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CONTENTS

The Twelfth Conference of the International Society for Fluoride Research will convene at the Hilton Hotel in St. Petersburg, Florida, May 16th to 18th, 1982. Transportation from Tampa Airport to the Hilton is available. The rate per room for double occupancy is $50.00 or $25.00 per person.

The Program Committee is soliciting abstracts (up to 300 words) of papers to be presented at the conference dealing with any phase of fluoride research. Kindly send abstracts to the Society's office, P.O. Box 692, Warren, Michigan 48090 prior to February 15, 1982.

FLUORIDE is published quarterly by THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH, INC.,

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

FLUORIDE is listed in
Current Contents Agricultural
Food and Veterinary Sciences

EDITORIAL

URINARY FLUORIDE AND FLUORIDE TOXICITY

Whether or not urinary fluoride excretion can serve as an indicator for the degree of toxic effects of fluoride is of interest to scientists engaged in fluoride research, to government agencies setting up standards, to the legal profession involved in litigation concerned with fluoride and to the clinician diagnosing fluoride intoxication.

Several authors have, in the past, equated urinary fluoride excretion with its toxic action. Hodge and Smith of the University of Rochester, N.Y. (1) stated: "Average urinary F concentrations not exceeding 5 mg/liter, which corresponds approximately to a daily intake of 5 mg, are not associated with osteosclerosis in such workmen, and such changes are unlikely at daily intakes of 5 - 8 mgF. The amount of fluoride which is retained by an individual inhaling air containing 2.5 mg of fluoride dusts per m^3 (the current TLV) is approximately 5 - 6 mg." Smith even considers high fluoride excretion "pathognomonic" of fluorosis (2).

Others have encountered low fluoride excretion in patients with nonskeletal fluorosis. In 10 such subjects with nonskeletal fluorosis residing close to an Ohio aluminum factory, Waldbott recorded 24-hour urinary fluoride levels ranging between 0.35 and 2.40 mg (3). Similarly in the early stage of hydrofluorosis due to artificially fluoridated water daily urinary fluoride excretion rarely exceeds 1.5 mg per day (4).

In several statistical surveys on workers exposed to atmospheric fluoride, a close parallel has been reported between 24-hour urinary fluoride excretion and fluoride intake. However, it must be recognized that fluoride excretion varies widely from one individual to another:

1. Growing children are known to retain more fluoride in their bones than adults and therefore excrete less.
2. The function of the kidneys alters the pattern of fluoride excretion, i.e. patients with kidney disease retain more fluoride (5).
3. During pregnancy, urinary fluoride excretion is reduced (6).
4. Simultaneous intake of substances which bind fluoride, such as calcium, aluminum and other metals accounts for greater fluoride elimination through the gastrointestinal tract and therefore decreases urinary excretion (7).
5. Persons previously exposed to high fluoride intake, who are on a low fluoride regime prior to sampling, eliminate more fluoride than they take into their bodies (8,9).
6. Other factors to be taken into account are the variability of the consumption of fluoride-containing food and water from day to day and from hour to hour and the extent of the activity of a fluoride-emitting factory at the time of sampling. All these factors render spot sampling less reliable than 24-hour sampling. Also the specific gravity(10) and the acidity (pH) of the urine (11) and possible contact of the urine with metal, glass or enamel will have to be considered.

Whereas all these factors account for wide individual differences

between one sample and another nevertheless, in general, urinary fluoride output rises in relation to fluoride intake.

However the question arises: Does urinary fluoride excretion parallel adverse effects due to fluoride?

In extensive surveys on workers in fertilizer and aluminum industries, Derryberry et al., the former health director of the TWA project (12), and by Kaltreider et al. (13), of the medical staff of the Aluminum Company of America, attempt to equate low fluoride excretion with no illness. In both studies, the results of physical examination, laboratory tests and urinary fluoride excretion of extensively exposed workers were compared with those of so-called controls, i.e. workers in the same factory who were presumably less exposed. Since it is doubtful that anyone employed in such factories can avoid temporary or longterm exposure to atmospheric fluorides, these surveys cannot be considered controlled.

In Derryberry's studies there was an extremely wide scatter of urinary fluoride values from 0.2 ppm to 44.0 ppm and no data were made available on the health of individual workers whose excretion was highest.

Similarly, in the more elaborate survey by Kaltreider et al. the controls were selected from other personnel of the factory rather than from subjects far removed from the fluoride-polluted area. The thoroughness of the examination must be questioned since the laboratory tests included only one enzyme. Certain abnormal findings were casually dismissed in the following manner: "... it is believed that the abnormal values for total bilirubin found in five individuals are fallacious, since they all occurred on the same day and none of these employees showed evidence of clinical jaundice." No details were provided about symptomatology in individual workers with advanced skeletal fluorosis. Both the Derryberry et al. and Kaltreider et al. studies employed spot sampling instead of 24-hour urine collection.

As in most statistics of this kind, data are meaningless unless special studies are presented on the individual subjects both at the top and at the bottom of the statistical scale. Furthermore we must consider that workers in a factory are likely to minimize their complaints in view of the fact that verbalizing them might threaten their job.

Experience with low-grade chronic intoxication from other toxic agents such as lead, mercury, cadmium, etc., has demonstrated the difficulties encountered in arriving at a diagnosis in the early stage of the disease when only subtle damage to liver, blood, kidneys, and other organs occurs and because few, if any, diagnostic laboratory tests are available. Such damage is rarely attributed to its source and can persist for prolonged periods before more typical signs of toxicity develop. This phase of fluorosis has been clearly outlined by Roholm, one of the keenest students of the disease (14) and is now being described as "side effects" in fluoride therapy of osteoporosis (15). It is impossible to diagnose a disease on the basis of even the most elaborate statistics. Instead, careful clinical observations of individual patients over prolonged periods of time are needed to establish the diagnosis.

Whereas there is some, but no consistent, correlation of urinary fluoride excretion with fluoride intake, it is fallacious to equate the level of urinary fluoride with fluoride-induced illness.

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CHEMICAL PROFILE OF HUMAN SERUM IN FLUORIDE TOXICITY AND FLUOROSIS: I. TOTAL PROTEIN-BOUND CARBOHYDRATES, SEROMUCOID AND FLUORIDE LEVELS

by

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SUMMARY: Sera of 15 patients, aged 10 to 50 years - afflicted with fluorosis were analyzed for fluoride, total protein-bound carbohydrates (namely, protein-bound hexose, hexosamine, fucose) and seromucoid contents. The level of the glycoprotein components declined significantly with increased circulating levels of fluoride. In the seromucoid fraction, the protein part was greatly affected by fluoride ions compared to its sugar component. Inhibition of glycoprotein biosynthesis is reflected in low circulating levels of glycoprotein. Interference of fluoride ions in glycoprotein biosynthesis is therefore suggested.

Introduction

Glycoproteins, the conjugated proteins with carbohydrate as the prosthetic group, play various important roles in the body (1). Their levels are altered in a variety of pathological conditions (2). The measurement of total glycoprotein levels in pathological conditions for diagnostic purposes has certain limitations because the individual glycoprotein fractions can vary independently. Therefore, the measurement of the levels of individual glycoprotein fractions are considered more meaningful (3).

Previous reports from this laboratory provide evidence to the effect that sodium fluoride affects the glycoprotein and fibrinogen levels in rabbit plasma (4, 5). The present report deals with the levels of fluoride, total protein-bound carbohydrates and seromucoid fraction in the serum of patients afflicted with fluorosis. The objective of this study was to assess the deviations, if any, in the level of individual glycoproteins, of value either in the prognosis or in understanding the pathogenesis of the disease.

Materials and Methods

Patients: Serum from patients afflicted with fluorosis was obtained from the endemic regions of Andhra Pradesh, one of the southern states of India where fluorosis was first reported by Short et al. (6). The sera subjected to analysis were obtained from Nalgonda district of Andhra Pradesh, where the potable water is known to contain 5 - 20 ppm of fluoride

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(7). The patients selected for the present investigation were of both sexes, aged 10 to 50 years. They had classical manifestations of fluorosis namely, mottled teeth, back pain, pain in the joints, rigidity of joints and spine, as well as, knock-knees (8). Control blood samples were drawn from normal healthy individuals, aged 25 to 55 years.

Protein-bound Hexose, Hexosamine and Fucose Determination: Serum was initially treated with 95% ethanol; protein precipitate thus obtained was assayed for protein-bound hexose, hexosamine and fucose.

Protein-bound Hexose: The precipitated protein was dissolved in 0.1 N NaOH and assayed by the orcinol sulphuric acid method. We used an equimolar mixture of glactose and mannose as standard (9).

Hexosamine: To estimate the hexosamine content, the precipitated protein was treated with 3N HCl for 4 hours in a boiling water bath. It was then neutralized by 3 N NaOH and assayed by the method of Winzler (9).

Fucose: Fucose was estimated by the method of Dische and Shettles (10) in the protein precipitates.

Seromucoid Fraction: The seromucoid content of sera were measured in terms of its hexose and protein contents. Sera were initially diluted with 0.85% saline and treated with 1.8 M perchloric acid. The samples were centrifuged and the supernatant decanted. The seromucoid present in the supernatant was precipitated by 5% phosphotungstic acid. The precipitate was dissolved in 0.1 N NaOH and known aliquots were treated with orcinol and Folin-phenol reagents respectively for the estimation of hexose (9) and protein (11) contents.

It is pertinent to point out the limitations of the methods. The alcohol and acid precipitates of glycoproteins are likely to be contaminated with other serum components. There is a possibility of contamination with proteoglycans and glycosaminoglycans which are also rich in hexosamine. However, our investigations on rabbit sera (unpublished data) and those on human patients (12), have shown that the glycosaminoglycan levels in sera are extremely low or negligible. These limitations can be overlooked in view of the fact that the same method of investigation has been used for both normal and fluorosed samples.

Fluoride Estimation: Fluoride content in the serum was determined by the method described by Hall et al. (13) using a fluoride ion specific electrode. The results are expressed as ppm fluoride.

Results

The results obtained are given in Table 1. The normal levels of fluoride, total protein-bound hexose, hexosamine, fucose and seromucoid contents in human sera are in fair agreement with the reports of other investigators (9, 14, 15). The fluoride concentration in the sera of patients afflicted with fluorosis increased significantly, as much as tenfold. On the other hand the total protein-bound carbohydrates (protein-

Table 1

Total Protein-bound Carbohydrates, Seromucoid and F^- in Serum of Normal and Fluorosis Patients

	Normal Mean S.D.	Fluorosis Mean S.D.	% Difference and P Value *
Fluoride	0.04± 0.02	0.48± 0.13	891.84 (+)
Protein-bound	163.71±11.32	138.07±11.09	15.66 (-)
Hexosamine	128.14±21.22	103.27±18.40	19.56 (-)
Fucose	13.50± 2.99	10.53± 3.25	22.00 (-)
Seromucoid Fraction			
a. as Hexose	25.17± 4.70	20.71± 4.04	17.72 (-)
b. as Protein	62.17±18.90	44.40± 4.70	28.58 (-)

Values are expressed as mg per 100 ml of serum except for fluoride which is expressed as ppm and are mean of 15 number of samples.

(-) Decrease; (+) Increase; (*) $P < 0.001$

bound hexose, hexosamine and fucose) and seromucoid levels are reduced in patient's sera. From the data obtained for total protein-bound carbohydrates in fluorosed sera, fucose shows the maximum percentage reduction, whereas protein-bound hexose shows the least.

The seromucoid fraction measured as hexose and protein has also shown a significant reduction in fluorosis. The seromucoid fraction measured as protein, shows a higher percentage reduction compared to that measured in terms of its hexose content.

The data shows that increased fluoride concentration reduces the glycoprotein levels in serum.

Discussion

The glycoprotein content of the sera of patients afflicted with fluorosis showed a reduction. It is interesting to note that in most diseases the serum glycoprotein levels are elevated except in a few pathological conditions where they are reduced (2, 16-21). From the results obtained in the present investigation, it is evident that fluorosis is yet another clinical condition wherein reduction in serum glycoprotein levels takes place. This indicates that fluoride ions interfere with the normal metabolism of glycoproteins. The lower levels of total protein-bound carbohydrates could be due to various reasons. The most likely explanation is that, during fluorosis, the concentration of individual gly-

coproteins could have been reduced. In the present study, the seromucoid fraction decreased significantly in fluorosis. Our previous reports (4, 5) also support this observation. During these studies it was observed that the seromucoid fraction and fibrinogen levels (both acute phase proteins) were also reduced.

In the seromucoid fraction, the ratio of protein to hexose was decreased in fluorosis. This indicates that in fluorosis the protein part of seromucoid is greatly affected compared to its sugar component and that the chemical composition of seromucoid has been altered in fluorosis.

The decreased glycoprotein content in fluorosis could be the result of the impairment of its biosynthesis. It is known that fluoride ions reduce the protein biosynthesis. The liver plays a vital role in glycoprotein biosynthesis and during fluoride toxicity the liver has been reported to be damaged (22, 23).

The reduction in the glycoprotein levels could also be due to increased catabolism, higher excretion and neo-bone formation (4), but evidence to support this view is inadequate.

The present report which has provided data on glycoprotein content, reveals significant deviations in the chemical profile of serum in fluorosis. The significant increase in serum fluoride and reduction in total protein-bound hexose, hexosamine and seromucoid fraction might possibly be used for the early detection of the disease.

Acknowledgements

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In a review of the history, causes and treatment of osteoporosis, the author recommends oral estrogen therapy combined with calcium supplementation. He comments on "disturbing reports" about fluoride administration which "rather than improving osteoporosis may instead lead to secondary hyperparathyroidism and osteomalacia." Such side effects as synovitis, plantar fasciitis, vomiting and anemia were noted by Riggs, et al. in 42% of the patients who were given fluoride for osteoporosis.

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EFFECT OF ADMINISTRATION OF SODIUM FLUORIDE ON URINARY EXCRETION OF TRYPTOPHAN METABOLITES

by

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SUMMARY: The effect of the oral administration of sodium fluoride on the urinary excretion of tryptophan metabolites was examined in rats. In relation to the creatinine excretion a single dose of 10 mg NaF caused a 50% decrease in the excretion of the metabolites of the serotonin pathway. No further changes were observed when the NaF administration was continued and the effect was reversible when the NaF administration was discontinued. These results suggest that fluorides interact with tryptophan metabolism.

Introduction

Knowledge about the effect of fluoride on the human and animal organism is of fundamental importance in view of the danger of pollution of the natural environment with the industrial emission of fluoride.

The use of fluoride-containing products has increased during the last few years. The varnishes prescribed in caries prevention have a fairly high fluoride concentration (about 2.5% expressed as NaF) and as they adhere to the enamel the fluoride exposure time is extended to several hours (1). On the other hand, defects in the equipment for water fluoridation have caused serious intoxication (2).

Although small amounts of fluoride are believed to be required for maintaining health, at least in the rat, (3) and to be beneficial in the prevention of dental caries (4), adverse effects may occur at any level of fluoride intake (5). Fluorides are known to affect enzymatic systems, either by activation or by inhibition (6) and the inhibitory effect of fluorides on DNA and protein synthesis has been described (7). However, little is known about the effect on the amino acid metabolism. Pandit and Rao (8) have mentioned an interference of fluoride with phenylalanine and tyrosine metabolism. Rapaport (9) has reported a fluoride-induced deviation of tryptophan (TRP) metabolism in the fruit fly. The observed acceleration of the kynurenine pathway when fluoride was incorporated in the nutrient medium was expressed by a high tumor occurrence and an abnormal eye color.

As the essential amino acid tryptophan and its derivatives play an

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important role in the metabolic regulation of the human and animal organism and in view of the increasing exposure to high concentrations of fluoride, the purpose of the present work was to study the influence of an oral load of sodium fluoride on the metabolic fate of extracerebral tryptophan by monitoring the urinary excretion of TRP-metabolites using reversed phase high pressure liquid chromatography.

Materials and Methods

Adult female Wistar rats (weight 250-270 g) were housed in individual metabolic cages (B.V. Metaalindustrie, Utrecht, The Netherlands). The temperature of the animal quarter was held between 20 and 22°C. The animals were kept under light from 7 A.M. to 7 P.M. During the first week of the experiment (control period), the rats daily received 20 g of powdered standard food (A.04, obtained from U.A.R., Villemoisson-sur-Orge, France) and had free access to water. The fluoride content of the standard food was determined after $HClO_4$ - digestion by means of a fluoride sensitive electrode (as modified by Vandeputte*); it amounted to 10 mg/kg. The fluoride concentration of the water ranged between 0.0 and 0.4 mg/L.

In a first series of experiments, the animals were given a single dose of 10 mg NaF, mixed with the top layer of the food (single administration experiment). In a second series, 10 mg NaF/day was added to the food during 5 consecutive days (multiple administration experiment).

The 24-hour urine samples were collected at pH^2 and at 4° (11) for at least 4 days in the single administration experiments and for at least 8 days in the multiple administration experiments. The urine collections were started at the moment when the animals began to have access to the food.

The main tryptophan metabolites present in the sample, namely tryptophan, xanthurenic acid, serotonin, 5-hydroxytryptophan, indican, 5-hydroxy-indole acetic acid, indole lactic acid, were separated by high performance reversed-phase chromatography on two μ-Bondapak C-18 columns in series (Waters Assoc. Milford, USA), with an eluent system described by Sentfleber et al. (12). In short, the low concentration eluent was a 0.025 mol/l sodium acetate buffer, pH 4.4, and the high concentration eluent a 0.1 mol/l acetic acid solution in methanol. The eluents were degassed with a water-vacuum line before use. The flow rate was 40 ml/h (corresponding to a linear velocity of 0.117 cm/s) and the temperature was ambient. The compounds were detected at 260 nm with a variable wave length U.V.-detector (Model LC 55, Perkin-Elmer, Oakbrook, USA) and by native fluorescence (Fluorimeter 3000, Perkin Elmer) (excitation wave length, 280 nm; emission wave length, 360 nm).

Urinary creatinine was colorimetrically determined with the Jaffe reaction (13).

The results were statistically analyzed (t-test) according to Snedecor and Cochran (14).

* - Personal communication

Table 1

Effect of NaF on Diuresis and Daily Creatinine Excretion

	Control (n = 24)	+ NaF (n = 18)	P (t-test)
Diuresis	9.54 ± 2.83	10.89 ± 4.14	>0.05
Creatinine (mg/day)	13.86 ± 1.78	8.80 ± 1.71	<0.005

Results

In Table 1, the influence of a single oral dose of NaF on diuresis and daily creatinine excretion is shown. From these results, it appears that the diuresis was not affected by the fluoride intake, but the daily creatinine excretion was reduced by 40% during fluoride intake, compared with the values obtained during the control period ($P<0.05$). For the other parameters measured (food and water intake, body weight) no significant differences could be observed (t-test, $P>0.05$).

Table 2

Values of Tryptophan Metabolites

	Control Period (n = 24)	After Single NaF Administration (n = 18)	After 5 Days NaF Intake (n = 18)	3 Days After Last NaF Intake (rehabilitation) (n = 18)
XA	1.670±0.471	1.484±0.377	1.521±0.351	1.608±0.426
SER	0.366±0.099	0.175±0.046***	0.180±0.053***	0.380±0.080
5-HTRP	0.675±0.305	0.374±0.150*	0.350±0.108**	0.701±0.325
IND	14.690±4.375	12.400±2.618	13.822±3.393	12.866±3.857
TRP	5.950±1.530	4.041±1.944	4.187±1.786	5.042±1.463
5-HIAA	3.311±0.466	2.606±0.484**	2.526±0.422**	3.241±0.384
ILA	0.599±0.141	0.521±0.112	0.533±0.121	0.554±0.126
IAA	2.180±0.671	2.645±0.927	2.620±0.828	2.465±0.741

Legend: XA = xanthurenic acid; SER = serotonin; 5-HTRP = 5-hyroxy-trytophan; IND = indican; 5-HIAA = 5-hydroxy-indole acetic acid; ILA = indole lactic acid; IAA = indole acetic acid.

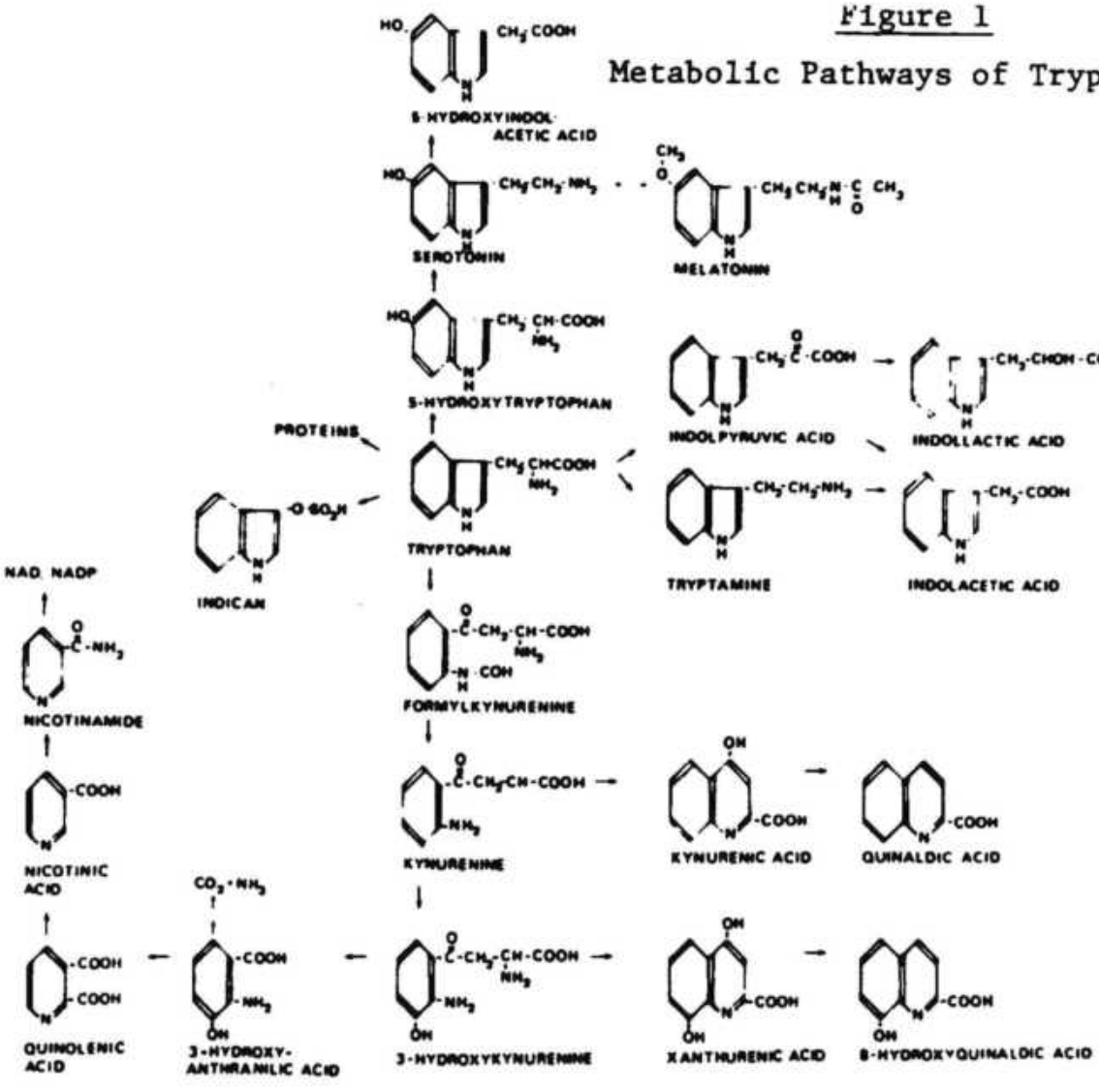
Values are expressed as Mean ± Standard Deviation

* - $P<0.05$; ** - $P<0.01$; *** - $P<0.001$

Table 2 summarizes the results obtained for the quantitative analysis of the main tryptophan metabolites. It appears that after a single oral administration of 10 mg NaF, the urinary excretion of the metabolites of the serotonin pathway (expressed in μ mole/day/ g creatinine) was significantly decreased ($P<0.001$, $P<0.05$ and $P<0.01$ for serotonin, 5-hydroxytryptophan and 5-hydroxyindole acetic acid respectively). These fluoride-induced changes were already detected in the first 24-hour sample. No further alterations in the excretion of the tryptophan metabolites could be observed when the daily administration was continued for four more days. Three days after the NaF administration was discontinued the urinary profile of tryptophan metabolites together with the creatinine excretion, gradually returned to normal.

Figure 1

Metabolic Pathways of Tryptophan



Discussion

Fluoride appears in the blood as early as 10 minutes after ingestion; its concentration reaches a maximum after 120 minutes (1). It was established by Margolis et al. (15) that at least 80% of the ingested fluoride is excreted in the urine within 24 hours. For this reason, and in order to measure accurately diuresis and daily creatinine excretion, 24-hour samples were collected.

The amount of fluoride given to the rats is fairly high but, in relation to the body weight, it is in the order of magnitude of fluoride intake in accidental intoxication in humans.

We have observed a decrease in the daily creatinine excretion during the NaF treatment. These results could confirm the lowering effect of fluoride on the basal metabolic rate (16). However, Ophaug et al. (17) did not notice such a decrease, but the doses of fluoride administered by these investigators were much lower than in our study.

The results of the present experiment concerning the influence of fluoride on tryptophan metabolism could suggest an alteration of an important pathway of this amino acid: the serotonin pathway (Fig. 1). During fluoride intake, the urinary excretion of 5-hydroxytryptophan, serotonin and 5-hydroxyindole acetic acid is less than 50% compared with the value obtained during the control period.

These results are in agreement with the suggestion of Rapaport (9) that fluoride might have an inhibitory effect on the serotonin pathway of tryptophan.

The observed effect of sodium fluoride on tryptophan metabolism could be due to a decreased synthesis or to an impaired excretion of the metabolites studied. Further investigations on regulating enzymes and physiological functions involved could elucidate the action mechanism of fluorides on tryptophan metabolism.

The importance of the observed changes in the tryptophan metabolism is to be related to the metabolic activity of the tryptophan derivatives. Some of the endogenously produced metabolites of the tryptophan-nicotinic acid pathway are carcinogenic and serotonin is known to have multiple activities (neurotransmitter, vasoconstrictor, psychotropic drugs, etc.) (18). Consequently some of the modifications observed in the general metabolism and the physiological state in cases of fluoride poisoning could be correlated with the changes in tryptophan metabolism.

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THE EFFECTS OF FLUORIDE ON FISH IN GABES GULF

by

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SUMMARY: Factories processing natural phosphate discharge fluoride-rich effluents into Gabes Gulf. The fish caught in the vicinity of the outflow reveal, in various tissues, fluoride levels which are four or five times higher than in fish caught in Tunis Gulf. In the mullets caught in Gabes Gulf the mean values of fluoride are: Fishbone: 320 ± 225 ppm; muscle: 9.6 ± 10.2 ppm; muscle plus skin: 14.6 ± 11.1 ppm.

The high values of the standard deviations show that there is a great disparity in contamination; some fish have accumulated much more fluoride than others.

Introduction

South Tunisia is very rich in natural phosphate. Part of it is exported in its crude form from the harbors of Sfax and Gabes; the rest is transformed into phosphoric acid and superphosphate on the spot. Two big plants for such transformation of phosphate have been built in Gabes: I. C.M. (Industries Chimiques Maghrebines) and S.A.E.P.A. (Société Arabe des Engrais Phosphatés et Azotés). They are located near the commercial harbor and discharge their effluents outside the harbor, in the corner limited by the shore and the south pier. The wastes are essentially cooling waters from the sulfuric acid plants, fluoride-containing scrub waters from the cleansing of gases from the phosphoric acid plant and wastes from the reaction of phosphoric acid on natural phosphates (phosphyogypsum), suspended in water again for disposal.

As shown by Ezzedine (1) and Darmoul (2) these effluents are responsible for three forms of pollution: an acid pollution restricted to the waters close to the beach, a deposit of phosphogypsum spreading over a 3 km distance, on a total area of about 2.3 km^2 (Fig. 1) where benthic life has practically disappeared, a fluoride pollution caused by scrub water and the fluoride contained in phosphogypsum. In this work, our objective was to study the effects of fluoride on fish caught close to the source of pollution.

Materials and Methods

Samples were collected from Gabes Bay at definite locations deter-

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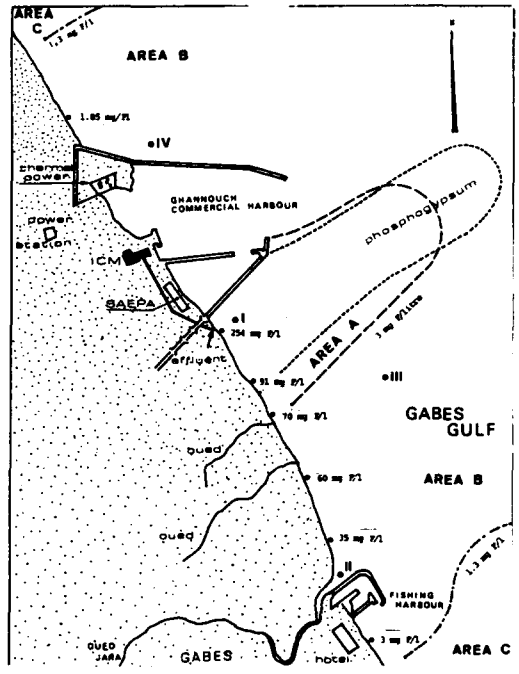
Figure 1



mined by the studies by Darmoul (2) on the fluoride contamination of the water along the beach and on the infralittoral area (Fig. 1). The water samples collected 1.5 m off the beach showed high fluoride concentrations: 250 to 300 ppm close to the effluent, 70 ppm at 700 m, 35 ppm at 2 km with phenomena of dilution at the mouths of the oueds. Infralittoral waters, beyond 200 m from the beach, can be divided into 4 areas separated by fluoride isoconcentration lines: 3 mg/l areas A-B; 1.3 mg/l areas B-C; 1 mg/l areas C-D (Fig. 1).

The fish were collected in December 1979 at four different sites, site I,200 m south of the effluent, 100 m from the shore, 5 m deep; site II,2300 m south of the effluent at the mouth of Jara oued, 10 m from the shore, 0.4 m deep; site III,half-way between the two piers of the commercial and fishing harbors, about 3 km from the shore, 6 m deep; site IV,100 m from the north pier of the commercial harbor, 5-6 m deep (Fig. 1). At that date, only I.C.M. I and II were in operation. Work at S.A.E.P.A. was officially started on January 4, 1980. As control samples, fish were caught in Tunis bay, 25 m from Gammarth beach, 1 m deep, in February 1980.

Immediately after being collected, the fish (essentially mullets, oblads and corbs) were identified, after which - within 24 hours - they were deep-frozen and refrigerated at -18°C in plastic bags. During a

period of 24 hours, the fish were thawed out at +4°C, weighed and measured in order to determine their age; their scales were scraped and washed in distilled water; their fins were removed; the livers and digestive tracts were collected; the skins were minutely dissected and the fillets separated from the bones. In some cases, the skin was not separated from the muscle. The bones were carefully cleaned.

The samples thus obtained were immediately weighed, ground and mixed with 1/10th of their weight of quick lime, so as to avoid loss of fluoride compounds during mineralization. The mixture thus obtained was dried in incubators until constant weight was obtained, then calcined in a stainless steel-coated oven, equipped with a heat programmer ensuring a temperature of 550°C reached in stages. The ashes thus obtained were weighed. Trial samples were very accurately weighed according to their nature (from 0.1 to 1 gr from fishbone, from 3 gr to 5 gr from fillets) and submitted to distillation (Willard and Winter's method) (3). To the distillate thus obtained, was added a special buffer solution (T.I.S.A. B.), the fluoride concentration was determined by means of a specific electrode by the method of dosed additions (Barnes and Runcie) (4). Fluoride was also measured in the quick lime which was added to the samples and the results were adjusted accordingly. The sensitivity of the method approximates 0.1 ppm and its accuracy is more or less 5%.

Results

The post-mortem examinations on the five batches of fish and on two other batches have been reviewed at length in the veterinary medicine doctoral thesis of one of us (5). Fish caught in Gabes Gulf are covered with a kind of brownish mud which looks like phosphogypsum. This compound is also found in the gills, the stomach and the cecum in the fish from sites I, II and III, whereas it cannot be found in the fish from site IV, collected north of the commercial harbor. The deposit is very marked at site I, moderate at site III and slight at site II, located at the mouth of Gabes oued. The fish caught in Tunis Gulf are clean; only sand is to be found in the digestive tract.

Table 1 shows the fluoride levels in muscle, skin, scales and fishbone of most fish caught at sites I, II, IV. Because the weight of the muscle and skin was known, it was possible to calculate the contamination of both muscle and skin. The table shows too, the approximate age of mugilides calculated according to their size (H. Ferrugio's method) (6). In the fish caught at site III and in the control fish, fluoride levels were measured in scales, fins, gills and in either liver and digestive tract or a mixture of the two samples (Tables 2 and 3).

Obviously fluoride concentrations in all organs of the fish caught in the vicinity of the effluent are much higher than those in the fish caught in Tunis Gulf. In mullets, the ratio is about 5 for fishbone and muscle and 4 for muscle + skin. In oblads, the ratio is about 5 for fishbone and 4 for muscle + skin, yet the absolute values are much higher, 1530 ± 860 ppm/wet weight (W.W.) for fishbone at site III versus 300 ppm/W.W. in controls; 95 ± 42 ppm/W.W. for muscle + skin at site

Table 1

F^- Levels (ppm/wet weight) in Fish Caught at Sites I, II, IV in Gabes Gulf

No. of Fish	Species	Muscle	Skin	Muscle + Skin	Scales	Fishbone	Age (Years)
			SITE I				
1	Mugil Auratus Risso	3.3	-	-*	180	105	2
2	Mugil Auratus Risse	2.8	19	4.2*	190	140	3
3	Mugil Cephalus L.	-	-	3	350	210	3
4	Mugil Cephalus L.	-	-	18	1260	630	1
5	Mugil Cephalus L.	26	-	-	1550	425	2
9	Mugil Cephalus L.	-	-	9*	7 -	310	1
14	Mugil Labrosus	4.4	40	10	495	530	2
15	Mugil Labrosus	6	30	9.2	405	140	2
Mean		8.5	30	9	630	310	
Values	Mullets	±9.9	±10.5	±5.3	±540	±200	
			SITE II				
8	Mugil Cephalus L.	4.8	36	9.8	940	405	1.5
10	Mugil Cephalus L.	8.8	47	15	1000	195	2
20	Mugil Labrosus	-	12	-	190	95	1.5
22	Mugil Labrosus	-	-	18*	715	350	1
23	Mugil Labrosus	5.1	23	9	-	260	1.5
24	Mugil Labrosus	-	-	23	1050	665	1.5
Mean		5.2	29.5	15	780	328	
Values	Mullets	±2.2	±15.2	± 5.8	±350	±198	
11(9)	Oblada melanura L.	6.3		44	445	260	
		2.2					
			SITE IV				
25	Mugil Labrosus	4.5	51	12*	655	390	2.5
26	Mugil Labrosus	15.3	86	27*	1250	940	2.5
Mean		9.9	68.5	19.5	950	665	
Values		±7.6	±24.8	±10.6	±420	±389	
27	Ombrina cirrosa L.	4.4	25	6.9*	475	360	
28	Ombrina cirrosa L.	-	-	11	355	465	
29	Ombrina cirrosa L.	5.7	17	7.6	400	260	
Mean		5	21	8.5	410	360	
Values		±0.9	± 5.7	±2.2	± 60	±102	

*: Values calculated from concentrations in muscle and skin

Table 2

F^- Levels (ppm/wet weight) in Fish Caught at Site III in Gabes Gulf

No. of Fish	Species	Muscle	Skin	Muscle+ Skin	Scales	Fins	Gills	Fishbone	Liver+digestive tract	Age (years)
44	Mugil Cephalus L.	44	84	55*	675	645	765	345	5685**	1.5
41	Mugil Cephalus L.	7.8	87	19*	1050	700	-	615	1985**	1.5
43	Mugil Cephalus L.	9	42	16*	530	470	250	350	62	2
39	Mugil Cephalus L.	9.7	40	16*	195	220	190	100	1280	2
38	Mugil Cephalus L.	4.4	12	5.8*	185	215	150	92	275**	2
42	Mugil Cephalus L.	3.4	16	5.7*	250	225	505	80	340	2
40	Mugil Labrosus	5.9	19	7.5*	430	415	200	145	78	2
37	Mugil Labrosus	7.7	42	14*	420	380	98	180	1220	2.5
Mean Values	Mullets	11.5 ±13.3	43 ±29	17.4 ±16	470 ±290	410 ±190	310 ±240	240 ±186	1560 ±1940	
32(7)	Oblada Melanura L.	-	-	105	1430	2090	1475	1275	130**	
34(5)	Oblade Melanura L.	-	-	115*	1885	3435	1945	1975	130**	
33(3)	Oblada Melanura L.	54	370	125*	1320	3330	1865	2410	125**	
35(3)	Oblada Melanura L.	-	-	33	615	565	55	445	70	
Mean Values				95 ±42	1310 ± 525	2350 ±1340	1335 ± 880	1530 ± 800	115 ± 30	

Table 3

F^- Levels (ppm/wet weight) in Fish Caught in Tunis Gulf

No. of Fish	Species	Muscle	Skin	Muscle + Skin	Scales	Fins	Gills	Fishbone	Liver + digestive tract	Age (Years)
T_1	Mugil Labrosus	2	17	4.5*	200	205	90	100	44**	3
T_2	Mugil Labrosus	1.5	7.3	2.8*	100	173	41	52	1.6	3
T_3	Mugil Cephalus L.	2.7	21	6.3*	290	230	125	130	4**	4
T_4	Mugil Cephalus L.	1.3	8.8	2.7*	135	100	45	45	0.9	3
T_5	Mugil Cephalus L.	1.3	10	2.5*	115	-	37	46	1.9**	3
Mean Values	Mullets	1.8 ±0.6	13 ±5.9	3.8 ±1.6	170 ±78.3	157 ±70.7	67.6 ±38.6	73 ±40.5	17.9 ±22.5	
T_6(13)	Oblada melanura L.	-		25	565	655	415	300	35**	

* Values calculated from concentrations in muscle and skin.
** Values calculated from concentrations in liver and digestive tract

III versus 25 in the controls. In mullets, the concentrations are the following in all fish caught in the area surrounding the effluent: fishbone, 320 ± 225; muscle, 9.6 ± 10.2; muscle + skin, 14.6 ± 11.1 ppm/W.W. whereas the levels in the controls are respectively 73 ± 40.5; 1.8 ± 0.6; 3.8 ± 1.6 ppm/W.W.

On account of the relatively limited number of fish analyzed, it is difficult to make comparisons between the different sites where the fish were collected in the vicinity of the effluent. These differences between fish from the same area seem greater than those existing between the various sites of collection whereas the difference between phosphogypsum accumulation in gills and in the digestive tract from one site to another is significant. This may be because fluoride accumulation depends on very slow exchanges between the fish and the environment, whereas phosphogypsum deposition is much quicker. Thus, this accumulation normally increases with age.

In the scales, the fins and the gills, which are in contact with the outside environment, fluoride levels are always higher; whereas lower values are obtained in the fishbones, skin and fillets. In the viscera, the levels vary according to the fish. In the mullets caught at site III, the levels in fishbone, skin and muscle represent respectively 58, 10 and 1.8 per cent of the scales, fins and gills taken together. In the control mullets, they represent 54, 10 and 1.3 per cent. Yet, there are considerable differences between the individual fish as shown in the tables.

Discussion

Whereas, as a rule, and according to Hemens and Warwick (7), fluoride accumulation in fish results from fluoride in solution in water in the vicinity of the effluent in Gabes Gulf, the deposition of phosphogypsum on the gills and the scales and its ingestion in large quantities must be taken into consideration since this compound contains, as mentioned by Klinghoter (8) and as we ourselves learned, from 1 to 1.5% fluoride.

In the assessment of hazards for fish one must take into account, not only the fluoride contamination of the sea environment near the outflow, but also the habits of living and of migrating of the fish. Hemens and Warwick (7) have shown that, comparatively, the mullet is not highly sensitive to the direct toxic effects of fluoride. A 96-hour exposure to a 100 mg F/l concentration has no effect on juvenile mullet "Mugil Cephalus (L.)". A 72-day exposure in an adequate environment to 52 mg/l concentration induces 3 deaths out of 10 in control and treated fish. The following differences are underlined: "of the 3 mullet deaths recorded in the control tank, two were due to accidental death by jumping out of the tank halfway through the experiment. At the end of the experiment the 7 survivors in the control were active and full-bellied, in contrast to those in the fluoride tank which were hollow-bellied and noticeably less active, two of which were suffering from a fungal infection. Had the experiment continued for an additional 2-3 weeks there is little

doubt that a higher mortality would have occurred in the fluoride tank than in the control."

On the other hand, trout is much more sensitive to the toxic effects of fluoride. According to Neuhold and Sigler (9) "the 48 h LC 50 for rainbow trout "Salmo gairdneri" at least one year old, is as low as 2.7-4.7 ppm. Herbert and Shurben (10) recorded a 48 h LC 50 of 8.5 ppm fluoride in yearling rainbow trout. Wright (11) has shown that the LC 50 for brown trout fry "Salmo trutta" was reached only when concentrations were higher than or equal to 20 ppm fluoride. He has also shown that temperature of and the calcium and chloride content in water played a major role.

Capacities of adaptation to high fluoride concentrations undoubtedly exist. Sigler and Neuhold (12) mention that, in Yellowstone National Park and in Nevada lakes, normal populations of rainbow trout survive in waters where the fluoride concentrations are 14 and 13 ppm respectively. As in the other species, an accumulation of fluoride in the organism of fish is observed before the appearance of toxic effects. Sigler and Neuhold (12), for instance, have noted fluoride concentrations in fishbone of 1600 ppm/sec in trout living in waters containing from 13 to 14 ppm.

Fluoride accumulation in fish which live in waters containing normal fluoride levels varies according to the species, as shown by the results obtained in fish caught in Tunis Gulf: muscle + skin, 3.8 ppm/wet weight in Mugilidis versus 25 ppm in Sparidis. The differences are smaller in the fish caught by Wright and Davison (13) off Northumbrian coast, where fluoride levels in sea water ranged from 1.2 to 1.5 ppm.

Fluoride concentrations in cod "Gadus morrhua", haddock "Gadus aeglifinus" and Dab "Pleuronetis limanda" vary between the following extreme values: Axial skeleton 18.2 at 99.7 mg/kg wet weight; skin 13.3 at 74.3 mg/kg wet weight; muscle 0.5 at 3.7 mg/kg wet weight. In different species of fish taken in a nonpolluted estuary in New Zealand, I.R. T.P.C. (14) reports the results of Stewart et al., who found fluoride concentrations from 509 to 2885 ppm/dry weight in fishbone.

At the conclusion of the above-mentioned experiment conducted by Hemens and Warwick to determine the possible toxic effects of fluoride after 72 days exposure in water with 52 ppm fluoride, all the mullets were investigated for fluoride accumulation. The treated fish had accumulated 7743 ppm F/ashes, i.e. 520 ppm/wet weight, whereas the control contained only 149 ppm/ashes.

These figures are hardly comparable with those we have obtained. Yet a few points must be emphasized:

1. As a rule, fish contain high levels of fluoride.
2. The parts of the fish which are in contact with the outside environment (scales, fins, gills) contain higher levels of fluoride than the bones.

3. The skin contains markedly higher levels than the muscle and the ingestion of muscle + skin by the consumer increases the intake of fluoride attributable to fish.

The sampling which was achieved fails to give a precise idea of the contamination of the fish caught in Gabes Gulf for the following reasons: Samples should be collected from all fish caught by the numerous fishermen who sail from the various small harbors on the Gulf. Also the part played by fish in consumers' daily intake should be assessed, along with fluoride intake from other sources such as water and tea.

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FLUORIDE LEVELS IN SOIL, WATER, PLANTS AND CATTLE IN THE DARMOUS ZONE OF MOROCCO

by

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SUMMARY: In the Darmous zone in Morocco, contamination of cattle by fluoride can be correlated with the duration of fluoride ingestion. From a practical point of view it can be accurately and easily evaluated by the dental lesions which, when correlated with bone fluoride levels, reflect the severity of contamination of the animal.

Introduction

In the Darmous area in Morocco, both men and animals are extensively affected by hydrotelluric fluorosis. In such zones, fluoride from soil, water and plants accumulates in animals, mainly in their bones and teeth (1,2) and to a lesser extent in soft tissues. The normal bone level in cattle ranges between 100 and 800 ppm (3,4). In fluorotic cattle between 2000 and 8000 ppm can accumulate in maxillary bone and caudal vertebrae (5,6).

The present study was designed to measure fluoride contamination of the environment and to correlate it with bone fluoride and dental lesions in cattle in the Darmous zone.

Experimental Procedure

The experiments were carried out in 40 two to four-year-old, locally bred cattle in 40 different range-breeding units. In each unit, the following samples were collected in polyethylene vials or bags: maxillary bone (after slaughter); approximately 50 g of straw and 50 g of barley; 100 ml of well-water. Three different random samples of soil were taken at each of the 3 following depths: 0 to 20 cm, 20 to 40 cm and 40 to 60 cm.

Fluoride was measured by use of an ion selective electrode (39600 Beckman) and of a digital 4500 Beckman pH-meter, in molar citrate buffer either directly for water or after acid ashing according to Armstrong (7) for bone, and after alkaline ashing according to Neil (8) for soil and plants. Moreover, the teeth were examined to determine the severity of the dental lesions which were classified from 0 to 4.

Results

As shown in Table 1, mean fluoride was higher in the upper layers of

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Table 1

F^- in Soil, Water, Plants and Bovine Maxillary

		Range (ppm)	Mean (ppm)
Soil	0 to 20 cm layer	410-11,850	3232
	20 to 40 cm layer	430-11,850	3637
	40 to 60 cm layer	410-12,630	2785
Water		0.42-1.90	1.17
Straw		52-147	104
Barley		10-43	22
Bone		1115-8350	3260

of the soil but the levels varied widely. The mean fluoride concentration of water was 0.91 ppm but was dependent at any depth upon the fluoride level of soil ($p<0.001$). Fluoride concentrations in barley correlated with that of soil ($p<0.05$) but not with fluoride in straw nor with that of water ($0.05<p<0.10$).

The mean fluoride level in bone was 3260 ppm. This concentration depended significantly ($p<0.001$) upon the duration of fluoride ingestion, i. e. upon age; but no correlation could be found between fluoride in bone and in soil, in water or in plants. Moreover, as shown in Table 2, the more severe the dental lesions, the higher was the fluoride level in bone.

Table 2

Correlations Between F^- (m±SD) in Maxillary Bone and Severity of Dental Lesions

Dental Fluorosis (Degree)	Number of Animals	F^- in Bone (ppm)				
0	6	842±447				
1	6	2562±1142	NS			
2	16	3705±1173	<0.05	NS		
3	9	5260±1182	<0.05	<0.05	NS	
4	3	6731±1893	<0.05	<0.05	<0.05	NS
			0	1	2	3 4

Discussion

In this zone, the fluoride levels in soil differ greatly from one place to another but the correlation between the concentrations observed and those reported in normal soil (160 ppm) is highly significant. In water, the fluoride ion concentration is relatively low but water may also contain fluoride-rich particles or dust as reported by Charnot (9). In barley, the fluoride content is slightly higher than normal although the correlation with fluoride in soil, mainly in its upper layers, is high. The fluoride contamination of straw is abnormally high, probably due to deposition of dust.

Our findings do not demonstrate any correlation between bone fluoride and the contamination of the environment. Such an observation can be explained on the following basis: Contamination of animals results from ingestion of plants, water and soil. Moreover dust can be either inhaled or ingested, mainly with fresh grass. In periods of drought, animals scratch into the ground in search of roots and therefore ingest some soil. This contamination can be reinforced by the geophagia, often observed in deprived animals.

Conclusion

In the Darmous zone in Morocco, contamination of cattle is directly correlated with the duration of fluoride ingestion; from a practical point of view it can be accurately and easily evaluated by dental lesions which correlate with bone fluoride levels and demonstrate the severity of animal contamination.

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INDUSTRIAL FLUOROSIS

by

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SUMMARY: In 1242 apparently healthy and actively employed workers of a Canadian aluminum facility, the history of musculoskeletal symptoms, of the incidence of fractures, of neck and back surgery, as well as the x-ray findings were reviewed. A highly significant relationship of exposure to fluoride was established with the frequency of back and neck surgery, fractures, symptoms of musculoskeletal disease and past history of diseases of bones and joints in the absence of the typical findings of skeletal fluorosis.

Monitoring exposed workers for the early manifestations of "musculoskeletal fluorosis" is recommended prior to the development of destructive and degenerative changes of the skeleton.

Introduction

In Danish cryolite workers in 1932, Moller and Gudjonsson (1) found extensive involvement of the musculoskeletal system including radioopacity of the vertebrae along with extensive calcification of ligaments and fibrocartilaginous attachments. The most severe manifestations were always found in the vertebral column and pelvis. Since the appearance of Roholm's (2) classic treatise on fluoride intoxication, other scientific workers have reported additional groups of cases of bone abnormalities, in some instances with crippling effects, in men exposed to fluorides in aluminum smelters. CASAW, the Canadian Association of Smelter and Allied Workers, a labor union which represented the workers in a large aluminum smelter, concerned about the increasing numbers of smelter workers with back and neck problems and the possible excessive exposure to fluorides, undertook support of a health effects study of their members.

The overall objective of the study was to determine, by epidemiologic methods, whether exposure to toxic substances in the smelter had adversely affected the health of the workers. This presentation will limit itself to an examination of our findings on the effects of fluorides on the musculoskeletal system.

Material and Methods

The smelter produced in excess of 800 tons of aluminum per day. It emitted 4 - 5 kilograms of fluoride per ton of aluminum into the am-

From the University of Illinois Medical Center, Chicago, Illinois. Presented at the 11th Conference of the I.S.F.R., Dresden, GDR, April 1981.

bient air. Production started in 1954. The vertical stud Soderburg process is used.

Eligibility for Inclusion into Study: The cohort was selected from a seniority list of hourly employees at the date of onset of the study. Excluded from the study were those on disability leave and those who had worked at the smelter for three months or less. 1242 workers, 85% of those eligible by these criteria, participated.

Each individual filled out a self-administered questionnaire which provided name, birthdate, ethnic and racial background, previous work record and marital status. An interviewer-administered questionnaire collected information on past medical history, symptoms and cigarette smoking. Questions asked included:

Surgery:

Have you ever had spinal fusion? If so, what year?
Have you ever had low back surgery? If so, what year?
Have you ever had neck surgery? If so, what year?

Those answering yes to one or more of these questions in the years after starting work were categorized as having had back or neck surgery following employment. Workers who had surgery at multiple sites or more than once were counted only once.

Fractures: Those answering yes to having one or more fractures on one or more occasions following start of employment were counted only once.

Musculoskeletal Disease History: To examine the past history of musculoskeletal diseases, workers were asked if they had ever been told by a physician that they had arthritis, gout, back trouble, slipped disc or any other significant musculoskeletal medical problems. Workers were counted once if they answered yes concerning one or more of these five conditions.

Musculoskeletal Symptoms: Workers, questioned regarding symptoms of musculoskeletal problems, which included joint pain, back or neck pain, stiffness in the back, stiffness in joints, and swollen joints, were scored as zero for never, one if the problem occurred one to three times per month, two if more than three times per month but less than daily, and three if it occurred daily or was present all the time. The score, the sum of these responses, varied from zero, that is, none of the five symptoms present at any time, to a score of 15, representing all of the five symptoms occurring daily or present all of the time. A score of zero to seven was categorized as low frequency, whereas a score of eight to fifteen was considered a high frequency.

X-ray: A PA chest x-ray and a AP lumbar spine were done and were read by a certified radiologist who knew only the age and sex of the subject but nothing concerning exposure. For bone x-rays, those categorized as "abnormal" included increased density, whiteness, cortical

thickening, hyperostosis, blurring of margins and calcification of ligaments. Those considered possibly abnormal included fractures, evidence of bone surgery, renal calculi, other soft calcifications, "other" nonspecific abnormalities, scoliosis and lipping of vertebral bodies.

Exposure Risk Index: Two factors were used in establishing an exposure risk index for each worker. An estimate of the level of concentration of fluoride for each job category in the smelter was made and characterized as high, moderate or low. These were weighted as 0.25, 1, and 2 respectively and multiplied by the duration of exposure of each worker in each job over his entire employment at the smelter to arrive at an exposure risk index. For purposes of analysis, the frequency distribution of the musculoskeletal exposure risk index was used to categorize the entire cohort into low, medium, and high exposure groups.

Workers were divided into four age groups, 18-30, 31-40, 41-50, and 51 years or older. The group ages 18-30 were used because very few workers were under the age of 20 and these were, therefore included. The same is true of the category 51 years or older, since relatively few workers were aged 60 or more.

Results

History of Musculoskeletal Disease: Table 1 and Figure 1 examine the relationship between a history of musculoskeletal disease and exposure to fluoride and compare the frequency of a history of musculoskeletal diseases commencing after employment in the smelter in workers in the high, medium and low fluoride exposure groups. The Mantel-Haenszel chi-square test was used to examine the relationship between those in the highest compared to medium and to the lowest, and medium compared to the lowest category of exposure. The comparison between those heavily exposed with those with minimal exposure reveals striking differences in past medical history. The differences between high and medium groups and medium and low groups suggest a direct relation between an increase in a past history of musculoskeletal disease and an increase in exposure. This relationship is maintained across all age groups. As shown in Table 1, all differences were significant.

Musculoskeletal Symptoms and Complaints: Comparison of the frequency and number of complaints of musculoskeletal symptoms in the past year was made with the level of risk as a result of fluoride exposure as shown in Table 2 and Figure 2. The results were statistically significant, the differences showing themselves particularly in the older age group with the highest exposure.

Neck and Back Surgery: Table 3 and Figure 3 compare the frequency of back and neck surgery as previously defined and performed since commencing work at the smelter with the level of risk of exposure to fluoride. The results were strikingly positive. A comparison of the high to low risk groups revealed no surgery in the younger age groups. As age increased,

Figure 1
Frequency of history of musculo-skeletal diseases (N=1239)

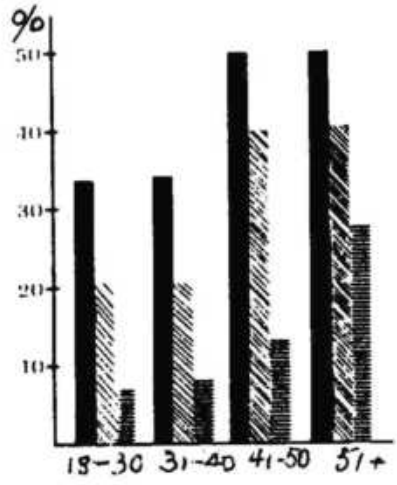


Figure 2
Frequency of symptoms of musculo-skeletal diseases (N=1195)

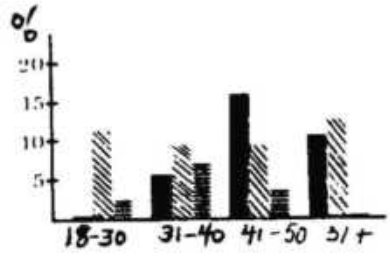


Figure 3
Frequency of back and neck surgery (N=1239)

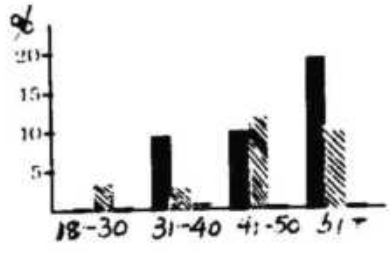
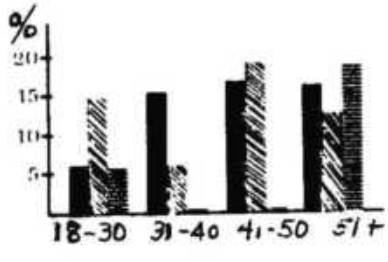


Figure 4
Frequency of one or more fractures (N=1239)



Fluoride Exposure
high
medium
low

FLUORIDE

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Table 1

Frequency of a History of Musculoskeletal Diseases
Commencing after Employment in the Smelter

Exposure Groups	Total Number	Age: 18 - 30 Positive History (%)	31 - 40 Positive History(%)	41 - 50 Positive History(%)	51+ Positive History(%)	Mantel-Haenszel χ^2
High	417	33.3% (5/15)	34.6% (27/78)	50% (96/192)	51.5% (68/132)	42.90
Low	407	7.1% (19/268)	8.9% (8/90)	13.2% (5/38)	27.3% (3/11)	$\chi<0.001$
Medium	415	23.0% (46/200)	22.4% (30/134)	40.8% (20/49)	43.8% (14/32)	37.43
Low	407	7.1% (19/268)	8.9% (8/90)	13.2% (5/38)	27.3% (3/11)	$\chi<0.001$
High	417	33.3% (5/15)	34.6% (27/78)	50% (96/192)	51.5% (68/132)	5.00
Medium	415	23.0% (46/200)	22.4% (30/134)	40.8% (20/49)	43.8% (14/32)	$\chi>0.05$ $\chi<0.025$

Table 2

Frequency of Complaints of Musculoskeletal Symptoms

Exposure Groups	Total Number	Age: 18 - 30 *High Frequency(%)	31 - 40 High Frequency(%)	41 - 50 High Frequency(%)	51+ High Frequency(%)	Mantel-Haenszel χ^2
High	410	0.0% (0/15)	5.1% (4/78)	15.9% (30/189)	10.2% (13/128)	1.954
Low	381	2.1% (5/243)	6.7% (6/89)	2.6% (1/38)	0.0% (0/11)	$\chi>0.05$
Medium	414	11.6% (23/199)	9.0% (12/134)	10.2% (5/49)	12.5% (4/32)	14.92
Low	381	2.1% (5/243)	6.7% (6/89)	2.6% (1/38)	0.0% (0/11)	$\chi<0.001$
High	410	0.0% (0/15)	5.1% (4/78)	15.9% (30/189)	10.2% (13/128)	0.159
Medium	414	11.6% (23/199)	9.0% (12/134)	10.2% (5/49)	12.5% (4/32)	$\chi<0.10$

* High frequence equals a score of 8-15 points

Table 3

Frequency of Back and Neck Surgery

Exposure Groups	Age: Total Number	18 - 30 % Surgery	31 - 40 % Surgery	41 - 50 % Surgery	51+ % Surgery	Mantel-Haenszel χ^2
High	417	0% (0/15)	9.0% (7/78)	9.9% (19/192)	18.9% (25/132)	10.62
Low	407	0% (0/268)	1.1% (1/90)	0% (0/38)	0% (0/11)	χ>.001 χ<.005
Medium	415	3% (6/200)	2.2% (3/134)	12.2% (6/49)	9.4% (3/32)	11.12
Low	407	0% (0/268)	1.1% (1/90)	0% (0/38)	0% (0/11)	χ<0.001
High	417	0% (0/15)	9.0% (7/78)	9.9% (19/192)	18.9% (25/132)	1.57
Medium	415	3% (6/200)	2.2% (3/134)	12.2% (6/49)	9.4% (3/32	χ>0.10

Table 4

History of One or More Fractures Occurring Since Employment at the Smelter

Exposure Groups	Age: Total Number	18 - 30 % Fractures	31 - 40 % Fractures	41 - 50 % Fractures	51+ % Fractures	Mantel-Haenszel χ^2
High	417	6.7% (1/15)	15.4% (12/78)	17.7% (34/192)	16.7% (22/132)	15.50
Low	407	5.2% (14/268)	0% (0/90)	0% (0/38)	18.2% (2/11)	χ<0.001
Medium	415	14.5% (29/200)	6.0% (8/134)	18.4% (9/49)	12.5% (4/32)	19.52
Low	407	5.2% (14/268)	0% (0/90)	0% (0/38)	18.2% (2/11)	χ<0.001
High	417	6.7% (1/15)	15.4% (12/78)	17.7% (34/192)	16.7% (22/132)	0.99
Medium	415	14.5% (29/200)	6.0% (8/134)	18.4% (9/49)	12.5% (4/32)	χ>0.10

surgical intervention in those at high risk due to exposure increased remarkably to almost 20% in the 51+ categories compared to 0% incidence in the same age group in those who had little or no exposure. A very significant increase in incidence of surgery is also noted when medium and low categories are compared. When the high and medium exposure groups were compared, the results were not statistically significant at the $p<0.5$ level although those in the higher category reported surgery in 19% as compared to approximately 9% in those moderately exposed.

Fractures: Table 4 presents a comparison of the frequency of the history of one or more fractures occurring since employment at the smelter, among those with a high, medium and low fluoride exposure index Table 4 shows that high and low exposure groups, and medium and low exposure groups have a different incidence of fractures at a statistically significant level. A comparison of the high to medium exposure group did not achieve a statistically significant level of association. The biggest differences were seen in the age groups from 31-40 and 41-50.

X-ray Findings: In contrast to the above findings, comparisons of the high to low, medium to low and high to medium groups revealed no significant differences in the frequency of bone x-ray abnormalities when standardized for the four age groups. Whereas many had nonspecific abnormalities, few had evidence of the dense bone described as "fluorosis" by Roholm and others in early studies.

Discussion

As a result of considerable effort by the union and due to workers' concern for their health, 85% of those eligible participated in the study. Of the 216 who failed to appear for testing, questioning, and examination, only 46 were due to outright refusals. The other 170 were on vacation, absent from work or, for other reasons, did not appear for the examination. This was a cross-sectional study, that is, it examined only supposedly healthy and actively employed workers. It was, therefore, in fact a study of a "survival" population. Any abnormal findings represent a most conservative estimate of the problem since those who left employment or were absent because of illness were not examined. Further, internal controls were used because the community is isolated and most of its able-bodied men work in the smelter. Since even the "controls" had some degree of exposure, differences between exposure groups would tend to be minimized so that when medical problems do appear, they can be assumed to be at least as serious as we found them.

The personal exposure risk index based on the entire job history and duration of exposure appeared to be a useful method for quantitating risk among the workers. We have also developed and previously published personal risk index for other organ systems which estimates levels of risk based on duration, intensity, and multiplicity of exposures, and intrinsic toxicity of the chemical agent, which appears useful in more precisely quantitating risk where multiple agents act on the same organ system (3).

The classic cases of fluorosis described by Møller and Gudjonsson (1) and by Roholm (2), and the epidemics of fluorosis found in India are not seen very often today although Kaltreider et al. (4) found that 96% of the 79 potroom workers he examined had varying degrees of skeletal fluorosis. Where fluorosis or exposure to fluorides has been studied, there is considerable evidence that the back and neck are among the first and most severely affected skeletal areas. Moller and Gudjonsson, and Roholm in their studies remarked on the stiffness of the back and complaints by the workers of rheumatic pains. Agate et al. (5) found abnormal x-rays in more than 25% of heavily exposed potroom workers with symptoms of musculoskeletal disease but without the classical signs of fluorosis. Vischer et al. (6) found in 17 heavily exposed potroom workers ossification of spinal ligaments and outgrowths of bony spurs on the vertebrae but in only nine was density of the pelvis and lumbar spine increased on x-ray. All except one complained of pain and stiffness of the extremities, shoulder, neck and lower back.

Similar findings of musculoskeletal changes without classic x-ray signs of fluorosis in workers exposed to high levels of fluorides have appeared in a number of other studies. Of special importance is the large prospective study by Zislin and Girskaya (7). They followed 2738 workers from the time they first came to work in an aluminum smelter and compared them with 1700 others employed in a nonfluoride producing industry. They found that nonspecific bone changes, musculoskeletal symptoms and other findings antedate the classic x-ray changes of fluorosis in the bones by five to seven years and concluded that the changes of fluorosis described by Roholm represent the late stage of the disease.

Our findings demonstrate a highly significant relationship between the frequency of back and neck surgery, fractures, symptoms of musculoskeletal disease and a past history of diseases of the bones and joints. In the absence of so-called classic fluorosis, a disease complex was established which involves much more than merely the radiologic appearance of dense bone. Since more stringent regulations in many countries have resulted in reduced exposure to fluorides, it is reasonable to examine workers and watch for these findings instead of waiting for dense bone to appear which is related to massive exposure to fluoride. This conclusion is supported by our findings of a statistically significant, direct correlation between back and neck surgery and a past history of other bone and joint disease, with a high fluoride exposure risk index. The relationship between those having back and neck surgery following employment and increased fluoride exposure was highly significant when compared within and among age groups based on the Mantel-Haenszel chi-square analysis.

Various theories have been advanced to explain the concentration of fluoride in the lumbar and cervical spines. Two factors should be considered. In the study by Pandit (8) in 1940 of Indian basket weavers exposed to fluoride, it was observed that the much used left arm and wrist were particularly susceptible to fluorotic exostosis. Additionally, Ascenzi (9) found that the pattern of ^{18}F distribution in the skeleton is determined by the supply of blood to a bone with increased deposition in those bones receiving the most blood. If this is true, the areas suffer-

ing repeated or constant stress or trauma, and as a result requiring ongoing repair, may be areas of increased circulation and metabolism and, as a consequence, increased deposition of fluorides.

The highly significant correlation between exposure to fluoride and fractures which we found has not, as far as we know, been reported previously in the literature. Because of concern about this possibility, studies were carried out to examine whether this might occur. An early study by McClure (10) in 1944 compared high school teenagers and young armed forces recruits from communities with varying amounts of fluoride in the water. The levels of exposure were considerably less than those in the smelter and the groups were much younger. McClure found no differences in fracture experience. Our findings are important in view of administration of fluorides in fairly high doses in treatment for osteoporosis, particularly in the elderly, for the purpose of increasing bone density and bone strength. In light of our findings, further consideration should be given as to whether such treatment truly increases bone strength as opposed to bone density. The increased density noted on x-ray often appears to be due, not to a true increase in bone mineralization, but rather to exostotic thickening of the bone. We feel that one cannot equate the x-ray findings with the degree of calcification or physical strength of bone. In fact, multiple studies have suggested that frequently the bone in fluoride intoxication is less dense and less mineralized than normal and that there is evidence that high levels of absorption of fluorides are associated with decreased mineralization. Thus, whereas the bone may appear more dense radiologically, it may, in fact, be more fragile and more susceptible to fracture. Our findings of a highly significant increase in fractures in the high fluoride exposure group suggests that this may indeed be the case. Further, in one study, those receiving fluorides as treatment complained of joint pain and tenderness, symptoms similar to those experienced by the workers we examined.

In conclusion it would appear that classic skeletal fluorosis, is in fact a far advanced manifestation of a disease which may present a variety of musculo-skeletal symptoms including abnormal fracturing of bone and of much earlier occurrence pathology of the cervical and lumbar vertebrae. Given the improvements in the work environment and air quality in the past decade, it is more appropriate to monitor exposed workers for the early manifestations of the disease complex which we have described, and which we call musculoskeletal fluorosis, before the degenerative and destructive changes of the vertebrae, joints and other bony structures make their appearance.

Acknowledgement

We acknowledge the assistance of Jim Brisebois, Field Project Manager, who was essential to the carrying out of the field examinations.

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FLUORIDE BRIEF

Enflurane (CHF_2OCF_2CHClF) undergoes oxidative dehalogenation in the liver to form difluoromethoxydifluoroacetic acid ($CHF_2OCF_2CO_2H$) together with chloride ion and renally toxic amounts of fluoride ion. When the C-H bond in the -CHClF group of enflurane or in the -CHCL- group of isoflurane ($CHF_2OCHClCF_3$) is replaced with the more resistant C-Cl bond, almost no fluoride ion is released from either anesthetic in rat liver microsomes. These results suggest a way to design safer, less toxic fluorinated anesthetics.

Burke, T.R., Jr., Branchflower, R.V., Lees, D.E., and Pohl, L.R.: Mechanism of Defluorination of Enflurane. Drug Metab. Dispos. 9: 19-24, 1980.

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A.W.B.

FLUOROSIS: GEOGRAPHICAL PATHOLOGY AND SOME EXPERIMENTAL FINDINGS

by

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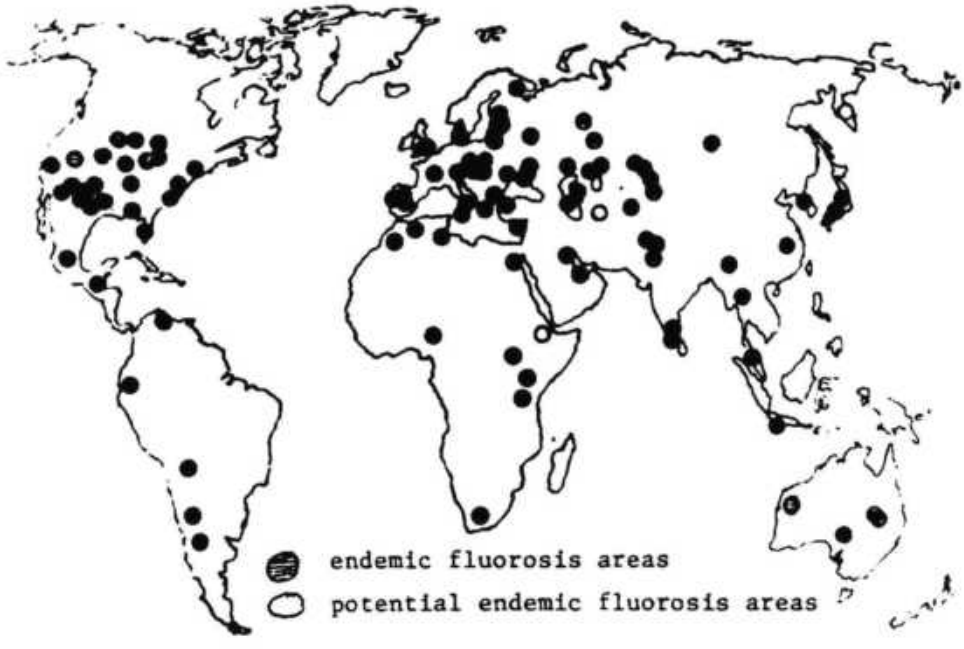
Endemic fluorosis is related to a high concentration of fluoride in water. Such springs are found in regions of disintegrating granite, in regions of former or current volcanic activity and in natural phosphate zones. A certain role in the development of endemic fluorosis is also attributable to the consumption of food grown on soils rich in fluoride.

The actual prevalence of endemic and industrial fluorosis is unknown. However a search of the relevant literature covering 15 years allows one to conclude that not less than 20 million people in the world are affected by this disease. Areas of endemic fluorosis occur in all inhabited continents (Fig. 1). They are being studied most extensively in North

Figure 1

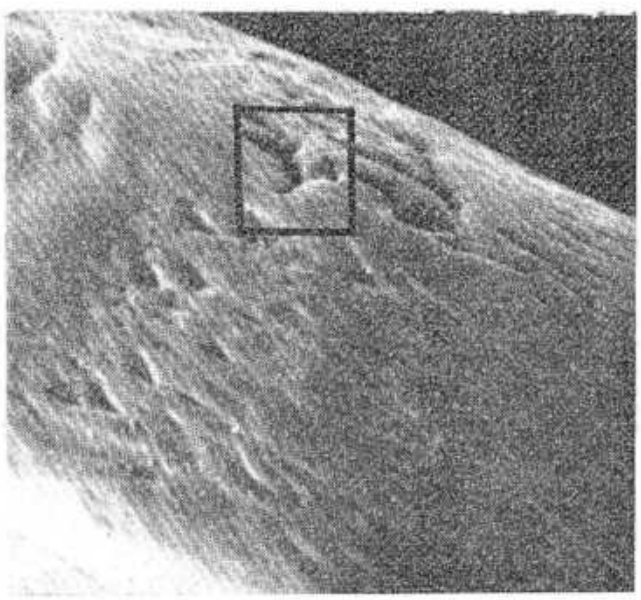
Geographical Distribution of Endemic Fluorosis Throughout the World



From the Institute of Human Morphology, Tsyurupa Street 3, 117418 Moscow, USSR. Read at the 11th I.S.F.R. Conference, Dresden, GDR, April 8-10,1981.

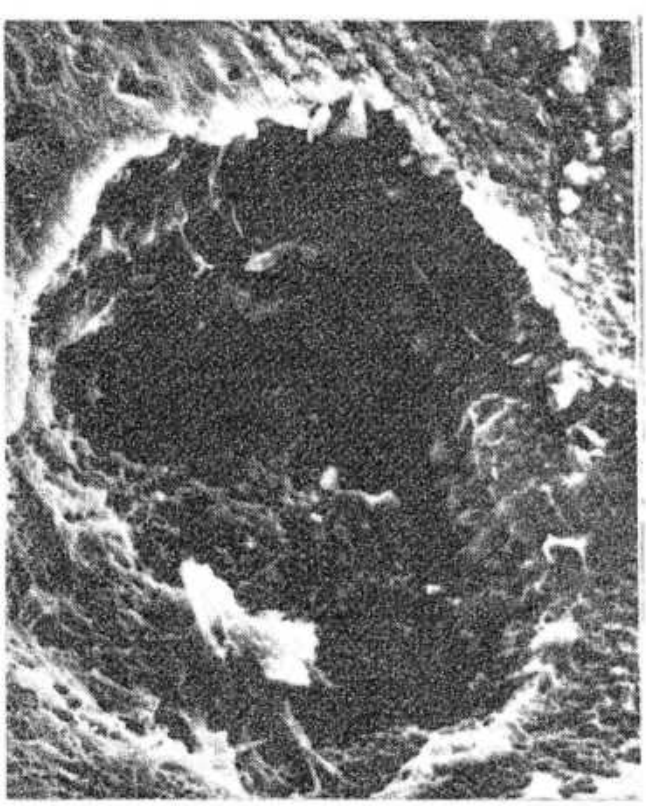
Figure 2

Enamel Changes of Rat Incisors in Experimental Fluorosis



a. Enamel showing numerous fissures and erosions (x40).

b. Same (x400).



c. Deep erosion of enamel(x800).

d. Eroded enamel surface "cobblestone road" (x210).

America (USA, Canada), Europe and those Asiatic countries where the problem is widespread as, for example in India. In the last-mentioned, the zone of fluorosis is designated "fluorosis belt."

In the Northern European part of the Soviet Union, in Siberia and in the Far East, the drinking water is low in fluoride. The vast majority of the world population consumes water low in fluoride (less than 0.5 ppm).

An extensive survey of an area of endemic fluorosis in the North Kazakhstan region of the Soviet Union revealed that the fluoride content of rocks was twice as high as that in clay. The concentration of fluoride in lake water was as high as 11 ppm and in drinking water, 4 ppm. The maximal concentration of fluoride in milk was 0.5 ppm. In locally grown cabbage, the fluoride content was up to 3 ppm. The maximal concentration of fluoride in human blood was 0.62 ppm, in the teeth, 776 ppm. In hair the maximal level is 72 ppm which is 10 times lower than in a nonendemic area.

In children born in the endemic area, consuming water with 4 ppm fluoride, dental fluorosis occurred in 91.8%, and dental caries in 40.7%. In children who came to the endemic area after birth, dental fluorosis was recorded in 47.2% and caries in 55.8%. Adults were affected by fluorosis in 46.8%, by caries in 72.4% and by a combination of both (fluorosis and caries) in 31.4%.

Experiments on white rats receiving daily 12 mg of sodium fluoride per kilogram body weight revealed specific changes of the incisor enamel pigmentation. The scanning electron microscopic study of the incisors showed pronounced alteration of the enamel in the form of irregular structure of enamel prisms and the presence of fissures and erosions. The normal prismatic structure of the enamel was replaced by a globular structure and the surface of enamel resembled a "cobblestone road" (Fig. 2).

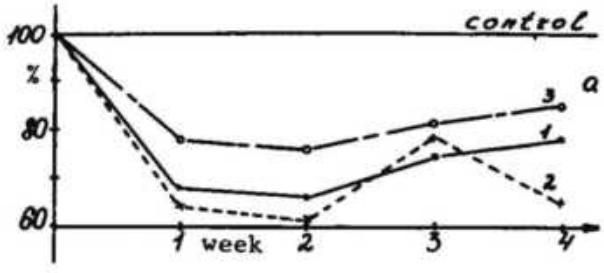
Next we studied the dynamics of RNA and protein synthesis in some organs of CBA-mice. The animals were decapitated 1, 2, 3, and 4 weeks after daily subcutaneous injections of 12 micrograms of sodium fluoride per gram of bodyweight. One hour before slaughter, the animals received intraperitonealy ^{3}H-uridine and ^{3}H-leucine. The scintillation was performed after dissolving the pieces of tissues in concentrated formic acid and the data obtained were processed on a computer.

The studied organs were classified into three groups according to the intensity of rapidly tracing RNA synthesis. In testicles and in different parts of the brain no significant changes of the RNA metabolism were revealed. In the ileum and rectum the synthetic activity broadly varied in time. In such organs as the heart, liver, and knee joint a marked decrease of RNA transcriptions was recorded as early as during the first week of intoxication (Fig. 3a). In adrenals, in the spleen and in the muscles, a marked decrease in isotope insertion was revealed at the first stages of the experiment. Later, in spite of continuing intoxication (Fig. 3b), it was replaced by increased synthetic activity almost to the control level. In the lungs, kidneys, pancreas, and duo-

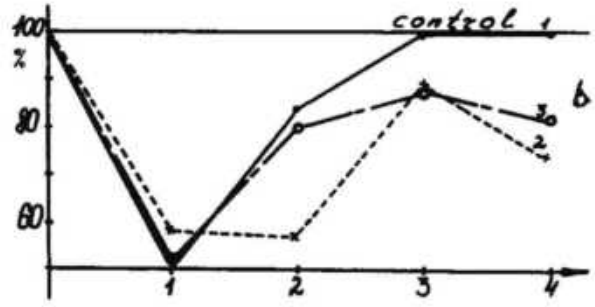
denum, a gradual decrease of RNA synthesis took place which reached its maximum at the termination of the experiment (Fig. 3c).

Figure 3

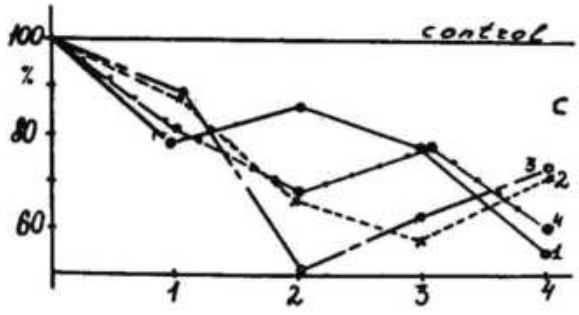
Decrease of RNA Synthesis in Mouse Organs During NaF Intoxication (% of control)



a. 1-heart, 2-liver, 3-tissues of knee joint.



b. 1-muscle tissue, 2-adrenal gland, 3-spleen.



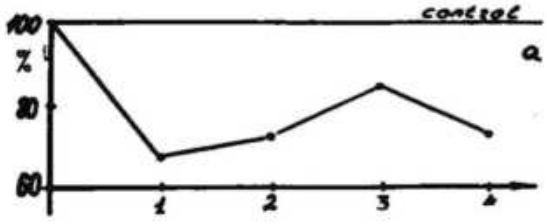
c. 1-lung, 2-kidney, 3-pancreas, 4-duodenum.

Fluoride intoxication had a different effect also on protein synthesis which accounted for a classification of the mouse organs into three groups. In different parts of the brain, in testicles, and in the ileum, the fluctuations in the isotope did not differ significantly from the control values. In the spleen, adrenals, liver and duodenum pronounced fluctuations of protein biosynthesis were directly correlated with the increase in fluoride levels. However in most of the other organs, the development of fluorosis was accompanied by a marked decrease of the protein synthesis in comparison with the control level. Whereas this decrease became manifest in the tissues of the knee joint during the first weeks of the experiment (Fig. 4a), in muscle tissue and in the colon inhibition of protein synthesis occurred only after 3 - 4 weeks of fluoride administration (Fig. 4b). In the kidney and the pancreas,

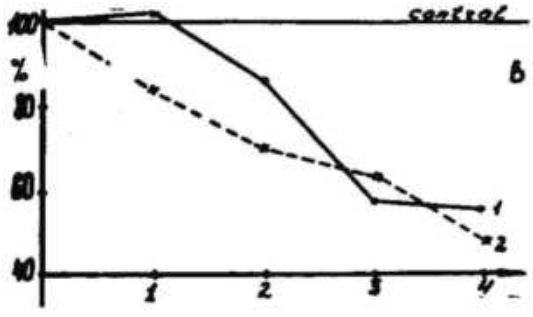
the inhibition of the precursor insertion in the beginning of the experiment was replaced later by an increase of the synthetic activity almost to the control level. In the heart and the lungs, the intensity of protein synthesis decreased in the middle of the experiment (Fig. 4c, d).

Figure 4

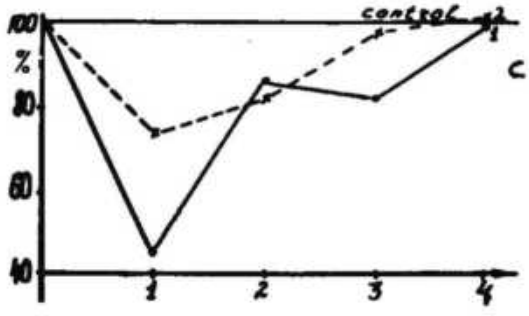
Inhibition of Protein Synthesis During NaF Intoxication



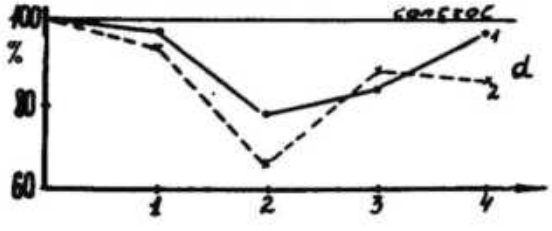
a. knee joint



b. 1-muscle, 2-rectum



c. 1-kidney, 2-pancreas



d. 1-heart, 2-lung

The depression of protein synthesis is probably related to a decrease in the RNA transcription, although a strict parallelism between these two processes in most of the studied organs was not established. The disturbance of the protein-synthetizing system in fluorosis also is attributable to a decrease in activity of a group of enzymes, catalyzing the key processes of cellular metabolism. The group includes enzymes catalyzing certain stages of biosynthesis of nucleotides and nucleic acids (5^1-nucleotidase, adenosidedesaminase), enzymes catalyzing certain stages of

aminoacid synthesis (glutaminesynthetase) and enzymes catalyzing certain stages of protein synthesis (methionin-activating enzyme of the liver).

Administration of sodium fluoride inactivates also certain enzymes of glycolysis and of the tricarboxilic acid cycle. The action of fluoride on these enzymes, which in most cases are magnesium-dependent, is related to production of weakly dissociating complexes of magnesium-fluoride and enzymes. Consequently the activity of these enzymes, in which magnesium is a cofactor, sharply declines.

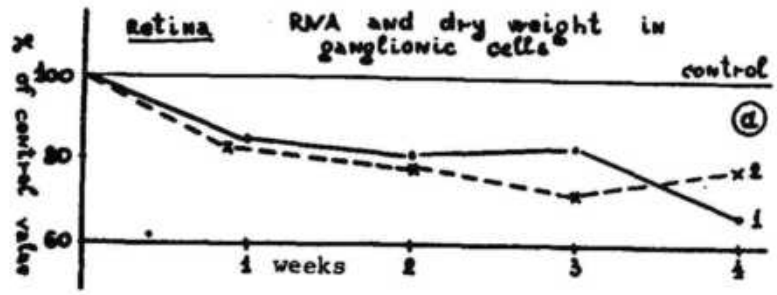
The degree to which fluoride complexes with magnesium ions can vary in different cell compartments causing local changes in the concentration of the activator. This in turn affects the function of the RNA-polimerase system, the free energy of ATP hydrolysis and the conformational structure of some types of RNA and proteins.

In the same series of experiments, the metabolic changes in certain organs of the mouse were studied by morphological methods. In the ganglion cells of the retina, the content of rRNA was estimated by the cytophotometric method and the dry weight was calculated by means of interpherometry. Administration of fluoride produced a progressive decline in the rRNA content and in the dry weight (Fig. 5a). By means of the use of ^{3}H-uridine and ^{3}H-leucine this event was found to be associated with a decrease of synthetic processes in such types of cells. A similar decrease in precursor insertion was revealed by autoradiography also in the other layers of the retina. The inhibition of RNA synthesis was more pronounced in the ganglion cells and the decrease of protein synthesis in perikaryons of the photoreceptors (Fig. 5b, c). During the course of the development of pathologic changes in the blood vessels of the retina, the penetration of fluoride through the hemato-ophthalmic barrier increases (3). This in turn intensifies the process of inhibition in accordance with the "cascade" principle. A hypothesis is suggested correlating the disturbance of the ascorbic acid metabolism and the development of fluoride retinopathy in fluorosis. It is known that fluoride can inhibit the action of certain enzymes in the epithelium of the ciliary body which are essential for the transport of the oxidized form of ascorbic acid into the eye. Fluoride also changes the metabolism of the ascorbic acid in the body. The "vicious circle" in the ascorbic acid metabolism by the feed-back mechanism increases necrotic processes in the retina. Thus this vitamin may be indicated as a prophylactic remedy in fluorosis.

A.P. Tarinsky (4) revealed a 2-3 fold increase of symptoms of oligospermia and azoospermia in male workers suffering from industrial fluorosis compared with healthy men of the same age. Tokar (5, 6) found an association between fluorosis and hypogonadism. These data made it necessary to study the changes of synthetic processes in the testes of mice in experimental fluorosis. A total scintillation method of probe computing on the counter revealed certain shifts of RNA and protein metabolism in this organ, but the data were not statistically significant. More informative, was the cytochemical investigation of certain cell types in the testes on the separate stages of spermatogenesis, particu-

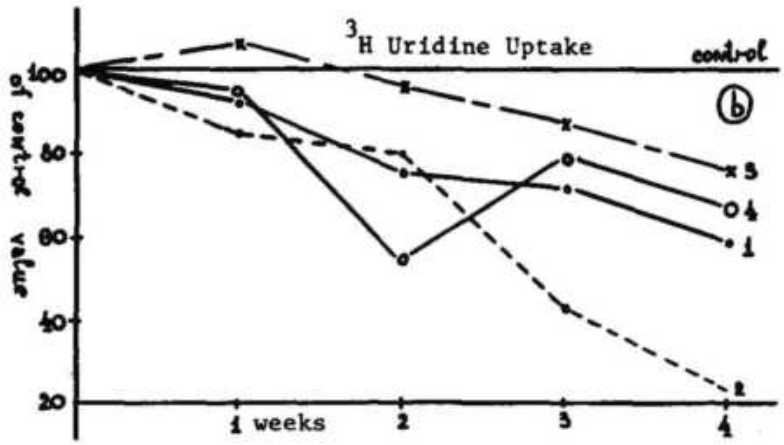
Figure 5

Metabolic Changes in Mice Retina During NaF Intoxication

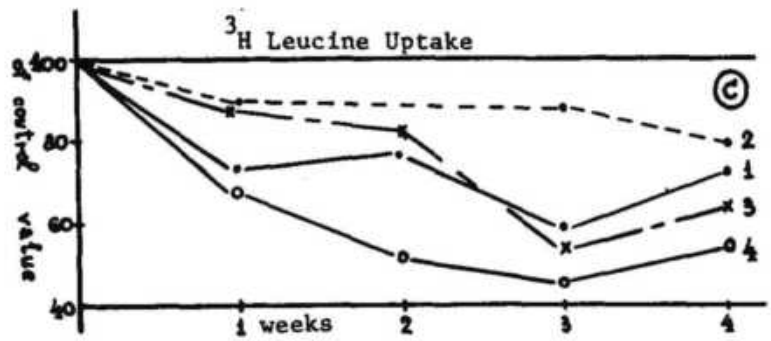


a. Progressive decrease of RNA content (1) and of dry weight (2) of ganglion cells.

RNA Synthesis in Different Layers of Retina



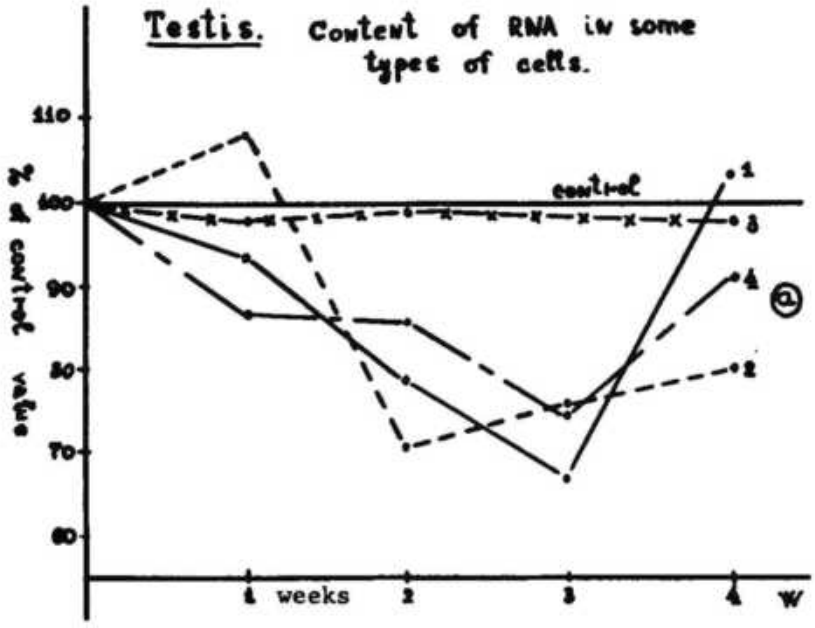
Protein Synthesis in Different Layers of Retina



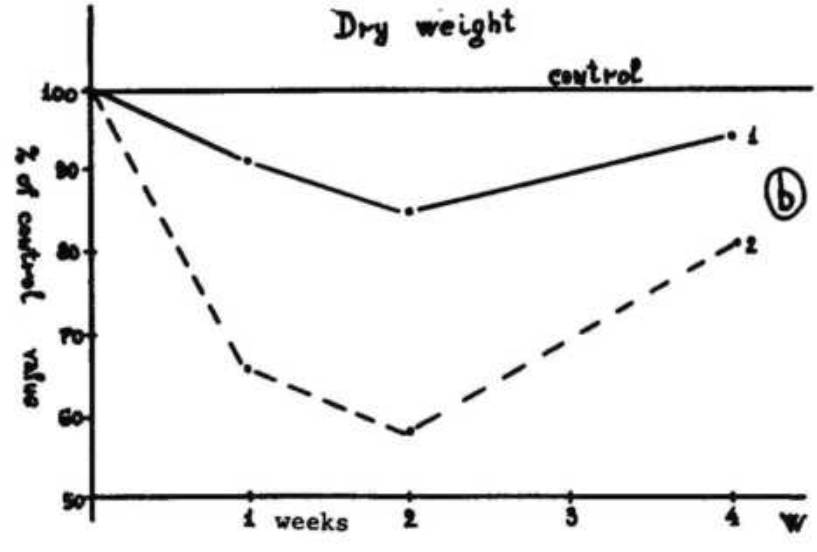
b. and c. 1-total uptake of H uridine in the retina, 2-ganglionary cells, 3-photoreceptor perikaryons, 4-bipolar perikaryons.

Figure 6

Metabolis Changes of RNA and Protein in Different Cells of Mice Testes During NaF Intoxication



a. 1-Leidig cells, 2-Sertoli cells, 3-spermatogones A, 4-spermatides.



b. Decrease of dry weight of spermatides (1) and of Leidig cells (2) on the 7th stage of spermatogenesis.

larly on the 7th stage. The cells of spermatogenic epithelium with fairly stable DNA content - Sertoli cells, spermatogones of A type, spermatides and also interstitial cells of Leidig - were studied. Between the second and third week after the beginning of the experiment there was a decrease of rRNA in the basal part of the Sertoli cells, in Leidig cells and in spermatides.

In the spermatogones this index was not significantly changed (Fig. 6a). During the course of hyperfluoridation a decrease of the dry weight appeared in Leidig cells and in spermatides (Fig. 6b). An adaptation of the cells to the toxic influence of fluoride is suggested since at the termination of the experiment a tendency to normalization of the rRNA content developed and the dry weight of the spermatides and the interstitial cells reached almost the control level. It is known that Sertoli cells constitute a part of the hematotesticular barrier and are actively attacked by fluoride ions, which is perhaps the cause of the decrease of rRNA in these cells. On the other hand, these cells play a trophic role for spermatides and probably supply them with rRNA. Consequently the decrease of the rRNA content and of the dry weight in spermatides is caused by the disturbance of the metabolism in Sertoli cells and therefore presents a secondary event.

The noted cytochemical alterations in Leidig cells and in the basal parts of Sertoli cells reflect the disturbances in the protein synthesizing system of these cells in fluorosis and to a certain degree explain the hormonal imbalance in this disease, since Leidig cells synthesize testosterone and Sertoli cells produce protein-binding androgens. The high resistance of the spermatogones to the influence of fluoride in comparison with Sertoli cells is difficult to explain since in most cases the unfavorable factors affect primarily the sperm cells.

Since the various stages of spermatogenesis are controlled by different hormones (e.g. testosterone controls the process of meiosis) (2) the present findings allow one to develop a more complete concept of the alteration of the germinative epithelium in fluorosis - not only through the disturbance of the functioning of the cell enzyme systems but also by the way of induction of hormonal imbalance in the body.

In conclusion we can state that fluorosis affects the whole organism with elective lesions of the teeth and the skeleton. The pathogenesis of fluorosis in many aspects remains unclear. Intrinsic mechanisms of the influence of fluoride on the body take place on the cellular and molecular levels as illustrated in this report by demonstration of certain cellular alterations and disturbances of systems in fluorosis from fluoride in water naturally and in experimental fluorosis.

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6. Tokar, V.I., Savchenko, O.N.: The Influence of Inorganic Fluorine Compounds of Functional Condition of the Hypophysis-Testes System. Problems of Endocrinology, 23:104-107, 1977.

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FLUORIDE CONTENT OF BONE SAMPLES IN DOGS

Untersuchungun zum Fluorgehalt in
Knochenproben von Hunden

by

Loeffler, K., Brehm, H., Oelschlager, W.
Schenkel, H. and Freyler, L.

(Abstracted from Kleintier Praxis 26:147-150, 1981)

Bone specimens from 36 dogs, ranging from 3 months to 11 years in age, were examined for their fluoride content. They were derived from bone splinters obtained in the treatment of fractures and from animals that were autopsied; an additional ten specimens were exostoses which had been removed surgically. In the ashed bone of the autopsied animals, the fluoride content ranged from 280 to 1700 ppm; in the exostoses of the ten animals, from 990 to 2670 ppm. Fluoride levels were higher in the older animals. Likewise the fluoride content of the spongiosa and of the exostoses was more elevated than that of compact bone.

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FLUORIDE IN DOGS

Fluorose Beim Hund

by

Loeffler, K., Brosi, C., Oelschlager, W., and Feyler, L.

(Abstracted from Kleintier Praxis 24:167-171, 1979)

Three female dogs at the workplace of a carpenter exhibited typical dental fluorosis in their deciduous teeth. A 1-1/2-year old female was born on the place, the other two, age 5-1/2 and 4 months, who showed only minor lesions had been in the possession of the carpenter for 7 and 8 weeks respectively. The deciduous teeth of one of the dogs contained between 230 and 770 ppm ashed fluoride. In two control animals, the fluoride content of the deciduous teeth ranged between 113 and 847 ppm. In 10 adult dogs used as controls, fluoride in the range of 412 to 1620 was found in bones with the highest levels in the older animals, and in the spongiosa as compared to the compact bone. The tibia of one of the dogs contained 1596 ppm of fluoride in the bone ash of the compact as compared to 2078 in the spongiosa.

The source of fluoride in the carpenter's establishment was sawdust in his work area which contained 24% fluorine, present in chromium trifluoride which was used to impregnate large roof beams. It was believed that the dogs' food became contaminated with the sawdust. Another source of fluoride intake in dogs is commercial dog food, samples of which contained between 4.5 to 80.4 ppm of fluoride dry weight.

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THE EFFECT OF FLUORIDE INTAKE ON THE TOTAL LIPID, CHOLESTEROL AND VITAMIN E LEVELS IN SERA AND LIVER OF GUINEA PIGS ON HIGH FAT DIET

by

Vatassery, G.T., Ophaug, R.H., and Singer L.
Minneapolis, Minnesota

(Abstracted from Life Sciences, 27:1961-1966, 1980)

The authors studied the total lipids, cholesterol and Vitamin E levels in guinea pigs which were raised on a diet containing 18% fat, as well as either 25 or 0 ppm fluoride in drinking water. The determinations were made after the animals were sacrificed at the end of 0, 3, 6, 9, and 13 weeks.

In the high fluoride groups after 9 to 13 weeks, the serum total lipids, cholesterol and alfa tocopherol levels increased. The total lipids and tocopherol also increased in the liver. After 13 weeks the rise in serum cholesterol levels in the high fluoride group compared to controls was significant. The total lipids in the sera of animals also increased significantly during the same period. There was a definite increase in serum alfa tocopherol of the high fluoride group between 9 and 13 weeks. Elevation of total lipids and tocopherol was observed in the liver of the animals.

The animals in the high fluoride group showed elevated serum ionic fluoride concentration between 0 and 3 weeks after which they decreased to a stable level. The results of this study indicate a specific effect of fluoride on the serum alpha tocopherol levels in the high fluoride animals and have confirmed earlier observations of increased toxicity of fluoride in association with high dietary fat.

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FLUORIDE-INDUCED DAMAGE TO DOMESTIC ANIMALS WITH SPECIAL REFERENCE TO FEEDING AND PRODUCTIVITY

Zum Problem Fluorschädigung bei landwirtschaftlichen Nutztieren unter besonderer Berücksichtigung von Fütterungsbedingungen und wirtschaftlicher Nutzung

by

W. Oelschlager, E. Moser, L. Feyler and K. Loeffler
Stuttgart und Rheinfelden

(Abstracted from Staub-Reinhalt. Luft 40:448-453,1980)

The authors comment on the recent directive by the DBR government regarding the top limit of fluoride permissible in feed for domestic animals. They consider fluoride assays in soft tissue, blood, milk, urine and bones less useful as indicators of fluoride damage to animals than the determination of fluoride levels of fodder. However, even at the maximal allowable concentration as established in 1974 by the U.S. National Research Council (NRC) of 40 ppm (as dry substance) in animal feed, heifers prior to their first pregnancy can contract dental fluorosis and minor periosteal hyperostoses.

For beef cattle, the NRC tolerance limit is 100 ppm in forage and for lambs, 60 ppm. These values are higher than for milking animals in view of the short stay of the animals on the farm (about 18 months). The NRC tolerance limit for pigs is 150 ppm and for poultry - which is more fluoride-resistant than other domestic animals - up to 300 ppm. These limits pertain to soluble fluoride such as sodium fluoride.

In addition to fluoride in hay, cattle consumes fluoride present in phosphate supplements, levels of which range from 9 to 2275 ppm. In contaminated areas, especially in late summer and fall, fluoride uptake in cattle increases considerably because the animals consume soil contaminated by fluoride along with their feed. Near highways, soil contains between 450 and 490 ppm fluoride. Fourteen samples of dust derived from four areas in the contaminated Ruhr Valley contained from 1250 to 7500 ppm fluoride. Near an aluminum smelter, dust contained 9.2% sodium fluoride and 13.3% less soluble aluminum fluoride.

Under these conditions fluoride levels in hard tissue which were considered "normal" twenty years ago, are now appreciably higher. Nowadays, ashed pelvic bones of cattle contain approximately 2500 ppm of fluoride.

The authors emphasize the importance of considering total fluoride intake, the state of the animals' productivity and the degree of atmospheric contamination when evaluating fluoride damage to cattle.

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FLUORIDE CYCLES IN AN ESTUARINE ECOSYSTEM

by

F. Murray
Newcastle, N.S.W.

(Abstracted from The Science of the Total Environment, 17:223-241,1981)

The author sampled the fluoride content of vegetation, soil and wildlife at Kooragang Island near the estuary of the Hunter River of New South Wales. Three fluoride-emitting facilities situated in that area release approximately 176 tons of fluoride yearly. The fluoride assays were compared with those of a relatively unpolluted area at Karuah.

In December 1976, 1188 µg F/g dry weight was recorded. The possibility of damage to grazing wildlife by consumption of contaminated foliage was investigated. In leaves of the plant termed Avicennia Marina, fluoride concentrations ranged from 1.8 to 1250 ppm fluoride. The highest values occurred in July at the height of the growing season. Grass on the island contained from 24.4 to 56.4 ppm (dry weight), the bones of house mice (Mus musculus) on the order of 2000 to 3800 ppm, the femors of five pelicans 3495 ppm, bones of deer from 610 to 6809 ppm. Among insects, spiders showed fluoride levels of 63.0 - 126, flies from 24 - 80, grasshoppers from 5 - 10 ppm.

The annual means of monthly values of ambient fluoride concentrations ranged from 0.16 - 0.34 $\mu g/m^3$ in Fern Bay and from 0.24 - 0.75 $\mu g/m^3$ in Stockton; approximately 1/3 were in gaseous form and 2/3 as particulate.

"Evidence is presented which indicates that fluorides are transmitted from vegetation to herbivores and on to carnivores along food chains. The possibility of fluoride cycling from vegetation to soils and from vegetation and soils to some elements of the wildlife is discussed."

****

FLUORIDE BRIEF

In order to determine whether or not organic fluoride in human plasma is due to industrial exposure to fluorochemicals, serum of 8 individuals residing in a rural Chinese area was assayed for organic fluorine and inorganic fluoride. The samples contained low levels (0.004-0.017 ppm) of organic fluorine as well as inorganic fluoride(0.044-0.076 ppm). The author concluded that there is no conclusive evidence that the prevalence of trace amounts of organic fluorine in human blood is primarily the result of industrial fluorochemicals.

Belisle, J.: Organic Fluorine in Human Serum: Natural Versus Industrial Sources. Science, 212:1509-1510, 1981.

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IN MEMORIUM

Clarence Conrad Gordon
July 26, 1928 - July 11, 1981

During his all-too-brief lifetime Clancy Gordon had gained worldwide repute, notably as coordinator of the Environmental Studies Laboratory at the University of Montana's Botany Department where extensive studies were conducted on the phytotoxic aspects of environmental fluoride.

I first met Clancy Gordon in 1968, when he presented a paper at a meeting convened by Don Chant, Head of the University of Toronto's Zoology Department. At that time, the topic of concern was fluoride pollution in the Port Maitland region of Ontario. In his presentation Clancy came across as knowledgeable, forthright, and with just the right nuance of leprechaun-ish Irish humor. Whenever he wanted to emphasize a particular point, he had a very engaging way of resorting to a touch of Irish brogue and this added to the uniqueness of his phraseology.

Our next meeting was at a 1976 symposium organized by the American Chemical Society in San Francisco where both of us were speakers. Aside from the excellence of the symposium, there were attractions such as the cable cars and Fisherman's Wharf in that beautiful Bay City.

One of Clancy's last major projects was in connection with fluoride pollution on Cornwall Island, situated midway between Canada and the U.S. on the St. Lawrence River. One of his major concerns was whether the U.S. Customs officials would allow him to re-enter the U.S.A. with his collection of vegetation samples, some of which could be mistaken for narcotics.

Our last meeting was on June 23, 1980, when we both attended a meeting at New York City's Mount Sinai Hospital. After the meeting, he and I strolled along the streets of Manhattan. I could tell that he was preoccupied about something. Finally, he told me that he had recently been told that he had cancer; moreover, it was incurable, inoperable, and terminal. To my question as to what life expectancy he had been given, his reply was "about one year." As things turned out, this was a precise estimate; he lived only 2 weeks longer than a full year. And yet, Clancy

was undaunted and maintained his perspective and his sense-of-humor. He had decided to take a sabbatical of several months in Europe, and did this during the Fall of 1980. His tremendous capacity for work, his high ideals and dedication to principles of respect for the environment earned him the gratitude of friends and professional associates throughout the Nation.

He will be missed, not only by his family and students, but also by all his colleagues who respect the truth in science. The many farmers he helped will miss him, as will the St. Regis Mohawks. He was most certainly "a white man who spoke with a straight tongue."

John Marier
Ottawa, Canada

The following articles on fluoride were authored or co-authored by Prof. Gordon:

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