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FLUORIDE
Quarterly Reports

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EDITORIAL

Takamori's Research

This issue presents the second installment of abstracts from the articles by the late Professor Tokio Takamori and his school at Tokushima University, Japan, which appeared during the years of 1950 to 1957. Much of this research pertained to massive doses of fluoride (up to the 500 ppm range). Nevertheless, it constitutes a basic guide to the student of fluoride toxicology.

Some of Takamori's findings have not as yet been followed up by other investigators. For instance, the observation of myocardial damage in skeletal fluorosis as well as in children with dental fluorosis has received little attention by other clinicians. Takamori's teams supported their clinical data by experiments on rabbits. Although the large doses of sodium fluoride used in these experiments do not reflect conditions encountered in clinical medicine, a careful reappraisal of Takamori's studies might stimulate further research.

Kawahara's system for classifying mottled enamel based on the degree of discoloration, streak formation and pitting of enamel permits a succinct description of the kind and severity of existing mottling and a differentiation of fluoride mottling from non-fluoride enamel defects.

Like subsequent investigators, Takamori et al. observed a direct correlation between the degree of mottling and the level of fluoride in drinking water. On the other hand, they found that the extent and severity of skeletal fluorosis were not related to the fluoride content of water supplies.

With low fluoride intake (up to 0.5 ppm in drinking water) Takamori's team found no impairment of skeletal growth but children with extensive dental fluorosis (P4 and P5 of Kawahara's classification) from fluoride in water (up to 3.3 ppm) showed a decline in growth, a slight anemia, a shift to the left in the distribution of leucocytes and toxic granulation of white blood cells.

In acute fluoride intoxication due to large doses (250 mg/kg NaF) glycogen and phosphorylase values in the liver and skeletal muscle were below normal limits whereas those in the heart showed no essential changes.

An increase in alkaline phosphatase seemed to be an indicator of fluoride intoxication, but small doses of fluoride failed to induce a significant change in the activity of this enzyme.

A macrocytic hyperchromatic anemia and a rise in reticulocytes occurred following massive doses (10 to 50 mg/kg) of sodium fluoride. This condition was reversible after the termination of the experiment.

Large doses of NaF (0.05 to 0.15 mg per gram of body weight) inhibited recovery from muscle fatigue in the gastrocnemius muscle of the frog. Previous administration of magnesium sulfate mitigated this action.
The kidney showed only minor changes in rabbits intoxicated with low doses of fluoride. However, when the animals were given large doses of the order of 10 to 50 mg/kg fluoride, extensive damage was done not only to the filtering system, but also to the glomeruli. The kidney function was greatly impaired.

A notable feature in the publications of Takamori and his group is their attempt to relate the degree of damage not only to the doses of fluoride, but also to the period of time which had elapsed following its administration. This approach renders their research particularly valuable today, when fluoride intake throughout a person's lifetime is much higher than it was during the early fifties.

THE FOURTH ANNUAL I.S. F.R. CONFERENCE

The Fourth Annual Conference of the International Society for Fluoride Research was held at The Hague, Holland, October 24th to 27th. The wide variety of subjects by prominent scientists from thirteen countries, the excellence of the papers and discussions made this conference the most outstanding one since the inception of The Society. Most European countries, as well as India and Israel were represented at the conference with predominance of German and U.S.A. participants.

A brief evening session on October 24th was devoted to the significance of fluoride as an air pollutant. The same subject was further expanded the following morning. A vivid description of the effect of airborne fluorides on livestock following the 1970 eruption of the volcano Hekla in Iceland was the high point of the opening session. The topic of fluoride as an air pollutant was rounded out by papers on the prevention of industrial fluoride emissions and of fluorosis in cattle as well as on the assessment of atmospheric fluorides.

Among botanical subjects, papers pertaining to the mechanism of structural changes in plants damaged by fluoride and to the distinction between leaf damage by fluoride and that from other airborne pollutants were presented. A scientist from the U.S. Dept. of Agriculture in Beltsville Maryland elucidated the effect of fluoride on growth retardation and aging of plants and on RNA contents of leaves.

Leaders in the new area of research known as liquid breathing participated in a highly stimulating symposium composed of three papers: A fluorocarbon of the tributylamine series carries oxygen into organs and removes carbon dioxide. The research on this subject centers about four areas: 1. Oxygenation of blood outside of the body; 2. Perfusion of isolated organs; 3. Artificial blood and 4. Breathing in a liquid medium (rather than in air).
A team of Swiss scientists presented data on fluoride's action upon the content of the cow's rumen, determined by studying rumen fermentation, rumen flora and production of volatile fatty acids. The diagnostic aspects of acute fluoroacetamide poisoning in sheep, particularly with reference to serum dehydrogenase activity and to citric acid formation, were presented.

Another series of papers dealt with human fluorosis encountered in industry and in natural fluoride areas of India. Reports on histological changes following NaF therapy for osteoporosis, on biochemical data from fluorotic individuals in India, on calcium balance studies, on the development of osteoporosis in natural fluoride regions followed. The activity of the parathyroid glands in hydrofluorosis was discussed.

Other papers pertaining to the effect of fluoride on humans, pointed to the possible relationship of fluoride to certain kinds of arthritis and to calcification of seminal vessels. New evidence was presented indicating that Chizzola Maculae, skin lesions encountered near Italian aluminum factories, are due to fluoride.

Statistical data pointing to a direct relationship between the incidence of lung cancer and the magnitude of atmospheric fluoride emissions from steel plants were accompanied by a large array of material collected from that area. One paper presented data on hepatitis and its fatal outcome following anesthesia by the fluorocarbon halothane.

Two papers reported statistical data on the effect of 17 years of fluoridation in the experimental Dutch city of Tiel.

All presentations will be published in FLUORIDE beginning with the April 1972 issue. If feasible, they will be accompanied by the most pertinent features of the discussions.

The Fifth Conference will be held in Oxford, England, in spring 1973 rather than in October when it is difficult for faculty members to absent themselves from their universities, March 19 to 22, 1973 has been selected.
EXPERIMENTAL STUDY OF THE TOXICITY OF A FLUOROALKENE DERIVATIVE, 
THE HEXAFLUORODICHLOROBUTENE (HFCB)

by

R. Truhaut, C. Boudene, J. M. Jouany and A. Bouant
Paris, France

SUMMARY: Pulmonary toxicity of hexafluorodichlorobutene (HFCB) 
has been experimentally studied on the rabbit according to a general 
technique proposed by the authors. Acute toxicity is induced by tra- 
echotomy and ventilation assistance. A diagram called "Physiogram" 
is established by recording EEG, EGG and arterial pressure. For chron- 
ic studies, a "mask-cage" is used as a means of administration of the 
toxic atmospheres; EEG is then recorded.

The mode of administration appears to be very important. Delay be- 
tween the absorption and the first symptoms is a main feature of in- 
toxication. Primarily, the first symptoms are extensive pulmonary in- 
volvement with necrosis of the tissues which precede cardiovascular 
and central nervous system changes.

HFCB can be assayed by a modified Fujiwara reaction or by ultra-vio- 
et spectrum to follow its localization in the body. One of its trans- 
formations leads to a compound which appears to be trifluoroacetic 
acid formed in pulmonary tissue, which would explain, in part, this 
powerful compound's necrotic action on tissues.

In the class of organic fluoroderivatives, especially fluoroalkenes, toxicity 
of Hexafluorodichlorobutene (HFCB) was brought into focus when accidents oc- 
curred during its manipulation for synthetic purposes in industry. The respiratory 
tract was the route of its penetration. The toxic action of this compound had al- 
ready been studied on animals following its administration through the respiratory 
tract. As anticipated, its toxicity seems to increase with the duration of exposure to its vapors.

In the rat, for instance, the LC$_{50}$ can be estimated at 100 ppm for one 
hour, 50 ppm for three hours' and 16 ppm for four hours' exposure (Table 1).

| Table 1 |

| Lethal Concentrations (LC$_{50}$) for HFCB According to Various Exposure Periods Determined on Different Animals |
|-----------------|-----------------|-----------------|
|                 | LC$_{50}$ (1 hr.) | LC$_{50}$ (3 hr.) | LC$_{50}$ (4 hr.)  |
| Rats            | 100 ppm          | 50 ppm           | 16 - 100 ppm       |
| Mice            | 61-75 ppm        |                 | 26 ppm             |
| Dogs            | 200 ppm          |                 | 182 ppm            |
| Monkeys         | 90 ppm           |                 |                   |
| Rhesus          | 54 ppm           |                 |                   |

From the Faculté de Pharmacie, Université de Paris, France.
Truhaut et al.

Fig. 1
UV Spectrum of HFCB

Fig. 2
Control Curve of Increasing Amounts of HFCB Dosed by Absorption in UV Light at 222 nm

FLUORIDE
the determination of such lethal concentrations leads to heterogenic results on a
single animal species, according to various experimental conditions. The LC₅₀ for
a four hour exposure has been estimated to be 16 ppm, 52 ppm and even 100 ppm fol-
lowing which death occurred after 4 to 14 days. Sometimes, however, minor patho-
logical changes occurred at 50 ppm. Furthermore, the results differed according
to the animal species (1, 2, 3).

Two facts must be pointed out:

1. Respiratory failure occurs regularly. Pulmonary irritation with or
without pneumonia, pulmonary congestion and sometimes acute pulmonary edema
are always found post-mortem.

2. Death occurs after a delay of 6 to 20 hours by respiratory failure,
even when high concentrations (840 ppm for 3 hours) are employed. Concentrations
of 350 or 400 ppm are not always fatal to the rhesus. Should an animal survive as
long as 5 days following intoxication, there is a possibility of recovery.

Changes in the renal tubuli have been described in rats with concentrations
of 100 ppm for 4 hours as well as centrolobular necrosis in the liver of dogs (4, 5).

Method

We studied the pulmonary toxicity of this compound on the rabbit, on our
own, according to the general technique established to evaluate this kind of intol-
cation by Truhaut et al. in 1969 (J. Europ. Toxicol. 4:200-206). This technique is
composed of two parts:

1. Acute Toxicity: For the study of acute toxicity, intoxication is in-
duced by tracheotomy and ventilatory assistance and a "physiogram" is established.
As shown in figures 1 to 3, the physiogram is a diagram in a system of three coo-
dinates, obtained by simultaneous and repeated measurements of arterial pressure,
cardiac pulse and EEG frequency. One point in the physiogram corresponds to the
behavior of the animal at each time. Its displacement from the normal zone shows
immediately any abnormal variations of the measured constants and their evolution
throughout the experiment. Variations of the cardio-vascular system correspond
to vertical displacement of the point, variations of the central nervous system to
the horizontal (Fig. 4). A catheter introduced into the femoral artery allows blood
sampling in order to follow biological changes such as alkaline reserve, ions, trans-
aminases, phosphatases, etc.

2. Chronic Toxicity: Chronic toxicity cannot be studied by the above tech-
nique because of the slow recovery from the tracheotomy. Thus, a different means
of administering toxic atmosphere has been developed by the "mask cage". The
anterior part of a special cage is a small chamber in which the animal places its
head. A rubber membrane fits tightly enough to prevent exchange of air but not
tight enough to strangle the animal. The toxic atmosphere is distributed in small
volumes directly to the head of the animal (Fig. 3). The same method can be used
also in acute toxicity experiments and represents another route of administration
of polluted air. The EEG is recorded continuously and blood sampling is done.
Figure 5 shows variations in frequency and amplitude of the EEG.

**Fig. 3**

"Mask-Cage" for Repeated or Chronic Intoxications

Toxicological studies of a compound must also include its chemical characteristics and the dosage necessary for the control of atmospheric concentrations as well as for the study of its behavior in the body.

**Determination of HFCB**

a) Ultra-violet spectrum: Because of its double-bond, HFCB is absorbed in a particular region of the UV spectrum. The maximum absorption is quoted at 222 nm in alcoholic solutions; 5 micrograms can be detected (Fig. 1).

b) Fujiwara reaction: HFCB in contact with pyridine produces, within a few minutes, a brown color even at normal temperature. If aqueous KOH 10 M is added immediately after pyridine, by heating in a boiling water bath a pink-red color develops which indicates the positive pyridino-alkaline reaction of Fujiwara. Consequently, the following technique has been developed:

2 cm³ of water are added to 0.5 or 1 cm³ of an alcoholic solution of HFCB. After addition of 2 cm³ of pyridine and 4 cm³ of aqueous 10 M KOH, the mixture is placed for 3 minutes into a water bath at 60°C and then cooled in ice. The intensity of the pink color of the supernatant can be measured photometrically. To increase the sensitivity, 0.2 cm³ of formic benzidine (3 g of benzidine in 100 cm³ of 80% formic acid) can be added to 1 cm³ of the supernatant; after 30 minutes' contact, photometry can be done at 530 nm. According to the Beer-Lambert law, 5 micrograms can be detected (Fig. 2).

c) Determination in the atmosphere: Vapors of HFCB can be trapped in Ethanol 96 and the UV spectrum or the Fujiwara reaction can be applied; as a last possibility, the pyridine can be used to trap the vapors but prolonged contact between the compound and the solvent produces a brown discoloration which induces errors.

FLUORIDE
HFCB Toxicity

1 Pulmonary Toxicity of HFCB

a) Physiogram: When the animal is intoxicated during one hour and observed for the following three hours, no definite changes can be noted up to 500 ppm.

A concentration of 1950 ppm of the compound for one hour is required to produce a narcotic reaction which becomes evident during the first 10 minutes of the experiment. During the recovery period some hypotension occurs; the central nervous system (CNS) does not return to normal activity (Fig. 4).

Concentrations higher than 3600 ppm cause death within one to two hours of the beginning of the experiment; hypotension occurs first, EEG activity decreases subsequently. The intensity of hypotension seems to be directly related to the HFCB concentration.

Macroscopically, the lungs often appear barely altered; pulmonary edema occurs only from higher concentrations.

Biological changes are not noticeable: SGOT increases only from high levels of HFCB; SGPT remains normal; hyperkalemia and hyponatremia are associated with concentrations higher than 1000 ppm; the alkaline reserve decreases dramatically. Alkaline phosphatase activity increases irregularly. It does not reflect the degree of pulmonary edema (Table 2).

**TABLE 2**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Exposure time</th>
<th>500 ppm</th>
<th>200</th>
<th>100</th>
<th>200</th>
<th>200</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1 hour</td>
<td>1 hour</td>
<td>1 hour</td>
<td>30 minutes</td>
<td>15 minutes</td>
<td></td>
</tr>
<tr>
<td>Delayed Death</td>
<td>85 min, to 3 1/2 hours</td>
<td>12 hours</td>
<td>4 days</td>
<td>3 days</td>
<td>0</td>
<td></td>
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</table>

b) Mask-Cage: Death occurs from much lower concentrations (200 ppm) than with the previous technique but is considerably delayed indicative of the dangerous insidious toxicity of HFCB (Table 3). When animals survive longer than 8 days, the risk of accidents seems to be very small.

At autopsy, pulmonary edema is noted. The lung tissue often resembles liver tissue. When concentrations up to 1000 ppm are administered, fluid is found in the pericardium. At higher levels, hemorrhagic zones appear in the spleen with splenomegaly. The kidneys have a marbleized appearance.

At concentrations of 500 and 1000 ppm, neurological abnormalities develop namely degenerative changes of mature neurons which appear to be atrophic and sometimes show chromatolysis. These histological changes can be related to
PHYSIOGRAMS Obtained During and After Intoxication by HFCB at increasing Concentrations in Atmosphere
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<td>NO. 301</td>
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<th>Biochemical Data in HFCB Intoxication</th>
<th>SCOT</th>
<th>SGPT</th>
<th>SGOT</th>
<th>Transaminase</th>
<th>ALK Phosphatase</th>
<th>Sodium</th>
<th>Potassium</th>
<th>Alkaline Phosphate</th>
<th>Reserve Reserve</th>
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**TABLE 3**
Fig. 5

Modifications of EEG, in Frequency and Amplitude During and After Intoxications by HFCB

9500 ppm

1025 ppm

FLUORIDE
pains in the radicular lumbo-sacral area which are encountered by physicians (6).

During intoxication, EEG amplitude increases as the concentration of HFCB rises; a decrease in frequency occurs but is not related invariably to the increased amplitude (Fig. 5).

II Metabolism of HFCB

After a 1 hour exposure by tracheotomy at a concentration of 1000 ppm (0.667 mg of HFCB), the content of the rabbits' expired air showed that roughly 50% of the compound remained in the body. Afterwards, only minimal amounts of HFCB are eliminated through the lungs.

It is well known that this compound can evolve into trifluoroacetic acid (TFA, CF₃-COOH). We have tried unsuccessfully to assay this acid in the lungs of intoxicated animals. Its presence could easily explain the severity and the delayed occurrence of the necrotic changes induced by HFCB.

Lungs of rabbits intoxicated by a concentration of 3000 ppm of HFCB were homogenized in isotonic NaCl solutions. After acidifications of the medium to pH 1 to 1.5, two methods were employed to isolate TFA:

a) Extraction by steam vapors followed by extraction of the distillate by ether in acidic medium.

b) Direct extraction of the tissue homogenate by ether in acidic medium, with occasional cleaning by charcoal.

Since TFA fails to give the Fujiwara reaction, Chelnokova's (7) method of identification was used. A thin layer on chromatogram silica-gel is developed with the mixture n-butanol-NH₄OH water (5 to 2 to 3). The plates are identified by bromocresol green. Within half an hour pink spots of Rf 0.40 can be obtained. Figure 6 shows that minimal quantities of TFA are recovered by either means of extraction, accompanied by other acids. The Rf of one of them corresponds to that existing in an old TFA. The composition of this peculiar acid is unknown.

Conclusions

The rabbit, like other animals, seems to be very sensitive to the toxic action of HFCB vapors. Because of the great discrepancies in the results obtained after intoxication by tracheotomy (direct administration into the lungs) and by mask (spontaneous ventilation of the animal), it appears that the way of administration is very important. Whereas in 1 hour exposures 1500 ppm are required to induce rapid death by tracheotomy, by the mask method 500 ppm are sufficient. The question arises whether this difference in the lethal dose is due to ventilatory assistance or to the combined effect of HFCB and pentobarbital.

The main feature of the intoxication is the delay between the absorption of the toxic agent and the appearance of the first symptom and death. This delay
may be for several hours or for several days. A 12 hour delay occurs frequently and is in agreement with clinical observations by physicians. Should no alarming symptoms appear for 8 days, survival is possible.

The most important manifestation is an extensive pulmonary involvement with "hepatisation" and necrosis of lung tissue. Pulmonary edema may or may not be observed while the animals are alive but it is always present at autopsy.

Hypotension occurs generally before the changes in the EEG occur. The animals' blood pressure rarely returns to normal levels during the recovery period. Aggravation of the hypotension is the rule.

SGOT rises frequently in relation to a severe acidosis. Hyponatremia and hyperkalemia occur regularly. Disturbances of liver function are rare or non-existent when low and medium concentrations of HFCB are used.

Pains in the lumbo-sacral area, which were described by physicians, can be related to the histological changes observed in neurons of the corresponding area of rabbits intoxicated with 1000 ppm of HFCB.

HFCB can be determined easily by the Fujiwara reaction or by UV spectrum with good sensitivity. After HFCB intoxication a compound, which appears to be trifluoroacetic acid, has been found in the pulmonary tissue. It explains the powerful irritant and necrotic effect of the halogen derivative and the delay between the administration of the poison and the onset of the disease. Trifluoroacetic acid is probably not the only metabolite, other acids of unknown formula have been found.

FLUORIDE
HFCB Toxicity

Bibliography


HISTOLOGY OF "CHIZZOLA MACULAE"

by

S. Steinegger
Bolzano, Italy

SUMMARY: The histological findings of "Chizzola Maculæ", skin lesions resembling traumatic bruises which occur near fluoride-emitting factories, are presented on patients from a polluted area in Bolzano. Pericapillary lymphocytic infiltration and proliferation of endothelial cells of capillaries were the principal pathological features. They were interpreted as a toxic inflammatory process.

During recent years several authors (1, 2) described skin lesions resembling traumatic suffusions which have occurred in epidemic proportion in Chizzola, a village of about 1000 inhabitants near Trentino in northern Italy and in the city of Bolzano. Both communities are situated in narrow valleys down-wind from aluminium factories. In Chizzola the lesions cleared up in 1935 after the factory had installed antipollution equipment. They recurred in 1964 when production of the factory tripled. In Bolzano, Steinegger (2) observed and described the same kind of skin eruption in June, 1967 for the first time. His patients were residing in the

From Ufficio Sanitario, Bolzano, Italy.
city of Bolzano (formerly Bozen) at the southern border of which the aluminum factory and three other fluoride emitting factories are located. Prior to 1967 the lesions had attracted little or no attention.

Morphologically, the lesions are round or oval in shape, not sharply demarcated. They have a reddish-brown color when they originate and change only slightly to bluish-brown prior to their disappearance. There is no break in the skin nor any other evidence of damage to the epidermis. The lesions clear up spontaneously after 5 to 7 days only to recur in other areas of the body, mainly on extremities. The afflicted individuals, mostly women and children, exhibited muscular pains, gastro-intestinal disorders and headaches. In some, the skin lesions were not associated with any other complaints.

Cavagna, et al., (3) assumed that permeability of capillary blood vessels were the principal pathological feature of the lesions, whereas Cecchioni and Waldbott (4) who observed the maculae in Canada and the U.S.A., failed to note extravasation of blood into pericapillary tissues.

In order to further elucidate the pathology of the lesions, skin biopsies were made on three children with characteristic maculae for histological studies, the findings of which are herewith presented.

Case 1, M. N., a 4 year old farmer's son had resided all his life in the same area about three kilometers down-wind of the aluminum factory. The area is surrounded on three sides by mountains approximately 1000 meters high. The family had been eating food grown in a field near the factory. The child was in good health until age 2 when the maculae occurred on the thigh, on the deltoid muscle and the forearm.

On microscopic examination (Fig. 1), the squamous epithelium of the epidermis appeared slightly thinned. The upper layers of the corium exhibited slight inflammatory changes around the capillary blood vessels. There was moderate proliferation of endothelial cells and of pericytes.

Case 2, P. K., a 4 year old farmer's son, had maculae on both arms and legs. The family resides about 200 meters south of the factory. Their vegetables were grown near their home. The child complained of muscular pains in arms and legs, particularly at night. Subsequent to May 1968, when antipollution equipment was installed in the factory, the maculae did not occur as frequently or as extensively as previously.

A microscopic examination of skin* biopsied on 7/12/67 from the anterior aspect of the right lower leg, revealed distinct proliferation of the endothelial cells of capillaries at the upper layer of the corium. There was also evidence of pericapillary inflammation with the presence of lymphocytes, histiocytes and pericytes. In distinction to case 1, the lesions were found at the central portion of the corium. There was no extravasation of the blood. A slight edema of the papillary bodies was noted.

*As reported by the Histopathology Institute, Univ. of Innsbruck, Austria

FLUORIDE
Thinning of epithelial layer, lymphocytic pericapillary inflammation.

Endothelial proliferation (pericapillary inflammation).

Skin Biopsy of Case 1

Histology of Maculae

Skin Biopsy of Case 2

Fig. 1

Fig. 2
Case 3, B. J., 2 1/2 year old son of a farmer had been living 1000 meters from the factory. The biopsy was taken on January 20, 1968. Most lesions were distributed on the legs. They decreased in number subsequent to 1968.

Histologically, inflammatory changes were noted on the surface of the cutis but also at the subcutis with epithelial proliferation and the presence of lymphocytes. It appears that the presence of cellular elements, mostly lymphocytes around the capillaries of the cutis and histocyte formation constitute the principal pathology.*

The absence of neutrophiles suggests that the inflammatory process may be of a toxic rather than infectious origin. There was no evidence of extravasation of blood as is also borne out by the gross appearance of the lesions, which do not show the greenish-blue color of suffusions.

Bibliography


*Reported by the Histopathology Institute, University of Innsbruck, Austria.
A VOLATILE FLUORIDE GENERATOR FOR FUMIGATING PLANTS

by

D. N. Rao and D. Pal
Varanasi, India

SUMMARY: A simple apparatus for generating volatile fluoride in controlled amounts is described. The apparatus is portable and is convenient for fumigating plants in greenhouses. A suitable dilution of HF or H₂SiF₆ is atomized and passed through a heating tube maintained at 150°C in order to obtain the gaseous fluoride.

Among the methods which are employed for exposing plants to fluorides, the simultaneous use of atomization and thermal action for producing volatile fluoride is most common (1-4). The equipment used for this purpose is generally bulky and not easily portable. Therefore some smaller outfit which could be conveniently used in the field is needed. The authors have described here a portable fluoride generating equipment which is suitable for fumigating plants at any desired location.

Assembly of the Apparatus

A 1000 ml polythene reservoir is mounted over an atomizer and a feeder cup containing a solution of hydrofluoric acid (HF) or hydrofluosilicic acid (H₂SiF₆), as a source of volatile fluoride (Fig. 1). A syphon tube, fitted with a flow regulator, connects the reservoir with the feeder cup. The atomizer consisting of a platinum capillary tube with an outlet of 3.5 mm in diameter, a compressed air nozzle and a basal tube, converts the F⁻ solution into a mist of fine droplets. The upper narrow end of the atomizer is connected with a heating tube in which the liquid droplets are converted into gas. The heating tube is made of copper and is 75 cm long and 1.0 cm wide. It has two right angle bends towards the outer end and two small copper tubes welded on its upper side. Teflon tubing can be used instead of copper but we prefer the latter because of its high thermal conduction property. The two welded tubes are partially filled with mercury to accommodate separately the stem of a temperature regulator and the bulb of a thermometer. The entire surface of the heating tube is covered with a thin asbestos sheet and is then overlaid with a coil of Nichrome wire. The length of the Nichrome wire is determined in order to obtain an intake of 1 amp at 220/230 V supply. The exposed surface of the Nichrome coil is insulated electrically and thermetically with asbestos and an overall metallic cover is provided to protect the heating tube assembly from weather effects. All the parts, except the heating tube, are made of hard plastic or polythene material and housed in a 40 x 30 x 15 cm wooden box (Fig. 2).

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Volatile - Fluoride Generating Unit

Fig. 1

Schematic Sketch

Fig. 2

Photograph of the Unit
Working of the Apparatus

The heating element is connected with the electric supply and the temperature on the regulator (thermostat) is set at 150°C with the aid of the thermometer. The flow regulator of the syphon tube is opened slowly to maintain a regular supply of the fluoride solution to the feeder cup. The compressed air nozzle is connected with a nitrogen or air cylinder and the pressure regulator valve is opened to deliver the gas at a pressure of 0.5 to 1 kg/sq cm. When atomization starts, fine droplets of fluoride solution pass into the heating tube but the heavier ones condense on the atomizer cover and the resulting liquid is brought back to the feeder cup through the tube at the base of the atomizer. The supply of the solution is so regulated that the level of the liquid in the feeder cup remains more or less constant due to the fresh solution coming from the reservoir and the solution returning from the atomizer. This arrangement helps in maintaining a constant concentration of the atomizing solution. The bends in the heating tube cause a partial restriction on the direct flow of the atomized droplets and thus the contents pass through the tube more slowly. They facilitate a complete conversion of droplets into gas despite the fact that the heating tube is reduced in length. The compressed air entering into the atomizer unit helps to keep its nozzle unobstructed. The gas content of the heating tube is injected into the air stream entering a plant exposure chamber.

The concentration of HF or $\text{H}_2\text{SiF}_6$ solution is determined on the basis of utilization of the solution by the atomizer and the volume of the air diluting the gas per minute. Once the proportion of the amount of solution (as pure acid) being mixed with the unit volume of air is established, any gas concentration in the chamber may be obtained by diluting the solution accordingly.

In a more recent method (5) hydrogen fluoride is produced by passing a measured stream of air through a bottle of dilute hydrofluoric acid of definite concentration and at a fixed temperature. The air stream charged with hydrogen fluoride is then added to a large air volume passing through the fumigation chamber. Though the dispensing of hydrogen fluoride is greatly facilitated in this method, the temperature control of the HF solution is a problem under tropical conditions. During summer months when outside temperatures at Varanasi are in the range of 100-120°F, an appreciable amount of water is evaporated into the system. Under this situation humidity in the fumigation chamber increases which ultimately affects the rate of fluoride absorption by plants. We feel that in very hot and humid environments the method of atomizing is better than bubbling for generating volatile fluoride.

Acknowledgement

The authors wish to express their appreciation to Prof. R. Misra, Dr. K. C. Misra and Dr. G. N. Choudhury for their valuable suggestions and to the Indian Council of Agricultural Research, New Delhi, for financial support.
DENTAL FLUOROSIS IN AN INDIAN AREA WITH HIGH NATURAL FLUORIDE CONTENT OF WATER

by

A. H. Siddiqui
Hyderabad, India

SUMMARY: Details of dental changes in 32 adults and 110 children in an area of endemic fluorosis (2.5 to 11.8 ppm) are described. The factors responsible for a severe grade of mottling and a low caries incidence in India are discussed.

Ingestion of water fluoride causes dental fluorosis which has been reported from many parts of India (1, 2, 3, 4). Susceptibility to mottled enamel is restricted to a sharply defined age group because fluoride is deposited in the crown of the permanent teeth during calcification. This period extends from infancy, when the central incisors may be affected, until age 16 when calcification of the last teeth, the third molars, has been completed*. Both sexes are adversely affected with equal frequency. A well balanced diet does not prevent mottling, although a high calcium intake lessens the incidence of mottling (5).

*Formerly of Osmania Medical College and Osmania General Hospital, Hyderabad, India. Current address: P.O. Box 377, MECCA, Saudi Arabia.
The villages of Kamaguda and Yellareddyguda in Nalgonda District of Andhra Pradesh were selected for investigation. These villages lie close to each other and are known areas of endemic fluorosis (6). The climate is hot and the temperature in the shade reaches as high as 115°F (46.1°C) in summer. The population of Kamaguda and Yellareddyguda is 95 and 1100 respectively, the former has two and the latter eighty four wells. The concentration of fluoride varied between 2.5 and 11.8 ppm.

Adults: 32 adults admitted to the Osmania General Hospital for detailed neurological investigations were examined dentally. Of these, 15 had mottled enamel (dental fluorosis), three were edentulous, the remaining 14, who were more than 16 years of age when they migrated from non-endemic areas, had normal teeth.

Children: Dental examination was made of 110 children aged 3 to 14 years. The following classification was adopted in grading mottled enamel (7).

Mild: White opacities or patches on the enamel; very faint yellow line across the enamel.

Moderate: A distinct brown stain.

Severe: Besides well-established brown stain, considerable pitting all over the enamel, sometimes with chipped-off edges.

The condition was largely confined to the permanent teeth although in two cases the deciduous teeth were affected, which points to placental and mammary transference of fluoride. The most commonly affected teeth were the premolars and the second molars. The surfaces subjected to attrition showed marked lesions. Some of the mottled teeth exhibited discrete or confluent pitting; they often appeared corroded. Mottling was rare in the age group 3 to 6 years, was evident in the age group 7 to 9 years, and was definite in the age group 9 to 14 years. The degree of mottling was directly related to the level of fluoride in water. With increasing concentration the effect was more pronounced, so that at 6 ppm the incidence of mottling was 100% (Fig. 1 and 2).

Frequent reference has been made in the literature to the possibility that fluoride may reduce the incidence of dental caries. The condition of the first molar was studied with particular attention because of the high rate of caries in this tooth. The co-existence of mottled enamel and caries in the same molar was noted in two cases only. On the whole, the incidence of dental caries was very low in areas of endemic fluorosis.

**Discussion**

Consumption of fluoride-contaminated water gives rise to dental fluorosis as has been reported from many parts of India (1, 2, 3, 4). The degree of mottling is related to the concentration of fluoride in water and to meteorological factors, especially temperature.
In the United States, manifest mottling is reported to be associated with 3 to 4 ppm fluoride, a level at which many workers in India have recorded cases of skeletal fluorosis. Venkateswarlu et al (4), and Siddiqui (6) found 0.9 to 1 ppm in drinking water to be associated with mottled enamel, the concentration considered optimal for domestic water supplies in Europe and in the United States for the prevention of dental caries. In India, hot weather not only increases water intake, but also increases the concentration of fluoride and leads to the ingestion of unusually high amounts of sediment. Lower temperatures and ingestion of small quantities of water are undoubtedly factors responsible for the absence of dental disfigurement in Europe and in the United States at levels of fluoride in water higher than those in India.
Dental Fluorosis in India

The incidence of dental caries is much lower in India than in the Western countries. Therefore, the existence of other factors which contribute to the low caries incidence should be recognized. Dental caries may be minimized by paying attention to adequate and proper dental care after every meal as well as to the type of food consumed.

Bibliography

A HISTOCHEMICAL STUDY ON ALKALINE PHOSPHATASE OF EPiphyseal CARTILAGE IN EXPERIMENTAL FLUOROSIS

Part I

CHANGES IN THE ALKALINE PHOSPHATASE OF EPiphyseal CARTILAGE OF GROWING NORMAL RATS

by

Kasaburo Abe
Tokushima, Japan

(Abstracted from Shikoku Acta Medica, 14:56-60, January 1959)

Many reports have dealt with the relation between ossification and alkaline phosphatase. However, no data are available on how the alkaline phosphatase is related to the age of the epiphyseal cartilage. The current study on growing albino rats constitutes a histochemical investigation of alkaline phosphatase in the epiphyseal cartilage related to differences in its age.

The following results were obtained (Fig. 1 to 4):

1) The zone of proliferation of the proximal epiphyses of long bones in growing albino rats 30 to 45 days old, showed no alkaline phosphatase activity. At the distal site of the zone, a trace of the enzyme was demonstrable, but no phosphatase activity was detected in the matrix.

2) In the zone of hypertrophy, an abundance of alkaline phosphatase activity was found. In the majority of the cells which were examined, the enzymatic reaction was detected in the cytoplasm, but some of the cells failed to take up the dye for the enzyme.

3) In the zone of provisional calcification, the enzyme was present only in the matrix.

4) In the nucleus of the proliferating cells, alkaline phosphatase activity decreased with advancing growth of the animal; no enzyme was detectable 150 days after birth.

5) The maximal amount of alkaline phosphatase appeared in the zone of hypertrophy in animals which were 50 to 60 days old. After 90 days, however, its content decreased to a considerable degree and after 150 days it diminished markedly.

6) From these experiments, it is evident that the alkaline phosphatase in epiphyseal cartilage decreases with the growth of the animal.

From the Department of Internal Medicine, School of Medicine, Tokushima University, Japan.
Alkaline Phosphatase in Growing Epiphyseal Cartilage

Fig. 1
Rat No. 3 - 30 Days Old (x 100)

Fig. 2
Rat No. 14 - 60 Days Old, 30 Days on Standard Diet (x 100)

Columnar cell layer
Foam cell layer
Layer of beginning calcification

Fig. 3
Rat No. 16 - 60 Days Old, 30 Days on Vitamin A Deficient Diet (x 100)

Fig. 4
Rat No. 23 - 150 Days Old, 120 Days on Standard Diet (x 100)
PART II

CHANGES IN THE ALKALINE PHOSPHATASE OF EPiphyseAL CARTILAGE OF GROWING RATS FED VARIED LEVELs OF FLUORINE

by

Kasaburo Abe
Tokushima, Japan

(Abstracted from Shikoku Acta Medica 14: 61-70, January 1959)

In previous reports from our department, morphological and histological changes in bones, particularly in epiphyseal cartilage in experimental fluorosis, have been described. However, no histochemical studies have been recorded.

Growing albino rats of the same sex and same litter were divided into two groups. One group was fed a standard diet, the other received the standard diet supplemented by varying amounts of fluoride. Changes in the alkaline phosphatase in the upper epiphyseal cartilage of the tibia were recorded after 30 days, 60 days and 120 days.

The results are summarized as follows:

1) Administration of large amounts of fluoride of the order of 100 ppm and 375 ppm caused a disturbance of bone growth and an increase in the alkaline phosphatase activity of the epiphyseal cartilage,

2) Smaller doses of fluoride (5 ppm) accelerated bone growth, but the alkaline phosphatase of the epiphyseal cartilage remained unchanged,

3) There is evidence that the increase in the alkaline phosphatase of the epiphyseal cartilage following administration of large doses of fluoride represents a compensatory mechanism in the delayed ossification by fluorosis.

From the Department of Internal Medicine, School of Medicine, Tokushima University, Japan.
Alkaline Phosphatase in Epiphyseal Cartilage

Fig. 1
Rat No. 26; 5 ppm Fluoride
30 Days

Fig. 2
Rat No. 54; 5 ppm Fluoride - 60 Days

Fig. 3
Rat No. 85; 5 ppm Fluoride - 120 Days

Fig. 4
Rat No. 27; 100 ppm Fluoride - 30 Days

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THE EFFECT OF FLUORIDE UPON THE SERUM-PHOSPHATASE

by

Setsuo Tanaka
Tokushima, Japan


The morphological and functional changes observed in bones, bone-marrow, heart, kidneys, liver, spleen and in the blood-picture in experimental chronic fluorosis have been thoroughly studied in our department and by others. It is well known that fluoride is an enzyme poison. It is also established that phosphatase is involved in the ossification mechanism, especially the calcification of bone. Significant changes were found in plasma alkaline phosphatase in rickets and in other bone diseases. Phillips and Smith and Lantz reported briefly on plasma alkaline phosphatase in fluorosis. Their results differed from those obtained by the author.

The author studied the effect of fluoride on serum phosphatase. To date, no studies on alkaline phosphatase in blood plasma in fluorosis have been carried out.

Chronic fluorosis was experimentally induced in three to four-month old white uterine rabbits weighing 1300 to 1600 grams. Divided into four groups they received 1, 3, 10, 30 and 50 miligrams of sodium fluoride per 1 kg of body weight, by mouth up to 115 days. In two other groups, acute fluorosis was induced by injecting 70 to 150 mg/kg of sodium fluoride into the muscles of their backs until they died. Quantitative analysis for alkaline phosphatase was carried out according to Bodansky's method.

Results

1) The control rabbits showed a gradual decrease in serum alkaline phosphatase in accordance with their physiological growth.

2) In the acute fluorosis groups, a gradual increase in the serum alkaline phosphatase activity occurred.

3) In the chronic fluorosis group given 50 mg NaF/kg, the serum alkaline phosphatase gradually increased in relation to the duration of the experiment. In the rabbits which received 30 mg/kg, the enzyme increased gradually until the 24th day after which it began to decline. However, the values were higher than those of the control rabbits (Fig. 1).

From the Department of Internal Medicine, School of Medicine, Tokushima University, Japan.
Fig. 1

Mean Alkaline Phosphatase Activity in Rabbits at Levels of 1, 3, 10, 30, and 50 mg/kg NaF

Fig. 2

Mean Alkaline Phosphatase Activity in Rabbits Given 1, 3 mg/kg NaF
In the group given 1.3 mg/kg, a decrease of alkaline phosphatase activity occurred after 115 days compared with the control group (Fig. 2).

4) The serum acid phosphatase showed no significant change in the fluorotic animals.

ODONTOLOGICAL OBSERVATIONS ON FLUOROSIS IN MT. ASO-VOLCANO DISTRICT

by

Haruyuki Kawahara
Tokushima, Japan

(Abstracted from Shikoku Acta Medica 4:32-38, 1953)

Our current survey showed that the water in artesian wells in villages of Imamachi, Kuronagare and Narikawa of the Mt. Aso district contained 0.9 to 3.5 ppm of fluoride. A relationship between the fluoride content of water and the incidence of mottled teeth was established in this area as in other high fluoride districts. Mottled teeth, which represent one of the cardinal symptoms of the so-called "Mt. Aso-Volcano Disease" (named by Hatano), are caused by fluoride in drinking water. They are clinically and genetically identical with mottling in other districts. The P-system changes, one of the 4 systems in the classification of mottled teeth, are based on the fluoride content of drinking water (Fluoride 4:172-175, 1971). They become more severe as the fluoride level in water increases. An investigation of the relation between the P-system changes and the children's physical state, yielded the following results:

1) In children with mild changes (P₁, P₂) of mottled teeth, no changes in the blood picture, the radiograph of the carpal bones or in the dentition were found. Their height, weight and chest, however, were slightly better than those of the control group i.e. of children without mottling in Yunoura village with 0.0 to 0.2 ppm fluoride in drinking water.

2) Where the water was high in fluoride and severe mottling (P₄, P₅) was present, the symptoms of chronic fluorosis prevailed namely a decline in children's growth during the period of their development. Variations in the radiographs of carpal, metacarpal and finger bones, a slight anemia, a shift to the left of the nuclei of leucocytes and the appearance of toxic granules in the blood picture were recorded; dentition was delayed.

From the Department of Internal Medicine, School of Medicine, Tokushima University, Japan.
From the above results it appears that considerable fluoride in drinking water induces fluorosis which has been designated the Mt. Aso-Volcano disease. Our studies indicate that a combination of fluorosis plus an X factor are the cause of the morbid changes of residents in the Mt. Aso district.

As the X factor a deficiency of amino acid (s), an unbalanced diet and deficiency of Vitamin A, D and Panthotenic acid are being suspected, factors which are being investigated by animal experiments.

The most notable inhibition of dental caries is believed to be brought about by fluoride uptake through the bloodstream by enamel and dentin through the ameloblasts and odontoblasts during the period of tooth development (Table 1 and 2).

### TABLE 1

**Significance of % of Caries Experience in Children with Mottled Teeth (Narikawa) and without Mottling (Yunoura).**

<table>
<thead>
<tr>
<th>ppm F⁻ in Water</th>
<th>Caries With</th>
<th>Caries Without</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Narikawa 0.9 - 3.5</td>
<td>4</td>
<td>33</td>
<td>37</td>
</tr>
<tr>
<td>Yunoura 0.0 - 0.2</td>
<td>12</td>
<td>25</td>
<td>37</td>
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<tr>
<td>Total</td>
<td>16</td>
<td>58</td>
<td>74</td>
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</tbody>
</table>

$t^2 = 3.9; \ t = 1.96; \ gt = 0.49; \ ax = 0.3$

### TABLE 2

**Level of Significance in % of Caries Experience**

<table>
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<tr>
<th>Conditions</th>
<th>% of Molars Showing Caries</th>
<th>Level of Significant Difference</th>
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<tbody>
<tr>
<td>NFM</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>EFM</td>
<td>27%</td>
<td>0.049</td>
</tr>
<tr>
<td>FM</td>
<td>28%</td>
<td>0.45</td>
</tr>
<tr>
<td>FM</td>
<td>35%</td>
<td>0.025</td>
</tr>
</tbody>
</table>

NFM: Children born in fluoride areas with mottled teeth,

EFM: Children born in nonfluoride areas; no mottled teeth; moved into the fluoride areas at age 3 to 7.

FM: Children born in "nonfluoride" areas; no mottled teeth. who have been drinking fluoride water frequently.

FM: Children born in nonfluoride areas; no mottled teeth, who had no chance to drink fluoride water.
These results revealed that addition of small amounts of fluoride to drinking water is an effective method of caries inhibition. However, upon failure to accurately control the concentration, the morbid changes of fluorosis will appear. The experience with fluorosis in the Mt. Aso district serves as a warning regarding caries inhibition by fluoride. Dean reported that the optimal concentrations of fluoride in water for decay prevention is 1.0 to 1.5 ppm of NaF in America. In Japan, judging from our statistical surveys made in December 1951, the permissible doses of fluoride should be about 0.7 ppm.

BLOOD PICTURE OF EXPERIMENTAL FLUOROSIS

PART I

CHANGES OF ERYTHROCYTE, HEMOGLOBIN, COLOR INDEX, RETICULOCYTE, BLOOD PLATELETS, AND THE SIZE OF ERYTHROCYTE

Mitsugi Hirao
Tokushima, Japan

(Abstracted from Shikoku Acta Medica, 5:344-353, October, 1954)

In a previous publication (1), the author recorded the blood pictures in dental fluorosis, a condition which is associated with chronic fluoride poisoning. The results were similar to those reported by Roholm (2) and Flemming (3) in cryolite workers in Scandinavia. Additional reports dealing with the blood picture in experimental fluorosis were presented by McClure (4), Ginn and Volker (5), Greenwood (6), Valjavee (7), Roholm (2), Schwyzer (8) Shimada (9) and Takamori (10).

The purpose of the following experimental approach was to determine whether or not anemia occurs in fluoride intoxication and how it should be classified, to investigate the influence of fluoride on bone marrow and to determine experimentally whether or not the changes in the blood picture which were observed in dental fluorosis are due to chronic fluoride poisoning.

Method

Experimental rabbits were given 1%, 3% and 5% solutions of sodium fluoride orally by Nelaton's catheter for 2 to 4 weeks. This provided a daily fluoride intake of 10 to 50 mg per kg body weight. The following examinations were made: Routine red blood cell counts, hemoglobin estimation by Sahli's method, brilliant-cresylblue Giemsa double-staining method for reticulocyte counts, Fonio's method.

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Fig. 1

Mean Values for Each Experimental Group


B Group: (10 mg F)

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Hirao

Results

The results can be summarized as follows:

In the rabbits which ingested sodium fluoride, a marked reduction in body weight was noted. A hyperchromatic macrocytic anemia and an increase in reticuloocytes and platelets were noted in the rabbits exposed to fluoride during the course of the experiment.

The mechanism of the above-mentioned blood changes will be further investigated by studying differential counts of bone marrow smears.

Bibliography


Part II

LEUCOCYTES IN RABBITS INGESTING SODIUM FLUORIDE

by

Mitsugi Hirao
Tokushima, Japan

(Abstracted from Shikoku Acta Medica, 5:64-69, October 1954)

In the previous paper the author reported data on the blood picture of rabbits to which sodium fluoride had been administered in their ration. The parameters which were studied were erythrocyte, hemoglobin, color index, reticuloocyte, blood platelet and the size of the red blood cells.

In the current investigation the influence of sodium fluoride on leucocytes of rabbits was investigated.
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<td>13</td>
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<td>10 mg Fe</td>
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<td>11</td>
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<td></td>
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<td>11</td>
<td>11</td>
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<td>13</td>
<td>19</td>
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<td>0 mg Fe</td>
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<td>10</td>
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<td>19</td>
<td>25</td>
<td>32</td>
<td>37</td>
<td>43</td>
</tr>
</tbody>
</table>

**Note:** The table shows the number of animals with decreased (↓) or increased (↑) values. The columns represent different treatments and the rows represent different time points.
Schwyzer reported that 55 to 60 percent of leucocytes of the blood of the normal rabbit were multinuclear but that, in the blood of rabbits which had been given fluoride, the leucocyte values ranged from 10 and 34%.

Contrary to Schwyzer's results, Valjavee noted an increase in multinuclear leucocytes with a marked shift to the left after daily intravenous injections of a 1% sodium fluoride solution over a period of 105 to 159 days. This provided 10 to 30 mg fluoride per kg of body weight.

Miyazawa observed an increase in toxic granulation and a shift to the left of multinuclear leucocytes in young rabbits and dogs which received 30 to 50 mg sodium fluoride per kg body weight per day orally during a period of 7 to 8 weeks.

The author encountered the same changes as those observed by Schwyzer and Miyazawa, namely

1) a marked increase of toxic granulation and a shift to the left of the nucleus in heterophile leucocytes,

2) leucopenia, due to a decrease of heterophile leucocytes and lymphocytes, during the last half of the experimental period (Table 1).

Part III

INFLUENCE OF SODIUM FLUORIDE INGESTION ON THE BONE MARROW OF RABBITS

by

Mitsugi Hirao
Tokushima, Japan

(Abstracted from Shikoku Acta Medica 5:376-383, October 1954)

The author reported in Part I and II the changes of the peripheral blood picture in rabbits ingesting sodium fluoride. In order to investigate the origin of these changes, the total nucleated cell counts, differential counts of marrow smears and the histological observations of bone marrow in rabbits exposed to fluoride were performed.

Schwyzer noted partial changes of the yellow marrow into red and an irritation or an inflammation of the bone marrow. A degeneration of myeloid parenchyma was observed by Roholm. Shimada and Kono described marked changes of the gelatinous marrow due to fluoride.
However, no reports are available of the total nucleated cell counts, the differential cell counts of smears and the systematic histological observations of bone marrow compared with the peripheral blood picture in experimental fluorosis.

We obtained the following results:

1) The total nucleated cell counts decreased markedly in a large number of experimental rabbits.

2) At the beginning of this experiment a slight hyperplasia was observed, but at the end or during its course hypoplasia with maturation or an arrest in production of marrow cells were recognized.

3) Histologically, fatty marrow in the A group, gelatinous marrow in another group and the congestion of sinusoid in each group were observed.

4) The changes of the peripheral blood picture in Parts I and II can therefore be explained on the basis of the changes in the bone marrow.

STUDIES ON THE GLYCOGEN AND PHOSPHORYLASE VARIATIONS IN MYOCARDIUM, SKELETAL MUSCLE AND LIVER IN EXPERIMENTAL FLUOROSIS

Part I

INFLUENCE OF FLUORINE ON GLYCOGEN

by

Tadashi Iwase
Tokushima, Japan

(Abstracted from Shikoku Acta Medica 12: 616-623, April 1958)

Fluoride is considered a violent enzyme-poison. In minute amounts, it inhibits several enzymes. A considerable number of reports have been published on the inhibition of glycolysis of muscle by fluoride which is due to its action upon the glycolytic enzyme. No data are available on how glycogen, the starting compound for glycolysis, changes within the living body during this process.

From the Department of Internal Medicine, School of Medicine, Tokushima University, Japan.

Volume 5 Number 1
January, 1972
The current studies were, therefore, undertaken to determine glycogen in the myocardium, in the skeletal muscle and in the liver in acute and chronic fluorosis. Acute fluorosis intoxication was induced experimentally in rabbits by injecting large doses of fluoride subcutaneously, the chronic phase of the disease by administering low doses of fluoride for prolonged periods orally.

Methods

**Acute fluorosis** was precipitated in rabbits by the subcutaneous injection of 250 mg/kg of sodium fluoride in 50 cc of distilled water. The rabbits were sacrificed 2 hours after the injection.

**Chronic fluorosis** in rabbits was induced by forced oral administration of an aqueous solution of sodium fluoride equivalent to 30 mg/kg per day in one group of rabbits, 10 mg/kg per day in another for variable periods of time ranging from 15 to 169 days. Subsequently, the animals were sacrificed and histochemical determinations of the glycogen in the myocardium, the skeletal muscle and the liver were made.

Results

**Acute fluorosis**: Glycogen concentrations of the skeletal muscle and the liver were significantly lower in the rabbits which had received fluoride than in the controls. In the myocardium, however, very little quantitative difference was found between the rabbits which had received fluoride and those which had not. Therefore, fluoride's effect on the skeletal muscle differed from that on the myocardium.

**Chronic fluorosis**: The administration of sodium fluoride over extended periods of time caused degenerative changes of varying degrees in the myocardium of the groups which had received 30 mg and 10 mg of sodium fluoride/kg orally. These changes were followed by changes in the localization of glycogen. Glycogen was absent or decreased in the necrotic foci and in the highly degenerated areas. Conversely, the amounts of glycogen remained normal or were slightly increased in the mildly degenerated areas.

Almost no degeneration was found in the skeletal muscle, where no change in the localization of glycogen was noted. However, in some animals which received sodium fluoride over longer periods of time, glycogen concentrations were lower in the skeletal muscle than in the control animals.

In those subjected to administration of sodium fluoride over prolonged periods of time, degenerative changes were also found in the liver, though to a lesser degree than in the myocardium. Degeneration of liver cells was followed by changes in the localization of glycogen. These changes corresponded to those of the myocardium where either a decrease or an entire loss of glycogen in the severely damaged areas occurred. However, the glycogen concentrations of undamaged hepatic cells were within normal limits.
Part II

Influence of Fluorine on Phosphorylase

by

Tadashi Iwase
Tokushima, Japan

(Abstracted from Shikoku Acta Medica 12:624-269, April, 1958)

In Part I of our report dealing with the changes of glycogen in fluorosis, fluoride's effect upon glycolysis differed in the myocardium, skeletal muscle and liver. It was therefore of interest to study the changes in the activity of phosphorylase which catalyzes the breakdown and synthesis of glycogen in fluorosis, a subject on which, to date, no data have been reported.

Biochemical determinations and histochemical examinations of phosphorylase were performed in the myocardium, skeletal muscle and the liver in rabbits which were subjected to experimental fluorosis.

Methods

Acute fluorosis was induced in rabbits by subcutaneous injections of 250 mg/kg of sodium fluoride in 50 cc of distilled water. The rabbits were sacrificed 2 hours after the injection. Chronic fluorosis was induced in one group of rabbits by forced oral administration of 50 mg/kg, 30 mg/kg and 10 mg/kg per day respectively of sodium fluoride in water for about 3 months. The animals were sacrificed when the stomach was empty and submitted to examination of phosphorylase.

Results

Acute fluorosis: From biochemical determinations and histochemical studies, phosphorylase in the skeletal muscle showed lower activity in the fluoride animals than in the controls whereas phosphorylase activity in the myocardium and the liver was not diminished. Thus, the effect of fluoride on phosphorylase in the myocardium differed from that in the skeletal muscle. This is similar to the difference of glycogen levels in heart and skeletal muscle tissue.

Chronic fluorosis: Various degrees of degenerative changes ranging from severe to mild, according to the duration of fluoride administration, were observed in the myocardium and the liver. There were also individual variations in the animals. The changes in the localization of phosphorylase coincided with the areas of degeneration. Phosphorylase activity was found to be diminished or lost in the severely degenerated areas. By means of biochemical determination, the myocardium and the liver of the fluoride animals showed no statistically significant difference from those of controls. No degeneration was observed in the skeletal muscle and consequently no changes in the localization of phosphorylase was found. The biochemical determination signified no statistically significant lower activity of phosphorylase in the skeletal muscle of the fluoride animals than in the controls.
Phosphorylase in Heart

Fig. 1
Heart Phosphorylase
Rabbit No. 86; Normal Control

Fig. 2
Heart Phosphorylase
Rabbit No. 81, NaF 50 mg/kg (87 Days)

Fig. 3
Liver Phosphorylase
Rabbit No. 86; Normal Control

Fig. 4
Liver Phosphorylase
Rabbit No. 81, NaF 50 mg/kg (87 Days)
ELECTROMYOGRAPHIC STUDIES ON THE INFLUENCE OF SODIUM FLUORIDE ON MUSCLE FATIGUE

by

Jiro Imura
Tokushima, Japan

(Abstracted from Shikoku Acta Medica, 14:145-172, Feb, 1959)

The sciatic nerve of Rana nigromaculata with blood circulation stimulated supramaximally at frequencies of 10, 20 and 50 cycles per second, and the muscle action potential (M Wave) was recorded from surface electrodes placed at the gastrocnemius muscle. In one group the stimulation was interrupted during the three minute experiment, in the other it was continuous. The stimulation for three minutes before and after rest was designated "stimulation before rest" and "stimulation after rest" respectively. Employing the muscle action potential as an index, the author determined the muscle fatigue, the influence of NaF (a known inhibitor of glycolysis) on muscle fatigue and, finally, how the inhibitory activity of NaF was altered by administration of MgSO₄ and ATP. The results can be summarized as follows:

I Findings on Muscle Fatigue

After fifteen minutes' rest following stimulation of the right leg, the excitability on the left leg was not altered significantly, irrespective of the frequency of the stimulus employed. With this in mind, the following experiments were performed first on the gastrocnemius muscle of the right leg of the frog and subsequently on the left leg. No difference in action potential due to variations of the body weight was noted in frogs weighing from 20 to 50 g (Fig. 1)

II Influence of NaF on Muscle Fatigue

NaF was administered in doses of 0.15 mg, 0.10 mg and 0.05 mg per gram of body weight.

a) The initial action potential caused by "stimulation before rest" of the leg of the NaF animal was not significantly different from that of the leg in the control frogs which had not received NaF with the exception of the group which was given 0.15 mg NaF/g and stimulated at a frequency of 50 cycles per second (c. p. s.) (Fig. 2).

b) The action potential caused by "stimulation after rest", however, dropped more markedly in the legs of NaF groups than in those of the control (Fig. 3).

From the Department of Internal Medicine, School of Medicine, Tokushima University, Japan.
Fig. 1

Change of Action Potentials in Response to One Series of Stimulation

Upper records: Before Rest, Lower records: After Rest.
ia = initial action potential; Beginning of stimulation
15, 30, 60, 120 and 180 seconds after stimulation began

Fig. 2

Muscle Action Potentials in Response to "Stimulation before Rest" at the Third Series of Stimulation.

Upper: Control; Lower: After 0.15 mg/g NaF
ia: Beginning of stimulation

Fig. 3

Muscle Action Potentials in Response to "Stimulation after Rest"

Upper: Control - Lower: After 0.15 mg/g NaF
ia: Beginning of stimulation
c) The initial action potential caused by "stimulation after rest" in the NaF groups at various frequencies showed higher values in the group stimulated at a frequency of 50 cps than in the animals which received stimulation of 10 and 20 cps.

From these findings it can be concluded that muscle fatigue due to NaF administration constitutes contraction fatigue.

d) Fifteen minutes after NaF was administered, immediately after completion of one series of stimulation, another series of stimulation was given at the homolateral leg and at the contralateral leg respectively. At the homolateral leg, the action potential decreased more markedly than in the contralateral leg.

e) Thirty-six minutes after completion of the second series of stimulation (given on the homolateral leg to the first series) the third series of stimulation was given on the homolateral leg. With this procedure, an even greater reduction of the action potential was noted.

From these findings it appears that NaF inhibits recovery from muscle fatigue when one series of stimulation is repeated. The amount of NaF is another significant factor in the response.

III Effect of MgSO₄ on Induced Muscle Fatigue

a) Administration of MgSO₄ was followed by the first series of stimulation. Immediately thereafter, NaF was given followed fifteen minutes later, by the second series of stimulation. MgSO₄ completely neutralized the reduction of excitability (muscle fatigue) when it was given prior to administration of 0.05 mg/g of NaF. When MgSO₄ was given before 0.10 mg/g and 0.15 mg/g NaF, it mitigated the decrease of excitability to some degree.

b) The effect of MgSO₄ before NaF administration was compared with that of MgSO₄ after NaF induced muscle fatigue had become evident. MgSO₄ mitigated muscle fatigue due to NaF when given before NaF, but showed no significant effect when given after the muscle excitability had appeared.

IV Effect of ATP on the Influence of NaF on Muscle Fatigue

Fifteen minutes after NaF was given, immediately after completion of one series of stimulation following ATP (Adenosintriphosphate) administration, another series of stimulation was given on the contralateral leg.

a) The action potential caused by "stimulation after rest" tended to show higher values in the group given ATP before 0.15 mg/g of NaF than in the group given NaF alone.

b) The action potential caused by stimulation after rest was significantly higher in the group which had received ATP administration of 0.10 mg/g NaF than in the group given NaF alone. The exogenously given ATP is believed to have mitigated the inhibition of recovery from fatigue due to NaF.
EXPERIMENTAL STUDIES ON THE CHANGES OF THE KIDNEY DUE TO FLUOROSIS

Part I

INFLUENCE OF SODIUM FLUORIDE ON THE URINE CHANGES AND NON-PROTEIN NITROGEN, CREATININE AND SODIUM CHLORIDE IN SERUM OF RABBITS

by

Hitoshi Kawahara
Tokushima, Japan

(Abstracted from Shikoku Acta Medica 8: 266-272, May 1956)

So far no systematic experimental studies have been carried out on the effect of fluoride upon renal function. The following experiments were conducted in order to determine possible renal changes by fluoride.

Method

Mature male rabbits weighing over 1.5 kg were given orally 1%, 3%, 5% sodium fluoride solutions which provided 10, 30 and 50 mg respectively of sodium fluoride per kg body weight.

During the course of the experiment, urine examinations were performed at intervals of 1 to 2 weeks for protein and sediments and the serum was checked for nonprotein nitrogen, creatinine and sodium chloride in serum.

Results

1) Urine Examination: In all animals which received sodium fluoride, albuminuria occurred either temporarily or persistently. The urinary sediment contained numerous leucocytes and renal epithelium. In the severe cases erythrocytes, granular and hyaline casts were found (Table 1).

2) Serum Non Protein Nitrogen: In seven of twelve animals, which were given varying doses of sodium fluoride, a significant increase of serum NPN was observed with a maximum of 69.5 mg/100 cc. These values were most pronounced in the group which received 50 mg per kg body weight (Table 2).

3) Serum Creatinine: In seven of twelve cases which received varying doses of sodium fluoride, a definite reversal in the serum creatinine concentration was noted. These changes were marked especially in the group which received 50 mg NaF per kg in which the creatinine value reached 4.13 mg/100 cc.

From the Department of Internal Medicine, Tokushima University, School of Medicine, Tokushima, Japan.
TABLE 1

Albumen and Microscopic Examination of Urine

<table>
<thead>
<tr>
<th>No. of NaF Rabbits</th>
<th>Albumen in Weeks or Months</th>
<th>Sediments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 week</td>
<td>2 weeks</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>+</td>
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<td></td>
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<tr>
<td>28</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>50 mg</td>
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<tr>
<td>29</td>
<td>-</td>
<td>+</td>
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<tr>
<td>30</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>34</td>
<td></td>
<td>++</td>
</tr>
</tbody>
</table>

Eryth = Erythrocytes; Leuco = Leucocytes; Ren, Ep = Renal Epithelium; Hy, Casts = Hyaline Casts; Gr, Casts = Granular Casts.

TABLE 2

Samples of Serum NPN, Creatinine and NaCl

<table>
<thead>
<tr>
<th>Rabbit #12 Given 10 mg NaF/kg</th>
<th>Concentrations in mg%</th>
<th>Rabbit No. 50 Given 50 mg NaF/kg</th>
<th>Concentrations in mg%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
<td>Weight (gr)</td>
<td>NPN</td>
<td>Creatinine</td>
</tr>
<tr>
<td>------</td>
<td>-------------</td>
<td>-----</td>
<td>------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1600</td>
<td>34.7</td>
</tr>
<tr>
<td>15</td>
<td>1640</td>
<td>31.6</td>
<td>1.77</td>
</tr>
<tr>
<td>14</td>
<td>1680</td>
<td>37.7</td>
<td>1.90</td>
</tr>
<tr>
<td>21</td>
<td>1670</td>
<td>29.4</td>
<td>1.77</td>
</tr>
<tr>
<td>31</td>
<td>1780</td>
<td>22.1</td>
<td>2.05</td>
</tr>
<tr>
<td>44</td>
<td>1740</td>
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</tr>
<tr>
<td>60</td>
<td>1680</td>
<td>33.0</td>
<td>2.10</td>
</tr>
<tr>
<td>72</td>
<td>1860</td>
<td>35.0</td>
<td>1.87</td>
</tr>
<tr>
<td>90</td>
<td>1710</td>
<td>31.2</td>
<td>1.90</td>
</tr>
<tr>
<td>105</td>
<td>1830</td>
<td>34.8</td>
<td>2.40</td>
</tr>
<tr>
<td>120</td>
<td>1820</td>
<td>26.7</td>
<td>2.17</td>
</tr>
<tr>
<td>135</td>
<td>1600</td>
<td>50.2</td>
<td>3.00</td>
</tr>
</tbody>
</table>

Mean values prior to NaF administration:
NPN: 34.8 ± 3.65 mg%; Creatinine: 2.01 ± 0.26 mg%; NaCl: 606 ± 33.61 mg%

FLUORIDE
4) Serum Sodium Chloride: In two of four cases belonging to the group to which 10 mg per kg NaF had been administered, a slight decrease of the serum NaCl was observed. In one of four cases of the 50 mg/kg group, however, the NaCl values were elevated.

The above results of urine and blood suggest that renal damage occurs in fluorosis.

Part II

INFLUENCE OF SODIUM FLUORIDE ON RENAL CLEARANCE IN RABBITS

by

Hitoshi Kawahara
Tokushima, Japan

(Abstracted from Shikoku Acta Medica 8: 273-282, 1956)

In previous papers the author reported disturbances of renal function, especially changes in the urine, serum NPN, serum creatinine and serum chlor-natrium of rabbits due to ingestion of fluoride. The current investigation deals with the effect of sodium fluoride on renal clearance, particularly on plasma urea clearance, on renal plasma flow (RPF) and glomerular filtration rate (GFR) in rabbits.

Method

1) Plasma urea clearance: Twelve healthy male rabbits weighing from 1800 to 3000 grams were given orally 10 mg, 30 mg and 50 mg per kilogram body weight of an aqueous solution of sodium fluoride daily for 2 weeks to 2 months. During the course of this experiment, plasma urea clearance was measured by the Van Slyke method at intervals of 7 to 10 days.

Urine was collected by catheter every half hour and blood samples were taken twice between the urine collections. Plasma and urinary urea concentrations were determined by the bi-acetylmonoxime method.

2) Renal Plasma Flow and Glomerular Filtration Rate: For this study 15 adult rabbits weighing from 2100 to 2600 grams received intravenous injections of 10 mg, 30 mg and 50 mg per kg of a 1% NaF isotonic saline solution. The glo-
merular filtration rate was measured by exogenous creatinine clearance the the renal plasma flow by p-aminophippuric acid clearance (Table 1).

Urine collections varied from 15 to 30 minutes depending upon the urinary flow. The blood samples were taken from the jugular vein. Creatinine was determined by the Folin's method and p-aminophippuric acid by the technique of Smith et al.

Results

Plasma urea clearance was significantly below normal in nine of twelve animals; in one animal it was above normal and in two it was unchanged. The low values were most marked in the group which had received 50 mg per kg (Table 1).

| TABLE 1 |

Plasma Urea Clearance: Continuous Oral Administration of 10 mg NaF per Kilo of Body Weight - Rabbit No. 25

<table>
<thead>
<tr>
<th>Days</th>
<th>Body Weight (gr)</th>
<th>cc of Urine per hour per minute</th>
<th>Urea Concentration (cc/m)</th>
<th>Urea Clearance (average) (cc/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Plasma</td>
<td>Urine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>2340</td>
<td>20.4</td>
<td>0.34</td>
<td>17.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18.2</td>
<td>0.303</td>
<td>18.0</td>
</tr>
<tr>
<td>8</td>
<td>2320</td>
<td>31.5</td>
<td>0.525</td>
<td>22.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50.8</td>
<td>0.846</td>
<td>22.9</td>
</tr>
<tr>
<td>14</td>
<td>2330</td>
<td>32.0</td>
<td>0.533</td>
<td>28.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>31.5</td>
<td>0.525</td>
<td>29.7</td>
</tr>
<tr>
<td>20</td>
<td>2180</td>
<td>22.5</td>
<td>0.375</td>
<td>28.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22.0</td>
<td>0.367</td>
<td>29.2</td>
</tr>
<tr>
<td>31</td>
<td>2140</td>
<td>18.0</td>
<td>0.3</td>
<td>30.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27.2</td>
<td>0.453</td>
<td>30.0</td>
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<tr>
<td>38</td>
<td>2060</td>
<td>18.0</td>
<td>0.3</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>29.4</td>
<td>0.49</td>
<td>24.7</td>
</tr>
</tbody>
</table>

The renal plasma flow increased in 1 of 4 animals which were given 10 mg NaF per kg. No decrease occurred in any of them. A marked reduction was observed however in all animals which had received 50 mg NaF/kg. In the 30 mg group no change was noted.
No significant changes in the glomerular filtration rate were observed in the 10 mg/kg group whereas in 3 out of 4 animals, which had received 30 mg/kg, a tendency to lower values was found.

In the 50 mg/kg group, all animals showed a marked decrease in the glomerular filtration rate. A slight increase in filtration fraction was noted in two of four animals which had received 10 mg/kg. There were no other significant changes in the other groups.

No consistent changes whatsoever in the percentage of renal tubular resorption were observed in any cases.

The authors concluded from the experimental data presented here that the administration of fluoride in the above doses impairs the kidney function.

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Part III

MORPHOLOGICAL STUDIES ON THE CHANGES OF THE KIDNEY OF RABBITS AND GROWING ALBINO RATS DUE TO SODIUM FLUORIDE

by

Hitoshi Kawahara
Tokushima, Japan

(Abstracted from Shikoku Acta Medica, 8:283-288, May 1956)

In previous papers (Part I and II), the author reported impairment of renal function in rabbits due to fluorosis. The current study presents morphological renal changes of rabbits and young albino rats due to fluorosis.

Method

1) After the experiments reported in the first and the second report, all rabbits were sacrificed and their kidneys examined.

2) Young albino rats 30 to 50 days after birth were fed with Scherman's standard ration mixed with sodium fluoride which contained 375, 100, 50, 10, 5 ppm of sodium fluoride respectively. The animals were killed at various periods extending from 16 to 234 days and their kidneys were compared with those of control littermates.
Results

1. Changes in Rabbits

Macroscopic and histological changes of kidneys of RABBITS receiving NaF orally for 14 to 150 days (Fig. 1-3):

On gross examination, no marked changes were observed. However, in both groups which had been given 30 and 50 mg of NaF per kg of body weight, inflammatory changes in the glomeruli with increased cellularity, capillary hyperemia, exudation, hypertrophy or atrophy, tubular degeneration with cloudy swelling, vascular degeneration and protein casts or blood in the tubular lumens were seen microscopically.

**Fig. 1**
Rabbit No. 6 - Kidney (400x)
30 mg/kg NaF; 120 Days

**Fig. 2**
Rabbit No. 36 - Kidney (140x)
50 mg/kg NaF; 14 Days

Capillary hyperemia and hypertrophy of glomeruli, protein casts in tubules.

Round cells infiltration in interstitium and protein casts in tubules.

**Fig. 3**
Rabbit No. 60 - Kidney (200x)
30 mg/kg NaF; Intravenously

Exudate (E) in glomeruli, cloudy swelling of tubules.

FLUORIDE
2. Changes in Rats

Similar histological changes occurred in animals which received intravenous injections of 30 and 50 mg/kg NaF.

In young albino RATS, the following morphological kidney changes occurred (Fig. 4-8):

**Fig. 4**

Albino Rat No. 33 - Kidney
375 mg/kg NaF; 234 Days

Granular irregularity of the surface and atrophy.
Lower: Normal control kidney

Marked nephrosclerosis and numerous granular irregularities of the kidney surface were observed macroscopically. In the group, which had received 375 ppm of fluoride, progressive fibrosis and round cell infiltration was detected in the interstitium.

Inflammatory changes in glomeruli and tubular degeneration were noticeable in the two groups which had received 100 ppm and 375 ppm.

The above-mentioned morphological changes, combined with impairment of renal function described in the previous reports, indicate that fluoride causes serious damage to kidneys.
**Fig. 5**

Albino Rat No. 33 - Kidney 375 ppm NaF; 234 Days

Fibrosis and infiltration of round cells in the interstitium. Contraction and compensatory hypertrophy of the tubules.

**Fig. 6**

Albino Rat No. 33 - Kidney (400 x) 375 ppm NaF; 234 Days

Protein casts in the tubules (P).

**Fig. 7**

Albino Rat No. 34 - Kidney (280 x) 375 ppm NaF; 189 Days

Marked round cell infiltration in interstitium.

**Fig. 8**

Albino Rat No. 6 - Kidney (200 x) 10 ppm NaF; 120 Days

Severe cloudy swelling of tubules.
GIANT CELLS IN BONE MARROWS OF PATIENTS ON HIGH-DOSE FLUORIDE TREATMENT

By

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In the bone marrow of three patients who received 16 to 150 mg of sodium fluoride daily during periods of 1 to 36 months for treatment of osteoporosis, the authors observed giant cells of the kind not heretofore described.

A 73 year-old woman, with severe osteoporosis of the spine and multiple compression fractures, developed weakness and pallor with low hemoglobin (4.3 gm/100 ml), a hematocrit of 15% and a platelet count of 400,000/mm³. Sizable iron was absent in the bone marrow. The giant cells, called monocytoïd, were suggestive of a malignancy. Treatment of the patient's anemia with ferrous sulfate brought the hemoglobin up to 14.4 gm/100 ml in 7 weeks. The abnormal giant bone marrow cells, however, persisted for 14 weeks. At this time, about 50% of the giant cells appeared to represent a transition between a megakaryocyte and monocytoïd cell. They were distinctly different from the normal appearing megakaryocytes, an increased number of which are usually found in bone marrow following hemorrhages.

At first it was felt that sodium warfarin (Coumadin) and two other drugs, one of which contained aspirin might have been responsible for the abnormal giant blood cells. When the symptoms of osteoporosis had improved and the dose of fluoride was decreased to 50 mg/day, the number of monocytoïd giant cells decreased considerably but many transitional giant cells were observed. At this time, the authors were inclined to attribute the unusual giant cells to a malignancy, but also considered the possibility that the giant cells might be related to the fluoride treatment. Experiments on two additional patients established that they were indeed due to fluoride.

The second case was a 65-year old woman with osteoporosis who received 150 mg sodium fluoride daily. The abnormal cells were found as long as the full dose was being administered. They decreased in number following reduction of the dose to 50 mg per day. Three months after the fluoride treatment had been discontinued, the bone marrow was normal.

The third case was a 72-year-old woman who had been taking 16 mg of sodium fluoride daily for three years without medical supervision. A few giant mono-

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Abstract

cytoid cells and some transitional giant cells were found in her bone marrow, which was examined 10 days after discontinuance of therapy. This patient had two benign ulcers, anemia and an diffusely distributed increase in bone marrow cells.

In an Indian with typical findings of advanced skeletal fluorosis, who had spent most of his life in Gila Bend, Arizona (with a fluoride content in drinking water of 5 to 10 ppm), a few giant cells were found after he had been in a Tucson, Arizona tuberculosis sanitorium (fluoride in water 0.3 ppm) and away from its reservation for 9 months.

The cells differed in appearance from those in the sodium fluoride-treated patients. They were oval in shape and measured between 30 and 60 microns. The nuclei had indentations and projections similar to those in monocytes but were much larger and filled most of the cell. The cytoplasm showed dark basophilic granules and in some instances, pseudopods. Some of the cells showed phagocytosis of red and white cells.

The authors pointed out that the bone marrow of some patients with osteoporosis shows an increase in mast cells but these cells are readily distinguishable from the above-described giant cells. They are smaller in size with purplish cytoplasmic granules and a round nucleus.

In their search of the literature, the authors failed to find reference to the kind of giant cells in bone marrow identical with the cells described here.