.3	1080.5	532.2	403.7
85+	1569.9	1384.5	499.1	608.2

Table 6

Age-sex-ethnic structures (per cent in the ten fluoridated and ten nonfluoridated U.S. central cities, in three census years, in 100,000 of population

Age	Ethnic group	Fluoridated 1940		1950		1970		Nonfluoridated 1940		1950		1970	
		M	F	M	F	M	F	M	F	M	F	M	F
0–4	W	3.045	2.937	4.457	4.279	2.413	2.313	1.418	1.372	2.486	2.396	2.005	1.914
	NW	0.446	0.446	1.047	1.042	2.002	1.995	0.208	0.206	0.467	0.450	1.056	1.053
5–14	W	6.655	6.476	6.199	6.062	5.165	4.992	3.032	2.998	3.334	3.264	4.278	4.129
	NW	0.956	0.983	1.420	1.451	4.557	4.584	0.448	0.459	0.621	0.631	2.236	2.238
15–24	W	8.269	8.781	6.521	6.972	5.470	5.954	3.742	4.105	3.502	3.824	4.729	5.158
	NW	0.902	1.086	1.302	1.593	3.163	3.775	0.460	0.550	0.572	0.697	1.608	1.917
25–34	W	8.459	9.031	8.179	8.671	4.196	4.037	4.026	4.340	4.398	4.651	3.557	3.415
	NW	1.053	1.161	1.744	2.031	2.279	2.812	0.516	0.601	0.759	0.872	1.247	1.462
35–44	W	7.598	7.735	7.386	7.966	3.477	3.512	3.675	3.838	3.958	4.299	2.891	2.930
	NW	1.074	1.060	1.559	1.669	2.071	2.512	0.491	0.536	0.695	0.748	1.007	1.200
45–54	W	7.057	6.791	6.511	6.820	4.094	4.710	3.243	3.311	3.466	3.720	3.096	3.457
	NW	0.789	0.698	1.215	1.137	1.832	2.090	0.351	0.333	0.516	0.515	0.859	1.001
55–64	W	4.426	4.379	5.405	5.471	3.908	4.738	2.228	2.412	2.735	3.002	2.591	3.123
	NW	0.372	0.342	0.631	0.584	1.263	1.441	0.187	0.173	0.282	0.277	0.611	0.716
65–74	W	2.218	2.593	2.865	3.298	2.512	3.622	1.215	1.435	1.612	2.040	1.622	2.437
	NW	0.171	0.194	0.302	0.337	0.748	0.900	0.088	0.095	0.159	0.178	0.354	0.511
75–84	W	0.663	0.897	0.896	1.274	1.176	1.963	0.402	0.563	0.560	0.848	0.756	1.389
	NW	0.036	0.050	0.079	0.101	0.238	0.329	0.019	0.026	0.043	0.052	0.122	0.179
85+	W	0.092	0.150	0.124	0.226	0.225	0.442	0.056	0.094	0.086	0.158	0.173	0.365
	NW	0.009	0.016	0.013	0.022	0.059	0.096	0.005	0.008	0.007	0.012	0.034	0.054

or for any site associated with additional daily fluoride intake of about 1 mg. Although adjustment for population mobility and differences in age, ethnic background, and socio-economic status has been achieved to varying degrees, the absence of an effect related to fluoride is a convincing conclusion." Unfortunately, this "convincing conclusion" results from deliberate exclusion in the WHO-IARC monograph, and indeed in many other reports before and since, of the vast amount of evidence to the contrary in a host of scientific papers, U.S. Congressional Hearings, U.S. Court cases, etc., too numerous to cite here. The Danish data reported (1) can itself scarcely have any statistically significant bearing on cancer from drinking fluoridated water since the number of individuals is too small for this purpose by several orders of magnitude (cf. Table 2).

In any event, the paper of Grandjean, Juel, and Jensen has a plethora of observations and reflections that highly recommend it to a large readership, who will find it both thoughtful and thought-provoking.

Dean Burk
Dean Burk Foundation, Inc.,
4719 44th St.
Washington, D.C. 20016

References

1. Grandjean, P., Juel, K., and Jensen, O.M.: Mortality and Cancer Morbidity after Heavy Occupational Fluoride Exposure. Amer. J. Epid. 121:57-67, 1985.
2. Graham, J.R., and Burk, D.: "Lord Jauncey and Justice Flaherty: Opposing Views on the Fluoridation-Cancer Link. Fluoride, 17:63-74, 1984.
3. Grandjean, P. Occupational Fluorosis through 50 years: Clinical and Epidemiological Experiences. Am. J. Ind. Med. 3:227-236, 1982.
4. International Agency for Research on Cancer. Some Aromatic Amines, Anthroquinones and Nitroso Compounds, and Inorganic Fluorides used in Drinking Water and Dental Preparations. IARC Monograph, 27:302, 1982.

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The Fifteenth Conference of the International Society for Fluoride Research will be held on the Utah State University campus, July 30—August 2, 1986. Our host will be Professor G.W. Miller, Department of Biology, UMC 45, Utah State University, Logan, Utah 84322.

BORON AS AN ANTIDOTE TO FLUOROSIS?
PART I: STUDIES ON THE SKELETAL SYSTEM

by

J. Franke, H. Runge, R. Bech, W. Wiedner, W. Kramer, W. Kochmann, A. Hennig, H. Ludke, W. Seffner, W. Teubner, M. Franke, W. Moritz, L. Barthold, and D. Geinitz

Erfurt, Bitterfield, Halle, Jena, Leipzig, GDR

SUMMARY: The antidote action of boron and aluminum sulphate in chronic fluorine intoxication was tested on 8 groups of domestic pigs over a period of 13 months. To the diet of pigs, given 7 mg NaF/kg/d, 4 and 8 mg B/kg/d and 0.1 g of aluminum sulphate was added as an antidote. NaF feeding caused chronic bone fluorosis. Although boron did not lead to reduction in fluorine storage in the skeleton, it exerted a certain detoxicating effect due to formation of less toxic boron fluorine complexes. Respecting the skeletal system, direct F action (increase of bone mass) is, at least partially, compensated by direct boron action upon bone (decrease in bone mass with reduced parathyroid activity). Aluminum sulphate reduces F absorption and F retention in the skeleton by 25 to 29%; concurrent inhibition of calcium absorption from the intestine results in secondary hyperparathyroidism and decrease in bone mass.

According to these experiments, boron or aluminum sulphate are unsuitable prophylactically in humans chronically exposed to fluoride because of individual reaction to fluoride and because of the toxic action of boron and aluminum sulphate upon bone. Short-term administration of boron for more rapid detoxication in fluorosis cases may be permissible during exposure to fluoride and after exposure to the pollutant has been discontinued.

KEY WORDS: Aluminum; Antidotes; Boron; Experimental fluorosis; Pigs; Prophylaxis; Skeletal fluorosis; Sulphate; Therapy.

Introduction

Since 1973 the authors have been searching for an antidote to reduce or prevent the toxic effects of inorganic fluoride compounds or to complex or displace the fluorine ion at its place of action, either in the metabolic process or the skeletal system. Initially, antidotes were tested such as vitamin C, aluminum, magnesium, calcium, molybdenum, copper, iron, boron (B) vanadium, selenium, glutamine, cysteine and glycocorticoids on green algae *Chlorella fusca* var. vacuolata, human erythrocytes and albino mice.

Because in all three tests (1) iron and boron compounds seemed to be par-

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Department of Orthopedic Surgery, Medical Academy, Erfurt, DDR 5010, Regierungsstrasse, 42A. Presented at the 13th conference of the International Society for Fluoride Research, Nov. 14-17, 1983, New Delhi, India.

ticularly effective they were more intensively explored on rabbits. F^- action on teeth and skeleton was clearly reduced by concurrent administration of borax. Iron compounds were ruled out because of unfavorable side effects (2,3).

In the first experiment in the current series 48 domestic pigs were given for 1 year, 0.5 mg and 5 mg NaF/kg body-weight and antidotes B (0.15 and 3.0 mg as borax/kg body-weight), serpentine (6.75 mg/kg body-weight) and Mg 0 (2.75 mg/kg body-weight). F action on the skeletal system was reduced by serpentine and, particularly, by high boron doses. The F content in bone ash, however, was not affected by the antidotes. Boron alone caused osteoporosis (4 and unpublished data). A new experiment with higher F and B doses was initiated to provide conclusive answers, especially with regard to the isolated effect of high doses of boron on the skeletal system.

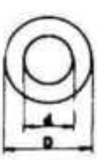
Material and Methods

The experiments were performed on 68 castrated male domestic pigs (mean initial weight, 59 kg) over a period of 13 months. Twelve animals (group 1) served as controls. Groups 2 - 8 (8 animals each) received the following doses of NaF plus an antidote: Group 2: 7 mg NaF/kg; Group 3: 7 mg F/kg + 4 mg B^{+++}/kg as boric acid (H_3BO_3); Group 4: 7 mg F/kg + 8 mg B^{+++}/kg; Group 5: 4 mg B^{+++}/kg; Group 6: 8 mg B^{+++}/kg; Group 7: 7 mg F/kg + 100 mg Al-sulphate: $Al_2(SO_4)_3 \times 18\ H_2O$; Group 8: 7 mg F/kg + 100 mg Al-sulphate + 4 mg B^{+++}/kg.

Aluminum sulphate, an agent known to reduce fluorosis in grazing cattle (5), was additionally included. The F/B ratio was related to $BF_2(OH)_2$, with a calculated excess of boron over fluorine of 100 or, resp., 300%.

Fasting morning samples of blood and urine were taken the 5th, 10th 12th, and 13th month on the day of sacrifice. Only analyses dealing with the skeletal system are presented here. Bone mineral content was determined by ^{125}I-photon absorptiometry on the cleaned metacarpal bones 2 and 3 and the femur according to the Cameron and Sorensen method (6) in the bone center. X-ray photographs were taken of all 3 bones in two planes, and of the lumbar vertebral column. On the roentgenograms of metacarpals 2 and 3 and femur, the corticalis index was computed according to Exton-Smith et al. (7) (Fig. 1).

Figure I



$$\frac{\text{Cortical Area}}{\text{(bone) surface area}} = \text{Cortical Index}$$

According to Exton-Smith et al. (1969)

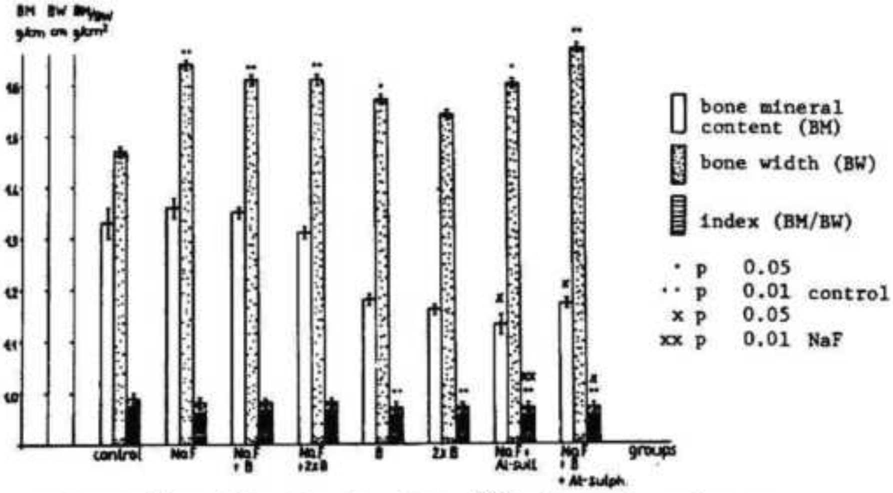
Pieces of bone, 1.5 - 2 cm long, which served for histological and histomorphometric studies, were sawed out of the right iliac crest (tuber coxae), alcohol-fixed, embedded in methacrylate and cut in non-decalcified condition. The sections were stained according to Goldner and with toluidine-blue. The spongiosa and osteoid volume were histomorphometrically determined by the point-counting method according to Chalkey (8). F in serum, urine, iliac crest and rib ash was determined by the ion selective electrode. For boron determination, bone samples were ashed in the presence of lithium carbonate: all boron was converted to $(BF_4)^-$ complexes and photometrically determined after enrichment by extraction. To determine parathyroid function, areas of 150 nuclei of the parathyroid were measured histomorphologically per animal. Increasing sizes of the nuclei indicate a stimulation of function.

Results

^{125}I photon absorptiometry (Fig. 2a and b): In group 2. the effect of fluoride was an increase in bone mineral content (BM) and bone width (BW) at

Figure 2 a

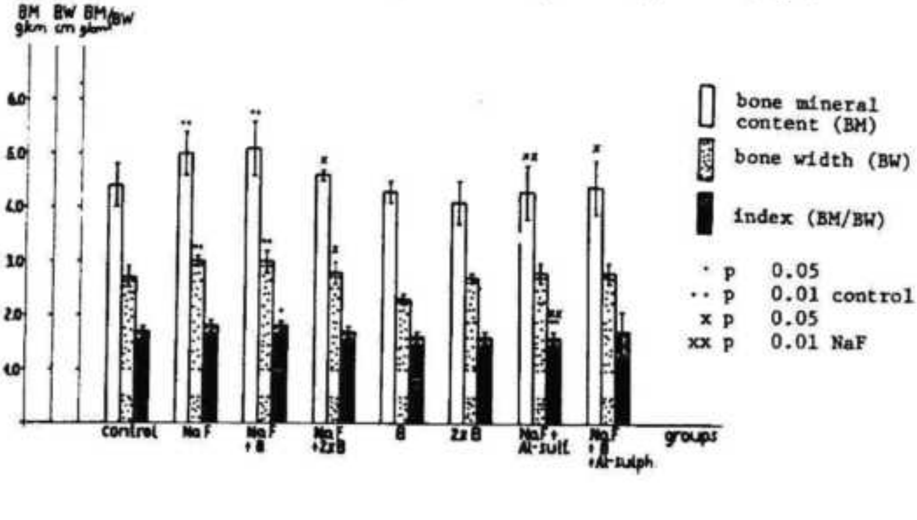
Results of the ^{125}I photon absorptiometry - metacarpal bone II



metacarpal bone II   Results of the ^{125}I photon absortiometry

Figure 2 b

Results of the ^{125}I-photon absorptiometry, Femur (a.p.)



all measuring points. Addition of 4 mg B/kg (Group 5) to the diet did not prevent F action. Doses of 8 mg B/d (Group 6) almost eliminated fluoride action in all groups. Boron by itself (groups 5 and 6) caused a decrease on bone mineral content in all bones, compared to controls. Bone width remained unchanged which was more evident in group 6 (8 mg B). Addition of Al-sulphate as an antidote (Group 7) led to a significant decrease of BM compared to Group 2. All values were still below those of controls. Group 8 (F + 4 mg B + Al--sulphate) showed no substantial changes compared to Group 7.

Roentgenological findings: Pathological findings in line with fluorosis were absent. Bone tissue density varied in each group.

Cortical index: Table 1 shows the mean values from computations dealing with the femur in antero-posterior and lateral direction; the two metacarpals were also measured in two planes. Compared to controls, the administration of boron by itself (Groups 5 and 6) caused a significant decrease in the index on all bones. The action was similar in Group 7 (Al-sulphate). Fluoride alone (group 2) also caused, at least in the metacarpals, a decrease in the cortical index. No significant antidote action was apparent.

Table 1

Results of Cortical Indices According to Exton-Smith et al. (7)

Group	Femur ap. and lateral in %	Metacarpal bone II and III ap. and lateral in %
1	40.68±3.97	34.01±4.19
2	39.60±2.90	27.41±3.30 **
3	41.01±2.35	30.40±5.09
4	36.69±5.72	25.85±3.47 **
5	35.96±4.51 *	30.38±5.03
6	30.03±5.58 **	27.96±4.95 *
7	34.60±3.72 **xx	28.51±3.16 **
8	40.66±6.20	30.43±5.38

* $p<0.05$; ** $p<0.01$ to control group; xx $p<0.01$ to NaF group

Table 2

Histomorphometric Results of the Iliac Crest Bone (Tuber Coxae)

Group	Volume of Spongy Bone %	Volume of Osteoid %	n
1	21.8±4.0	2.9±1.2	6
2	23.2±4.0	10.0±1.6 **	3
3	20.6±3.8	7.8±1.5	4
4	21.9±2.0	6.5±1.3 x	4
5	22.4±2.3	2.3±2.2	4
6	15.2±3.7 **	1.7±1.7	4
7	17.7±3.9	3.5±0.8 xx	3
8	19.3±4.2	4.2±1.3 xx	4

** $p<0.01$ to control group; x $p<0.05$; xx $p<0.01$ to NaF group

Histological findings - Histologically, and histomorphometrically, in Group 2, osteoid seam thickness, osteoid surface, trabeculae volume increased: bone resorption increased slightly. When boron was added, osteoid mass remained normal. Boron groups 5, 6, especially group 6, caused a strong diminution of bone and osteoid mass corresponding to osteoporosis. Administration of Al-sulphate plus NaF reduced bone mass below that of the control group. The osteoid seams were similar to those of the control group. As apparent from table 2, administration of NaF led to increased osteoid and bone mass. Additional B reduced spongiosa mass but affected osteoid quantity only slightly. Spongiosa density was reduced markedly by high boron dose (group 6) with concurrent osteoid diminution. Al sulphate distinctly diminished fluoride action; it even reduced spongiosa volume to a level below normal (Table 2).

Fluoride determination: The fluoride serum values rose, as shown in Fig. 3, in all groups in the course of the study. The serum level rose about six-fold due to NaF by itself (Group 2). With concurrent administration of boron and fluoride, F values were significantly higher (up to 40%) detectable in groups 3 and 4 only during months 5 and 10. When Al-sulphate is administered as an antidote (groups 7 and 8), the fluoride serum level dropped markedly. F concentrations in urine (Fig. 4) varied widely in the individual groups of animals with the following trends: When F was administered, F excretion rose ten to fifteen-fold, whereas excretion in each group almost doubled from the 5th to the 12th month. The constantly increasing excretion in the boron antidote groups (5 and 6) compared to NaF group 2, ranged from 3 to 71% - not statically significant. By addition of aluminum sulphate (group 7), urinary fluoride decreased insignificantly. Conversion of the F concentration to the creatinine excretion in urine (Table 3) shows the same trend. F content in pelvic bone ash (Fig.5) was, on an average, 25% above rib content. Fluoride administration during 13 months induced a rise up to ten times normal values. F values in bone of boron antidote groups 3 and 4 are in the same order of magnitude although slightly higher. Al-sulphate administration (groups 7 and 8) lead to a highly significant 25-30% decrease in F content in ash. The ash content (Table 4) was reduced in all groups compared to con-

Figure 3

Fluoride Content in Serum During Experiment

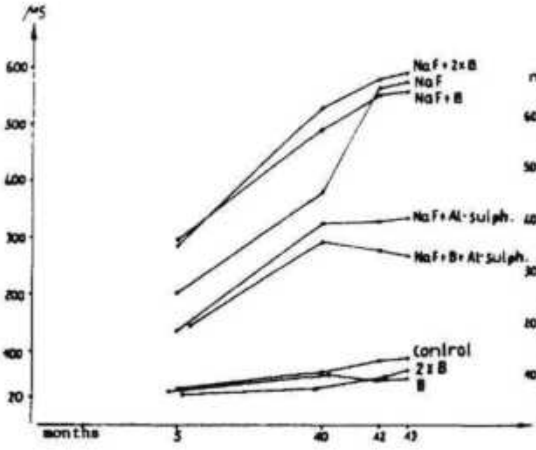
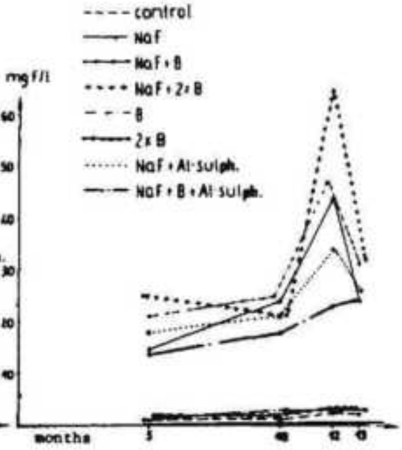


Figure 4

F⁻ Content in Urine During Experiment



trols and to NaF group 2; reduction was most marked in boron groups 5 and 6 and in Al-sulphate groups 7 and 8 (Table 4). Boron content in bone failed to increase in any of the groups. The marked fall in activity of the parathyroid glands (Table 5) in boron groups 5 and 6 is highly significant and the increase in activity in the two Al-sulphate groups 7 and 8 statistically significant. No hyperactivity in NaF group 2 was observed (Table 5).

Discussion

The 13-month duration of administration of 7 mg F /kg/d to young pigs resulted in mild skeletal fluorosis. Macroscopically some exostoses and hyperostoses were observed. Apposition rings in the subperiosteal region were ap-

Table 3

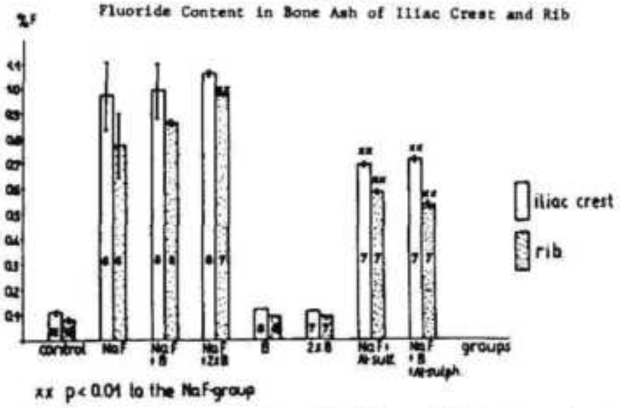
F Content in Urine Calculated to Creatinine Content in Urine (mg F /mmol creatinine)

Group	5th Month	n	10th Month	n	12th Month	n	13th Month	n
1	0.149±0.062	12	0.216±0.042	11	0.109±0.019	10	0.137±0.043	12
2	1.578±0.659**	8	2.114±0.897**	6	2.470±1.562	7	1.472±0.309	8
3	2.254±0.725	8	2.272±1.491	8	2.014±0.499	8	1.748±0.529	7
4	2.468±0.507xx	8	2.330±1.079	8	3.969±2.719	7	1.807±0.291x	8
5	0.112±0.038	8	0.212±0.045	8	0.149±0.031	7	0.153±0.045	7
6	0.124±0.038	8	0.211±0.047	8	0.178±0.063	8	0.195±0.089	8
7	1.650±0.476	8	1.115±0.526x	8	1.527±0.527	7	1.368±0.960	8
8	2.217±1.084	8	1.043±0.261x	8	1.711±0.571	7	1.376±0.456	8

**p<0.01 to control group; xp<0.05; xxp<0.01 to NaF group

Figure 5

Fluoride Content in Bone Ash of Iliac Crest and Rib



xx p<0.01 to the NaF-group

parent on the humerus cross-section (9,10). I^{125}-photon absorptiometry showed an increase in mineral content and bone width, which is also typical of human industrial fluorosis (11). Morphometrically obtained cortical indices decreased since the medullary canal is enlarged by increased endosteal resorption. Radiography revealed no reliable fluorosis signs at the femur, the lumbar vertebral column and the metacarpals. Histologically the increase in surface osteoid and osteoid seam thickness at the iliac crest with a slight increase in trabecula thickness and of resorption, is typical of beginning fluorosis (10, 12-15). At a dosage of 0.5 mg F/kg/d, the F level in serum, namely 575 µg/l is almost three times that recommended by some for osteoporosis therapy (16-18). Fluoride values in iliac crest bone ash reached 0.97%, a level equivalent in humans to stage III fluorosis, according to Roholm (10, 13, 14, 19, 20).

Table 4

Ash Content in % to Dried Bone

Group	Iliac Crest%	n	Rib%	n
1	28.3±3.6	12	53.4±4.0	12
2	28.7±3.8	6	52.0±2.5	6
3	27.3±2.9	8	50.3±3.3	8
4	27.6±3.6	8	50.6±5.1	7
5	26.7±3.9	8	48.6±5.6	8
6	26.4±2.3	7	46.0±6.1 *	7
7	26.2±2.2	7	47.0±5.5 *	7
8	29.8±3.4	7	47.7±4.6 *	7

*$p<0.05$ to control group

Table 5

Parathyroid Activity: Average Area of 150 Parathyroid Nuclei per Animal

Group	μm^2	n
1	18.65±1.79	12
2	18.87±1.23	5
3	19.50±2.57	8
4	18.47±2.60	8
5	13.23±1.08 **	8
6	15.97±1.44 **	7
7	21.05±1.56 **	7
8	21.72±2.08 **	7

**$p<0.01$ to control group

Boron given in two different dosages as an antidote yielded the following results in the skeletal system. At bone seams, photon-absorptiometrically, histologically and chemically (ash content), fluoride action was cancelled or alleviated by concurrent administration of boron, especially at high dosage (group 6). Elevated F level in serum, associated in both boron groups 5 and 6, with constantly increased F excretion in urine, pointed towards the antidote action of boron. The formation of F-B complexes, which are preferably excreted through the kidney and are less markedly stored in bone (21), theoretically might explain this phenomenon. $[BF(OH)_3]^-$, $[BF_2(OH)_2]^-$, $[BF_3OH]^-$ and $[BF_4]^-$ are the complexes involved: the first three form fairly rapidly but are less stable than the fourth. F analyses of iliac crest and rib ashes, however, failed to show any antidote action of boron. In keeping with the higher F values in serum, F values in bone were slightly higher than those in F group 2. Boron analysis did not prove that boron was stored in bone. This contradiction is explained by analysis of boron action on bone in boron groups 5 and 6 which showed clearly reduced mineral values in femur and metacarpals. A similar effect was shown by the decrease in cortical indices in the roentgenogram and, histologically, by osteoporosis with diminished bone formation, especially in group 6 (8 mg B). Boron causes osteoporosis by its immediate action on bone metabolism. This direct boron action on bone (decrease in bone mass) cancels, at least partially, the direct F^- action on bone. Since fluoride storage in bone is not affected by it, no genuine antidote action of boron by formation of B-F complex in bone is involved. The raised F levels in serum, the slightly raised F content in bone and the increased F excretion in urine suggest boron-improved F absorption from the intestine, perhaps as (B-F) complex. Elsair et al. (22) proved by acute fluoride intoxication in rabbits (60 mg F /kg/d), an increased F digestive utilization coefficient with increased F excretion in urine and increased fluoride storage in bone, by the use of fluoride balances. The decrease in parathyroid activity in the two boron groups

(5 and 6) may be the key to boron action. An increase in parathyroid activity due to a relatively high NaF dose (15.4 mg/kg/d) could not be proved by determination of the average area of nuclei, though a secondary hyperparathyroidism was actually found in animal experiments involving 200 ppm (23, 24) and in South India in human endemic fluorosis (25-27). In the last-mentioned cases malnutrition, calcium and vitamin D deficiencies associated with a high F intake (up to 65 mg F/d) are some of the causes involved. Secondary hyperparathyroidism in rabbits, acutely and subacutely intoxicated by 60 or 40 mg F/kg, is caused by inhibition of calcium absorption from the intestine (22). Nevertheless, the toxic effect of fluorine seems to be alleviated by concurrent administration of boron (28), as shown in the literature and by our former studies due to varying toxicity of NaF and BF complexes. The oral LD_{50} for potassium fluoroborate in rats is 2,000-3,000 mg/kg according to Hodge and Smith (29); for NaF, the authors found an LD_{50} of 250 mg/kg in rats (30), for fluoroborates toxicity was ten-fold lower. With the ion-sensitive electrode, fluorides as $(BF_4)^-$ complexes can be determined only after destruction of this complex (total fluorides). In the rabbit experiment, after feeding boron and fluorine, ionized fluoride content and total fluoride content coincided; $(BF_4)^-$ complexes were not being formed but rather the stages $[BF(OH)_3]^-$ to $[BF_3OH]^-$ might be assumed.

The following findings from earlier experiments as well as the results of part II confirmed that BF complexes reduced the toxic effect of fluoride. The growth-retarding effect of NaF on green alga Chlorella fusca var. vacuolata is markedly reduced by sodium borate. The glycolysis rate of human erythrocytes, which is reduced by NaF, is raised again by borate addition (1), but normal values were not attained. In the rabbit experiment (2) the reduction in serum iron and iodine level by NaF is prevented by concurrent administration of boron; the same behavior was observed with cholinesterase but boron addition failed to induce normal values. In our first experiment with yearling domestic pigs during 12 months, lowered serum iron level and cholinesterase activity as well as increase in the glucose-6-phosphatase activity caused by 5 mg NaF/kg/d were restored to normal by 0.35 mg B/kg/d.

Administration of boron concurrently with NaF aerosol inhalation in Syrian golden hamsters induced a decrease in cholinesterase and caused transaminases to increase (31). Elsair et al (32) also found that hemostasia disorders in the rabbit, caused by 40 mg F/kg/d, were inhibited by 15.4 mg B/kg/d. The same team, testing liver homogenates in vitro, observed a correction of increased oxygen consumption caused by NaF (33). In acute F intoxication of rabbits (60 mg F/kg/d), administration of boron – after discontinuation of fluoride administration – accelerates F excretion (detoxication) (34). Negative calcium and phosphorus balances, due to high F doses leading to hypocalcemia and secondary hyperparathyroidism, are corrected by concurrent boron doses: F storage in the skeleton is unaltered or increased (22,28,35). As early as 1965, Hasek (36) found that 3 g of boric acid/100 kg of body weight, administered to fattened bulls, failed to prevent dental fluorosis. On the other hand, the prevailing opinion in the literature is that aluminum salts reduce fluorine retention in bone. For grazing cattle, Gründer (5) reported a reduced absorption quota of 30 - 40% due to various Al-compounds; the bone fluorine content was reduced by about 20%. In poultry, F absorption due to Al-sulphate was significantly reduced (F content in intestine increased by 63%) (37). In humans, F absorption was reduced by 30% due to high F doses of aluminum hydroxide and by 57.6% with low F doses (38).

In our experiments, F storage in bone was reduced 25-29% by Al-sulfate. A distinct pathological change in bone was apparent: bone mass decreased in comparison to the control group. Increased parathyroid activity in both Al--sulfate groups might provide an explanation. Al-sulfate in the intestine leads to precipitation of calcium and phosphorus. The resulting calcium deficiency may then trigger secondary hyperparathyroidism and/or phosphorus deficiency of 1α-hydroxylase in the kidneys. Combinations of Al-sulfate and borate failed to yield any additional information.

Conclusion

Boron acts as an antidote in F intoxication, probably due to formation of less toxic boron-fluoride complexes; F content in bone, however, is slightly increased with a concurrent increase in F serum level and in urinary F excretion. Boron by itself, in the doses used, leads to osteoporosis associated with reduction in parathyroid activity. The boron effect, decrease in bone mass also, at least partially, cancels the F effect on bone, namely, increase in bone mass.

Boron prophylaxis is not recommended. It may even be dangerous, since strong individual differences were observed in chronic fluoride intoxication of humans caused by industry or by high F content in drinking water, and since only a certain proportion of exposed persons are affected by fluorosis (39-41). According to Elsair et al (22) boron is only suitable for short-term use to attain rapid detoxication (curative) after discontinuance of exposure to fluoride. The situation is similar when aluminum sulphate is involved. By reducing F absorption from the intestine and F storage in the skeleton, it leads to secondary hyperparathyroidism due to deterioration in calcium absorption. The mechanism of boron and Al-sulphate action on the development of chronic fluorosis differs. Al-sulphate reduces F absorption; boron acts directly on bone and forms less toxic boron fluoride complexes.

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References

1. Kochmann, W., Bech, R., Grunewald, A., Domesle, E., Kramer, W., Hentschel, H., Wiedner, W., Böhm, H., Schrandt, D., Franke, J., Runge, H., and Brox, u.D.: Ergebnisse experimenteller Untersuchungen an verschiedenen biologischen Testobjekten mit anorganischen Fluoriden und organischen Fluorverbindungen unter Einwirkung mehrerer Gegenmittel. Wissenschaftl. Hefte d. Pädagog. Inst. Köthen, 2:283-302, 1973.
2. Baer, H.P., Bech, R., Franke, J., Grunewald, A., Kochmann, W., Melson, F., Runge, H., and Wiedner, W.: Ergebnisse tierexperimenteller Untersuchungen an Kaninchen mit Natriumfluorid unter Einwirkung von Gegenmitteln. Z. ges. Hyg. 23:14-20, 1977.
3. Grunewald, A., Kochmann, W., Bech, E., and Wiedner, u.W.: Untersuchungen über das Verhalten von Natriumfluorid im Warmblüterorganismus unter Einfluß von Bor und Eisen. Z. ges. Hyg. 24:526-528, 1978.
4. Seffner, W., and Tuebener, W.: Antidotes in Experimental Fluorosis on Pigs: Morphological Studies. Fluoride, 16:33-37, 1983.

5. Gründer, H.D.: Fluorimmissionswirkung auf Rinder. Zbl. Vet. Med. A 19:229-302, 1972.
6. Cameron, J.R., and Sorenson, J.: Measurement of Bone Mineral in vivo. Science, 142:230-232, 1963.
7. Exton-Smith, N.A., Millard, P.H., Payne, P.R., and Wheeler, E.:Method for Measuring Quantity of Bone. Lancet, 2:1153-1157, 1969.
8. Chalkley, W.H.: Method for the Quantitative Morphologic Analysis of Tissue. J. Nat. Cancer Inst. 4:47-53, 1943.
9. Shupe, J.L.: Fluorine Toxicosis and Industry. Amer. Ind. Hyg. Assoc. J., 31:240-247, 1970.
10. Roholm, K.: Fluorine Intoxication. A Clinical-Hygienic Study. H.K. Lewis & Co., London, H.K., 1937.
11. Runge, H., Franke, J., Geryk, B., Hein, G., Fengler, F., Paul, H., Bismarck, M., and Schmidt, C.W.: Bone Mineral Analysis in Persons with Long-Time Fluoride Exposure. Fluoride, 12:18-27, 1979.
12. Franke, J.: Histological Changes of Human Fluorosis, Experimental Fluorosis in Animals and Osteoporosis Following Sodium Fluoride Therapy. Fluoride, 5:182-198, 1972.
13. Franke, J., Rath, F., Runge, H., Fengler, F., Auermann, E., and Lennart, G.: Industrial Fluorosis. Fluoride, 8:61-83, 1975.
14. Franke, J., Runge, H., and Fengler, F.: Endemic and Industrial Fluorosis. In: B. Courvoisier, A. Donath and C.A. Baud (Eds.), Symposium CEMO II: Fluoride and Bone, Genève: Editions Medecine et Hygiene, 1978, pp. 129-143.
15. Shupe, J.L., Miner, M.L., Greenwood, D.A., Harris, L.E., and Stoddard, G.E.: The Effect of Fluorine on Dairy Cattle: II. Clinical and Pathological Effects. Amer. J. Vet. Res., 24:964-979, 1963.
16. Taves, D.R.: New Approach to the Treatment of Bone Disease with Fluoride. Feder. Proc. 29:1185-1187, 1970.
17. Fuchs, C., Dorn, D., Hauswald, C., Henning, H.V., Köbberling, J., Kubosch, J., McIntosh, C., Unger, H.D., and Scheler, F.: Fluorid-Spie--gel im Serum bei der Osteoporose-Behandlung mit NaF. Verh. dt. Gesellsch. Inn. Med., 82:910-912, 1976.
18. Sluys Veer, J.J., vanKesteren, G., Backer-Dirks, O., and Flissebaalje: Serum Fluoride Concentrations in Osteoporotic Patients During NaF Treatment. In: B. Courvoisier, A. Donath, and C.A. Baud (Eds.), Symposium CEMO II: Fluoride and Bone, Genève, Editions Medecine et Hygiene, 1978, pp. 293-397.
19. Franke, J., and Auermann, E.: Die Bedeutung der Beckenkammpunktion mit histologischer und mikroanalytischer Untersuchung des gewonnenen Knochenmaterials bei der Diagnostik der Fluorose. Int. Arch. Arbeitsmed, 29:85-94, 1972.
20. Hodge, H.C., and Smith, F.A.: Occupational Fluoride Exposure. J. Occupat. Med., 19:12-39, 1977.
21. Largent, E.J.: Fluorosis, Columbus Ohio State Univ. Press, 1961.
22. Elsair, J., Merad, R., Denine, R., Reggabi, M., Benali, S., Hamrour, H.M., Azouz, M., Khalfat, K., Tabet Aoul, M., and Nauer, J.: Action of Boron Upon Fluorosis, An Experimental Study. Fluoride, 15:75-78, 1982.
23. Faccini, J.M., and Care, A.D.: Effect of Sodium Fluoride on the Ultrastructure of Parathyroid Glands of the Sheep. Nature, 207:1399-1401, 1965.
24. Faccini, J.M.: Fluorine and Bone. Calcif. Tissue Res., 3:1-16, 1969.
25. Teotia, S.P., and Teotia, M.: Endemic Skeletal Fluorosis in Children

Evidence of Secondary Hyperparathyroidism. In: Frame, B., Parfitt, A.M., and Duncan, H. (Eds.): Clinical Aspects of Metabolic Bone Disease, Excerpta Medica, Amsterdam, 1973, pp. 232-238.

26. Teotia, S.P.S., and Teotia, M.: Secondary Hyperparathyroidism in Patients with Endemic Skeletal Fluorosis. Brit. Med. J. 1:637-640, 1973.

27. Makhni, S.S., Sidhu, S., Singh, P., and Singh, G.: The Parathyroid in Human Fluorotic Syndrome. Fluoride, 14:17-19, 1980.

28. Elsair, J., Merad, R., Denine, R., Reggabi, M., Alamir, B., Benali, S., Azzouz, M., and Khelfat, K.: Boron as a Preventive Antidote in Acute and Subacute Fluoride Intoxication in Rabbits: Its Action on Fluoride and Calcium-Phosphorus Metabolism. Fluoride, 13:129-138,1980.

29. Hodge, H.D., and Smith, F.A.: Biochemical Effects of Inorganic Fluorides. In: Simons, J.H. (Ed.), Fluorine Chemistry Vol. IV., Acad. Press, New York, London, 1965.

30. Franke, J., Runge, H., Fengler, F., and Wanka, Ch.: Beitrag zur experimentellen Knochenfluorose. Int. Arch. Arbeitsmed. 30:31-48,1972.

31. Rathmann, D., Bech, R., and Baer, H.P. Beitrag zur tierexperimentellen Fluorose nach Inhalation von NaF-Aerosolen. Z. ges. Hyg. 23: 632-633, 1977.

32. Elsair, J., Merad, B., Denine, R., Reggabi, M., Benali, M., Alamir, B., and Ali Rachedi, M.: Effects of Fluoride Intoxication of Several Months on Hemostasis in Rabbits in the Presence and Absence of an Antidote (Boron). Fluoride, 12:136-143, 1979.

33. Elsair, J., Merad, R., Denine, R., Reggabi, M., Alamir, B., Benali, M., Khelfat, K., and Ali Rachedi, M.: Effect of Fluoride and an Antidose (Boron) on Respiration of Liver Tissue in Rabbits, Fluoride. 12:172-176, 1979.

34. Elsair, J., Merad, R., Denine, R., Regabbi, M., Benali, S., Azzouz, M., Khelfat, K., and Tabet Aoul, M.: Boron as an Antidote in Acute Fluoride Intoxication in Rabbits: Its Action on the Fluoride and Calcium-Phosphorus Metabolism. Fluoride, 13:30-38, 1980.

35. Elsair, J., Merad, R., Denine, R., Azzouz, M., Khelfat, K., Hamrour, M., Alamir, B., Benali, S., and Reggabi, M.: Boron as Antidote to Fluoride Effect on Bones and Claws in Subacute Intoxication of Rabbits. Fluoride, 14:21-29, 1981.

36. Hasek, A.G., Narozny, J., Hluchan, E., and Blaho, U.: Erforschung der Maßnahmen zur Einschränkung der Fluorose bei Mastbullen. Veterinarni Med., 10:605-614, 1965.

37. Cakir, A., Sullivan, T.W., and Mather, F.B.: Alleviation of Fluoride Toxicity in Starting Turkeys and Chicks with Aluminum. Poultry Sci., 57:498-505, 1978.

38. Spencer, H., Kramer, L., Norris, C., and Wiatrowski, E.: Effect of Aluminum Hydroxide on Fluoride Metabolism. Clin. Pharmacol. Ther. 28:525-539, 1980.

39. Franke, J.: Wirkungen von Fluor auf das Skelettsystem unter besonderer Berücksichtigung der Industriefluorose und der Natriumfluorid - behandlung der Osteoporose. Med. Diss. (B), Halle/S., Martin Luther Universität, 1976.

40. Franke, J.: A New Concept of the Effect of Fluoride on Bone. Fluoride, 12:195-208, 1979.

41. Fluoride Effects on Vegetation, Animals and Humans. Eds. Shupe, U.L., Peterson, H.B., Leone, N.C., Paragon Press, Salt Lake City, 1983, pp. 227-232.

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FLUOROSIS IN NALGONDA DISTRICT IN RELATION TO CHEMICAL CHARACTERISTICS OF POTABLE WATER AND STAPLE FOOD

by

K. Rajyalakshmi, and N.V. Ramamohana Rao
Hyderabad, India

SUMMARY: Nalgonda district in Andhra Pradesh is one of the areas in the state which is affected by fluorosis. Several thousand individuals are crippled and lead a vegetative life. Epidemiological, nutritional, clinical, radiological and laboratory investigation have been carried out to correlate the onset and severity of the disease with the chemical characteristics of potable waters and staple foods. The investigations have revealed the prevalence of cases of severe fluorosis associated with fluoride concentrations as low as 4.0 mg/l in drinking water. Low calcium intake through foods and low calcium and excessive bicarbonate content of drinking water are important factors which influence the severity of the disease. Some of the staple foods grown in the area contribute significant amounts of fluoride.

KEY WORDS: Endemic fluorosis; F^- in food; F^- in water; Nalgonda district; Skeletal fluorosis; Water hardness.

Introduction

In Andhra Pradesh a large number of people, in as many as 17 districts, suffer from fluorosis. In Nalgonda district thousands of people suffer from advanced fluorosis. Previous studies indicate that fluorosis is associated with fluoride naturally in drinking water as low as 0.6 ppm (1-4). Preliminary epidemiological surveys by the authors showed that other chemical constituents in water influence the water fluoride-disease relationship (5). Reports in the literature on animal experiments suggest that dietary constituents may also affect the incidence of the disease and the severity of symptoms (6-10).

Detailed epidemiological, clinical, radiological, and dietary investigations as well as laboratory studies have been undertaken at the Institute of Preventive Medicine, to determine what factors influence the incidence and severity of fluorosis and the measures needed for solving this chronic health hazard.

Material and Methods

On the basis of results of a preliminary evaluation of water fluoride levels in different parts of the district, 410 sources were resampled in known endemic areas for complete chemical analysis, including all available water sources

From the Institute of Preventive Medicine, Hyderabad, India. Presented at the 13th conference of the International Society for Fluoride Research, Nov. 14-17, 1983, New Delhi, India.

in 20 villages. Samples, collected in 2-liter polythene cans, were analyzed for pH, conductivity, alkalinity, hardness, calcium,magnesium, iron, aluminum, sodium, potassium, chloride, fluoride, nitrate, nitrite, phosphate, sulphate, and silica. 100 samples were also analyzed for trace elements - copper, arsenic, lead, chromium, and iodide. All analyses were carried out according to standard procedures (11). Fluoride was determined by the electrode method on an ion-selective meter (Orion USA). Samples of raw foods grown in endemic areas were analyzed for fluoride after ashing and distillation (12).

Epidemiological studies were conducted to determine the incidence of dental and skeletal fluorosis. 680 children, 6-14 years old, were studied to ascertain the degree of dental fluorosis according to the following classifications: Grade 1 - white opacities, faint yellow line; Grade 2 - changes of grade 1, plus brown stain; Grade 3 - brown line, pitting and chipped edges; Grade 4 - brown-black, plus loss of teeth. Skeletal fluorosis was studied in adolescents and adults under the following classifications: Mild - asymptomatic radiographs with increased bone density; Moderate - symptomatic - stiffness, rigidity, pain, aches; Severe - symptomatic with moderate fluorosis - osteophytosis, exotosis. To ascertain the radiological profiles of the fluorosis cases, X-ray photographs of the spinal cord (dorsolumbar), forearm, and knee were taken.

Biochemical Investigations: Samples of blood and urine were collected from patients consuming water containing fluoride in the following ranges: 1 - 4.0, 4.1 - 8.0 and above 8.0 mg/l, as well as from healthy persons for controls.

Dietary surveys were conducted to record the amounts of food materials consumed by the householder and to determine the total amount of a) proteins, b) fat, c) carbohydrates, d) iron, e) calcium, and f) phosphates. The daily consumption of water and the duration of use of a particular water source were also ascertained.

Results and Discussion

In every village surveyed water sources, even when close to one another, contained differing amounts of fluoride. Wide variations have been observed in the distribution of fluoride in the endemic areas in Nalgonda district ranging from 0.4 - 20.0 mg/l with a mean of 3.32 mg/l in water. In those villages where the maximum level of fluoride is about 4 mg/l a significant number of well waters contain 1.5 mg F/l or less. Clinical examination of children revealed dental fluorosis in varying degrees of severity, the incidence of which could be associated not only with the fluoride level in drinking water but also with the alkalinity. Table 1 shows the degree of mottling in relation to fluoride content and alkalinity of water. Increasing amounts of alkalinity are associated with higher degrees of mottling at identical concentrations. Clinical examination and radiological profiles revealed that definite cases of osteosclerosis could be associated with fluoride in drinking water as low as 4.0 mg/l (Fig. 1).

Besides the fluoride concentration of drinking water, the degree of skeletal fluorosis correlated with the calcium content and alkalinity of water. The calcium content in waters ranged from 4.5-50.5 mg/l with an average of 30.0 mg/l and alkalinity ranged from 48.0 to 1125 mg/l with a mean of 387 mg/l. In addition to the low level of calcium in water, the economically backward people,

Figure 1

whose diet is deficient in calcium, were more susceptible to fluoride toxicity. Alkalinity of water was usually related both to incidence of fluorosis and severity of symptoms. The relation between alkalinity of water sources and the incidence of skeletal fluorosis is presented in Table 2. The results indicate that the alkalinity of drinking waters has more influence on the incidence of disease than the fluoride concentration.

Dietary Surveys: Dietary surveys revealed that laborers and harijans* in the villages normally consumed only jawar, bajra, tamarind, chilies, onion, and, rarely, rice and grains. All severe cases of fluorosis were from the sections of society with low nutritional status. The quanitity of each food material consumed by individuals of low economic status suffering from fluorosis was ascertained, and total amounts of essential nutrients were computed by reference to I.C.M.R. manual on nutritive value of Indian foods (13) as recorded in Table 3 which shows that these people have one or more nutritional deficiencies. The total daily calcium intake was as low as 300-400 mg/l, less than half the recommended daily requirement.

Table 1

Water Quality vs. Incidence of Dental Fluorosis

Range of F^- (mg/1)	Range of Alkalinity in Water (mg/1)				
	100 - 200 1 2 3 4 Grade	210 - 300 1 2 3 4 Grade	301 - 400 1 2 3 4 Grade	401 - 500 1 2 3 4 Grade	501 and above 1 2 3 4 Grade of Mottling
0.5 - 1.0	- - - -	+ - - -	+ - - -	+ + - -	Samples not available
1.1 - 1.5	+ - - -	+ - - -	+ + - -	+ + + -	Samples not available
1.6 - 2.0	+ - - -	+ + - -	+ + - -	- + + -	- + + +
2.1 - 2.5	+ + - -	+ + - -	+ + + -	+ + + +	+ + + +
2.6 - 3.0	Samples not available	- - + -	- - + +	- - + +	- - + +
3.1 and above	Samples not available	- - + +	- - + +	- - - +	- - - +

Table 2

Incidence of Skeletal Fluorosis vs. Alkalinity of Drinking Water

Village	Range of F^- (mg/1)	Alkalinity (mg/1)	% of Skeletal fluorosis
1. Madhavayadavalli	4.4 - 7.5 (5.6)	436 - 600 (540)	24%
2. Sivannagudem	3.2 - 5.6 (4.8)	244 - 900 (536)	32.5%
3. Yellareddygudem	2.8 - 20.0 (6.9)	244 - 900 (530)	35%
4. Batlapally	3.2 - 8.6 (7.8)	360 - 920 (720)	71.6%
5. Velagapally	4.2 - 9.7 (8.2)	560 - 820 (760)	77.8%

Data in parenthesis indicates mean value.

* Lowest caste in India - They lack education and are underprivileged.

Radiological Profile: Examination of X-rays of fluorosis patients showed osteosclerosis, osteophytosis, and calcification of ligaments and interosseous membranes. Radiological profiles showed several afflictions associated with the lowest level of fluoride in drinking water, namely 4.0 ppm (Fig. 1).

Serum Fluorides: The serum fluoride values of the control group whose drinking water contained F^- in the range of 0.5-1.5 ppm and the fluorosis cases are recorded in Table 3 The serum fluoride concentration increased more than 10 fold in fluorosis patients. However, no linear relationship is discernable between water and serum fluorides and degree of illness. From the results, it appears that the serum fluoride concentration can be used as a useful tool in detecting fluorosis.

Table 3

F^- Content of Water, Serum, and Degree of Fluorosis

	F^- in Water (mg/l)	Serum F^- (mg/l)	Degree of Fluorosis
1.	0.8	0.01	Nil
2.	1.5	0.03	Nil
3.	2.0	0.02	Nil
4.	1.8	0.04	Nil
5.	3.5	0.15	Mild
6.	3.0	0.18	Mild
7.	5.0	0.4	Severe
8.	6.5	0.3	Moderate
9.	7.5	0.4	Severe
10.	8.2	0.4	Mild
11.	8.4	0.6	Moderate
12.	8.5	0.4	Moderate
13.	9.0	0.3	Severe
14.	9.2	0.2	Mild
15.	9.4	0.4	Severe
16	9.8	0.4	Severe
17.	10.2	0.3	Severe

Table 4

F^- in Drinking Water and Urine

	F^- in Drinking Water (mg/l)	F^- in Urine (mg/l)
1.	1.0	1.2
2.	1.2	1.5
3.	1.5	1.8
4.	1.8	2.6
5.	2.0	2.9
6.	3.5	4.2
7.	4.9	6.4
8.	5.8	7.2
9.	7.0	8.2
10.	8.4	10.0
11.	8.8	12.2
12.	9.2	14.5
13.	12.0	16.2

Urinary Excretion of Fluoride: The urinary fluoride excretion was excessive, corresponding to increasing concentration of F^- in drinking water; it varied from hour to hour. Since it was difficult to collect 24-hour pooled urine, samples were collected 2 hours after eating. The results presented in Table 4 show that no linearity was found between intake and output of fluoride. McClure and Kinser (14), Likens et al. (15) and Zipkin et al. (16) reported near equality of fluoride concentrations in drinking water and urine. Contrary to these findings, our investigation indicates that urinary fluoride concentration in fluorosis patients is usually higher than that in drinking water.

Trace Metals: Trace metals were absent in most of the samples. In a few samples, they were present in amounts well below the permissible concentration (0.05 mg/l) in potable water. Almost all samples contained iodide in the desirable concentration. No correlation was observed between the concentrations of fluoride and iodide.

Fluoride Content of Raw Foods: 40 samples of raw foods grown in severe endemic fluorosis areas have been analyzed for F^- content (Table 5). Some of the foods, especially yellow jawar, grains, and pearl millets are high in fluoride, whereas paddy was uniformly low in fluoride. Thus foods grown in endemic areas contribute significant amounts of fluoride.

Table 5

F^- Content of Food Grown in Endemic Fluorosis Areas in Nalgonda

	Commodity Food	Village	F^- (mg/Kg) dry weight
1.	Yellow jawar	Batlapally	41.6
2.	Pearl millet	"	74.0
3.	Paddy	"	2.1
4.	Okra	"	1.4
5.	Green grain	Sivannagudem	21.2
6.	Pearl millet	"	37.0
7.	Paddy	"	6.2
8.	Brinjal	"	2.3
9.	Paddy	Yellareddyguda	1.3
10.	White jawar	"	9.4
11.	Red grain	Kammaguda	52.8
12.	Yellow jawar	"	39.0
13.	White jawar	"	23.0
14.	White jawar	Sarampet	4.8
15.	Paddy	"	2.6
16.	Pearl millet	Kankanalapalli	14.8
17.	Paddy	"	2.4
18.	Vegetation	"	1.3
19.	Paddy	Marigudem	2.1
20.	Tomato	"	0.2

References

1. Ramamohana Rao, N.V., Rajyalakshmi, K., Krishna Sastry, K.R., Subbarao, P., Krishnarao, V.V., and Narasimharao, K.: Distribution of Fluoride in Water Sources in Andhra Pradesh, Proc. Symposium Fluorosis, Ind. Acad. Geo. Sci. Hyderabad 222235, 1974.
2. Daver, M.B.: Occurrence of Fluorosis in Endemic Form in Hyderabad State, Ind. Med. Gaz ., 80:332-336, 1945.
3. Siddiqui, A.R.: Fluorosis in Nalgonda District, Hyderabad Deccan.Brit. Med. J., 2:1408-1451, 1955.
4. Pandit, C.G., Raghavachari, T.N.S., Subba Rao, D., and Krishnamurti, V.: Endemic Fluorosis in South India: A Study of Factors Involved in Production of Mottled Enamel in Children and Severe Bone Manifestations in Adults, Indian J. Med. Res., 28:533-558, 1940.
5. Ramamohana Rao, N.V.: Endemic Fluorosis in Andhra Pradesh, Distribution of Fluoride in Water Sources: Proc. Symposium Comm. Water Supply, NEERI, Nagpur, 123-132, 1966.
6. Ramamohana Rao, N.V., and Rajylakshmi, K.: Studies on Water Quality and Incidence of GeoScience, Hyd., 273-284, 1974.
7. DeEds, F.: Chronic Fluorine Intoxication, Medicine 12:1 -60, 1933.
8. Majumdar, B.N., and Ray, S.N.: Determination of Fluoride in Biological Materials and Its Application in Fluorine Intoxication, Studies in Cattle in India. Ind. J. Med. Res., 35:323-334, 1947.

9. World Health Organization: Fluoride and Human Health. Monograph Series No. 59, 1970, 177-179.
10. Venkateswerlu, P., and Narayanarao, D.: Ameliorative Effects of Vitamin C in Fluorosis, Ind. Med. Gaz. 138-262, 1953.
11. Amer. Public Health Assoc., Amer. Water Works Assoc. of Water Pollution Control Federation, Standard Methods for Examination of Water and Waste Water, 14th Ed., 1976.
12. Association of Official Analytical Chemists; Official Methods of Analysis, 12th Ed., 1975.
13. I.C.M.R., Nutritive Value of Indian Foods, 1970.
14. McClure, F.J., and C.A. Kinser: Fluorine Content of Urine in Relation to Fluorine in Drinking Water, Public Health Rep., 49:1575-1591, 1944.
15. Likins, R.C., McClure, F.J., and Steere, A.C.: Urinary Excretion of Fluoride Following Defluoridation of a Water Supply, Public Health Report, 71:217-220, 1956.
16. Zipkin, I., Likins, R.C., McClure, F.J., and Steere, A.G.: Urinary Fluoride Excretion with Use of Fluoridated Water, Public Health Rep., 71: 767-772, 1956.

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SEQUENCE OF FLUOROTIC CHANGES IN LONG BONES OF MALES AND FEMALES
(An Experimental Study in Rabbits)

by

R.L. Mittal, S.S. Makhni, and D.K. Markan
Patiala, India

SUMMARY: The higher incidence of fluorosis in males than in females, to date, has not been explored. In the present study, the sequence of fluorotic changes in the long bones of male and female rabbits has been noted. Eighty rabbits were divided into two batches of forty each, twenty males and twenty females in each batch. Half of the animals of each sex served as controls. The experimental animals received 2 mgm NaF per kgm body weight per day subcutaneously. One batch was sacrificed after four months; the second, after eight months. Long bones were removed; the weight per unit length of these bones was calculated. Radiological and histological studies were carried out. In females, osteosclerotic changes took place from the very beginning whereas, in males, osteoporosis is followed by osteosclerosis, a significant finding not reported previously in clinical fluorosis in this part of the country which is an endemic fluorosis belt.

KEY WORDS: Fluorotic bone changes; Male-female bone differences; Rabbits

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From the Government Medical College Rajendera Hospital, Patiala, India.

Introduction

Whereas the fluorotic syndrome can affect almost all organs of the body, predominantly the skeletal system is involved. The changes of osteoporosis and osteosclerosis have been reportod. Mainly, however, osteosclerotic type of changes lead to crippling deformities. The incidence of fluorosis has been reported to be lower in females than in males. According to Kumar and Harper in Aden (1), the male and female ratio is 18:1; to Jolly et al. (2) 45.2:26.5; to Krishnamachari and Krishnaswany (3) 15:1. This difference may be due to greater consumption of water by male manual workers and possible to sex hormones. The gross difference in how fluoride affects male and female has not heretofore been investigated.

Material and Methods

Eighty rabbits of four to six months of age were divided into two batches of forty each. Each batch contained twenty male and twenty female rabbits. The batches were subdivided into 10 controls and 10 F^- rabbits.

Half of the animals of each group served as controls. To the rest of the animals, sodium fluoride was given subcutaneously. Distilled water was injected into the control animals. All were kept in separate cages and fed a similar diet. For drinking purposes, all animals received tap water which contained 0.6 ppm of fluoride.

At the end of four months, the first batch of animals was sacrificed, their long bones were taken out and the left femur of each was preserved in formalin solution for histological study. The rest of the long bones were subjected to radiological examination and their weights per unit length were calculated.

The experiment continued on the second batch of animals for another four months after which these were also sacrificed and their long bones were investigated in a manner similar to animals in batch one. The data were analyzed statistically and the two batches were compared.

Results

Weight per unit length of long bones: In the animals of Batch I, weight per unit length of long bones of the fluoride group compared with the control group, showed an increase in females but a decrease in males. In a similar comparison of the animals of the second batch, the weight per unit length increased in both females and males (Table 1). Comparing the fluoride groups, of two batches, although weight per unit length had increased significantly in both sexes of the second batch, the increase was statistically less significant in males than in females (Table 2).

Radiological Study of Long Bones: Definite osteosclerotic changes were observed in the form of thickening of cortices of femora and tibia, of five female animals of the second batch (Fig. 1-4).

Histological Study: In the first batch of six female animals, the number of outer and inner circumferential lamellae had increased with the new Haversian system formation in them. However, in four males, marked areas of resorption were noted.

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In the second batch of seven females, the outer and inner circumferential lamellae had increased more than the first batch with the new Haversian system formation in them. However, two males still showed some areas of resorption which were much less marked than in the first batch (Fig. 5-7).

Table I

Means of Weight per Unit Length of Long Bones mgm/mm

	Femur	Right Tibia Fibula	Left Tibia Fibula	R. Humerus	L. Humerus	Right Ulna	Left Ulna	R. Radius	L. Radius
Batch I									
Female Control	30.63	25.81	25.81	19.61	19.61	8.48	8.49	7.85	7.35
Female Fluoride	32.45	27.96	27.96	22.12	22.12	8.89	8.89	8.33	8.33
Male Control	38.29	31.83	31.83	22.84	22.84	10.27	10.27	8.98	8.98
Male Fluoride	33.03	29.51	29.51	22.66	22.76	9.46	9.29	8.68	8.60
Batch II									
Female Control	37.23	30.85	30.85	25.26	25.26	10.15	10.15	9.57	9.57
Female Fluoride	40.47	33.52	33.52	27.66	27.66	12.04	12.04	11.53	11.53
Male Control	41.24	33.60	33.60	28.68	28.68	12.17	12.17	11.05	11.05
Male Fluoride	41.25	34.36	34.36	28.73	28.73	12.07	12.07	11.14	11.14

Figure 1

Radiograph: Femur of Female Control (FC) Normal

Figure 2

Radiological Comparison of Femora (females), First and Second Batch

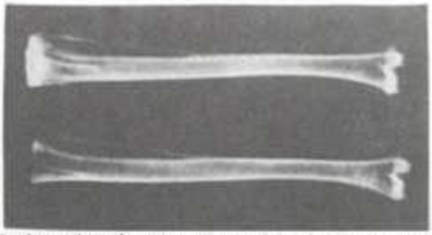
Condensation of cortex, after eight months, clearly visible

Figure 3

Radiograph Tibio-fibula FC Normal

Figure 4

Comparison of Tibiofibula of Female First and Second Batch



Condensation of cortex, of second batch, clearly visible

Table 2

Statistical Comparison of Two Batches of F^- Groups

Bone	't' value	P	Significance
Females			
Femur	3.58	0.01	++
Tibia fibula	3.25	0.01	++
Humerus	4.12	0.01	++
Ulna	4.51	0.001	+++
Radius	4.48	0.001	+++
Male			
Femur	3.12	0.05	+
Tibia fibula	2.85	0.05	+
Humerus	2.61	0.05	+
Ulna	3.20	0.05	+
Radius	3.35	0.05	+

In the second batch of seven females, the outer and inner circumferential lamellae had increased more than the first batch with the new Haversian system formation in them. However, two males still showed some areas of resorption which were much less marked than in the first batch (Fig. 5-7).

Figure 5
Microphotograph FC First Batch Normal

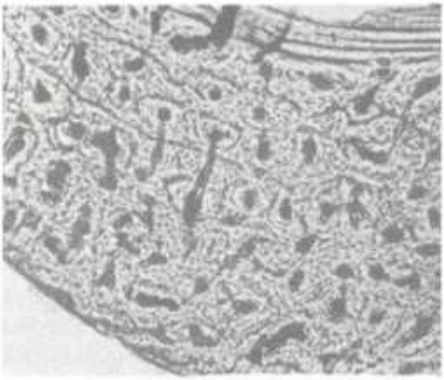
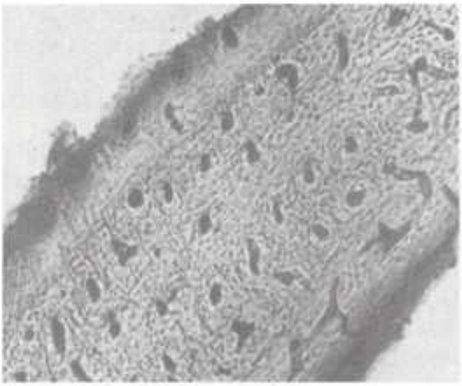


Figure 6
Female Fluoride (FF), First Batch

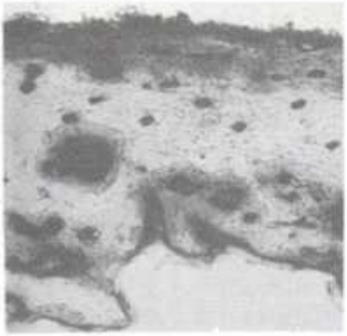


Increased I.C.L.* and O.C.L.** and Haversion system formation in O.C.L.

* I.C.L. inner circumferential lamellae
** O.C.L. outer circumferential lamellae

Discussion

Figure 7
Microphotograph Male Fluoride (MF)



First batch showing resorption

The weight per unit length of long bone, after four months of study, increased in females but decreased in males. After eight months, however, it increased in females as well as in males, although the increase in males was much less marked. This difference in fluoride effect according to sex could be attributed to the different sex hormones since laboratory conditions were similar for all animals and all were fed a similar diet. The findings were statistically significant in radius and ulna of females of the second batch. By that time, sufficient changes must have been produced indicating that prolonged exposure to the fluoride-polluted atmosphere is necessary for intoxication (4). The other bones may have shown significant changes had the exposure been more prolonged. Regarding the males, the changes were insignificant because preliminary decrease was followed by an increase in weight per unit length causing changes insufficient to be statistically significant. Additional exposure would probably have produced significant changes.

Radiologically, apparent osteosclerotic changes in females of the second batch could be due to the fact that these changes start at the beginning. On the other hand, in males, such changes could not be seen because in them osteoporosis occurs first followed by osteosclerosis which is clear from the findings of weight per unit length of bones. The osteosclerotic changes were not apparent in males because the experiment was not prolonged sufficiently for these changes to be visible. Histological study further supported these findings.

In an experimental study on rabbits by Makhni et al. (5), although osteoporotic changes were reported associated with 2-4 months duration of fluoride exposure, yet in that study no attention was paid to the differences between sexes.

In a survey by Krishnamachari and Krishnaswamy (3) in Andhra Pradesh, osteoporotic changes were noted in male limb bones with a 15:1 ratio to females. The cause of osteoporotic changes in males at the age of adolescence and young adults (10-30 years), was attributed to the low calcium content of the diet of these individuals and to the male hormones. In older age groups, mainly osteosclerotic changes, especially in the spine, were noted.

In the present study, the osteoporotic changes were followed by osteosclerosis in males whereas, in females, osteosclerotic changes were noted from the very beginning, a significant finding unreported to date in this part of the country. Sex hormones, either male or female, might be responsible for this difference in fluorotic manifestation. In Punjab, the most prosperous state of India, osteoporosis is not seen because the average diet consumed by rural folk is high in calcium and vitamin D.

References

1. Kumar, S.P., and Harper, R.A.K.: Fluorosis in Aden. Brit. J. Radiol., 36:497-502, 1963.
2. Jolly, S.S., Singh B.M. and Mathur O.C.: Endemic Fluorosis in Punjab (India). The Amer. J. Med. 47:553-563, 1969.
3. Krishnamachari, K.A.V.R., and Krishnaswamy, K.: Genu Valgum and Osteoporosis in an Area of Endemic Fluorosis. Lancet, II:877, 1973.
4. Singh, A., Jolly, S.S., Bansal, B.C., and Mathur, O.C.: Endemic Fluorosis, Medicine, 42:229, 1963; 44:97, 1965.
5. Makhni, S.S., Singh, P., and Thapar, S.P.: Long-term Effect of Fluoride Administration, an Experimental Study. Fluoride, 10:82-86, 1979.

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DEPOSITION VELOCITY OF AMBIENT FLUORIDES ON EXPERIMENTAL GRASS CULTURES

by

L. De Temmerman, H. Baeten, and E.L. Raekelboom
Institute for Chemical Research
Museumlaan 5, B-1980 Tervuren, Belgium

SUMMARY: Fluoride deposition velocities were calculated on experimental grass cultures in containers. The calculated values reached a maximum during the summer when they were based on "ground surface units" covered by plants. However, when the same values were recalculated based on "units of leaf surface", the deposition rates in relation to ambient fluoride concentrations were fairly constant over the growing season, despite the changing growing conditions and the different activity of the plants. Therefore the absorption of fluoride by plants must be principally a physical process.

The general deposition velocity on the leaf surface of grass amounted to an average of 1.20 mms^{-1}. In a limited period of time this value was strongly influenced by climatic conditions.

KEY WORDS: Ambient fluoride; Deposition velocity; Grass cultures.

Introduction

The comparison of atmospheric fluoride pollution and accumulation of fluoride by grasses is, in most cases, based on ambient concentration measurements and determination of the fluoride concentration in the vegetation. However the concentration of fluoride in plant material depends on specific plant properties such as growth, biomass per unit ground surface and the specific exposure characteristics of the leaves. These parameters are more or less enclosed in the deposition velocity which is determined by $vd = \frac{F}{\chi}$ in which vd = deposition velocity (ms^{-1}); F = total vertical flux or deposition rate (μgm^{-2-1}); mean concentration at a specified reference height above the canopy (μgm^{-3}). Deposition velocities have been used by Chamberlain (1) for the deposition of spores, but have also been calculated for sulphur dioxide (2 - 5) as well as for hydrogen fluoride deposition (6, 7).

Two different methods can be used to calculate the deposition rate on experimental grass cultures. It can be calculated per unit of ground-surface covered by plants or per unit of leaf surface. For the latter method the definition of "Specific Leaf Weight" is introduced (SLW = g dry weight per m^2 of leaf surface using both sides of the leaf).

The specific leaf weight is a very important parameter because, the greater the exposed leaf surface per unit ground surface, the greater the contact surface with the polluted air. This is very important for the accumulation of fluoride. The relationship between the leaf surface and the ground surface on which the plants are growing is the leaf Area Index, LAI, m^2m^{-2}.

From Institut de Recherches Chimiques, Ministere de l'agric, Tervuren, Belgium

Materials and Methods

Perennial raygrass, Lolium perenne, cv. Melino RVP, was sown in plastic containers, grown in the greenhouse and, after acclimatization, placed in experimental fields by the end of April. Permanent cultures, with integration periods of 28 days and alternating cultures with integration periods of 14 days were used. In the first method mentioned, the containers remained the whole growing season on the experimental fields. The grass was cut every 4 weeks, watersupply was provided by filter candles and, after each cut, fertilizers were added.

For the second method, every 14 days new grass cultures were sown in a reference area and, after growing, the containers were exposed 14 days in the polluted area. After each cut the container was replaced by another, which had been cut 5 days previously. The main difference between the two methods is that, by alternating cultures, there was always sufficient grass in the container to integrate the pollution whereas the permanent cultures, after each cut, needed 2 to 5 days for the formation of new leaves. Permanent cultures also suffered long term pollution effects which is not the case with alternating cultures. The containers used were 48 X 30 cm and 20 cm deep. The vertical sides were covered by 1 cm insulation material to avoid warming up of the peat soil in the containers. A water-reservoir (25 l) was available underground (8). The samples from each cutting were weighed and dried. Fluorides were measured by a specific electrode after extraction with 0.1 N nitric acid and the addition of a buffer solution (9).

Knowing the calculated fluoride concentration in the plant mater' (dry weight), the grass yield of one container (g dry weight), the surface of a container (m^2) and the exposure time, the deposition velocity could be calculated. To calculate the deposition velocity per unit leaf surface, the data were recalculated knowing that 20 g dry yield is equal to 1 m^2 leaf surface for Lolium perenne when both leaf sides were taken into consideration (10). Because of the lack of grass growth in the containers during the first week, only the last 3 weeks of the integration period for permanent cultures were taken into account. The fluoride-concentration of the air was measured with the single filter method (membrane filter impregnated with sodium formate) as described by Elfers and Decker (11) and slightly modified by Verduyn et al., (12).

Results and Discussion

The results of the deposition velocities on grass cultures, summarized in Table 1, show slightly higher values that, however, are not significant for alternating cultures due, possibly, to the fact that, during the whole growing season, young grass is available in alternating cultures. The deposition velocities of fluoride on grass cultures, calculated per unit of ground surface, reached a maximum during the summer months (fig. 1). In a period of rapid grass growth, the accumulation of fluoride (deposition rate) is apparently the highest. However, this can be explained by a much higher leaf area index. Indeed, when the deposition velocity is calculated per unit of leaf surface, the values are rather constant except for an increase toward the end of the growing season. This increase could be due to a change in specific leaf weight. The used value (20 gm^{-2}) is an average over the growing season

Table 1

Deposition Velocities
(vd 150/30 on grass cultures (mms^{-1}))

permanent cultures (n = 26/year)

PER UNIT GROUND SURFACE				PER UNIT LEAF SURFACE		
year	min - max	$\bar{x}$	s	min - max	$\bar{x}$	s
1979	4.2 - 11.4	7.2	2.8	0.7 - 1.85	1.38	0.5
1980	3.85- 20.2	10.0	6.0	0.76- 2.33	1.13	0.5
1981	4.52- 15.7	10.4	4.4	0.75- 2.06	1.17	0.5
1982	7.5 - 22.1	12	5.3	0.44- 2.10	1.17	0.6
mean		10.1	4.9		1.20	0.5

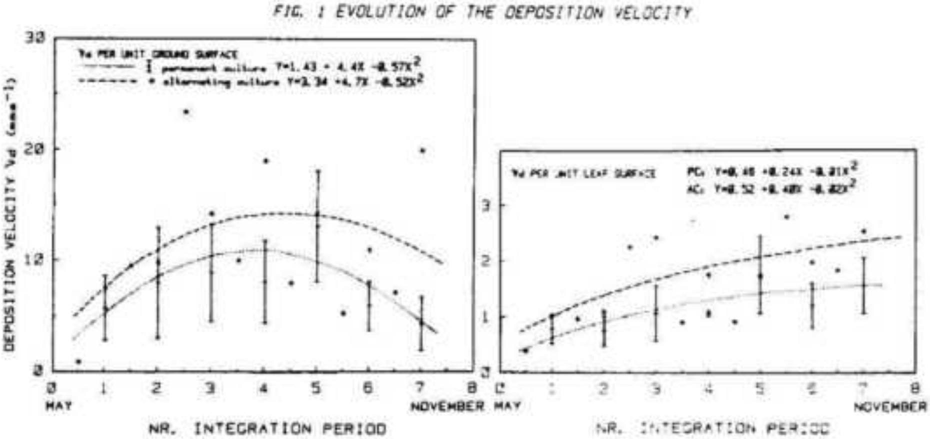
alternating cultures (n = 28/year)

PER UNIT GROUND SURFACE				PER UNIT LEAF SURFACE		
year	min - max	$\bar{x}$	s	min - max	$\bar{x}$	s
1981	0.77- 25.6	12.3	6.7	0.32- 4.55	1.8	1.2
1982	2.7 - 21.2	10.8	6.8	0.36- 2.83	1.4	0.9
mean		11.6	6.7		1.6	1.1

vd 150/30: immission measurement level at 150 cm, plant canopy level at 30 cm.

and is certainly not constant. Indeed, the leaves are thicker toward the end of the growing season and the proportion of shoots compared to leaves changes during the vegetation period. Davison (10) found a "Specific Leaf Weight" for leaves of perennial ray grass of about 10 gm^{-2} and for shoots, 56 gm^{-2}. For the whole plant, including approximately 20% of shoot material, 20 gm^{-2} was taken. The real value would be lower at the beginning of the growing season and higher at the end (more shoot material). This is also reflected in the evolution of the dry weight of grass, which is 10% at the beginning and 25% at the end of the vegetation period. When taking a value of 15 gm^{-2} at the beginning and 25 gm^{-2} at the end of the growing season as Specific Leaf Weight, the deposition velocity on the basis of leaf area was 1.17 in May and 1.24 in November, which indicated that the deposition velocity was constant over the whole vegetation period.

FIG. 1 EVOLUTION OF THE DEPOSITION VELOCITY



Taking into account the changing specific leaf weight during the summer period, the deposition velocity per unit of leaf surface of grass cultures was 1.20 mms^{-1} (containers at 30 cm and immission measurements at 150 cm above ground level). This value was constant during the growing season and was also constant when measured over different years (Table 1). Fluctuations in between individual periods were due to changing climatic conditions. Indeed, a large amount of fluoride could be eliminated by rain; this phenomena results in a lower deposition velocity (13). However, when climatic conditions were balanced out over the growing season, constant mean values resulted.

Conclusion

As the leaf Area Index is the most important factor determining the deposition velocity or, in other words, in determining the relation between ambient fluorides and their uptake by plants, the absorption of fluoride must be a physical process, independent of metabolic activity. Indeed, the larger the leaf surface, the higher the uptake of fluoride by plants, independent of growth rate.

References

1. Chamberlain, A.C.: Transport of Lycopodium Spores and Other Small Particles to Rough Surfaces. Prod. Roy. Soc. Ser. A., 296:45-70, 1966.
2. Owers, M.J., and Powell, A.W.: Deposition velocity of Sulphur Dioxide on Land and Water Surfaces using a 35S Tracer Method. Atmosph. Env., 8:63-67, 1974.
3. Garland, J.A., Atkins, D.H.F., Readings, C.J., and Caughey, S.J.: Deposition of Gaseous Sulphur Dioxide to the Ground. Atmosph. Env., 8:75-79, 1974.
4. Shepherd, J.G.: Measurements of the Direct Deposition of Sulphur Dioxide into Grass and Water by the Profile Method. Atmosph. Env., 8:69-74, 1974.
5. Wesley, M.L., and Hicks, B.B.: Some Factors that Affect the Deposition Rates of Sulphur Dioxide and Similar Gases on Vegetation. J.A.P.C.A., 27:1110-1115, 1979.
6. Israel, C.W.: Deposition Velocity of Gaseous Fluorides on Alfalfa. Atmosph. Env., 8:1329-1330, 1974.
7. Schwela, D.H.: An Estimate of Deposition Velocities of Several Air Pollutants on Grass. Ecotoxicology and Environmental Safety, 3:174-189, 1979.
8. De Temmerman, L.O., Baeten, H., Raekelboom, E.L.: Étude Biologique d'une pollution fluorée dans une zone industrielle. Revue de l'agriculture, In: press, 1985.
9. Roost, F., and Sigg, A.: Erfahrungen mit einer potentiometrischen Fluor--Bestimmungsmethods für biologische Materialen. Staub-Reinhalt. Luft 38: 363-366, 1978.
10. Davison, A.: The Effects of Fluorides on Plant Growth and Forage Quality. In: Effects of Gaseous Air Pollution in Agriculture and Horticulture. Ed. Unsworth M.H. & D.P. Ormrod. Butterworth Scientific, London 267,291, 1982.
11. Elfers, L.A., and Decker, D.: Determination of Fluoride in Air and Stock Gas Samples by use of an Ion Specific Electrode. Anal. Chemistry, 40: 1658-1661, 1968.
12. Verduyn, G., Muylle, E., and Legrand, M.: Luchtverontreiniging door fluoriden in de omgeving van Brugge van 1 juli 1975 tot 31 januari 1976. Rapport Instituut voor Hygiëne en Epidemiologie Juliette Wytsmanstraat 14, Brussells, 1976
13 De Temmerman, L.O.: Involed van atmosferische fluorideverontreiniging op kulturen van Engels raaigras, Lolium perenne L. en op artificiële graslandassociaties. Docoraatsthesis, State University Gent, Faculty of Agronomy, 1984.

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FLUORIDE CONTAMINATION IN RELATION TO LAND FAULTS IN LA RIOJA, SPAIN

by

I. Jiménez Domínguez*
La Rioja, Spain

SUMMARY: 170 samples from water supplies, wells, springs, and rivers in different parts of the province of La Rioja in the northeastern part of Spain were analyzed to determine the fluoride content and conductivity. This area, which covers 5,034 sq. mi., has a population of 237,000 inhabitants. In general, elevated fluoride values coincide with the geological land faults in the region.

KEY WORDS: Fluoride; Land faults; Water contamination

Introduction

The majority of the natural waters of La Rioja Province in northeastern Spain are known to be low in fluoride. Our observations of natural waters from thermal and nonthermal springs and underground wells of medium depth corroborate studies by Teotia and Teotia (1) on decreased fluoride content of waters from wells of medium depth. The four areas in the province of La Rioja which have their own geological characteristics (2) are Low Rioja, Middle Rioja, High Rioja, and mountain range. Of the 170 samples analyzed, the most significant fluoride values were found in Low and Middle Rioja; at isolated points, the majority come from wells and springs in other far distant areas. The areas of significant fluoride levels conform to a geological map of quarternary faults of Low Rioja (3,4). These relationships seem to indicate that significant fluoride levels occur in those places where the land support of these waters coincides with a land fault.

Materials and Methods

Nonchlorinated water, sampled uniformly in the majority of the towns of the province, was introduced into polyethylene flasks and analyzed by the selective fluoride ion electrode (5-8). The standard solution and the reagents had the same temperature as during analysis. Calibration of the apparatus was done daily. Results are expressed in ppm for the fluoride concentration and conductivity results are given in µS/cm.

Results

The most significant values of fluoride, which are typical of the entire province, together with their source and conductivity, appear in Table 1.

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*From the author's M.S. thesis performed under the direction of the faculty of Analytical Chemistry, University of Zaragoza, Spain. Address correspondence to: Duquesa de la Victoria 20 - 2º 26003 Logroño (La Rioja) Spain.

Discussion

The fluoride content of the majority of natural waters in LaRioja is below 0.2 ppm. The highest values were found in Torrecilla in Cameros 0.9 ppm (spring), Pradejón 1.1 ppm (spring) and Arnedillo 3.2 ppm (spring). The first and third are thermal waters. The values shown on the table correspond geologically with a study on quaternary faults of Low Rioja (3,4) as shown on Fig. 1. The concentration at significant points, presented on the map of the region (Fig. 2), shows a parallel between fluoride in water and land faults, which are active in this case due to a continuous rupture of the land brought about by endogenous forces that cause pressure and temperature increases in the space occupied and in the distance covered by underground waters. Due to these ruptures, big rocks, underground in the past, may have access to the

Table 1

F^- Values Typical of the Province, Their Source and Conductivity

Towns & Cities	Source	F^- ppm	Conductivity µS/cm
	Low Rioja		
Rincon de Soto	Well	0.5	1.700
Arnedillo	Thermal Springs Resort	3.2	9.720
Arnedillo	Cidacos River	0.9	3.250
Arnedo	Cidacos River	0.23	1.000
Bergasa	Fountain	0.28	514
Quel	Water Supply	0.22	460
St. Eulalia	Water Supply	0.25	870
Calahorra	Water Supply	0.24	1.740
Calahorra	Fountain	0.26	1.250
Pradejon	Spring	1.1	573
	Middle Rioja		
Agoncillo	Leza River	0.24	2.090
Arrubal	Well	0.36	5.190
Clavijo	Spring	0.5	1.050
Leza de rio Leza	Water Supply	0.28	370
Logrono	Well	0.7	2.500
St. Engracia DeJubera	Water Supply	0.3	569
Trevijano	Water Supply	0.3	280
	High Rioja		
Brinas	Spring	0.36	933
Cenicero	Well	0.42	1.110
Cuzcurrita	Spring	0.4	927
Fonzaleche	Spring	0.3	1.520
Haro	Ebro River	0.24	680
Sajazarra	Water Supply	0.26	853
San Asensio	Water Supply	0.52	1.000
Treviana	Fountain	0.32	600
Villalba de Rioja	Water Supply	0.45	1.300
	Mountain Range Rioja		
Soto De Cameros	Spring	0.35	530
Torrecilla En Cameros	Spring	0.9	788

The concentration of fluoride shown on the table is the average value of 5 determinations with 1% standard deviation. The remaining values, which are not shown on the table , were less than or equal to 0.2 ppm.

exterior and provide some fluoride compound, mineral dissolution of which could be favored by abnormal conditions of pressure and temperature. The map likewise shows an accumulation of significant points which are less extensive geographically in contrast to those geologically under study.

High concentrations of fluoride in the majority of samples analyzed are accompanied by high conductivity values. This does not imply however that high conductivity is invariably associated with high fluoride levels.

Figure 1

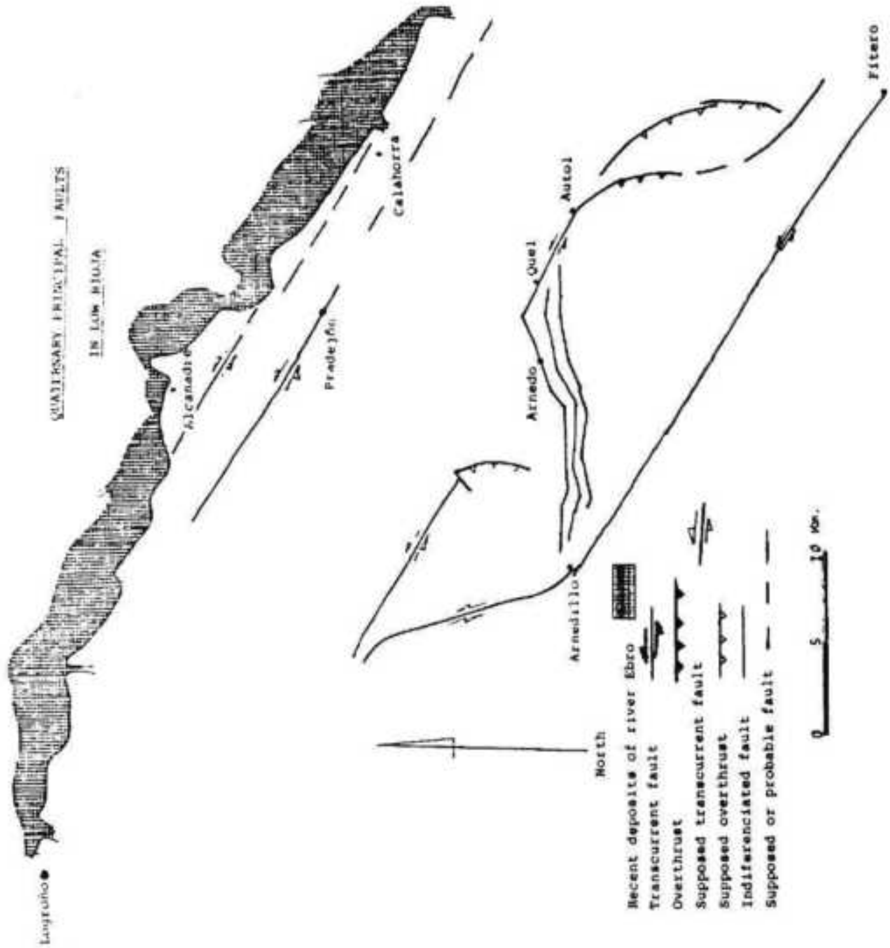
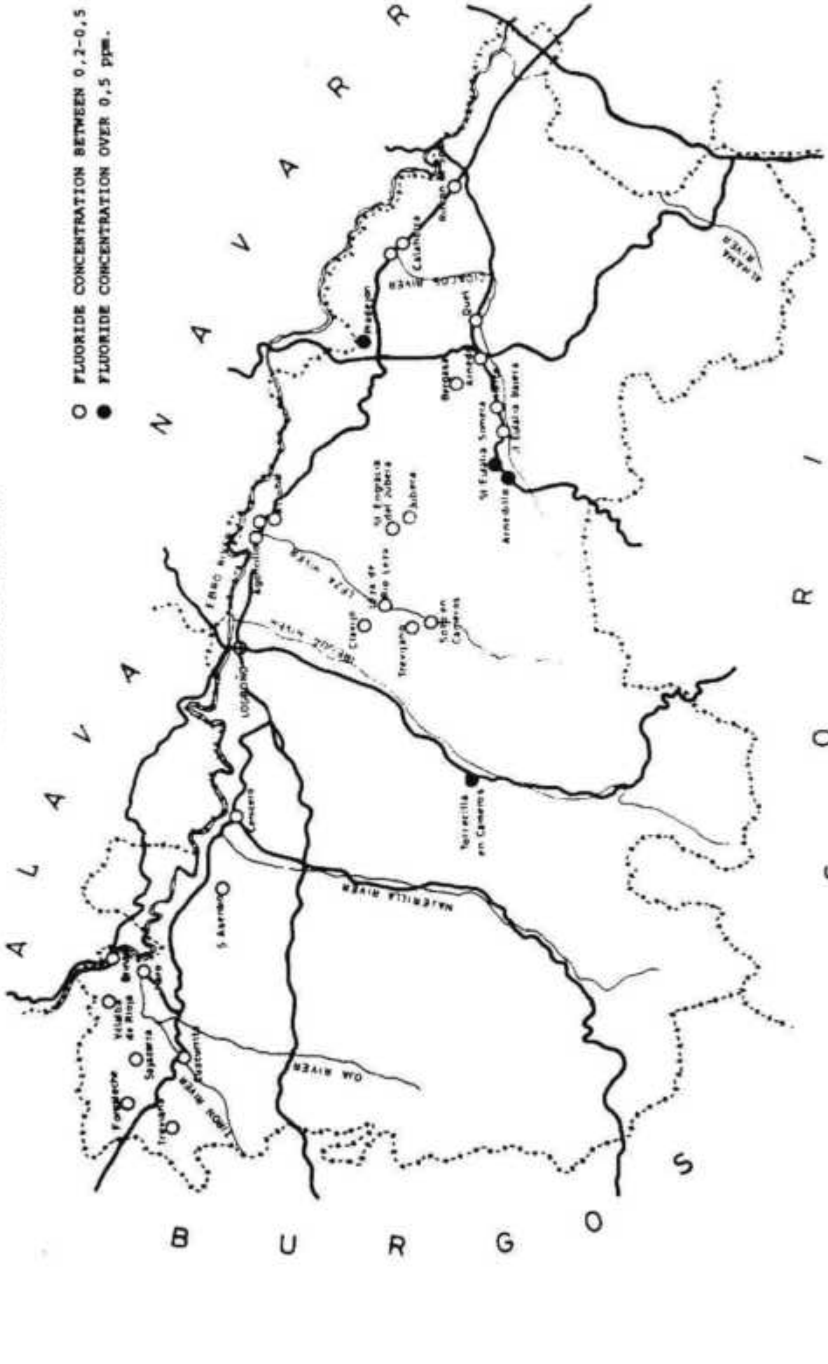


Figure 2

PROVINCIA DE LA RIOJA

○ FLUORIDE CONCENTRATION BETWEEN 0,2-0,5 ppm.
● FLUORIDE CONCENTRATION OVER 0,5 ppm.



References

1. Teotia, S.P.P., Teotia, M., Singh, D.P., Rathour, R.S., Singh, C.V., and Tomar, N.P.S.: Endemic Fluorosis: Change to Deeper Bore Wells as a Practical Community Acceptable Approach to its Eradication, Fluoride, 17:48-52, 1984.
2. Martin Losa, F. y Otros: Encyclopedia of LaRioja. Caja de Ahorros y Monte de Piedad de Zaragoza Aragón y Rioja. La Rioja (Espana) 1, 2 y 3, 1983.
3. Atares, A., Oretga, A., Perez, Lorente, F.: Quaternary Faults Near Alcanadre and in the Ebro Basin in Low Rioja. Cuadernos Investigacion Geográfica. Colegio Uversitario de La Rioja. Tomo 9, Vol. 1 y 2:29-40, 1984.
4. Perez Lorente, F.:Rioja Neotectonic. Some Morphological Peculiarities. Iª Jornada de Geografía en La Rioja (Espana) 1984.
5. Perez Olmos, R.: Use of an Ion-Selective Electrode for Water Analysis. Química e Industria, 29, 9:583, 1983.
6. Aznarez Alduan, J.:Instrumental Analysis II. Cátedra de Química Analítica. Universidad de Zaragoza. Espana, 1980.
7. Harwood, J.E.: The Use of an Ion-Selective Electrode for Routine Fluoride Analyses of Water Samples. National Institute for Water Research of the South African Council for Scientific and Industrial Research. P.O. Box 395, Pretoria (South Africa). Water Research, Pergamon Press, 3:273, 1969.
8. Instruction Manual Fluoride Electrodes "Orion" model 94-09. Research Inc. (U.S.A.).

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F^- EFFECT ON CALCIUM AND PHOSPHATE CONTENT IN FEMUR OF THE PREGNANT RAT AND ON CALCIUM TRANSFER THROUGH THE PLACENTA

by

L. Stosser, and R. Heinrich
Erfurt, GDR

SUMMARY: Pregnant Wistar rats, which had received distilled water or water to which 100 ppm F^- was added were decapitated at two intervals during pregnancy in order to determine the calcium, phosphate and fluoride content in the femur. Especially the calcium content of the bones obviously reveals the influence of fluoride on the mineral metabolism. A decrease in the calcium content, due to pregnancy, is apparent only in control animals. In fluoride-loaded animals the calcium content remained constant. Furthermore, the phosphorus content of the bones seems to be elevated in the fluo-

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ride group. No effect of fluoride on the transport of calcium through the placenta was found.

KEY WORDS: Bone metabolism; Fluoride; Pregnancy; Rat.

Introduction

In view of the use of fluorides early in life to prevent dental caries, the question of fluoride application to pregnant women continues to receive attention (1-2). On the one hand, there seems to be a fluoride barrier in the placenta, at least in some experimental animals (3, 4). On the other hand, some reciprocity cannot be denied between calcium and fluoride due to their high chemical affinity. Thus fluoride effect on calcium metabolism in parent animals and in fetuses has been examined in the gravid rat (5, 6).

Materials and Methods

Female Wistar rats (200 days old) which had been given water containing 100 ppm F^- or distilled water for 200 days were given a Ca^{45} injection on the 14th or 20th day of pregnancy (in each subgroup respectively). Twenty-four hours later the Ca^{45} content of the femur of the parent animal and of the whole fetuses was examined. The definition of the subgroups of animals employed was based on the day of injection and killing (Table 1). The biological material (maternal femur, fetuses) was incinerated in platinum trays for the determination of the Ca^{45} content, and the amount of Ca^{45} was determined in a liquid scintillation counter "Tricarb" (Model 3375-01 Packard) in the ash after its decomposition in 1 molar hydrochloric acid. One part of acidic solution was mixed with 10 parts of Insta-Gel (Packard) before counting. The quench caused by different biological materials was calculated. The amount of Ca^{45} established was then determined by calculation as percentage value of the individual dosage of Ca^{45} in each maternal animal (20 µCi/kg). In addition to the Ca^{45}, the total content of calcium was determined in the same bone material, using an atomicadsorption spectrometer (ASS-1, Carl Zeiss Jena). The phosphate was estimated according to Fiske and Subbarow (7); the fluoride according to Singer and Armstrong (8) using an ionsensitive electrode (Radelkis, Hungaris). All chemicals used were "analytical grade quality.

Table 1

Groups and Subgroups of Female Gravid Rats

F^-group	No. of Animals	Controls	No. of Animals
14-15*	10	14-15	10
20-21	10	20-21	10

*14-15 represent days of ^{45}Ca-injection and killing within pregnancy.

Results and Discussion

The fetuses of the fluoride group were statistically significantly lighter as to their ash and fresh weight (Table 2). The amount of radioactive calcium that was transferred to whole fetuses within 24 hours' time was 0.1% on the 15th day and 30% of the individual maternal injected dosage on the 21st day. Ca^{45} transport from fluoride-leaded animals to their whole litter was significantly lower, but no difference was observed in the Ca^{45} content per mg ash of the fetuses of the fluoride and control group. The Ca^{45} accumulation on the 21st day reflects the high demand of calcifying tissues for mineral ions at the end of grafidity.

Table 2

Weight and Ca^{45} Transfer from Gravid Rat to Fetuses (Mean SD)

	Groups of Rats			
	14-15		20-21	
	F	Control	F	Control
Fresh weight (g)	0.10±0.01	0.12±0.02	3.15±0.15	3.65±0.20
Weight of ash (mg)	1.13±0.21	1.39±0.20	44.40±2.4	51.10±3.43
Ca^{45} transfer (% of injected dosage)				
per whole fetuses (litter)	0.07±0.01	0.07±0.02	29.40±4.09	31.80±5.78
per 1 mg of ash	0.005±0.001	0.005±0.0007	0.064±0.004	0.063±0.007

In the maternal femur, the Ca^{45} content of the injected dosage per 100 mg femur ash shows a clear decrease from the 15th to the 21st day of pregnancy (Table 3). No differences of the tracer content that might have occurred due to fluoride in the experimental animals can be confirmed. This is entirely different when the total content of calcium in the bones is taken into consideration. The parent animals given 100 ppm F^- show a stable percentage of calcium in bone ash which scarcely changes despite the high need of calcium by mineralizing fetuses. Controls given distilled water differ significantly only at the 21st day of pregnancy. On the 15th day of gravidity, these bones contain the same amount of calcium (40 - 41%). Six days later this value is significantly lower (37.9%) in the controls than in the fluoride animals. The proportion of phosphate of the same femur correlates with the calcium values (Table 3). The percentage of phosphate, however, seems to be higher in fluoride-loaded bones (26 - 27%) than in control bones (20 - 21%). The Ca/P ratio decreases from 1.8 to 1.5 in the femur ash of control and fluoride rats respectively. The fluoride content of the bones clearly reflects fluoride supply (Table 3). The femur ash content of fluoride was 20 times higher than in controls.

To summarize the fluoride content in bones corresponds to results obtained by other authors both regarding the experimental group (9 - 72) and the control group (13). Indications of the influence on mineral metabolism by fluoride are given by the total content of calcium in the bones that remains unchanged in the fluoride group of the pregnant animals, whereas a decrease in the values from 41.6 to 37.9% reflects the need for calcium by the fetuses in the control group. This finding can be considered an indicator of a positive calcium balance by fluoride or sounder binding of calcium in the fluoride-loaded bony tissues. Such a difference cannot be detected by means of radioactive calcium within a period of 24 hours. Messer and co-workers (14) stated that total calcium content gives clearer indications of changes in the bone than examination using radioactive isotopes.

Table 3

Ca^{45}, Total Calcium, Phosphorus and Fluoride Content of Femur Ash of Gravid Rats (Mean±SD)

	Groups of Rats			
Days of Pregnancy	14-15		20-21	
	F	Control	F	Control
Ca^{45} content (% of injected dosage per 100 mg femur ash	0.28±0.04	0.24±0.04	0.19±0.03	0.17±0.03
total Ca content (per 100 mg femur ash)	40.19±3.10	41.60±2.30	40.62±2.70	37.88±2.60
P content (per 100 mg femur ash)	26.70±4.90	22.90±2.40	25.40±3.80	21.20±3.40
Ca/P ratio (mg/mg)	1.57	1.82	1.60	1.79
F^- content (ppm)	2000±185	150±10	6700±324	290±16

Estimation of the phosphate content of maternal femur ash shows a slight increase associated with fluoride without clear influence of the duration of gravidity. On the other hand, many authors (15 - 18) have reported that fluoride causes no changes in phosphate content. Bang (9), however, found a higher level of phosphate after 100 ppm fluoride application on mice. The reason for this increase may be a higher content of organic bound phosphate which functions in mineralization of hard tissues (19, 20). Raised phosphate levels are responsible for the changes in the Ca/P ratio but both results are in agreement with values of 1:1 to 2:1 reported by Kaufmann and Kleinberg (21) about hydroxyapatite of bone.

It seems that the transfer of calcium through the placenta is not easily influenced by fluoride whereas, in general, high amounts of fluoride inhibit growth in utero (22, 23).

References

1. Gedalia, I., and Bindermann, I.: The Composition of Developing Deciduous Dentition in Relation to Fluoride in Tissue Fluids. J. Dent. Res., 48:761-765, 1969.
2. Held, H.R.: Der Durchtritt des Fluors durch die Plazenta und sein Übertritt in die Milch. Schweiz. med. Wochenschr. 82:297-301, 1952.
3. Ericsson, Y., and Ullberg, S.: Autoradiographic Investigation of the Distribution of F-18 in Mice and Rats. Acta Odontol. Scand. 16:363-374, 1958.
4. Ericsson, Y., and Malmäs, C.: Placental Transfer of Fluoride Investigated with F-18 in Man and Rabbit. Acta Obst. et Gynecol. Scand. 41: 144-157, 1962.
5. Stösser, L., and Schmidt, H.: Kalziumtransport via Plazenta bei gleichzeitiger Gabe von Fluorid bei der Ratte. Zahn-, Mund-u. Kieferheilkd, 65:176-185, 1977.

6. Stösser, L., and Heinrich, R.: Der Einfluß des Fluorids auf den Kalzium and Phosphatgehalt im Femur der trächtigen Ratte. Zahn-, Mund- u. Kieferheilkd, 69:12-17, 1981.
7. Fiske, C.H., and Subbarow, Y.: The Colorimetric Determination of Phosphorus. J. Biol. Chem., 66:375-400, 1925.
8. Singer, L., and Armstrong, W.D.: Determination of Fluoride in Bone with the Fluoride Sensitive Electrode. Analyt. Chem. 40:613-614,1968.
9. Bang, S.: Effects of Fluoride on the Chemical Composition of Inorganic Bone Substance. In: Courvoisier, B., Donath, A., and Baud, C.A. (Ed.) Symp. CEMO II "Fluoride and Bone", 1977, Geneva: Editions Medicine et Hygiene 1978, pp. 56-61.
10. Baylink, D., Wergedal, J., Staffer, M., and Rich, C.: Effects of Fluoride on Bone Formation, Mineralization, Resorption in the Rat. In: Visher, T.L. (Ed.), Fluoride in Medicine, Bern-Stuttgart-Wien, H.Huber, 1970, pp. 37-70.
11. Guggenheim, K., Simkin, A., and Wolinsky, I.: The Effect of Fluoride on Bone of Rats Fed Diets Deficient in Calcium and Phosphorus. Calcif. Tiss. Res., 22:9-17, 1976.
12. Lengemar, F.W., and Comar, C.L.: Fluoridated Water and the Skeletal Uptake of Sr-85 and Ca-45 by Rats. J. Nutr., 79:195-197, 1963.
13. Patz, J.: Pharmakokinetische Untersuchungen zum Fluoridstoffwechsel. Stuttgart, G. Thieme, 1975.
14. Messer, H.H., Armstrong, W.D., and Singer, L.: Fluoride, PTH and Calcitonin: Interrelationship in Bone Calcium Metabolism. Calcif. Tissue Res., 13:217-225, 1973.
15. Deshmukh, D.S., Meranger, J.C., and Shah, B.G.: The Effect of Dietary Fluoride on Calcium and Phosphorus Metabolism of Rats. Canad. J. Physiol., and Pharmacol., 48:503-509, 1970.
16. McClure, F.J., and Mitchell, H.H.: The Effect of Fluorine on Calcium Metabolism of Albino Rats and the Compositions of Bones. J. Biol.Chem. 90:297-320, 1951.
17. Schmidt, H.: Der Fluorstoffwechsel. Dt. Zahn-, Mund- und Kieferheilkd, 59:289-317, 1972.
18. Wolinsky, I., Simkin, A., and Guggenheim, K.: Effects of Fluoride on Metabolism and Mechanical Properties of Rat Bone. Am. J. Physiol., 223:46-50, 1972
19. Boskey, A.C., Alperin, D.G., Ronner, H., and Posner, A.S.: A Calcium-Phospholipid-Phosphate Complex in Bone. J. Dental Res., 54:316, 1975.
20. DeSteno, C.V., Feagin, F., and Butler, W.T. Mineralization of Dentin, Bone and Tendon in Vitro. Calcif. Tissue Res., 17:161-163, 1975.
21. Kaufmann, H.W., and Kleinberg, I.: Conditions Favoring Brushite and Apatite Formation from Amorphous and Poorly Crystalline Calcium Phosphates. J. Dental Res., 54:124, 1975.
22. Forsyth, D.M., Pond, W.G. and Knock, L.: Effect of dietary calcium and fluoride levels on growth and reproduction of swine. Nut. Rep. Intern. 5:313-320, 1972
23. Loew, R.K., Fesseler A. and Ehrenberg-Kückebusch, W.: Der Fluoridgehalt nichtdurchgebrochener Rattenmolaren in Abhängigkeit vom mütterlichen Fluoridangebot während der Paarung, Trächtigkeit und Laktation. Deut. zahnärztl. Z.28:705-710, 1973.

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EFFECTS OF SUPPLEMENTAL VITAMIN E ON DENTAL FLUOROSIS IN RATS. A QUALITATIVE PRELIMINARY STUDY

by

Albert W. Burgstahler
Lawrence, Kansas, USA

SUMMARY: A daily oral supplement (10-15 mg) of d-α-tocopherol (vitamin E) given to rats consuming a standard laboratory diet with 23, 45, or 90 ppm fluoride in their drinking water did not prevent or delay the development of fluorosis in their incisor teeth. However, the fluorotic dental mottling in the vitamin E-supplemented rats, especially in the females, was less pronounced and gradually lost its alternating brown and white horizontal striations. Moreover, the lower incisors of the vitamin-E supplemented females showed more wear attrition than did those of either the nonsupplemented females or the supplemented or nonsupplemented males.

KEY WORDS: Dental fluorosis; rats; tooth wear; vitamin E.

Introduction

Administration of α-tocopherol (vitamin E) to rats has been reported recently to provide significant protection against fluoride-induced chromosome damage to their bone marrow cells (1,2). Earlier research has also shown that vitamin E deficiency in rats, especially in conjunction with an elevated-fat intake (3) or a low-protein diet (4,5), causes the incisor teeth to lose their normal orange-brown pigmentation and to become abnormally white or to develop brown and white mottling. Because fluoride (F^-) produces similar changes in rat teeth (6-9), it is of interest to learn what effects vitamin E might have on F^--induced disturbances of rat dentition (dental fluorosis). In the absence of elevated-F^- intake, excess vitamin E (2500 IU/kg diet) has no deleterious consequences to the teeth of rats, although it does raise the calcium and phosphorus content slightly (10).

Materials and Methods

Two series of experiments were performed, each beginning with 3- or 4-week-old Sprague-Dawley albino rats housed in 30x55x20-cm top-vented plastic cages (2 or 3 animals per cage) with aspen wood cuttings for bedding. Throughout the study the animals were kept on a 12-hr light/dark cycle in an air-conditioned room at a temperature of 22±2°C. Purina Rodent Chow (No. 5001) containing 65 IU of vitamin E/kg and 35 ppm F^- was supplied ad libitum, as was the drinking water. The latter was delivered by stainless steel tubes from inverted bottles.

The first series of experiments was conducted on 4-week-old females di-

From the Department of Chemistry, The University of Kansas, Lawrence, Kansas 66045, USA

vided into three groups. The first group, consisting of 3 rats, was initially given distilled water to drink while receiving orally, by dropper, 10-15 mg/day of d-α-tocopherol (Sigma T 3634, mixed isomers from vegetable oil) dissolved in an equal weight of corn oil. A second group, containing 8 rats, and a third group, containing 5 rats, were also placed on distilled water but without the vitamin E supplementation.

After 10 days the drinking water of the first and second groups but not the third group, was changed to 50 ppm NaF (23 ppm F^-) in distilled water and, after an additional 40 days, to 100 ppm NaF (45 ppm F^-). Daily administration of 10-15 mg of d-α-tocopherol to the first group of rats was continued throughout the study. The general appearance of the rats and the condition of their incisor teeth were monitored daily.

The second series of experiments was similarly conducted on 3-week-old males divided into two groups. The first group of 6 rats was given 10-15 mg/day of d-α-tocopherol and distilled water to drink. The second group of four rats was also supplied with distilled water but not the vitamin E. After 10 days the drinking water of half the rats in each group was changed to 100 ppm NaF (45 ppm F^-) in distilled water and the other half to 200 ppm NaF (90 ppm F^-) Again, the appearance of the rats and their incisors was monitored daily. During one of these inspections, after 80 days on the fluoridated water, photographs were taken of the lower incisors of each rat in both series of experiments. After 15 weeks on the fluoridated water, the rats were sacrificed and examined for gross changes in the liver and kidneys.

Results

The general health of all the rats appeared to be normal, and none of the animals became ill or died during the study. Overall, the vitamin E-supplemented (ES) rats grew at about the same rate as the nonsupplemented (NS) ones. The male rats consuming water containing 90 ppm F^-, however, gained weight slightly more slowly than their 45-ppm F^- cohorts. One of the NS males consuming 90 ppm F^- water developed overgrown upper incisors, as observed in earlier work (6), which prevented it from eating normally until the curved portion of these teeth broke off. Allowing for differences in body weight, water consumption appeared to be approximately the same for all the rats, although occasional diurnal variations were noted.

After consuming fluoridated water for about 40 days, both the ES and the NS rats began to display the characteristic transverse fluorotic banding pattern on the lower incisors, which, however, varied considerably in degree and prominence among the animals within each group. At 23 ppm F^- in the drinking water, in agreement with earlier work (7), the mottling was extremely faint but became conspicuous in about half the female rats 20-30 days after the change to 45 ppm F^- (see figures for representative examples). As expected, the enamel of the incisor teeth of the control rats on distilled water remained translucent and developed the normal orange-brown color.

In the females (but not the males) drinking F^- water, the characteristic brown and white mottling pattern of dental fluorosis was far more noticeable in the incisors of several of the NS rats than in any of the ES rats (see Table). After ca. 35 days on the 45 ppm F^- water, the ES but not the NS fe-

Table
Fluorotic Mottling and Lower Incisor Wear Attrition of
Vitamin-E Supplemented (ES) and Nonsupplemented (NS) Rats

Group	No.	F^- (ppm) in D.W.	No. with Degree of Mottling				No. with Wear Attrition		
			Nil	Faint	Moderate	Marked	Minimal	Slight	Marked
Females									
ES	3	23→45	0	1	2	0	0	1	2
NS	8	23→45	0	2	2	4	6	1	1
NS	5	0	5	0	0	0	5	0	0
Males									
ES	3	45	0	1	2	0	2	1	0
ES	3	90	0	1	2	0	2	1	0
NS	2	45	0	1	1	0	2	0	0
NS	2	90	0	0	2	0	1	1	0

males showed a gradual disappearance of the mottling with loss of the transverse banding and its replacement by an almost evenly dispersed white color in the incisors. Interestingly, this change was less marked in the ES males. Moreover, the lower incisors of the ES females showed more wear attrition than did those of either the NS females or the ES or NS males (see Figures).

Post-mortem examination showed normal appearance of the liver and kidneys in all the rats except in one of the NS males that had been drinking 90 ppm F^- water. The liver of this animal was enlarged and had necrosis of a small area of perihilar tissue.

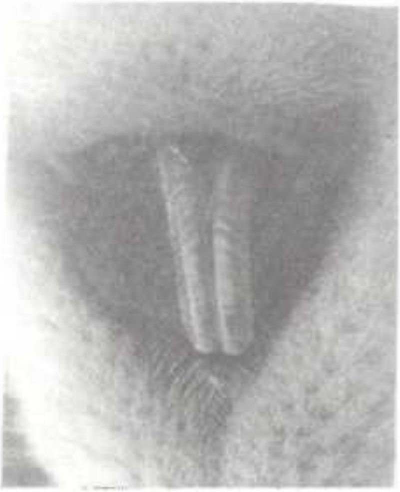
Discussion

Various adverse health effects of vitamin E deficiency in vertebrates are well documented, including disorders of the reproductive organs, skeletal muscles, nerves, blood, liver, and the cardiovascular system (11). Many of these effects are similar to those associated with fluoride intoxication, e.g., muscular weakness, capillary fragility, and elevated blood bilirubin (12, 13). Equally interesting is the fact that some of these disorders, such as the latter two, have been successfully treated in human subjects by administration of vitamin E (14, 15). At the cellular-molecular level, vitamin E is believed to exert its protective effect primarily through destruction of cell-damaging free-radical oxygen species (16). Since F^- adversely affects many cellular oxidation processes (17), and can also promote the formation of toxic free--radical oxygen (superoxide) species (18), it is reasonable for the protective role of vitamin E against F^--induced chromosome damage to bone marrow cells in rats to be ascribed to such a mechanism (1, 2).

Because vitamin E deficiency in rats can cause depigmentation of the incisor teeth similar to that seen in dental fluorosis, one might expect that supplemental vitamin E could affect the course of dental fluorosis in rats. Under the conditions of the present study, with a nutritionally adequate amount of vitamin E already present in the diet, extra vitamin E did not delay or protect against dental fluorosis in rats, although it did modify its course. The typical fluorotic transverse brown and white striations in the incisor enamel (6-9) were less prominent in the ES rats and gradually disappeared almost completely in the ES females and to a lesser extent in the ES males. In this connection, it is of interest that male rats of the Long-

Figure 1

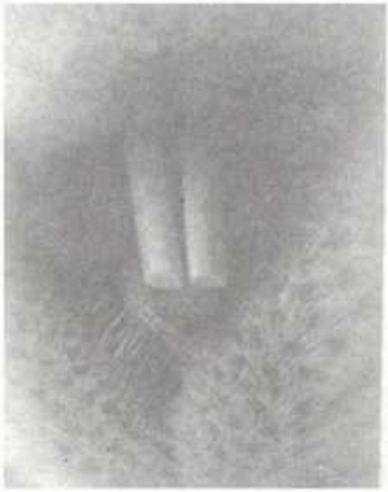
ES* Female (45 ppm F^-)



Moderate Mottling;
marked wear attrition.

Figure 2

NS* Female (45 ppm F^-)



Marked mottling;
minimal wear attrition.

Figure 3

ES* Male (45 ppm F^-)

Faint mottling;
minimal wear attrition.

Figure 4

ES* Male (45 ppm F^-)

Moderate Mottling;
minimal wear attrition.

*ES = Vitamin E-supplemented; NS = Nonsupplemented.

Evans strain have been reported to be less prone to undergo incisor depigmentation on a vitamin E-deficient diet than females (19). Similarly, young male albino rats have been found to be more resistant to F^- intoxication than females (20), and there is evidence that female rats retain more F^- than males (21).

Here, wear attrition of the lower incisors was more pronounced in the ES females than in the NS females, thereby indicating that supplemental vitamin E was decreasing the enamel hardness as well as changing the appearance of the dental fluorosis. Normally, most of the wear occurs on the leading edges of the upper rather than the lower incisors of rats, except when fluorosis is present (6). In contrast to rats with moderately fluorosed teeth, the whitened teeth of vitamin E-deficient rats show a significant elevation in ash content (22).

Although supplemental vitamin E did not protect rats against dental fluorosis in this study, what might occur with a vitamin E-deficient (or a minimally adequate vitamin E) diet remains to be investigated. The fact that vitamin E-deficient rats are markedly more sensitive to the toxic effects of enviromental pollutants such as lead (23) and chlorinated hydrocarbons (24) suggests that a study of the effects of a vitamin E-deficient diet on dental fluorosis in rats might be fruitful. In humans, endemic dental fluorosis has been shown to be more severe in proportion to lowering of the nutritional status (25).

Acknowledgements

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References

1. Aliev, A.A., Kul'gavin, A.E., and Sarina, T.N.: Effect of α-Tocopherol on the level of Chromosomal Aberrations Induced by Sodium Fluoride in Rat Femur Bone Marrow Cells. Izv. Akad. Nauk Az. SSR, Ser. Biol. Nauk, No. 1: 17-20, 1981.
2. Aliev, A.A., and Babaev, D.A.: Cytogenetic Activity of Vitamins in Bone Marrow Cells of Rat Femurs in Sodium Fluoride-Induced Mutation Conditions. Tsitol. Genet. (Kiev), 15(6):19-23, 1981.
3. Granados, H., and Dam, H.: Inhibition of Pigment Deposition in Incisor Teeth of Rats Deficient in Vitamin E from Birth. Proc. Soc. Exp. Biol. Med., 59:295-296, 1945.
4. Hove, E.L.: Interrelation between α-Tocopherol and Protein Metabolism: Body Weight and Tooth Pigmentation of Rats. Proc. Soc. Exp. Biol. Med., 63:508-511, 1946.
5. Moore, T.: The Significance of Protein in Vitamin E Deficiency. Ann. N.Y. Acad. Sci., 52:206-216, 1949.
6. McCollum, E.V., Simmonds, N., Becker, J.E., and Bunting, R.W.: The Effect of Additions of Fluorine to the Diet of the Rat on the Quality of the Teeth. J. Biol. Chem., 63:553-562, 1925.

7. Dean, H.T., Sebrell, W.H., Breaux, R.P., and Elvove, E.: Effect of Various Amounts of Sodium Fluoride on the Teeth of White Rats. Pub. Health Rep., 49:1075-1081, 1934.
8. DenBesten, P.K., and Crenshaw, M.A.: The Effects of Chronic High Fluoride Levels on Forming Enamel in the Rat. Archs. Oral Biol., 29:675-679, 1984.
9. Angmar-Mannsson, B., and Whitford, G.M.: Enamel Fluorosis Related to Plasma F Levels in the Rat. Caries Res., 18:25-32, 1984.
10. Alam, S.Q., and Alam, B.S.: Effects of Excess Vitamin E on Rat Teeth. Calcif. Tissue Int., 33:619-622, 1981.
11. Nelson, J.S.: Pathology of Vitamin E Deficiency. In Vitamin E: A Comprehensive Treatise, L.J. Machlin, Ed. Marcel Dekker, Inc., New York and Basel, 1980, pp. 397-428.
12. Waldbott, G.L., Burgstahler, A.W., and McKinney, H.L.: Fluoridation: The Great Dilemma. Coronado Press, Lawrence, Kansas, 1978, Chapters 9-11.
13. Lee, J.: Gilbert's Disease and Fluoride Intake. Fluoride, 16:139-145,1983.
14. Fujii, T.: The Clinical Effects of Vitamin E on Purpuras Due to Vascular Defects. J. Vitaminol. (Tokyo), 18:125-130, 1972.
15. Gross, S.J., and Landaw, S.A.: The Effect of Vitamin E on Red Cell Hemolysis and Bilirubinemia. Ann. N.Y. Acad. Sci., 393:315-322, 1982. See also ref. 16 below.
16. McCay, P.B., and King, M.M.: Vitamin E: Its Role as a Biological Free Radical Scavanger and Its Relationship to the Microsomal Mixed-Function Oxidase System. In Vitamin E: A Comprehensive Treatise, L.J. Machlin, Ed. Marcel Dekker, Inc., New York and Basel, 1980, pp. 289-317.
17. Borei, H.: Inhibition of Cellular Oxidation by Fluoride. Ark. Kemi, Minerol. Geol., 20(8):1-215, 1945.
18. Yiamouyiannis, J.: Disarming the Immune System, In: Fluoride: The Aging Factor. Health Action Press, Delaware, Ohio, 1983, pp. 50 and 172.
19. Telford, I.R.: Pigment Studies on the Incisor Teeth of Vitamin E Deficient Rats of the Long-Evans Strain. Proc. Soc. Exp. Biol. Med., 63:89-91, 1946.
20. Maynard, E.A., et al., cited in Fluorine Chemistry, Volume IV, by H. C. Hodge and F.A. Smith, J.H. Simons, Ed. Academic Press, New York and London, 1965, P. 41.
21. Muhler, J.C.: Retention of Fluorine in the Skeleton of the Rat Receiving Different Levels of Fluorine in the Diet. J. Nutr., 54:481-490, 1954.
22. Moore, T.: Dental Depigmentation in the Rat. Biochem. J., 37:112-115,1943.
23. Levander, O.A., Morris, V.C., Higgs, D.J., and Ferretti, R.J.: Lead Poisoning in Vitamin E-Deficient Rats. J. Nutr., 105:1481-1485, 1975.
24. Hove, E.L.: Interrelation Between α-Tocopherol and Protein Metabolism III. The Protective Effect of Vitamin E and Certain Nitrogenous Compounds Against CCl_4 Poisoning in Rats. Arch. Biochem., 17:467-474, 1948.
25. Massler, M., and Schour, I.: Relation of Endemic Dental Fluorosis to Malnutrition. J. Dent. Res., 44:156-165, 1952.

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Correction: Dr. Ilkka Penttila, Department of Clinical Chemistry, University of Kuopio, should have been included as a co-author of the article entitled Maternal Ionic Plasma Fuoride Concentrations During and After Delivery - Fluoride, (14: 4-9, 1981)

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SERUM BIOCHEMICAL EFFECTS OF FLUORIDE ON CATTLE IN THE DARMOUS AREA

by

M. Kessabi*, M. Khouzaimi, J.P. Braun, and A. Hamliri
Agdal, Maroc

(Abstracted from Vet. Human Toxicol., 25:403-406, 1983)

Chronic fluorosis in Morocco - named "Darmous" (dental fluorosis) - is a severe ailment of cattle in phosphate mining areas. The biochemical effects of Darmous have been poorly investigated. An experiment designed by the authors to study blood biochemical parameters and bone fluoride in cattle of the Darmous area was performed in Morocco around the cities of Kouribga and Qued-zem, where fluorosis is enhanced by an arid climate. Fluorosis affects more than 100,000 cattle and causes significant losses.

Fifty test animals taken from the Darmous area showed signs of dental fluorosis ranging from 4 to 5 by Dean's classification. Fifty controls were taken from a fluoride-free zone in Kasba Tadla near the Darmous area.

Animals from the Darmous area, especially older ones, had lower carcass weight than controls. Bone fluoride was about 3 times higher in cattle of the Darmous area than in controls: 5650 ± 1810 ppm vs. 1930 ± 630 ppm, respectively ($P < 0.001$).

For cattle in the Darmous area, serum calcium and magnesium were slightly but significantly lower than in controls, whereas potassium was higher. Glucose and cholesterol were lower in the animals from the Darmous area than in controls, whereas urea was higher and creatinine equal. Total proteins were lower in cattle from the Darmous area mainly due to low serum albumin, whereas gamma-globulins were higher. Most serum enzymes were higher in cattle of the Darmous area except for ALT which was lower. The most important differences were noted for LDH and ALP. Sodium and phosphate were unchanged. Serum potassium levels for animals of the Darmous area were higher than in controls and the normal levels reported in the literature. Bone fluoride for cattle from the Darmous area was much higher, reaching concentrations associated with severe fluorosis. The authors observed an inversion of the albumin/globulin ratio, which is a common finding in chronic liver disease.

The general disturbances induced by chronic fluorosis demonstrate important effects of fluoride on mineral and bone metabolism and on the functioning of kidneys and liver. Therefore in management of cattle herds in the Darmous area, attention should be focused on these organs.

KEY WORDS: Cattle fluorosis; Darmous area; Morocco; Serum, F^- effects on

Reprints: *Département de Toxicologie, Institut Agronomique et Vétérinaire Hassan II Rabat-Agdal, Maroc.

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A STUDY ON ANALYSIS OF FLUORIDE IN RICE

by

Shusaku Hara
Saitama, Japan

(Abstracted from Bull. Josai Dent. Univ. 12:83-88, 1983)

Rice samples of Nihonbare, Tonewase and Toyonishiki were taken from Saitama prefecture for analysis of fluoride content. Isolation and dry ashing rice samples were analyzed by the method of the Association of Official Analytical Chemists. For ashing, CaO was used as a fluoride fixative. Fluoride was isolated by steam distillation at 140 ± 5°C from 60% $HClO_4$ and determined by means of a fluoride electrode. Calcium and phosphorus levels were also measured, along with fluoride in nonglutinous unpolished and polished rice. The degrees of polishing of the rice samples were arbitrarily designated as 30, 50, 70, 90 and 100 (per cent) respectively.

The results:

1. Recovery test of fluoride in the sample was satisfactory by dry ashing 20 grams of the sample with 1 g. of CaO for 24 hrs. at 550-600°C.
2. The mean fluoride concentration of nonglutinous rice was 110 ppm (range 1.05-1.15 ppm) dry weight. The fluoride concentration of highly milled rice, at the polishing rate of 100%, ranged between 0.45 and 0.58 ppm (mean 0.53 ppm).
3. The calcium content of nonglutinous rice varied from 15 to 16 mg per cent with a mean of 16 mg percent. The content in the highly milled rice at the polishing rate of 100 per cent was 8-9 mg per cent (mean 8 mg per cent).
4. On the other hand, the phosphorus content in the nonglutinous rice varied from 334 to 393 mg per cent (average of 365 mg per cent), whereas that of highly milled rice at the polishing rate of 100 per cent ranged from 62 to 76 mg per cent (mean of 69 mg per cent).
5. The fluoride, calcium and phosphorus content in nonglutinous rice decreased during the process of refinement by 51.8, 50.0 and 81.1 per cent respectively, which indicated that rice bran contains a considerable amount of these elements.
6. A similar pattern of the decreasing linear gradient of the concentrations of fluoride and calcium was obtained during the processing of nonglutinous unpolished rice.
7. The fluoride concentration of the rice bran was 1.10-4.94 ppm dry weight. The present method of analysis seems to determine fairly accurately, the fluoride level in rice.

KEY WORDS: Calcium; Fluoride analysis; Phosphorus; Rice

Reprints: Department of Dental Health, Josai Dental University, Sakado, Saitama, Japan.

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GLYCOSAMINOGLYCAN ALTERATIONS IN RAT BONE DUE TO GROWTH AND FLUOROSIS

by

C.W. Prince, and J.M. Navia
Birmingham, Alabama

(Abstracted from J. of Nutr., 113:1576-1582, 1983)

There is evidence that alterations in bone glycosaminoglycans (GAGs) are at least partially responsible for increased bone density and trabeculae associated with skeletal fluorosis. The major objectives of the present study were to identify and quantitate the GAGs of bone from normal and fluorotic rats in order to understand the contribution of excess fluoride in drinking water to mineralization of the organic matrix of bones from growing young rats.

Weanling male rats were fed a semipurified diet, MIT 200, for 1 or 2 months ad libitum, and deionized water with and without 125 ppm fluoride. A third group of rats received the same semipurified diet at regular intervals thus constituting weight-matched controls for the fluoride-treated rats. GAGs were isolated from dry fat-free tibias and determined by digestion with chondroitinases and chondrosulfatases. Chondroitin-4-sulfate (C4S) comprised 90% of the bone GAGs, whereas chondroitin-6-sulfate (C6S), dermatan sulfate (DS) and hyaluronic acid (HA), together comprised about 10% of the total bone GAGs.

With increasing age, total GAGs, C4S and HA decreased, but DS remained constant. Fluorotic bone, which contained in excess of 5000 ppm of fluoride, had three times as much C6S and twice as much DS as bone from weight and age-matched control rats. Thus, compared to controls, specific alterations of bone GAGs result from fluorosis, independent of changes in body weight and age. Histological studies both of pig bones and teeth, and of rat bone, suggest that fluorotic-mineralized tissues contain decreased amounts of GAGs.

Fluoride-treated rats weighed significantly less ($P<0.05$) than ad libitum-fed control rats. Thus by this experimental feeding approach, effects of partial starvation are separated from effects of fluoride, an interaction not taken into account in other studies. Statistical analysis indicated significant increases in calcium and phosphorus concentrations with increasing age. No significant effect of pair-feeding or fluoride treatment on bone, Ca or P was detected, and no age-treatment interactions affected the mineral concentration in bone.

Mean concentrations of dermatan sulfate and chondroitin-6-sulfate were affected significantly by fluoride. Overall, fluoride-treated rats had increased amounts of these GAGs compared to control and pair-fed rats whose amounts did not differ from each other. One month after weanine, groups C and P had less dermatan sulfate than group F; 2 months after weaning,however, no significant differences were detected among the groups.

Abstract

Supplementation of drinking water offered to weanling rats, during a 2-month period, raised the fluoride concentration of tibias to about 5000 ppm F^-, a level as high as that found in fluorotic bone. Fluoride also affects the GAGs of bone matrix and thus contributes to the changes characertistic of fluorotic bone seen in humans who consume water with excess amounts of fluoride for prolonged periods.

In rats offered water with high concentrations of fluoride, initial changes in bone matrix are characterized by significant increases in chondroitin-t-sulfate and dermatan sulfate, alterations which might be partially responsible for the mineralization pattern of fluorotic bone.

KEY WORDS: Bone; Fluoride; Rat; Glycosaminoglycans

Reprints: Inst. of Dent. Research and Dept. of Nutr. Sciences, University of Alabama,Schools of Dentistry and Community and Allied Health, Univ. of Alabama, Birmingham, Alabama 35294

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A SURFEIT OF FLUORIDE?

by

Geoffrey E. Smith

(Abstracted from Science Progress (Oxford), 69: 429-442, 1985)

Individuals are now ingesting fluoride from a growing number of everyday sources including drinking water, food, dental health products and medicines, as well as insecticides, pesticides, and fertilizer residues, even from the air they breathe. Fluoride compounds are also incorporated into toothpastes, mouthrinses, and such dental filling materials as gels, varnishes, and paints used both in surgery and in the home. In addition, fluoride is topically applied to teeth, and fluoride drops, tablets and vitamin supplements are regularly prescribed; even fluoride-impregnated tooth-picks and dental floss can be purchased in pharmacies and supermarkets.

Babies in fluoridated areas, who drink milk formulas made with water containing 1 ppm fluoride, are ingesting up to 100 times the amount of fluoride that they would obtain from their mothers' milk. Scores of industries now pollute the total environment with fluoride emissions and solid wastes. Some of these industries are aluminum smelters, fertilizer factories, brick works, steel mills, and coal-burning power stations. Children aged 6-14 years who resided near a Czechoslovakian aluminum smelter ingested over 2 mg fluoride a day from air, water, animal foodstuffs and plants although their drinking water contained very little fluoride.

A pre-school child may swallow 0.3-0.4 g of paste at each brushing. Because most pastes contain 1000 ppm fluoride, a daily intake in excess of 0.5 mg fluoride from this source alone may be common. Tablets and drops for pregnant women, and for children from birth - still advocated by many - could result in daily intake of fluoride, 2-6 times the recommended dose. The F content of acidulated gels, commonly used by dental surgeons, varies between 0.5% and 1.28%. The patient is exposed to as much as 60 mg of fluoride for 4-5 min. Since they are both flavored and acidulated, salivation and swallowing of excess saliva and gel is stimulated during treatment. A child undergoing gel treatment, who weighed 22 kg, ingested fluoride equivalent to 1.8 mg F per kg body weight; plasma fluoride levels peaked at 76 μM/1, or almost 1.5 ppm fluoride. Following gel applications adverse reactions in both children and adults have been reported.

Recently simple and reliable methods for measuring blood levels of free ionic fluoride have been developed. It is now clear that even very small amounts of fluoride can cause normal plasma fluoride levels to surge and peak to potentially harmful values. In 1980, Swedish workers reported that, in a 25-year-old adult weighting 54 kg, plasma ionic fluoride levels of just over 1 ppm were reached 30 min after gel treatment, a level close to those which may result in impaired kidney function. After 14 hours the plasma fluoride concentration was about 10 times higher than the base line level. The authors of the study commented: "The plasma fluoride levels are close to those reported to give a reduction in maximum urine concentration ability".

In nonfluoridated areas, plasma fluoride levels have been found to range between 0.2 and 1 μM/1, or approximately 0.004-0.02 ppm F. In fluoridated areas they range between 1.0 and 2.0 μM/1 or 0.02-0.04 ppm fluoride. Plasma fluoride levels of 0.08 ppm F have caused moderate to moderately severe dental fluorosis. It is now clear that dental fluorosis, moderate to severe degree, can develop when plasma fluoride levels reach 0.05-0.1 ppm fluoride. Such levles may be reached by ingestion of submilligram doses of fluoride from many possible sources.

Noting that a number of physiological processes can be inhibited by fluoride at very low concentrations, the author asks "Can people ingest increasing amounts of fluoride, from a growing number of everyday sources, with impunity? Or has the time arrived for the medical and dental professions to review and even revise, their attitudes to fluoride?"

KEY WORDS: Fluoride intake (review); Fluoride sources; Fluoride surfeit

Reprints: 56 Surrey Road, South Yarra, Melbourne 3141, Australia

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DENTAL HEALTH STATUS AND ATTITUDES TO DENTAL CARE IN FAMILIES PARTICIPATING IN A DANISH FLUORIDE TABLET PROGRAM

by

E. Friis-Hasche*, J. Bergmann, A. Wenzel, A. Thylstrup, K. Miller Pedersen and P. Erik Petersen

(Abstracted from Community Dentistry and Oral Epidemiology 12:303-307, 1984)

The caries experience and dental fluorosis of 84 Danish children, whose average age at the time of examination was 6.8 years, were compared with those of a group matching in sex, age, place of residence and socio-economic status. They had used fluoride tablets 1-4 years during 1976-80. Mothers' attitudes toward dental care and candy, their knowledge about tooth-brushing, and number of teeth in maxilla showed no difference between the fluoride tablet group and the non-users group. Moreover, there was no significant difference between the two groups with respect to dental caries. Mothers in the fluoride tablet group apparently were more restrictive in candy consuming habits, in fact 30 children of this group had a fixed weekly day for candy in contrast to 17 in the non-users group.

On clinical examination, in the fluoride tablet group, 13 (12 + 1) out of 54 children examined showed slight degrees of dental fluorosis of the permanent first molar. In the non-users group all surfaces examined had a normal appearance. The two groups examined did not differ regarding dental fluorosis of the permanent central and lateral incisors. Since few enamel changes occurred in the primary second molars it was not possible to detect any difference between the fluoride tablet group and the non-users group. Nor has there been any significant difference in average caries experience in the two groups.

The similarity between the two groups in terms of current caries activity is clearly demonstrated by the fact that they did not differ in numbers of active and inactive lesions. The same picture was obtained with regard to the primary second molar. No difference in the prevalence of dental plaque and gingivitis was observed between the two groups. The difference between the groups, in terms of classical measures of caries experience or in current activity of caries, was significant.

Increasing awareness of the importance of dental hygiene and dietary patterns is likely to play a role in reduced caries progression.

KEY WORDS: Dental care; Dental caries; Dental fluorosis; Fluoride tablets

Reprints: Royal Dental College, Copenhagen, Denmark.

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EFFECT OF DOG FOOD CONTAINING 460 ppm FLUORIDE ON RAT REPRODUCTION

by

Thomas A. Marks, Diana Schellenberg, and Carl M. Metzler
Biomedical Science Department, Western Michigan University
Kalamazoo, Michigan

(Abstracted from J. of Toxicology and Environmental Health, 14:707-714,1984)

Since 1970, at least 115 litters of Shetland Sheepdogs at a kennel in Allegan County, Michigan, have been grossly deformed and/or died, generally within 3 days of birth. After it was discovered that fluoride in dog food had apparently caused mottled teeth and bony exostoses in dogs at this and at least two other kennels - both conditions believed to be due to fluoride - the fluoride content of dog-food and local well water were investigated as possible causes of reproductive problems.

In summer 1981, urine levels of fluoride were high; food fed in all three kennels contained 460 ppm fluoride due to rock phosphate added as a mineral source.

In March 1982 16 female and 4 male shelties, brought from outside Allegan County to the study kennel, were assigned to treatment with either high- or low-fluoride dog food and either local well or distilled water. Because dogs cycle only once or twice a year, a similar study was done in rats in the hope that information could be obtained more quickly with this species.

Nine male and 18 female rats were assigned to treatment with (1) high--fluoride dog food (460 ppm) and well water; (2) high-fluoride dog food and distilled water; (3) low-fluoride dog food (56 ppm) and well water and (4) low-fluoride dog food and distilled water. After 60 days in the kennel, the rats were mated. The first litter phase of the rat study produced no statistically significant differences between the groups. Teeth in the high-fluoride groups were whiter and larger than those in the low-fluoride groups; they tended to curve around and to press against other structures in the mouth. One dam in the high-fluoride-well water group continued to lose weight and died during the latter part of the breeding period. One dam in the low-fluoride--distilled water group, which had shown no obvious symptoms, also died near the end of the breeding period; this dam had eighteen normal-looking fetuses, 9 in each horn. Although mean litter size in the high-fluoride-distilled water group was significantly ($p < 0.05$) lower than that of the low-fluoride--distilled water group, the mean litter size of the low-fluoride-well water group was also significantly lower than this group. In addition, the mean litter size of the high-fluoride-well water group was higher than that of the low-fluoride-well water group.

The fact that reproductive problems seen in the dogs were not duplicated in the rats indicates that rats cannot be substituted for dogs in the contin-

uing search for the cause(s) of reproductive problems in dogs at this kennel.

KEY WORDS: F^- and rat reproduction; F^- in dog food

Reprints: Thomas A. Marks, Pathology and Toxicology Research, The Upjohn Company, Kalamazoo, Michigan 49001.

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EFFECT OF FLUORINE COMPOUNDS ON THE RESPIRATORY ENZYMES

by

Z. Machoy
Szczecin, Poland

(Abstracted from Postepy Biochem, 27:327-337, 1981)

Data are presented concerning the mechanism of interactions of fluorine compounds with respiratory enzymes. These enzymes contain iron or copper, or both of these elements simultaneously. The interaction of fluorine with iron and copper is generally believed to be due to inhibition of activity of the respiratory enzymes. Fluoride is an anion which complexes strongly with the atoms or ions of a number of metals.

Kinetic data showing the inhibitory effect of fluoride on cytochrome oxidase have been presented for enzymes of the mitochondrial respiratorv chain. The activity found for another one of the enzymes - succinate dehydrogenase - reflects inhibition not only by fluoride but also by a large group of organic fluorine derivatives applied as anesthetics. In regard to plant peroxidases fluoride's effect on peroxidase of radish, milk and cytochrome c has been studied. The mechanism of fluoride binding with cuproproteinic oxidases, lactase, ceruloplasmin, and ascorbate oxidase, are well known. However, data on the interaction of fluoride with other oxidoreductases are sketchy and sparse. Among those studied are such enzymes as catalase, malate dehydrogenase, citrate dehydrogenase and plant polyphenyloxidase.

The activity of respiratory enzymes is,as a rule, inhibited by fluoride. The group of cuproproteid oxidases has atoms of copper incorporated in a similar manner which display almost the same properties as far as the reaction with fluoride is concerned. On the basis of previous observations, we have failed to establish a direct dependence between the capacity of fluoride to form complexing of the metals and the changes in the activity of metaloproteidic enzymes.

The paper, written in Polish, cites 46 references to the literature.

KEY WORDS: Enzymes; Fluoride; Respiratory chain

Reprints: Pomeranian Medical Academy, Dept. of Biochemistry, 70-111, Al. Powstancow 72, Szczecin, Poland.

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FLUORIDE TOXICOSIS IN WILD UNGULATES

by

J.L. Shupe, DVM; A.E. Olson, MS; H.B. Peterson, PhD; J.B. Low, PhD
Utah State University, Logan Utah

(Abstracted from JAVMA 185:1295-1300, 1984)

The objectives of this study were to determine (1), the frequency of occurrence and degree of fluoride toxicosis in big-game animals of Utah, Idaho, Montana, and Wyoming; (2), the amounts and distribution of fluorides in the feed and water ingested by big game animals; and (3), the effects of fluoride on the general health of the big game animals.

Wild deer, elk, and bison have been exposed to a variety of sources of fluoride including water high in fluoride, forages contaminated by industrial effluents that were high in fluoride, vegetation contaminated with high fluoride-content soil by rain splash or wind, or a combination of these sources.

Factors influencing the expression or severity of chronic fluoride toxicosis include amount of fluoride ingested, duration of fluoride ingestion, solubility of fluoride ingested, species of animal, age of animal at time of ingestion, variations in amounts of fluoride intake (intermittent exposure), nutritional status (malnutrition intensifies toxicosis), health status of animal, simultaneous exposure to other toxic or potentially toxic substances, stress factors, and individual biological response.

Vegetation and water samples were collected from big game ranges where the animals lived in the vicinity of naturally occurring or industrially generated sources of elevated fluoride concentrations. Cache Valley in northern Utah, with no known increased amounts of fluoride, was selected as the control area from which vegetation, water, and animal tissues were collected for comparisons.

Findings from 1,125 mule deer, 105 elk, and 64 American bison were recorded as well as fluoride sources, both those naturally occurring and those from industrial operations in the immediate habitat of the big game species being studied. Fluoride-affected bones of all species examined had characteristic microscopic and radiologic changes which ranged from slight to severe. Irregular, poorly organized, and improperly mineralized bone was evident. Osteocytes were abnormally clumped together instead of being evenly dispersed throughout the osteons.

Fluorine content of vegetation varied greatly from area to area. In some mountainous habitats near certain industrial operations, even samples collected at the same time and close to each other varied greatly in fluorine content. Irregular terrain, variable weather conditions, and capricious winds were probably responsible for the inconsistent findings in these areas. Water samples in the same area were all low in fluoride.

The most distinctive primary lesions of chronic fluoride toxicosis appear in developing and mineralizing permanent teeth. Once the teeth have erupted, fluoride ingestion will have little or no effect on them. Whereas immature animals are most susceptible to dental fluorosis, severe cases of fluoride toxicosis have been correctly disgnosed in animals with normal dentition that have ingested excessive fluorides after completion of permanent dentition.

Bone changes occur when fluoride intake is excessive for any appreciable period, the type and expression of which depend on the 11 factors previously listed. They may include any of the following: sclerosis, porosis, periosteal hyperostosis, osterphytosis, and/or malacia. Prolonged slightly increased fluoride intake results in hardened or sclerotic bone. Extremely high long--term intake results in periosteal hyperostosis and porous or malacic bone, the changes most easily seen in cross sections of the affected bones. Seasonal migrations of big game animals often cause intermittent periods of ingestion of excessive fluoride and resultant variations in responses of the animals. These seasonal changes can also be noted in the bone cross sections.

Bones that are more active metabolically, such as those used in locomotion, chewing, or breathing, are more affected by osteofluorosis than bones that are primarily protective and less active in function. Also, more metabolically active portions of bone, especially near growth areas, have greater visible effects and higher content of fluoride than less active areas. Osteofluorotic lesions are not primarily associated with articular surfaces. Fluoride-induced effects are greater in areas of tendon or muscle attachment, than in nearby nonattachment areas.

Based on our knowledge of life habits and anatomic features of wild ungulates, we can assume that their tolerance for fluoride is probably similar to that for domestic ungulates.

KEY WORDS: Bison; Deer; Elk; Fluorosis in Western USA; Fluorosis in wild animals; Ungulates.

Reprints: Departments of Animal, Dairy, and Veterinary Sciences Agriculture and Irrigation Engineering and Fisheries and Wildlife, Utah State University, Logan, Utah 84322

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BOOK NOTICE

Avcyn, A.P., and Zahvoronkov, A.A.: Patologija fljuorosa (Path. of Fluorosis). Novosibirsk: Nauka 1981, 333 pages, 43 figs., 32 tables, Price:3.10 r.

A brief survey of the occurrence of fluoride in tissue and fluids of plants and animals, fluoride distribution in nature and occurrence of endemic fluorosis in all parts of the world were compiled geographically. Own investigations regarding the occurrence of endemic fluorosis in Northern Kazakhstan (Soviet Russia) complete the first portion of the book. The main portion includes pathological-anatomical investigations by the authors combined with a review of the literature covering all organ systems in experimental and human fluorosis, with emphasis on the changes in the glands of internal secretion and the nervous system.

KEY WORDS: Endemic human fluorosis; Experimental fluorosis; Pathological-anatomical and geographical investigations.

Translated from Russian by J. Franke, Erfurt

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SOME EFFECTS OF BONE MEAL SUPPLEMENTED DIET ON BONE AND TEETH OF GROWING RATS

by

P. Rasmussen, and G.R. Wesenberg
Bergen, Norway

(Abstracted from Acta Odontol. Scand., 39:313-320, 1981)

The aim of the present investigation was to evaluate incorporation of fluoride into bone and teeth of growing rats fed diets supplemented with 500 ppm F^- bone meal, amounts comparable with those recommended for children, in comparison with incorporation of fluoride from the same amount of fluoride ingested as NaF. Furthermore, the content of fluoride and calcium in serum and feces was estimated.

The final weight of males in the bone meal group and the NaF group was slightly, but not significantly higher ($0.3 < P < 0.4$) as compared to the controls, whereas in female rats the final weight of the NaF group was significantly lower ($P < 0.001$) than that of the control and bone meal groups. In females, serum-Ca was significantly increased in both bone meal and NaF groups.

Addition of bone meal or NaF to the diet increased the bone level of fluoride, always to a greater extent in the epiphyseal area. The F^- level was 2-4 times higher in the NaF group than in the bone meal group.

Addition of bone meal or NaF to the diet increased the amount of fluoride in feces, predominantly in the bone meal group. Regarding the calcium content in feces, no significant difference was observed between the control and NaF group, whereas in the bone meal group, feces-Ca was increased by about 40%.

In the present investigation, the Ca-content of the diet was adequate in the control diet and the NaF diet, and high in the bone meal diet. Serum-F increased in both the bone meal and the NaF group. The incorporation of fluoride into bones from the bone meal supplemented diet was considerably less than from the NaF supplemental diet.

Ingestion of fluoride, as bone meal or NaF, increased the amount of feces-F considerably. However, in the bone meal group, feces-F was elevated above the control level by 40% as compared to the NaF group. Feces-Ca which remained at control level in the NaF group, increased about 40% in the bone meal group. Whereas the bone meal added about 50% Ca to the control diet, only a negligible part of the additional calcium, supplied by the bone meal, has been absorbed.

KEY WORDS: Bone meal; Bone mineral; Fluoride; Teeth

Reprints: P. Rasmussen, Dept. of Anatomy, Arstadveien 19, N-5000 Bergen, Norway

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DISTRIBUTION OF FLUORIDE TO HUMAN BREAST MILK

Following Intake of High Doses of Fluoride

by

J. Ekstrand, C.J. Spak, J. Falch, J. Afesth, and H. Ulvestad
Stockholm, Sweden, and Oslo, Norway

(Abstracted from Caries Res. 18:93-95, 1984)

Concentrations of fluoride in breast milk collected from nursing mothers living in areas with either 1 or 0.2 ppm fluoride in the drinking water were similar: 0.36 ± 0.15 (±S.D.) and 0.30 ± 0.10 μM respectively. Following a single dose of 1.5 mg fluoride, administered to 5 mothers 5 days after delivery, there was a 10-fold increase in the plasma level.

In the present study, a high dose of fluoride was administered to a nursing mother suffering from osteoporosis. The experiment took place 7 months after delivery, and a normal breast milk production was recorded. Following an oral dose of 25 mg NaF (11.25 mg F) as tablets, venous blood was collected at 0, 2, 4, 5, and 8 h. Breast milk was sampled 0.25 h before and 0.5, 1, 1.5, 2, 4, 6, and 8 h after dose intake.

The slow but constant increase of fluoride in the breast milk during the first 2 h indicated a net flux of fluoride from plasma to the milk. After 2 h the fluoride concentration in the milk started to decline but did not return to the basal level within 8 h.

In conclusion, this study shows that there is a slight increase in the fluoride concentration in breast milk following intake of high doses of fluoride. In spite of this, the fluoride supply to the infant through the breast milk is very low compared to what it may receive from other sources, such as drinking water containing fluoride.

KEY WORDS: Breast milk; Fluoride; Plasma

Reprints: Department of Cariology, Karolinska Institutet, Stockholm, Sweden; Medical Department B. Aker Hospital, Oslo, and Department of Pedodontics and Caries Prophylaxis, School of Dentistry, Oslo, Norway.

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DENTAL FLUOROSIS CAUSED BY POLLUTED AIR DUE TO BURNING COAL IN THE ROOM

by

Li Shang
Hunan, China

(Abstracted from Chinese Journal of Stomatology 18:114-116, 1983)

The author investigated the incidence of dental fluorosis in children in two villages, 3 km distance from each other, in the suburb of Guiyang, southwest China. Altogether 909 pupils, 6-15 years old, were checked. Due to the differences in fuels used for heating and cooking food, the incidence of dental fluorosis in the two villages differed significantly. In village A, where coal was burned, the incidence of dental fluorosis was 597 in 610 native pupils (97.8%). In village B, where firewood was burned, of 299 native pupils only 37, (12.4%) had dental fluorosis (Table 1).

Table 1

Incidence of Dental Fluorosis* in Pupils 6-15 years old in Villages A and B (%)

Village	Total cases investigated	I	II	III	IV	V
A	610	11.0	16.7	43.3	23.1	3.7
B	299	10.0	2.0	0.3	0	0

* Mottled enamel classified by increasing severity according to Dean's scale.

In village A, in each family dwelling a big coal-burning round stove without a chimney is used for cooking, warmth, and baking; it burns constantly. The bedroom is directly connected with the room where the stove is located. Residents of village B burn firewood in their homes. Corn or some vegetables are baked in homes of both villages.

Eight samples of coal and air of 46 households, where coal or firewood was used for fuel, were analyzed for fluoride. The F^- levels in coal were 42.5-359.0 ppm (average 153.1ppm). HF_3 in the air of households in village A was 0.018-0.525 mg/m^3 (average $0.143/m^3$); in village B, it was 0.073-0.151 (average 0.091 mg/m^3). In addition, the fluoride content of 17 samples of drinking water, 7 species of foodstuff, and 6 samples of soil in the two villages were measured (Table 2).

The author concluded that dental fluorosis in village A children was caused by fluoride-polluted air due to burning coal in the house.

Table 2

Average Fluoride Values (ppm)

Village	Household air(mg/m^3)	Drinking water	Soil	Corn Fresh	Corn Baked	Hot pepper Fresh	Hot pepper Baked
A	0.143	0.15	1365	0.26	26.3	0.65	310.5
B	0.091	0.60	245	0.13	0.7	-	2.5

KEY WORDS: Air F^-; China; Coal F^-; Dental fluorosis; Mottled enamel

Reprints: Guiyang Hospital for Oral Diseases, Guiyang, Guizhou, China

Abstracted by Li Yumin

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OSSERVAZIONE DI CINQUE CASI DI "ASMA DA FLUORO" (5 Cases of Fluoride Asthma)

by

P. Maestrelli, G. Marcer, and E. Clonfero
Milano, Italy

(Abstracted from Med. Lavoro, 4:306-312, 1981)

Five cases of bronchial asthma in aluminium potroom workers with no previous occupational exposure to respiratory irritants, no history of asthma or respiratory heart disease, are described. The first attack of asthma occurred 7 to 36 months (average 1.5 years) after the subjects had started work in the potrooms. The symptoms appeared, both at the time of exposure, as well as after a work shift. In 4 cases, symptoms disappeared completely within a week of being transferred from the potroom; only in one worker occasional wheezing persisted after a year. In two subjects, skin tests for common allergens were positive. Six to 24 months after cessation of exposure, all workers had normal lung volumes but they showed high sensitivity to a bronchial provocation test with inhaled carbachol. On the basis of the patients' histories as well as clinical and respiratory function findings, the authors concluded that a causal relationship exists between potroom work and the onset of asthma.

KEY WORDS: Aluminum potroom exposure; Asthma, bronchial

Reprints: Instituto di Medicina del Lavoro dell'Universita, di Padova
Directore Paul M. Crepet, Milano, Italy.

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FLUORIDE IN SALIVA AFTER VARIOUS TOPICAL TREATMENTS

by

I. Rytomaa, and J. H. Meurman
Helsinki, Finland

(Abstracted from Proc. Finn. Dent. Soc., 79:172-177, 1983)

The concentration and clearance of fluoride in saliva were studied in a test panel of four dental students after application of the following products: 2% sodium fluoride solution; Elmex sol amine fluoride solution; Elmex gel sodium fluoride and amine fluoride gel; and Duraphat sodium fluoride varnish. Pretreatment control samples were taken by paraffin stimulation of salivary secretion. Subsequent post-treatment samples were taken 2, 4, 8, and 12 hrs. after the application of fluoride, before toothbrushing and breakfast the following morning, and on days 2, 4, 6, and 8 after treatment. Fluoride concentrations were determined using a fluoride electrode.

The Duraphat varnish gave higher peak fluoride values (mean F^- concentration 10 ppm, 2 hrs. after treatment) and a longer period of clearance from the saliva than the other products. The amine fluorides gave F^- means of 3 ppm, 2 hrs. after treatment, whereas the sodium fluoride solution gave F^- means a little above 1 ppm, 2 hrs. after application. The amine fluorides and sodium fluoride solutions were cleared to below 1 ppm of F^- during the first few hours after treatment.

From a toxicological point of view, the application of 5 ml of gel may be undesirable. Individual trays should be used to minimize the amount of gel needed. Suction apparatus has been recommended during treatment and patients should expectorate after treatment (2). Three times higher enamel fluoride concentration have been reported after Elmex solution treatment than after sodium fluoride treatment (4). The enamel fluoride concentration, however, is not always directly comparable with caries susceptibility or resistance (3). Unfortunately no long-term clinical evaluation of the caries-reducing potential of the products studied are available.

KEY WORDS: Fluoride, Saliva, Topical application

Reprints: Inkeri Rytomaa, Institute of Dentistry, University of Helsinki, Mannerheimintie 172, SF-00280 Helsinki 28, Finland.

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CORRECTIONS

Colquhoun, J., 17:234-242, 1984
(cn 18:121)

Hanhijarvi, H., 14:4-9, 1981
(cn 18:226)

INSTRUCTIONS TO AUTHORS

Fluoride, the official journal of the International Society for Fluoride Research (ISFR) is published quarterly (January, April, July, October). Its scope is the publication of papers and reports on the biological, chemical, ecological, industrial, toxicological and clinical aspects of inorganic and organic fluoride compounds. Papers presented at the annual ISFR conference are published in *Fluoride*. Submission of a paper implies that it presents original investigations and relevant bio-medical observations. Review papers are also accepted.

Preparation of Papers

1. **General** – No precise limit is given on the length of the paper; it should be written concisely in English, submitted in two copies, doublespaced with generous margins. Measures are given in metric system (SI).

2. **Title** – A concise but informative title should be followed by the name of author(s), the location and state (country) where the research was carried out. The name and address of the institution where the work was done should appear at the bottom of the first page.

3. **Summary** – The paper should begin with a brief, factual summary.

4. **Introduction** – Following the summary, a short introduction should state the reason for the work with a brief reference to previous works on the subject. References are given by numbers in parentheses.

5. **Materials and Methods** – should be condensed; however if the methodology is new or developed by the author(s) it might be more detailed.

6. **Results** – should contain the *direct conclusions* of the experimental work.

7. **Discussion** – should deal with the *general conclusions*. Reference should be made to other work on the subject with an indication whether the experimental results are in agreement or in disagreement with previous work. In short papers, results and discussion can be stated together.

8. Bibliography should be arranged according to the order in which the articles are cited in the text (not alphabetically). An example follows:

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

For books, the title, editor, publisher, location and year of publication, and pages should be given.

THE INTERNATIONAL SOCIETY for FLUORIDE RESEARCH
P.O. BOX 692 WARREN, MICHIGAN 48090