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FLUORIDE is listed in
Current Contents Agricultural
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高森時雄

TAKAMORI, Tokio

EDITORIAL

Tokio Takamori

In 1939, Kaj Roholm's classical book entitled "Fluorine Intoxication" (1) appeared. It represents the first comprehensive, and to this day the most valuable, contribution to fluoride research. Roholm's interest in the subject had been aroused by fluorosis in Danish cryolite workers and by the Meuse Valley disaster in 1930 in which fluoride emission played a major role.

His work elicited widespread interest, particularly in the two features of fluorosis which had but recently become associated with fluoride intake: mottling of teeth, a readily diagnosible sign of fluoride poisoning, and the skeletal changes of fluorosis. Subsequently fluorosis was investigated in vegetation, domestic animals and humans. In various medical centers throughout the world especially in India, North Africa, France, Italy and Argentina and in dental schools of the U.S.A., fluorosis became the subject of intensive studies.

Against this background Tokio Takamori, who at the time was professor at the Manchuria Medical College, carried out a series of remarkable investigations on fluorosis which, because of the language barrier and because of lack of communication during and after World War II, did not receive the attention in the English literature which they deserve.

Takamori had observed a peculiar bone ailment called Kaschin Beck Disease, which was endemic in remote mountainous communities of Manchuria, characterized by achondroplasia and by shortening of bones. His studies on this disease, carried out under trying and indeed dangerous circumstances, were published by his collaborators posthumously (2).

Following his appointment as head of internal medicine at Gifu University and subsequently of the medical department of Tokushima University he focussed his attention upon bone diseases in an area closer to home.

In the Aso volcano district, inhabitants had mottled teeth and their bones exhibited changes which were attributed eventually to fluoride in drinking water.

Takamori was not satisfied with merely reporting the clinical details on the patients whom he encountered. He and his students embarked upon an elaborate program of investigating many phases of the disease. They accumulated original data, some of which are still to this day most pertinent to the understanding of the biological action of fluoride. A portion of this vast amount of material is being republished or abstracted in this issue with the kind permission of the editors of Shikoku Acta Medica and Tokushima Journal of Experimental Medicine. Their courtesy is herewith gratefully acknowledged.

Takamori and his colleagues explored histologically and biochemically the growth of bones in the various stages of skeletal fluorosis. They compared the

development of bones in normal and rachitic subjects with that in fluorosis and supported all observations in humans by experiments with rabbits or rats.

The research of Takamori and Hirao, on the blood picture in experimental fluorosis, is still unequalled to this day. Of particular importance are his observations on cardiac abnormalities in fluorosis made in collaboration with Okushi. They noted heart changes in a significant percentage of children with mottled teeth, a subject to which few, if any, other investigators have thus far addressed themselves. Their findings in humans were corroborated by extensive experiments in rabbits, rats and dogs.

The work of Takamori's collaborators H. and K. Kawahara, on the genetic effect of fluorides in frogs, points to the strong possibility of teratogenic effects of fluorides in mammalians, including humans.

Although an extensive body of literature is available on how fluoride affects kidneys, it is doubtful whether anyone else has produced as accurate data on kidney function and kidney pathology as have Takamori and Kawahara.

The classification of mottled teeth by Takamori's collaborator H. Kawahara is unique. It affords an exact description of the extent and the appearance of mottled teeth.

Takamori's investigations confirmed that the incidence and severity of mottled teeth is related to the level of fluoride in water whereas the incidence of skeletal sclerosis is erratic and not solely linked with fluoride levels of water.

Takamori and his team tackled the important subject of the action of alkaline phosphatase in body tissue and glucose metabolism in fluorosis.

Jiro Imura made a valuable contribution to the explanation of muscle fatigue in fluorosis by studying the effect of stimulation of the gastrocnemius muscle of frogs under varying experimental conditions.

The current and next issue of FLUORIDE will honor the memory of Professor Tokio Takamori. At the same time, the two issues will fulfill another purpose: they will bring forth a variety of essential information which is not available in other current treatises concerned with the health effects of fluoride.

Regrettably space does not permit presentation of more than a limited amount of the vast material made available by Takamori and his collaborators.

RECENT STUDIES ON FLUOROSIS

by

Tokio Takamori
Tokushima, Japan

(Reproduced in part from the Tokushima Journ. of Exper. Med. 2:25-44, 1955)

I Introduction

For three years odontological and medical studies on fluorosis were carried out in our department in association with field work in several natural fluoride districts and with animal experiments. The characteristic changes of teeth, bones, blood picture and heart in fluorosis were investigated. Moreover, the interrelation between the amount of fluoride in drinking water and the children's physique (height, weight and chest) was explored. Experiments on rabbits, rats and dogs were carried out to supplement the clinical data.

The studies reported here were executed by members of the author's department and by H. Kawahara, M. D. and D. D. S.

II The New Method for Classifying Mottled Teeth Based on Fluoride Content in Drinking Water

In the past, various classifications of mottled teeth have been published, but most of them were unsystematic and inadequate. No attention had been paid to the differential diagnostic features of dental fluorosis. Therefore, Kawahara introduced a new system of recording mottled enamel based on the fluoride content of drinking water exclusively.

His classification (Fig. 1) takes into account the following features:

a) Presence of Streaks (Streifen) on Teeth: S-System

Chalky lines of the S-system appear when the enamel is abnormally calcified. When the lines cover the whole enamel surface, this change approaches the P-system mentioned below.

The lines are caused by intermittent attacks by fluoride during the period of enamel formation. In other words, the chalky lines represent enamel not completely calcified. They appear near the neck, edge or cuspid of tooth-enamel in response to the developmental stage when the tooth germ is attacked by fluoride. The width and shade of the chalky line is determined by the concentration of fluoride and by the length of time during which the tooth is affected by fluoride. This

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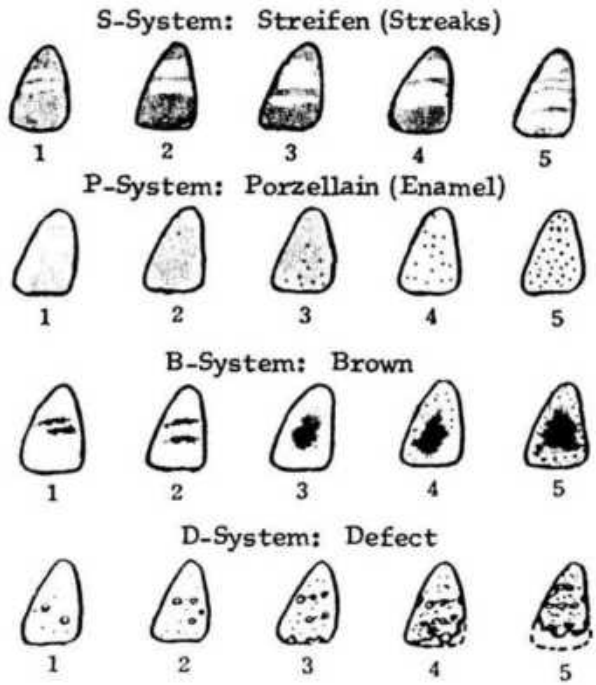
From the Department of Internal Medicine, School of Medicine, Tokushima University, Japan.

Fig. 1

Classification of Mottled Teeth Based on Fluoride Contents



concept can be documented by experiments on dogs, rats and mice which have been given NaF solution subcutaneously or orally.

Based upon the width and shade of the uncalcified lines, the S-system is divided into 5 degrees designated as S_1, S_2, S_3, S_4 and S_5.

S-system changes are found often in areas where drinking water contains comparatively small amounts of fluoride (less than 1.0 ppm). The effect of fluoride is dependent on additional factors as mentioned below.

b) Chalky or Porcelain-like Teeth: P-System (Porzellan or enamel)

The P-system changes tend to become more severe in proportion to the increase in fluoride in drinking water. The mild changes of the P-system (P_1, P_2) are caused by small amounts of fluoride in drinking water of the order of 0.4 to 0.8 ppm. Such mildly altered enamel has the appearance of a lustrous marble. The moderate P_3 change is caused by a greater amount of fluoride in the range of 0.6 to 2.0 ppm. The enamel changes resemble frosted glass. Severe lesions of the enamel (P_4, P_5) caused by large amounts of fluoride (over 2.5 ppm) appear chalky and are very brittle.

As mentioned above, the interrelation between P-system changes and fluoride content of drinking water is evident. Such changes are enhanced by unbalanced diets, deficiencies of amino acids and low intake of vitamin A, D, or B complex.

c) Brown-Spotted Teeth: B-System

Villagers in the affected regions believe that mottling progresses with the lapse of time. Some investigators have incorrectly assumed that mottled teeth become severe when they are attacked by fluoride even after dentition. It is certain that the brown or brown-black spots in enamel become more pronounced with the lapse of time. They must be considered as secondary changes superimposed upon the P-or S-system changes. Exogenic pigments derived from food and beverages penetrate into and lodge in the uncalcified parts of enamel thus accounting for B-system changes. We verified the occurrence of brown stains in our experiments on dogs. The brown pigment of the spotted teeth is likely to originate from endogenous factors.

As our field work shows, brown-spotted teeth are usually found in high fluoride zones. Whether or not the abnormal pigment formation in ameloblasts is related to fluoride, should be further explored.

According to the intensity of the brownish discoloration, the teeth are classified at five degrees, B_1, B_2, B_3, B_4, B_5.

d) Enamel Defect: D-System

Enamel defects are caused by various factors. D-system changes of dental fluorosis are accompanied by the severe changes of the P-or S-system (P_4, P_5, S_4, S_5). Enamel defects are divided into 5 degrees according to their extent. The differentiation of defects due to fluorosis from other abnormalities is relatively easy.

Method of Recording Mottled Teeth

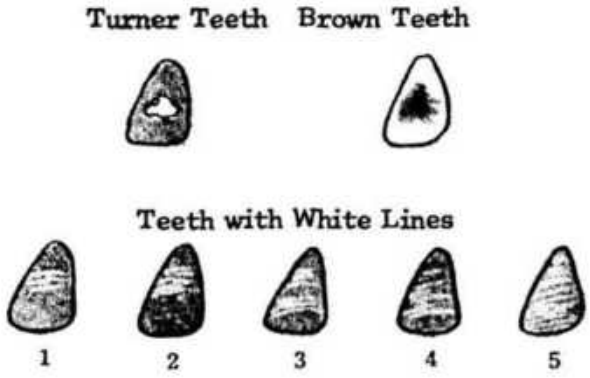
As outlined above, the changes of mottled teeth are classified into 20 fundamental kinds including 4 systems and 5 degrees. It is common that several changes can appear in a tooth simultaneously. As an example of recording the changes, the logogram, P_4, B_3 D_1 would indicate three kinds of changes of mottled enamel, namely the 4th degree of the P-system, the 3rd degree of the B-system and the 1st degree of the D-system in a tooth. If we employ this method we can record more than 300 different combinations.

Differential Diagnosis of Mottled Teeth

The principal enamel defects from which fluoride mottling must be differentiated are as follows (Fig. 2):

Fig. 2

Differential Diagnosis of Mottled Teeth



a) Turner Teeth:

Turner teeth are caused by local acidosis associated with apical periodontitis of deciduous teeth. The defect of enamel calcification is localized and well demarcated. It usually appears in the upper incisors of permanent teeth. A few early investigators assumed mistakenly that mottled teeth are identical with Turner teeth.

b) Brown Teeth:

There is no literature on Brown teeth in our country, and we have little experience with this disease. It is not impossible, however, to differentiate Brown teeth from the B-system changes of mottled teeth, because the brown discoloration of mottling is always preceded by P-or S-system changes.

c) Teeth with White Lines:

Characteristic of this condition is the fine white lines which appear in a part of enamel that is not completely calcified. As this symptom becomes more severe, the fine white lines cover the whole enamel. At this stage, the appearance of the teeth simulates that of the S-system change of mottled teeth and cannot be easily differentiated. Therefore we temporarily named it "false mottled teeth". In this severe form there are some differential diagnostic features: The uncalcified line in the S-system change has a homogenous chalky appearance; it is wider than that in the "Teeth with White Lines". The nonfluorotic "White Lines" consist of many fine white lines. However, the distinction between the mild change in S-system (S_1, S_2) and severe "White Lines" is difficult. Probably it will be differentiated in the near future by histochemical and electron-microscopic techniques.

The "White Line" defect occurs frequently in mountainous regions not readily accessible. According to investigations by our medical and odontological teams and to the survey of blood and water contents by Kuroda on affected children, it seems that "White Line Teeth" are caused by protein deficiency.

The method used by the Welfare Ministry of Japan distinguishes four groups of mottled enamel, namely M_1, M_2, M_3 and B. This classification is a simplified version of Kawahara's method. M_1 is equivalent to S-system changes; M_2, P-system changes; M_3, D-system changes; B, B-system changes.

III Relation of Mottled Teeth to the Fluoride Levels in Drinking Water and to Children's Physical Development

We made determination of fluoride in water by using the synthetic cation exchange resin column process and the aluminum-haematoxylin method improved by Shimizu in 1949. The accuracy of the method is $\pm$ 0.1 ppm. We obtained the following results.

1) Mottled Teeth and Fluoride Content of Drinking Water:

Throughout the Uchinomaki hot-spring district, mottled teeth are widespread and its main cause is believed to be the high fluoride content of drinking water (Table 1).

TABLE 1

Relation Between Mottled Teeth and Fluoride Content of Drinking Water in and Around Uchinomaki Hot-Spring District

Village	Kind of well	Fluoride in ppm	No. of wells	Mean fluoride content	No. of cases	Percentage of mottled teeth
Nakanomachi	ordinary well	0 -1.7	17	0.2	92	37%
		0.9-3.6	5	2.1		
Narikawa	ordinary well	0.2-1.4	2	0.8	16	3%
	artesian well	0.2-3.3	21	1.4	54	93%
Yunoura	ordinary well	0 -0.5	5	0.2	128	0%
Imamachi and Kuronagare	ordinary well	0 -1.7	8	0.3	10	3%
	artesian well	1.3-7.0	12	3.4	8	100%
Ogura	surface water	0	4	0	17	0%
	spring water	0	2	0		
	artesian well	0	4	0		
Namino and Akanita	ordinary well	0 -1.4	25	0.3	103	5%
	ordinary well	0	10	0	105	0%

2) Intensity of Mottling and Children's Height:

P-system changes (enamel defects of mottling) become more severe as the fluoride content in drinking water increases. P_1 and P_2 changes are caused by a little fluoride in drinking water (0.4 to 0.8 ppm). The moderate P_3 change, by a moderate amount of fluoride (0.6 to 2.0 ppm) and the severe changes P_4, P_5 are due to a relatively large amount of fluoride (above 2.5 ppm). Therefore, the relationship between the P-system changes and the fluoride content of drinking water is firmly established.

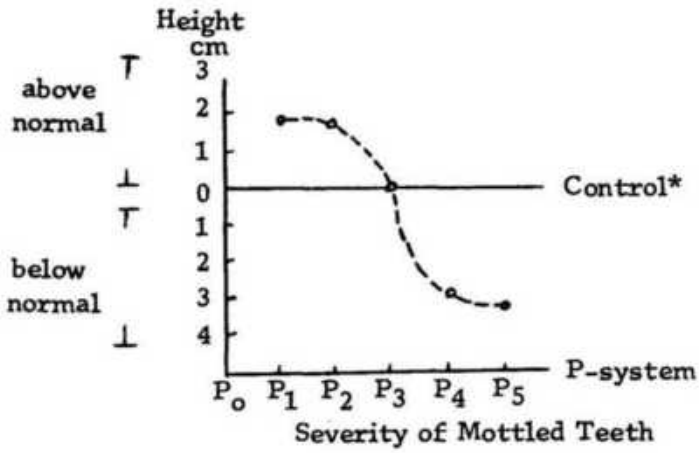
The author investigated the relationship between the severity of mottled teeth and the height of 52 children with various degrees of P-system mottling in Narikawa village.

Another 52 pupils of the same age from Yunoura village, where no mottled teeth were found, were chosen as controls. The results were tabulated.

It is obvious that the 25 children with a high degree of mottled teeth (P_4 and P_5) are inferior in height to the control children without mottled teeth in the nonfluoride area, and that 15 children with a low degree (P_1 and P_2) are superior to the control. Almost no significant difference was noted between 15 children with a moderate degree of mottling (P_3) and the controls (Fig. 3).

Fig. 3

Effect of Fluoride upon Childrens Growth



*Yunoura Village (Fluoride free)

Average height of cases with slight enamel change (P_1 or P_2 is increased significantly ($x = 0.045$),

Average height of moderate cases (P_3) is neither increased nor depressed,

Average height of severe cases (P_4 or P_5) is depressed significantly ($x = 0.014$).

3) Severity of Mottling and Body Weight:

Nine boys and girls with severe mottled teeth (P_4 and P_5) were selected at random among the first and second grade pupils of Uchinomaki intermediate school (born in 1936 to 1937) in Narikawa village, where mottled teeth are known to be widespread. The average weight of the pupils from the high fluoride zone (31.9 kg average) was less than that of 9 children from a non-mottled teeth area (34.6 kg average). Mathematical appraisal confirmed this relation ($\times a > 0.05$).

4) The Severity of Mottled Teeth and Chest Circumference:

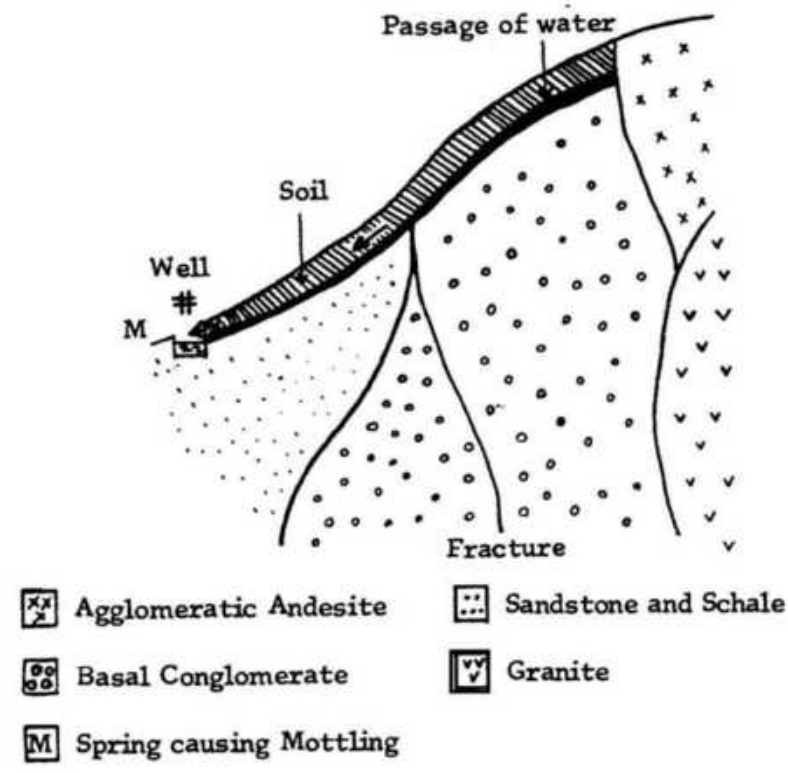
The chest circumference of nine pupils selected from each of two groups (those with and those without mottled teeth) was measured. The average values for the pupils residing in Narikawa village was 67.3 cm and that for those from Yunoura village (the control) was 69.7 cm. The group of pupils with severe mottled teeth, had a smaller chest circumference than the group without mottled teeth ($\times a > 0.05$).

5) Geological Considerations:

The author analyzed the soil in the Shionoe mineral spring district, one of the high fluoride areas in Shikoku province. It was established that the geo-

Fig. 4

Diagram of Vertical Cut Geology Near Well in Shionoe District



logical formation in the environs of Shionoe mineral spring originated at a latter stage of volcanic activity than the Aso-Volcanic district.

The geological studies revealed that fluoride in well water in this region is derived from the magma reservoir which erupted agglomeratic andesite. As one of the phenomena which happened during the last stage of the volcanic activity, the Shionoe mineral spring must have gushed out and subsequently become mixed with ground water which appeared in the well (Fig. 4).

IV Influence of Chronic Fluoride Intoxication Upon Bone Growth, Particularly Upon Epiphyseal Cartilage

A, Bone Changes of Growing Rabbits Given Large Doses of Fluoride

(This portion of the article contains the experiments by Kunimitsu Kono presented on page 180, Editor)

The findings obtained are in full agreement with the histological investigation. The impairment of the longitudinal growth of long bones (in fluorosis) appears to be due to retardation of synostosis of epiphyseal cartilage.

B, Changes in Growth in Body and Bones of Young Rats Fed at Various Levels of NaF

Growing 30 to 50 day old rats were given a standard ration mixed with 375, 100, 50, 10, 5 or 2 ppm of sodium fluoride. All groups which received more than 50 ppm NaF decreased in weight. Especially in the 375 ppm group, weight diminished markedly ($\alpha = 0.05$). However, in the 10 ppm group, 1.08 to 2.86 mg fluoride per kg, and especially in the 5 and 2 ppm groups the weight increased during the first six weeks.

2) Histologically, the bone trabeculae and cortical layers, of the groups fed more than 50 ppm, increased in thickness, turned into coarse fibrillar bone after which they were transformed gradually into osteoporotic bone with active osteoclastic resorption. On the other hand, following short-term administration of 50 ppm, 5 and 2 ppm, the bone trabeculae thickened and the length of bones increased. Regeneration of cortical layers was active.

Whereas a delay in synostosis occurred in the 50 ppm and more groups, a tendency to premature synostosis was noted in the10 ppm group. Premature synostosis occurred in every animal which received 2 and 5 ppm. In fact, in the metacarpi and metatarsi of these groups premature synostosis was already completed at a time when it had not as yet begun in the control animals.

3) In the groups receiving more than 50 ppm of fluoride, epiphyseal cartilage became thinner and developed into a wavy borderline or a saw-teeth shape; cartilage cells showed atrophy and pyknosis. On the other hand, with the smaller dosage, thinness and slight waving was seen but no change in the cells was detected.

4) The total bone width and the width of the epiphyseal cartilage zone were measured in microns. The percentage of zone width in relation to the total width of the bone, the ratio of $\frac{\text{zone width of hypertrophy}}{\text{zone width of proliferation}}$, the number of cells of every zone and the mechanism of normal synostosis in the control animals were determined. When the rats matured into the stage of synostosis of the epiphyseal cartilage, the following three conditions were noted:

a) A decrease of the total width of epiphyseal cartilage.
b) An increasing difference in the ratio of zone of proliferation to the percentage of hypertrophy.
c) A marked decrease in number of cells in the zone of proliferation.

Comment

Extensive literature on bone fluorosis as an occupational disease in cryolite factories and on endemic fluorosis in certain regions of the world is available. These papers were chiefly concerned with the bone changes of adults, but no data are available on the effect of fluoride upon bone growth.

The current study was undertaken in order to observe the effect of various amounts of fluoride upon bone growth in animals by experiment. Comparatively large doses of fluoride prevent bone growth and delay synostosis whereas small amounts of fluoride accelerate bone growth and the synostosis is completed at an earlier age. Such findings are of interest in interpreting the results of studies of children's growth in field work. Varying amounts of NaF administered to growing rats cause markedly different skeletal changes and influence the body weight in various ways.

The relationship of skeletal fluorosis to the magnitude of fluoride ingestion is not as marked as the relationship of fluoride to mottled teeth. Moreover, the bone changes in endemic skeletal fluorosis are not identical in different areas. The inhabitants in the fluoride districts which we investigated are generally of poor and needy circumstances. Our findings, therefore, suggest that the effect of fluoride in soil and water upon the development of bones is influenced by defective nutrition, especially a lack of vitamins or amino acids in the diet.

Other factors such as malnutrition, age, race, etc. appear to influence bone changes in high fluoride areas.

V. Blood Picture of Chronic Fluorosis

(The author summarizes the findings of his collaborator Mitsugi Hirao* as follows)

1) Erythrocytes and hemoglobin value decreased slightly.
2) The nucleus of neutrophiles showed a slight shift to the left.

*To be presented in detail in the next issue.

3) Toxic granules of neutrophiles and reticulocytes increased slightly.

4) The above-mentioned changes in the blood picture were observed not only in children with mottled enamel, but also among those without mottling residing in the high fluoride zone. The changes were more marked in the group with mottled teeth than in the latter.

5) There was no significant difference in the blood changes in the two lowest degrees of mottled enamel.

These results are similar to those reported by Flemming, Møller, Gudjonson and Roholm.

(For the purpose of classifying anemia due to fluoride, Takamori and his group investigated the blood changes in experimental animals with the following results, Editor):

1) A hyperchromatic macrocytic anemia and an increase in reticulocytes and platelets were noted in the experimental rabbits ingesting sodium fluoride.

2) A marked increase in toxic granulation and a shift to the left of the nucleus in the heterophile leucocytes occurred.

3) A leucopenia due to a decrease in heterophile leucocytes and lymphocytes was observed during the last half of this experiment.

The above-mentioned results support our observation that the changes in the blood picture of individuals with mottled enamel due to chronic fluoride uptake constitute a hyperchromatic macrocytic anemia.

Bone Marrow Studies in Experimental Fluorosis

The histology of bone marrow in experimental fluorosis was described only briefly by Schwyzer, Roholm, Shimada and Kono, but no reports are available on the total nucleated cell counts of aspirated bone marrow, the differential cell counts of marrow smears and the systemic histology compared with the peripheral blood picture in experimental fluorosis. Our results were as follows:

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

2) At the beginning of the experiment, a slight hyperplasia of the bone marrow occurred but during this experiment and at its termination, marrow hypoplasia with an arrest of the production of marrow cells was noted.

3) Fatty degeneration of the marrow was found in group A which had received 10 mg sodium fluoride per body weight daily. Gelatinous metaplasia of the marrow and congestion of sinusoids were observed histologically in all groups.

It appears that the changes of the peripheral blood picture in chronic fluorosis were brought about by bone marrow pathology.

VI. The Heart Muscle in Chronic Fluorosis

(The data of this investigation are presented on page 194, Editor)

1) Among residents of a high fluoride zone, electrocardiographic examinations revealed greater myocardial damage and X-rays showed a higher incidence of cardiac dilatation than in low fluoride areas.

2) A higher incidence of myocardial damage was found in children with mottled enamel than in those without mottling.

3) Children residing in non-fluoride districts showed no myocardial damage.

4) The myocardial damage appears to be due to fluoride in drinking water.

Experimental Studies on the Effects of Sodium Fluoride Upon the Heart Muscle of Rabbits

(The data of this investigation is reported by Okushi on page 199, Editor)

Summary

1) The complicated changes of mottled teeth were classified into 20 fundamental types including 4 systems: S (Stripes), P (Porcelain, enamel), B (Brown stain), D (Defect) and 5 degrees (1-5). By combining the basic systems and their degrees, more than 30 kinds of changes can be distinguished in mottled teeth. Dental changes have been described which simulate mottled enamel. They are liable to be confused with it. The classification used by the Welfare Ministry is a simplification of Kawahara's method which originated in our department.

2) The relationship of mottled teeth to the amount of fluoride in drinking water, furthermore to the physical development (height, weight and chest) was studied. Children with severe mottling showed a reduction in height compared to that of the controls (children without mottling in nonfluoride areas). The skeletal development among those with minor dental mottling showed no significant changes. It even appeared to be superior to the controls. Almost no significant difference was noted between those with a moderate degree of mottling and the control.

3) In experimental rabbits,given large doses of sodium fluoride, a decrease in weight and osteoporosis occurred in all cases,whereas fluoride ingestion for a long period (178 to 200 days) caused apposition of immature bone at the periosteal and endosteal surfaces. In the experiments on rats fed various fluoride levels the comparatively large amounts of fluoride seemed to retard bone growth and account for a delay in synostosis. Small amounts of fluoride, on the other hand, accelerated bone growth and completed synostosis at an earlier stage.

4) A decrease in erythrocytes and hemoglobin, a shift to the left of the nucleus of neutrophiles and an increase in toxic granules of neutrophiles and reticulocytes were observed not only in children with mottled enamel in high fluoride areas but also in the experimental rabbits which had received sodium fluoride orally. Our experiments also produced a hyperchromatic macrocytic anemia, an increase in reticulocytes, platelets and toxic granules, a shift to the left of the nucleus in the heterophile leucocytes, and leucopenia due to a decrease in heterophile leucocytes and lymphocytes. A review of our work on bone marrow discloses that the changes of the peripheral blood in chronic fluorosis are closely related to the changes in the bone marrow.

5) In residents of the high fluoride zones, myocardial damage and dilatation of the cardiac silhouette were noted. In experimental rabbits given sodium fluoride orally, we observed a pattern of myocardial damage characterized by depressed ST, inverted T, prolonged QT interval, multifocal ventricular premature contraction, bundle branch block and pulmonary P in the electrocardiogram. Histologically regressive degeneration, cellular infiltration, hyperemia, hemorrhages and thickening of vessel walls were found in the heart muscle.

Bibliography*

1. Black, F.S. and McKay, G.V.: Dent. Cos. 58:477, 1916.
2. Dean, H.T.: Dental Caries and Fluorine, 1949.
3. Hirata, M.: Saishin Igaku 6, 1951.
4. Wilson, R.H. and Floyd, D.: Arch of Ind. Hyg. and Occup. Med., 1950.
5. Phillips, P.H.: Amer. J. Physiol. 106:305, 1933.
6. Kraft, K. and May, R.: Hoppe-Seyler's Ztschr. Physiol. und Chem 246, 1937.
7. Shimizu, H.: Collective Determination of Fluorine in Water, 1949.
8. Takamori, T. et al.: The Tokyo Medical J. 67:4, 1950.
9. Baba, H. and Kawahara, S.: Shikoku Acta Medica 1:2, 1950.
10. Takamori, T., Baba, H. and Kawahara, S.: Shikoku Acta Medica 3:3, 1952.
11. Kawahara, S. and Kawahara, K.: Shikoku Acta Medica 5:3, 16, 1954.
12. Kono, K.: Shikoku Acta Medica 4:151, 1933.
13. Koni, K.: Shikoku Acta Medica 5:78, 1954.
14. Møller, F.: Acta Radiol. 13:269, 1932.
15. Roholm, K.: Arch. Gewerb, Path. 7:3, 1936.
16. Bishop, P.A.: Amer. J. Roentg. 35:577, 1932.
17. Kemp, F.H.: Lancet 2:93, 1942.
18. Shortt, H.E.: Ind. Med. Gaz. 72:396, 1937.
19. Shortt, H.E.: Ind. J. Med. Res. 25:553, 1937.
20. Galeazzi, M.: Rend. Ist. Super Sanita 10:739, 1947.
21. Ockerse, T.: S. Afr. Med. J. 15:261, 1941.

*This bibliography is presented as it appeared in the original paper without indicating the references in the text.

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22. Hatano: The Jap. Path. Soc. 33:332, 1944.
23. Takamori, T.: J. Jap. Soc. Int. Med. 39:131, 1950.
24. Takamori, T.: J. Jap. Soc. Int. Med. 40:232, 1951.
25. Takamori, T.: Tokyo Med. 67:7, 1950.
26. Hamamoto, E.: From Proceedings of the Japan Academy 1:53, 1954.
27. Roholm, K.: Arch. f. Gewerbepath. u. Gewerbehyg 7:255, 1937.
28. Roholm, K.: Klin. Wschr. 15:1425, 1936.
29. Roholm, K.: Fluorschädigungen. Arbeitsmedizin Heft 7, 1937.
30. Hirao, M.: Shikoku Acta Medica 3:30, 1952.
31. McClure, F.J.: J. Am. Med. Assoc. 139:717, 1949.
32. McClure, F.J.: J. Phar. and Exp. Ther. 89:77, 1947.
33. Ginn and Volker: Proc. Exp. Biol. Med. 57:189, 1944.
34. Greenwood, D.A. et al: Proc. Exp. Biol. Med. 31:1037, 1934.
35. Greenwood, D.A. et al.: J. Am. Vet. Med. Assoc. 39:28, 1935.
36. Valjavee, M.: Ztschr. f. d. ges. exp. Med. 85:382, 1932.
37. Schwyzer, F. Quoted by McClure: Phy. Rev. 13:277, 1933.
38. Schwyzer, F.: Bioch. Ztschr. 60:32, 1914.
39. Shimada, T.: Fukuoka Medical Journal, 32:121, 1937.
40. Takamori, T.: J. Jap. Soc. Int. Med. 37:148, 1948.
41. Tappeiner, H.: Arch. f. Exper. Path. 25:203, 1889.
42. Tappeiner, H.: Arch. f. Exper. Path. 27:108, 1890.
43. Larner, J.: Industrial Medicine and Surgery 19: 535, 1950.
44. DeNito, G.: Riv. di Path. Super. 3:294, 1928.
45. Gottdenker, F. and Rothberger, C.J.: Arch. f. Exper. Path. u. Pharmakol. 179:24, 1935.
46. Gottdenker, F. and Rothberger, C.J.: Arch. f. Exper. Path. u. Pharmakol. 179:38, 1935.
47. Chenowith, M.B., Gilman, A.: J. Pharmacol. Expt'l. Therap. 82:90, 1946.
48. Chatel, A. De: Ztschr. f. d. Ges. Exper. Med. 98:389, 1936.
49. Lepeschkin: Modern Electrocardiography 1, 1951.
50. Okuno, H.: J. Chem. Soc. Japan, 63:23, 1934.
51. Kawahara, H.: A Monthly Record of Dental Science 52:7, 1952.
52. Iowa Agric. Exper. Station Report for year ending June 30, 1928.
53. Okinaka, S.: Japanese Medical J. 3, 1951.
54. Smith, M.C.: J. D. Res. 15:281, 1936.
55. Smith, M.C., Lantz, M.E. and Smith, H.V.: J. Ind. and Eng. Chem. 23:996, 1931.
56. Brandl, J. and Tappeiner, H.: Ztschr. f. Biol. 28, 1891.
57. Tamai, I.: The Journal of the Wakayama Medical Society 3:23, 1952.
58. Shimizu, S.: N.J.A.C. 12:59, 1948.
59. Okushi, I.: Shikoku Acta Medica 5:55, 1954.
60. Okushi, I.: Shikoku Acta Medica 5:52, 1954.

PRELIMINARY REPORT ON THE INFLUENCE OF NaF SOLUTION UPON THE EARLY DEVELOPMENT OF TOAD-EMBRYOS

by

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(Abstracted from Tokushima Journal of Experimental Medicine 1:98-104, July 1954)

Experiments on fluorosis have proven that excess fluoride inhibits the development of an organism. Studies on various kinds of mammalia concerned with development during the early stages, including fertilization, egg-division, incubation, metamorphosis, have not been satisfactory because of the difficulties involved in observing these stages. In addition, these stages require a long period of observation and the necessary number of individuals could not be simultaneously obtained from the same mother. Also, it is difficult to obtain serial sections of whole organs in many experimental animals. In order to avoid the above-mentioned defects, in the present examination the authors used 1400 embryos acquired by artificially fertilizing toad-eggs from the same mother. The effect of NaF upon the early development of toad-embryos was observed satistically and histologically.

Method

The mature eggs were collected from the uterus of Bufo Vulgaris during breeding season. The sperm suspensions for insemination were made by mashing testicles in concentrations of NaF solution varying from 3 mg to 100 mg%. The eggs were fertilized by the dry method with the sperm suspensions previously prepared. The fertilized eggs were kept in various concentrations of NaF solution.

To observe histological changes, 10 embryos were selected at random from 200 embryos in each experimental group and in the controls. They were fixed with Bouin's fixer, sectioned serially and stained with hematoxylin eosin.

Results

A. Influence of NaF Solution upon the Fertilization and Egg Division:

In the fertilization rates in NaF solutions, there was no significant difference between the groups exposed to 25 mg% or less and the control group. However, in the groups exposed to 50 mg% and upwards, the inhibiting effect of NaF upon fertilization was recognized to a marked degree. However, once the mature

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eggs were fertilized in the NaF solution, their egg-divisions proceeded as fast as those of the control group until the 16th cleavage stage had been reached. At that point the cleavage furrows were not as deep and as clear as those of the control eggs.

B. Slipping Phenomenon and Hatching Stage

The slipping stage of young embryos was inhibited with NaF solution, especially in the groups of 50 mg% and above. This finding was probably due to retardation of the development of the embryos and to inhibition of the effect of the enzyme involved in the slipping of embryos from the jelly string.

The hatching stage of the embryos was remarkably accelerated with NaF solution. The acceleration became greater in proportion to the increase in NaF concentration. Such acceleration had been observed previously by Kawahara with KCN solution (1, 2). It is of interest that the hatching stage of the embryos is accelerated in conjunction with the marked inhibition of their development due to excess doses of NaF or KCN.

C. Early Development and Metamorphosis

The development of the 7 day-old embryos in the 100 mg% NaF solution group was delayed and a number of abnormal embryos appeared (Table 1). Most of them showed the so-called "Lordose-Form" i. e. the retroflexion of the cranio-caudal axis (Fig. 1). These abnormal embryos had 3 to 10 wrinkles on their dorsal side. From the histological studies, it seems probable that the lordosis deformity is caused by retardation of the dorsal development with degeneration of

TABLE 1

Appearance Rates of Abnormal Embryos 9 Days After Fertilization in NaF Solutions

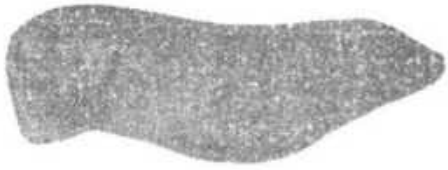
	Sampling No.	Abnormal Embryos No.	Abnormal Embryos %	Confidence limit
Control	50	0	0	0 - 4
3 mg	50	0	0	0 - 4
6 mg	50	0	0	0 - 4
12 mg	50	0	0	0 - 4
25 mg	50	0	0	0 - 4
50 mg	50	2	4	1.6 - 11.8
100 mg	50	12	24	15.8 - 35.1

the medullary tube, notochord, mesoblastic somites of the notochord, primordium of trunk muscles, etc. (Fig. 2). Almost all degenerate embryos in this group died about 10 days after fertilization (Fig. 3).

Fig. 1

Influence of NaF Solution Upon the Early Development of Toad Embryo.

9 - day old embryo in 0.1% NaF solution



9 - day old embryo in Contro (Pond water)

Fig. 2

Influence of NaF Solution Upon Hatching

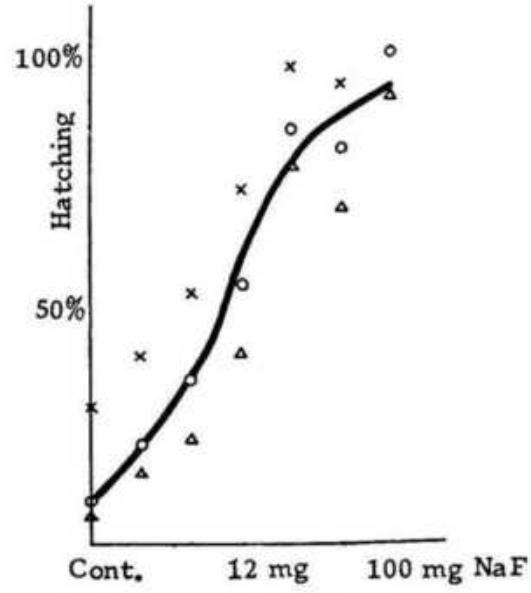


TABLE 2

Appearance Rates of Abnormal Tadpole 20 Days After Fertilization in NaF Solutions

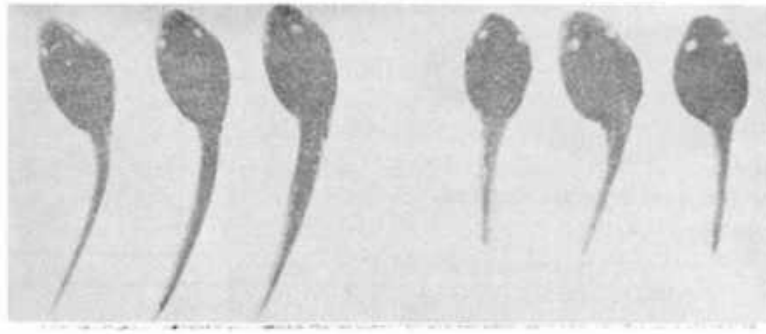
	Sampling No.	Abnormal Tadpole No.	Abnormal Tadpole %	Confidence limit
Control	50	0	0	0 - 4
3 mg	50	0	0	0 - 4
6 mg	50	0	0	0 - 4
12 mg	50	2	4	1.6 - 11.8
25 mg	50	4	8	5.5 - 17.0
50 mg	50	12	24	15.8 - 35.1
100 mg	All had died.			

In 1.6 to 11.8% the group exposed to 50 mg%, the same deformity which has been described above as having occurred in the 100 mg% group was observed but the appearance rate was not significantly different from that of the control group until 15 days after fertilization. However, 20 days after fertilization marked changes appeared in 15.8% to 35.0% of the abnormal embryos, namely

short tails and distended abdomens (Table 2)(Fig. 3). The short tail may be the result of arrested development due to degeneration of tail muscle fibers in which the disappearance of muscular striations and the kariolysis were observed microscopically and the muscular fibers run in wavy and irregular lines. It seems probable that the distended abdomen was mainly caused by the stagnation of intestinal contents due to dyspepsia which was observed histologically.

Fig. 3

Preliminary Report on the Influence of NaF Solution upon the Early Development



Control Smaller tail; distended abdomen

Among the embryos in the groups exposed to 25 mg% or less, only a small percentage were abnormal. The development and metamorphosis were markedly delayed with NaF solution.

Discussion

It is difficult to analyze fluoride's inhibiting mechanism upon fertilization, because the problem is very complex as reported by Hartmann (3). However, it is significant that egg divisions in mature eggs which were fertilized in NaF solution, proceded as fast as those of the control eggs. Also the position of cleavage furrows in eggs did not differ from that in the control eggs. Such a finding is of interest in relation to Dicken's observation of glycolysis in egg-division. In addition, it seems probable that the shallow and less pronounced cleavage furrows are related to the changes of the egg plasma structure particularly to fluoride's effect upon the Gel-sol in the cleaving egg.

Studies on the effect of NaF upon the development of organisms have been carried out by many previous investigators, but the studies on amphibians have not been clear. Arisono reported that NaF in a particular dose accelerated the development of frog embryos markedly. However, such an accelerating effect by NaF is only temporary. For example, when embryos immediately after incuba-

tion are placed in a 50 mg or a 25 mg% solution, they develop faster than the embryos of the control group but their development is inhibited later. Besides, there is much misunderstanding concerning the role of NaF solution in the development of embryos because NaF solution strikingly accelerates the incubation of embryos. The hatching stage is decided by the embryos' development as well as by the lowering of egg-jelly viscosity and egg-capsule solidity. Also the effect of hatching enzyme and slipping enzyme (4) must be considered. One of us, H. Kawahara, referred to many cases in which oxidation-reduction chemicals had accelerated the hatching stage, whereas an excess dose had inhibited the development.

The above-mentioned abnormal development seems to be caused by tissue degeneration due to inhibition of the Embden-Meyerhof glycolysis in young embryos. The percentages of deformity and mortality suggest many interesting features. For example, the 15.8 to 35.1% of deformities in the group exposed to 100 mg% solution is significantly different from that of the control group, but the 1.6 to 11.8% in the 50 mg% group is not significant. However, it is reasonable to assume that the abnormalities in the embryos receiving the 50 mg% NaF solution were caused by NaF because the deformity was similar to that observed in embryos of the 100 mg% group (Table 1).

As mentioned above, there are many cases in which the changes are closely related to the influence of fluoride, but these changes are not statistically significant. In addition, when fluoride is administered, the mean square (unbiased estimate of population variance) of measurement values widens in many other parameters, such as in the number of erythrocytes, the hemoglobin index, the number of white cells, height and weight of children, etc. Moreover, the sensitivity of embryos toward fluoride does not show a normal Gaussian distribution.

The rates of mortality in those exposed to solutions of 50 mg% and less were 0%, 10 days after fertilization but gradually increased with the passage of time. Eventually, almost all embryos died, especially in the metamorphosis and slipping stage (Table 2). Such a finding suggests that the appearance of fluorosis is closely related to the developmental stage. The effect of NaF which had accumulated in the body was also observed.

It seems that the current findings are related to many problems of vital investigations in the case of water fluoridation.

Bibliography

1. Kawahara, H.: Bull. Exp. Biol. 2, 119, 136, 1952.
2. Kawahara, H.: Bull. Exp. Biol. 3, 1-7, 1953.
3. Hartmann, M., Kuhn, R., Schartau, O. u. Wallenfels, K.: Naturw., 28, 144, 1940.
4. Kawahara, H.: Bull. Exp. Biol. 2, 41 (in Japanese) 1951.

ODONTOLOGICAL OBSERVATIONS OF MT. ASO-VOLCANO DISEASE

Part I

by

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(Abstracted from Shikoku Medical College Magazine, 1: 49-53, October 1950)

One of the most conspicuous symptoms of the so-called "Aso-Volcano Disease" is dental fluorosis or mottling of the tooth enamel. It is frequently found among the villagers near the Mt. Aso-Volcano.

Mottled teeth in the Mt. Aso district show macroscopically no special difference from those of other districts. Minoguchi determined that the water of an artesian well at Bochu, a village in the Aso district, contained 1.4 ppm of fluoride on a rainy day. According to my survey made at Kokuryu, Imamachi and Narikawa in the Aso district, the percentage of the patients with fluoride mottled teeth reached almost 100% among children who were using water from artesian wells, but no mottling occurred among those who were using ordinary wells. According to another survey made by the author in the Dogo district, Ehime Prefecture and in Osato-mura, Tottori Prefecture, the amount of fluoride in water and the incidence of mottled teeth were directly related. Mottled teeth in the Aso district, as in the other districts, must therefore be caused by fluoride.

Concerning the percentage of caries among M (children with mottled teeth) and $\underline{M}$ (children without mottling) at Osato-mura, Tottori Prefecture and certain towns and villages of the Aso district, the Yates' index showed x 90%. Thus, M and $\underline{M}$ did not differ significantly. Only in the intermediate school of Miyaji, where the highest percentage of caries was encountered x was 33%, a value closer to the significant standard ($x = 5\%$) in comparison with every other village school.

It appears that caries preventing factors other than fluoride predominate in places where the caries incidence is comparatively low. Although the percentage of mottled teeth in this school did not differ from that in other schools, it was recognized that mottling in the Miyaji school was less severe.

This fact suggests that a rise in degree of mottled teeth is associated with an increased percentage of caries. As staged above, the lack of a significant difference in the percentage of dental caries between M and $\underline{M}$ may be due to the fact that mottled teeth are already in existence before school age. Moreover, since most children of school age are often obliged to move from one place

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TABLE 1

Mottled Teeth and Caries at Uchinomaki Intermediate School

School Year		1	2	3	Total
No. of Cases		36	104	88	228
Classification of mottled teeth	I	2 (5.5)	1 (1)	2 (2.3)	5 (2.2)
	II	4 (12.9)	16 (15.5)	17 (12.3)	37 (16.2)
	III	4 (12.9)	10 (9.6)	10 (11.5)	24 (10.5)
	IV	0	0	1 (1.2)	1 (0.4)
	V	2 (5.5)	4 (3.8)	3 (3.4)	9 (3.9)
	VI	1 (2.8)	6 (5.8)	3 (3.4)	10 (4.4)
	VII	0	0	0	0
M		13 (36.2)	37 (35.6)	37 (42)	87 (38.1)
*M • C		1 (7.6)	10 (27)	6 (16.2)	17 (20.7)
$\underline{M}$		23	69	51	141
**$\underline{M}$ • C		1 (4.3)	11 (16)	11 (21.6)	23 (16.3)
C		2 (5.5)	21 (20.1)	17 (19.3)	40 (17.5)

TABLE 2

Mottled Teeth and Caries at Namino Intermediate School

School Year		1	2	3	Total
No. of Cases		28	41	29	98
Classification of mottled teeth	I	0	0	0	0
	II	7 (25)	8 (19.5)	7 (24)	22 (22.4)
	III	4 (14.3)	5 (12.2)	3 (10.3)	12 (12.5)
	IV	0	0	1 (3.4)	1 (1)
	V	0	2 (4.9)	0	2 (2)
	VI	0	2 (4.9)	0	2 (2)
	VII	0	0	0	0
M		11 (39.4)	17 (41.4)	11 38	39 (39.8)
*M • C		1 (9.1)	1 (5.9)	0 (0)	2 (5.1)
$\underline{M}$		17	24	18	59
**$\underline{M}$ • C		2 (11.7)	1 (4.1)	1 (5.5)	4 (6.8)
C		3 (10.7)	2 (4.9)	1 (3.4)	6 (6.1)

*M • C: Number (percentage) of children who have both mottled teeth and dental caries of first molar (6 6).
**$\underline{M}$ • C: Number (percentage) of children who have no mottled teeth, but dental caries of first molar (6 6).

Note: Classification of mottled teeth (by SATA): I, pearly-brilliant; II, chalky-spotted; III, chalky-linear; IV, map-like mottled; V, chalky; VI, brown; VII, enamel defective (enamel-hypoplasia).

to another, they have many chances to drink various kinds of water, in which the percentage of fluoride is not always constant.

In an investigation of the caries incidence at the Kuchiyama intermediate school, Kuchiyama-mura,Tokushima Prefecture, no fluoride in drinking water nor mottled tooth were found among villagers. This served as a control for comparison of the results with the previously obtained data at both the Uchinomaki and Namino intermediate schools in the Aso district. The living standard of the Kuchiyama children ranges approximately halfway between that of the two other communities.

The results of the investigation were as follows:

1) As to the difference of percentage of caries between

Namino ($C = 2$, $\underline{C} = 37$) and Kuchiyama ($C = 21$, $\underline{C} = 65$)

we found the following indices respectively (Table 1):

$$t = 2.5, \quad Gt = 0.49, \quad \alpha = 1.2\%$$

Namino therefore showed a significantly lower percentage of caries than Kuchiyama.

2) As to the difference between Uchinomaki ($C = 17$, $\underline{C} = 70$) and

Kuchiyama, a significant difference must be recognized (Table 2).

$$t + 1.64, \quad Gt + 0.45, \quad \alpha + 10\%$$

As mentioned above, we could not find a significant difference in the caries incidence between mottled and nonmottled teeth within the same environment of the Aso-district, but could clearly establish such a difference between two low fluoride places (Aso and Kuchiyama). This fact suggests that the caries-preventing effect of fluoride is not necessarily due to the entry of fluoride through the blood stream.

However, since the counts of acid-forming lactobacilli in the salivas of those children were also very low, it is difficult to ascertain whether the caries reduction can be attributed to lessened solubility of the teeth or to a bactericidal effect on the acid-forming organisms.

Whereas fluoride therefore acts to prevent dental caries as noted above, its excess in drinking water causes dental fluorosis which leads not only to mottled teeth, but to many other pathological symptoms.

The height and growth of children in the Aso district compared with that of children in Osato-mura, Tottori Prefecture, was below average in the Prefec-

ture and the surrounding area. In the Aso-district, I found radiographic changes on Ossa corpi, Ossa metacarpi and Ossa digitorum manus and, at the same time, a slight anemia, a slight shift of leucocytes toward the left and appearance of the toxic-granules in blood cells. The degree of these symptoms of fluorosis seems to be dependent on various secondary factors, especially intake of vitamins. Therefore, we must question whether or not the accepted concentration of fluoride in water of 1.0 ppm advocated in America is appropriate for prevention of dental caries in Japan.

(The data of Part II of this research is covered in the article by Prof. Takamori on pages 158 to 160, Editor).

X-RAY STUDIES ON THE DEVELOPMENT OF THE BONES OF THE HAND IN HEALTHY JAPANESE AND SKELETAL CHANGES IN "ASO-VOLCANO DISEASE"

Part I

STUDIES ON THE DEVELOPMENT OF THE BONES OF THE HANDS IN HEALTHY JAPANESE

by

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(Abstracted from Shikoku Acta Medica 10:442-455, June 1957)

Before undertaking studies on skeletal changes in the "Aso-Volcano Disease", it is necessary to record the normal bone development in healthy Japanese. Previous reports have dealt with the time of ossification and the development of pseudoepiphyses of the hand bones.

This investigation was therefore undertaken in order to obtain a more exact knowledge of the development of the hand bones in healthy Japanese.

The studies reported here are based on a careful appraisal of roentgenograms of 600 males and 650 females from 9 to 24 years of age in Tokushima city compared to 475 male and 536 female controls, 9 to 19 years of age in the mountains, Tokushima Prefecture. Among elderly persons, roentgenograms of 22 men and 18 women in Tokushima city and of 20 men and 20 women in the Tokushima Old People's Home from 69 to 99 years of age were examined.

The following observations were made:

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I. The ages when the process of ossification is completed and the cartilagenous disk has entirely disappeared, are as follows:

	Males	Females
1) In Tokushima city		
Phalanges and Metacarpals,	14 to 18	20 to 16
Distal extremity of Radius and of Ulna.	16 to 19	14 to 18
2) In the mountains		
Phalanges and Metacarpals,	14 to 19	13 to 18
Distal extremity of Radius and of Ulna.	17 to over 19	16 to 19

3) In young subjects, ossification was completed earlier in city dwellers than in subjects residing in the mountains.

4) In females, ossification was completed about two years earlier than in males.

5) The sequence in which the final stages of ossification occurs is as follows:

Distal phalanx; – Metacarpal I; – Proximal phalanx; – Middle phalanx; – Metacarpal; – Distal extremity of radius and of ulna.

6) The process of synostosis advances from the thumb towards the little finger.

7) In Japanese, ossification is completed much sooner than in the Europeans and Americans.

II. An irregular, wavy borderline of epiphyseal cartilage appears immediately before the synostosis commences. Only slight variations occur in the normal female and even less in the normal male.

III. Bone Atrophy: Both in Tokushima city and in the mountains, slight atrophy was observed in a few individuals.

IV. Osteosclerosis: The frequency of osteosclerosis in the male is relatively low. In the female, it is manifest about age 15 and increases with advancing age. A higher incidence of osteosclerosis was observed in the mountains than in the city.

V. Brachyphalangy (shortened phalanges): The order of incidence of brachyphalangy is as follows:

Middle phalanx of the 5th finger — Distal phalanx of the 1st finger — Distal phalanx of the 5th finger

Other phalanges and metacarpals also exhibited brachyphalangy but less frequently than the three phalangeal bones noted above.

VI. In the male, pseudoepiphyses were observed up to age 12. Thereafter they decreased gradually and disappeared at age 15 in Tokushima city and at 16 to 17 years of age in the mountains. In the female they are most frequently observed up to age 10; they disappear two years sooner than in the male. Pseudoepiphyses occur in the following order:

Metacarpal I; — Middle phalanx V; — Metacarpal II; — Metacarpal V; — Middle phalanx IV

With the exception of the distal phalanges, pseudoepiphyses may be found sometimes in other phalanges and metacarpals also. They appear to be a general physiological phenomenon.

VII. The incidence of atrophy and arthrosis deformans, which are recognized as senile changes of the bones, was higher among the inmates of the Tokushima Old People's Home than among residents of Tokushima city.

Part II

SKELETAL CHANGES IN "ASO-VOLCANO DISEASE"

by

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(Abstracted from Shikoku Acta Medica 10:456-463, June, 1957)

Mottled teeth are found among humans, cattle and horses in the Aso-Volcano district, Kumamoto Prefecture. In 1942, Hatano and co-workers observed both dental and skeletal changes. They named the disease "Aso-Volcano Disease". Takamori and his colleagues,who surveyed the same district in 1949, observed that the abnormalities in the skeleton and blood caused by the disease were similar to those which had been recorded as Kaschin-Beck's disease in Manchuria. Because of insufficient material, their investigations were not completed. Subsequently, they carried out five additional surveys in order to obtain a more exact knowledge of "Aso-Volcano Disease". The author, one of Takamori's co-workers, examined the skeletal changes as a part of these investigations.

Roentgen X-rays of the hand bones of 582 males and 610 females from 7 to 90 years of age in Aso district were compared statistically with those of healthy Japanese reported by the author in Part I.

The following conclusions were drawn:

1) The development of the bones of the hand among the inhabitants in Aso-Volcano district was very similar to that in the mountains, Tokushima Prefecture.

2) The changes corresponded to the third stage of the skeletal features in Kaschin-Beck's disease in the aged, but they are believed to be senile phenomena.

3) No significant difference was observed in the development of bones between persons having mottled teeth and those without mottled teeth. The development of the bones in the Aso district, therefore, is not believed to be influenced by fluoride in drinking water.

4) The present study revealed that the changes which were thought to be similar to "Kaschin-Beck's Disease" do not occur in "Aso-Volcano Disease".

Ossification of Bones in Hands

Fig. 1

Age 15 ♀ Incomplete

Fig. 2

Age 17 ♀ Complete

Note: Brachyphalangy (B)

ROENTGENOGRAPHICAL STUDIES ON SKELETAL CHANGES OF SCHOOL CHILDREN DRINKING PRIVATE SERVICE WATER IN OMUTA CITY

by

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(Abstracted from Shikoku Acta Medica 11:731-735, Dec. 1957)

Mottled teeth can be found among the inhabitants in the Aso-Volcano district, Kumamoto Prefecture. In 1942, Hatano et al. observed not only dental but also skeletal changes; they named the disease "Aso-Volcano Disease". In 1953, Takizawa et al. reported that the skeletal changes caused by "Aso-Volcano Disease" were similar to those of Kaschin-Beck Disease. The disease was observed not only in the Aso-Volcano district but also in Fukuoka Prefecture, Nagano Prefecture, Shizuoka Prefecture and various districts in Japan.

In November, 1956, we explored the skeletal changes of school children drinking private service water supplied by the Mitsui Coal Mine Company in Omuta city, which is practically fluoride free.

X-rays were taken of bones of the hands in 250 male students and 217 females, 10 to 18 years of age. The data thus obtained were compared statistically with those of the development of healthy Japanese reported by one of us (H.W.). The following conclusions are drawn:

1) The development of the bones of the hands among the school children drinking private service water in Omuta city is similar to that in Tokushima city where the fluoride content is low.

2) The kind of skeletal changes in the "Aso-Volcano Disease" which have been thought to be similar to those of Kaschin-Beck's Disease, were not encountered in the Aso-Volcano district nor in school children drinking private service water in Omuta city.

3) In drinking water in Omuta city, both in city water and in private service water, the fluoride content is only 0.1 to 0.2 ppm.

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Part I

INFLUENCE OF CHRONIC FLUOROSIS ON THE BONE GROWTH, PARTICULARLY ON THE EPIPHYSEAL CARTILAGE

by

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(Abstracted from Shikoku Acta Medica 4:151-162, August, 1953)

An extensive literature has accumulated on the bone changes due to fluorosis. However, little attention has been given to the influence of fluoride on bone growth, particularly on the epiphyseal cartilage which plays the most important part in the longitudinal growth of long bones.

Furthermore, the findings in spontaneous and experimental bone fluorosis are contradictory. In cryolite workers, the bone changes were recognized as sclerotic. In experiments, osteosclerosis has been reported in some instances, osteoporosis in many others. The current study has been undertaken in order to determine how a fluoride regime affects the growth of bones in animals.

Method

Rabbits, 30 to 60 days of age were given orally 30 to 140 mg/kg of sodium fluoride for 30 to 200 days. The animals were sacrificed at various intervals during this period and every long bone was studied both macroscopically and histologically.

Results

1) Fluoride poisoning due to the above dosage led to an extreme disturbance of the longitudinal growth of long bones and a reduction in bone weight compared with that of the controls (Fig. 1).

2) Bone trabeculae and compact cortical layers were reduced in thickness and increased osteoclastic resorption (osteoporosis) was recognized. A long-term fluoride regime caused apposition of immature (coarse fibrillar) bone adjacent to the periosteal and endosteal surfaces (Fig. 2 to 4).

3) The epiphyseal cartilage became thinner and showed a wavy borderline associated with a group of osteoclasts adjacent to the metaphysis (Fig. 5 and 6). Prolonged fluoride intake caused formation of the terminal plate at the zone of hypertrophy (Fig. 7). In the zone of proliferation, the cells were reduced in size

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Fig. 1

Size of Fluorosed Tibia and Femur
(Rabbit No. 37: Control; No. 33: NaF 30 mg/kg, 91 Days)

Normal bone (control)

Fluorotic bone (NaF)

↑Spontaneous Fracture

and number and their arrangement was irregular. Less proliferation and a decreased tendency to cell regeneration were noted. In this study a comparison of the age of synostosis, i. e. the closure of epiphyseal cartilage, in the control animals with that of the fluorotics showed a distinct retardation of closure in fluorosis.

By means of measuring total bone width and epiphyseal zone width in microns, we calculated the percentage of zone width to total width, namely

$$\frac{\text{zone width}}{\text{total width}} \times 100,$$ furthermore the ratio of $$\frac{\text{zone width of hypertrophy}}{\text{zone width of proliferation}}$$

and the number of cells of every zone. Thus the mechanism of normal synostosis of rabbits was observed (Fig. 2). The epiphyseal cartilage of early closing bones, i. e. phalanx, metacarpus and metatarsus, showed an increase in percentage of hypertrophy and a decrease in proliferation. Therefore the ratio was increased.

On the other hand, in epiphyseal cartilage of late-closing bones, i. e. proximal humerus, femur and tibia, there was little difference between the degree of hypertrophy and that of active proliferation of cells in order to grow. The resulting ratio was below or above one. The observation made upon the preparatory stage for synostosis even in the early stage of growth was of major interest.

The growth curves of the control animals were obtained in the same way. These curves were compared with those of the experimental animals. The zone width of proliferation in the experimental animals was reduced both absolutely (expressed in microns) and relatively (in percentage) as well as in the number of cells. The ratio was also reduced excessively compared with that in the normal control animals in which the ratio increased gradually from the time of birth. In other words, in fluorosis a retardation of synostosis occurred.

The decrease of zone width in microns and percentage and the number of proliferative cells suggests suppressed proliferation. A decrease of the ratio indicates retardation of synostosis. These observations are in agreement with the histological investigation. The impairment of the longitudinal growth of large bone seems to be due to the retardation of synostosis of every epiphyseal cartilage.

4) Cells in the bone marrow were decreased and the marrow showed gelatinous change.

Comparison of Normal and Fluorotic Bones of Rabbits

Fig. 2

Rabbit No. 14 - Control
Femur (proximal) (x 100)

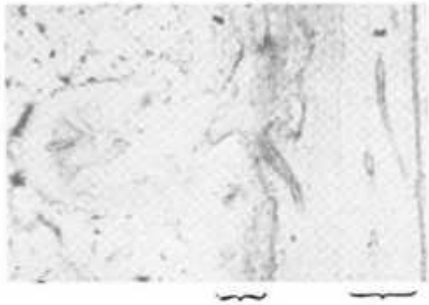
Epiphyseal cartilage

Fig. 3

Rabbit No. 13, 50 mg/kg
(143 days) Femur (proximal) (x 100)

Fig. 4

Rabbit No. 24, NaF 30 mg/kg (64 days),
Femur (x 100)



IE IP

Apposition of immature bone (coarse fibrillar) adjacent to the periosteal (IP) and endosteal surfaces (IE).

Fig. 5

Rabbit No. 29 - Control,
Humerus (proximal) (x 100)

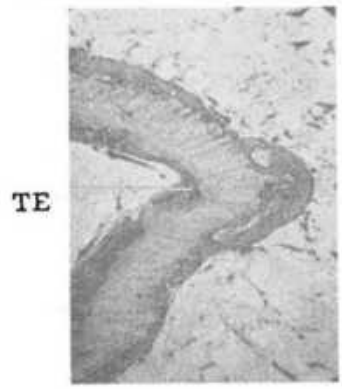
Fig. 6

Rabbit No. 27. NaF 30 mg/kg (31 days)
Humerus (proximal) (x 100)

P: Irregular arrangement of the proliferating cartilage cells.
W: Wavy borderline of the cartilage.

Fig. 7

Rabbit No. 32. NaF 30 mg/kg (91 days)
Tibia (distal) (x 100)



TE: Terminal plate of the epiphysis
TM: Terminal plate of the metaphysis

Part II

GROWTH AND BONE CHANGES OF GROWING RATS FED VARYING CONTENTS OF FLUORIDE

by

K. Kono
Tokushima, Japan

(Abstracted from Shikoku Acta Medica 5:30-39, April 1954)

Endemic dental fluorosis occurs in many parts of the world. However, cases of chronic fluoride intoxication with skeletal changes reported to date are comparatively rare. Endemic bone fluorosis was recently found where water contains fluoride naturally in India, Italy, Africa, South China and in Japan. These reports are chiefly concerned with the bone changes in adults but no data exist on how fluoride affects bone growth.

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

The effects of fluoride upon bone vary with the magnitude and duration of fluoride intake, age and species of animal. It is possible that varying amounts of fluoride affect bones differently in childhood during the active phases of growth.

Both in the Aso Volcano district and in other fluoride areas, Takamori (20) found that large quantities of fluoride inhibited height, weight and chest development in school children, but in some cases fluoride accelerated growth.

In this study, the effects of various amounts of fluoride upon bone growth and, especially, upon epiphyseal cartilage were investigated and a classification of the mechanism of bone fluorosis is presented.

Methods and Procedures

Growing rats, 30 to 50 days old, were given several kinds of food to which sodium fluoride was added in concentrations of 375, 100, 50, 10, 5 or 2 ppm. The animals received Sherman's standard ration and Morinaga dry milk which contained vitamins and mineral salts. The animals were sacrificed at intervals of about one month and were compared with littermate controls which were fed the standard ration without fluoride.

Sherman's standard ration consists of wheat flour 65.8%, powdered milk (Morinaga dry milk) 32.9%, NaCl 0.65%, $CaCo_3$ 0.65%.

Vitamin and mineral contents in 100 gram of Morinaga dry milk: Vitamin A, 2000 I.U.; Vitamin B_1, 0.63 mg; Vitamin B_2, 0.9 mg; nicotinic acid, 4.0 mg; Vitamin B_{12}, 2γ, Vitamin C, 40.0 mg; Vitamin D, 400 I.U.; Ca, 820.0 mg; phosphoric acid, 510.0 mg; Fe, 5.0 mg.

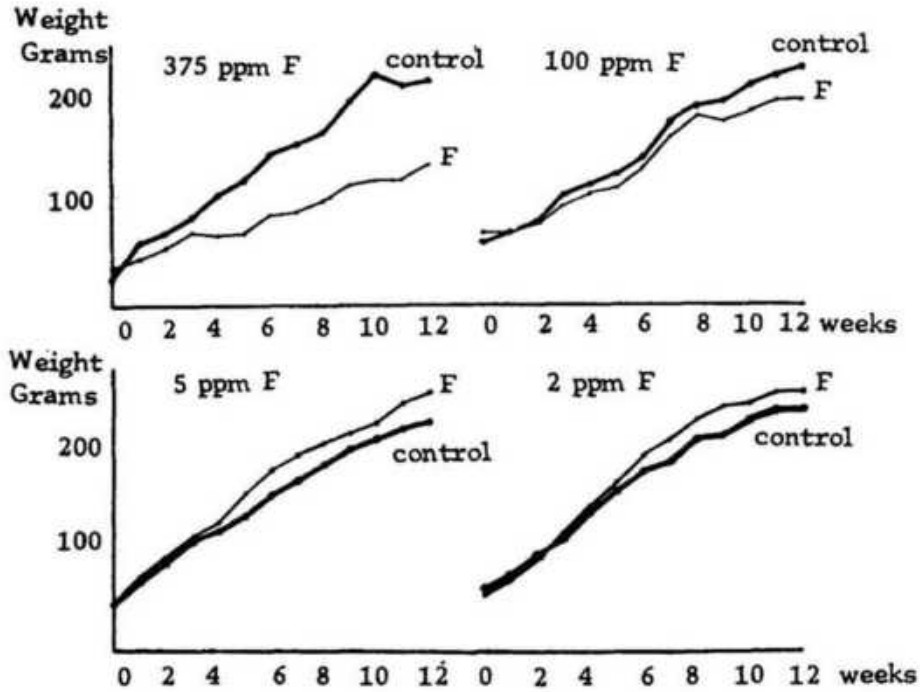
Results

1) In each group, which were given more than 50 ppm (F: 5.84 to 6.93 mg/kg) the weight decreased (Fig. 1). In the group receiving 375 ppm (F: 24.21 to 31.03 mg/kg) it decreased markedly ($\alpha = 0.05$). On the other hand, in the group receiving 10 ppm (F: 1.08 to 2.86 mg/kg), the weight showed a tendency to increase only during the first six weeks. Both in the 5 ppm group (F: 0.49 to 0.71 mg/kg) and the 2 ppm group (F: 0.18 to 0.44 mg/kg) the weight showed a marked increase ($\alpha = 0.05$) starting with the administration of fluoride, especially the 5 ppm group.

2) Histologically, in the groups which received more than 50 ppm, the bone trabeculae and cortical layers showed excess resorption. They turned into a coarse fibrillar substance and were transformed gradually into osteoporosis with active osteoclastic resorption. On the other hand, in the animals which received 50 ppm on a short term basis and those which received 5 and 2 ppm, the bones developed fairly well; bone trabeculae became thicker and longer than in the control; the regeneration of cortical layers was active.

Fig. 1

Average Weekly Body Weight of Rats Given 375, 100, 5 and 2 ppm NaF



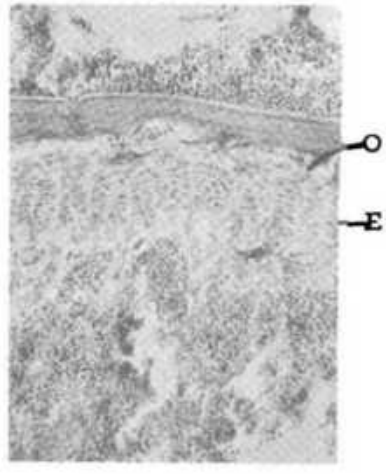
3) With respect to the synostosis of epiphyseal cartilage, the retardation of synostosis appeared in the groups which had more than 50 ppm of fluoride, whereas in the 10 ppm fluoride group, a tendency to premature synostosis was recognized. Premature synostosis occurred in every rat which received 5 and 2 ppm. In fact, synostosis was already completed in metacarpi and metatarsi, when it had not as yet commenced in the controls.

4) In the groups receiving more than 50 ppm of fluoride, epiphyseal cartilage became thinner and developed into a wavy borderline or a saw tooth shape; cartilage cells showed atrophy and pyknosis. On the other hand, in the small dosage groups, some degree of thinness and waving could be seen, but no atrophy and pyknosis of cells could be detected (Fig. 2 to 4).

5) By measuring the total and zone widths of epiphyseal cartilage in microns, percentage of zone width to total width, ratio of $\frac{\text{zone width of hypertrophy}}{\text{zone width of proliferation}}$ and the number of cells of every zone, the mechanism of normal synostosis of controls was observed when the rats matured into the stage of synostosis. The epiphyseal cartilage showed the following features:

Fig. 2

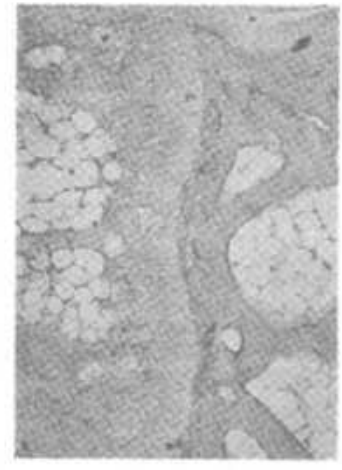
Rabbit No. 78 Fed 375 ppm of NaF 41 Days. Tibia (proximal) (x 100)



Epiphyseal cartilage (E) thinner; wavy borderline followed a group of osteoclast (O) adjacent to zone or hypertrophy. The cartilage cells show severe atrophy. Bone trabeculae are extremely scarce.

Fig. 3

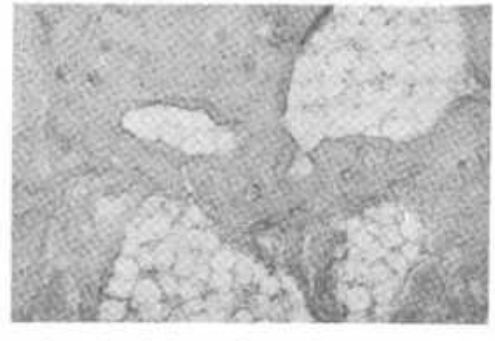
Rat No. 103 - Control



Beginning synostosis

Fig. 4

Rat No. 102 Fed 5 ppm of Fluoride for 100 Days, the Second Metacarpus (distal) (x 70)



Synostosis of epiphyseal cartilage completed.

a) Diminution of total width of epiphyseal cartilage.
b) Increasing difference of percentage of zone of proliferation from that of hypertrophy.
c) A marked decrease in number of cells in the zone of proliferation.

Comparing the curves of the fluoride groups, it is clear that in those which received more than 50 ppm of NaF, the difference in the percentage of zone of proliferation and that of hypertrophy was not increased. Consequently, the ratio was not higher and it did not occur in the pre-stage of synostosis. On the contrary, in the small dosage groups the three conditions were apparently shown.

Summary and Discussion

The literature on bone fluorosis as an occupational disease in cryolite workers and on endemic fluorosis in certain districts of the world is extensive. These reports were chiefly concerned with bone changes in adults, not with bone growth.

In Part I of this study, 30 to 140 mg/kg of sodium fluoride was given orally to growing rabbits during a maximum of two hundred days; diminution of weight and osteoporosis occurred in all cases. Long-term fluoride ingestion (178 to 200 days) caused apposition of immature (coarse fibrillar) bone adjacent to the periosteal and endosteal surfaces.

In the current study, it was shown that comparatively large amounts of fluoride prevent bone growth of rats and delay synostosis. In contrast, comparatively small amounts of fluoride accelerate bone growth and synostosis is completed in an earlier stage. It is of striking interest that fluoride when administered in differing amounts causes marked differences in bone alteration and exerts an influence upon the body weight of growing rats.

Skeletal fluorosis is not as directly related to fluoride intake as are mottled teeth. The amount of fluoride which usually causes marked bone changes in certain districts in India apparently rarely does so in America. It is noteworthy that in a given fluoride area, no bone changes were observed in patients with severe dental fluorosis, whereas deformities with only slight mottling were manifested in others. In general, the inhabitants in these fluoride districts are poor and in needy circumstances. The findings, therefore, suggest that fluoride in soil and water may influence the development of bone changes especially when it is associated with defective nutrition (vitamins or amino acids).

Takamori published studies on bone changes, i.e. premature synostosis of epiphyseal cartilage caused by acceleration of enchrondral ossification, bone atrophy and malformation of large bones and joints (Kaschin-Beck's disease) in the

natural fluoride areas in Manchuria. Hatano found bone changes in the Aso-Volcano district, which were considered identical with those in Kaschin-Beck's Disease. Takamori suggested that morbidity in these two diseases might be reduced by improving the nutritional standards. However, the bone changes in fluoride areas show marked differences due to such factors as malnutrition, age, race, etc.

EFFECTS OF SMALL QUANTITIES OF FLUORIDE ON THE GROWTH OF BONE, ESPECIALLY EPIPHYSEAL CARTILAGE, IN VITAMIN A DEFICIENCY

Part I

CHANGES IN THE GROWTH OF BONE, ESPECIALLY EPIPHYSEAL CARTILAGE, DUE TO DIETS DEFICIENT IN VITAMIN A

by

Hiroshi Kakihara
Tokushima, Japan

(Abstracted from Shikoku Acta Medica 12: 721-728, April 1958)

Impairment of bone growth in vitamin A deficiency has been well documented for many years. No systematic and detailed description, however, has as yet been forthcoming concerning the ossification period of the epiphyseal cartilage of long bone.

In order to observe the effect of fluoride in small doses on bone growth in vitamin A deficiency, it was necessary to make a preliminary study of the bone changes, especially the ossification period of epiphyseal cartilage in vitamin A deficiency. The purpose of the current investigation was to obtain knowledge upon which to base Part II of this study, namely the effect of fluoride in the diet in minute quantities upon the ossification period in vitamin A deficiency.

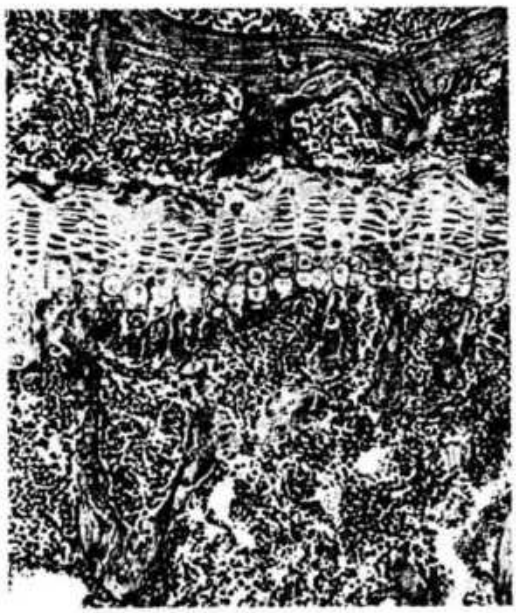
Material and Method

Young albino rats of the same sex and same litter were kept alive for 35 to 121 days and fed a Saheki and Fujimaki diet which is deficient in vitamin A. Histological observations were subsequently made on their long bones.

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

Fig. 1

Vitamin A Deficiency Epiphyseal Cartilage of Proximal Tibia (x 100)



Marked cellular infiltration, irregular outline of trabeculae (T), reduced thickness of cartilage (C)

Fig. 2

Control Normal Tibia (x 100)

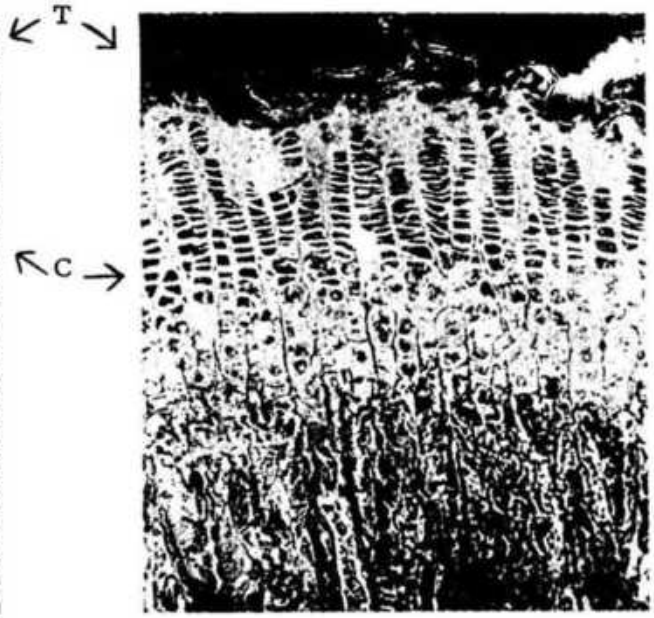


Fig. 3

Vitamin A Deficiency, Metacarpus

Ossification beginning

Fig. 4

Control - Metacarpus Ossification

Ossification completed

1) The body weight was considerably decreased in the group of absolute and relative vitamin A deficiency compared with that in the control group. A difference was also noted between the group of absolute and of relative vitamin A deficiency.

2) All long bones showed a marked decrease in length in the experimental groups compared to the bones of the controls.

3) Histologically, the epiphyseal cartilages became markedly thin; the columns of cells were arranged disorderly; the cells in general were atrophied and markedly decreased in number (Fig. 1 to 4).

In the deeper layers of the epiphyseal cartilage, an invasion by newly formed blood vessels occurred. The endochondral bone growth was markedly impaired.

4) That the ossification period of the epiphyseal cartilage was markedly delayed, was confirmed by measuring the width of the epiphyseal cartilage and by the cell count.

5) The cortex of bone was severely thinned and was devoid of trabeculae in the sections examined. The osteoblasts were less active and osteoporosis was present. The bone marrow showed pronounced hyperemia.

Part II

CHANGES IN THE GROWTH OF BONE, ESPECIALLY EPIPHYSEAL CARTILAGE, DUE TO DIETS DEFICIENT IN VITAMIN A AND TO FLUORIDE ADDED IN SMALL QUANTITIES

by

Hiroshi Kakihara
Tokushima, Japan

(Abstracted from Shikoku Acta Medica 12:729-742, April 1958)

A previous report dealt with the changes induced in bone by vitamin A deficiency. This investigation delineates the results of experiments concerned with the influence of fluoride, added in small quantities to a vitamin A deficient diet, upon the growth of bones, especially upon the epiphyseal cartilage which is of primary importance for growth of bone in length.

Method

Vitamin A deficient diet, devised by Saheki and Fujimaki, was given to

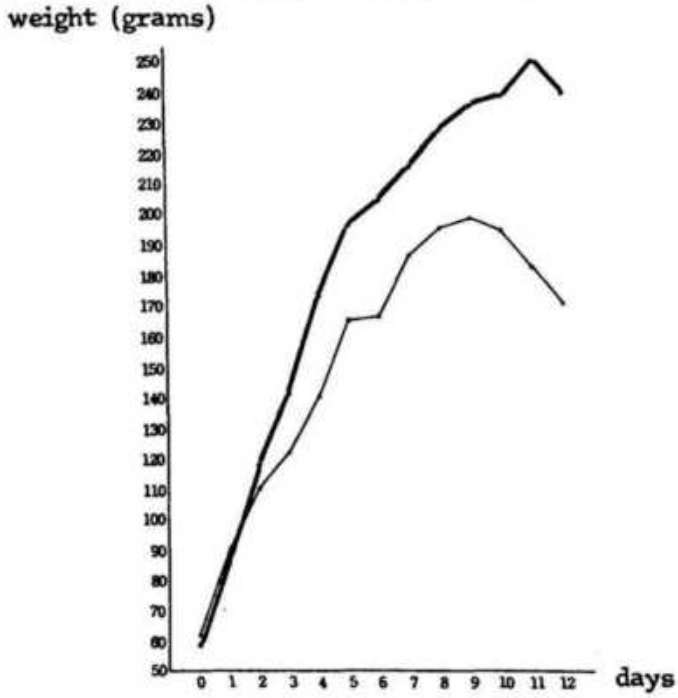
young albino rats of the same sex and the same litter. Sodium fluoride was added in such a way that the total fluoride intake did not exceed 5 ppm or 10 ppm. The rats were kept on this diet for 39 to 103 days, after which they were sacrificed and their long bones examined histologically.

Results

1) All experimental animals showed a decreased body weight compared with that of the controls (Fig. 1). The rats given 10 ppm of fluoride combined with the vitamin A deficient diet, however, weighed generally more than the rats which received the vitamin A deficient diet without supplementation of fluoride.

Fig. 1

Weight Curves of Vitamin A Deficient Diet



Rat No. 110: Vitamin A deficient diet.
Rat No. 108: Added 10 ppm NaF plus Vitamin A deficient diet.

No significant difference was obtained in the weight between the group which received 5 ppm of fluoride in conjunction with the vitamin A deficient diet and the group kept on the vitamin A deficient diet without added fluoride.

2) In all experimental animals, impaired growth of long bones was confirmed by measuring the length of the long bones.

Fig. 2

Metatarsal Ossification, Control (Normal Bone)

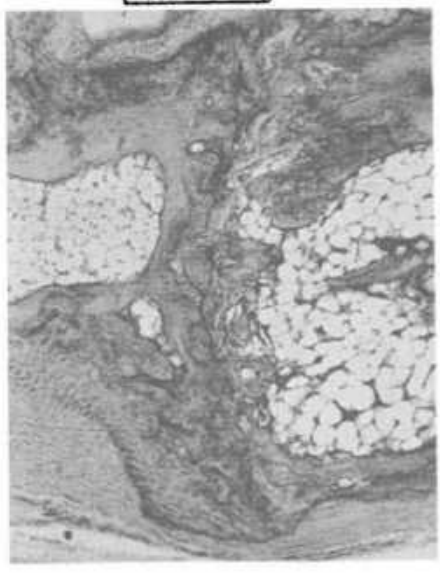


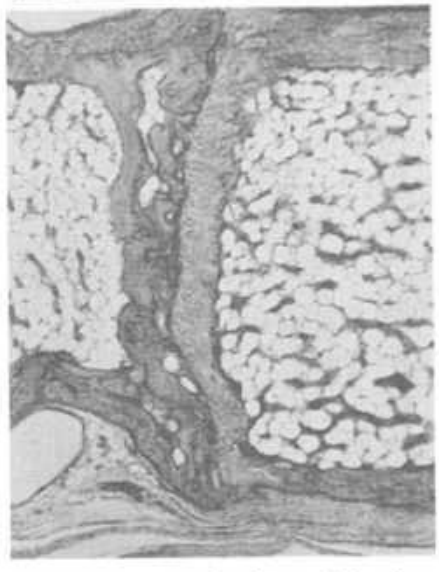
Fig. 3

Vitamin A Deficiency, Radius (x 40)

Beginning of ossification

Fig. 4

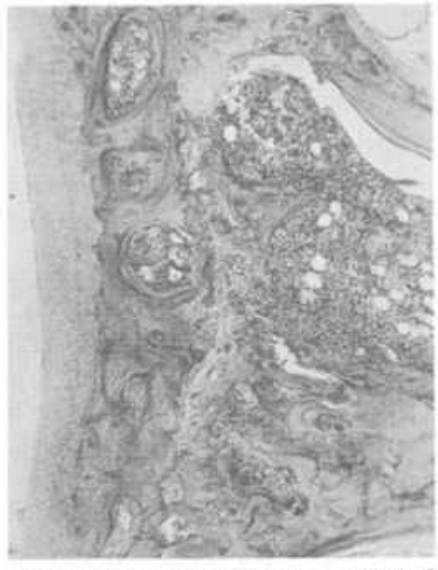
Metatarsal Ossification, Vitamin A Deficiency Fluoride plus 10 ppm (x 40)



Increase in bone density; cellular infiltration and hyperemia of marrow.

Fig. 5

Vitamin A Deficiency plus 10 ppm NaF



Completion of ossification. Marked osteoclastic activity. Irregular border of trabeculae.

3) Histologically, endochondral bone growth in the epiphyseal cartilage was notably impaired. This growth impairment was less pronounced in the group given 10 ppm of fluoride together with the vitamin A deficient diet than in the group submitted to vitamin A deficient diet alone (Fig. 2 to 5).

A distinct difference in the degree of growth impairment was observed between the group given 5 ppm of fluoride in combination with the vitamin A deficient diet and the group given the vitamin A deficient diet without fluoride.

4) The ossification period of the epiphyseal cartilage was delayed in all experimental animals as compared with that of the controls. Ossification was detected earlier in the group given 10 ppm of fluoride combined with the vitamin A deficient diet than in the animals on the vitamin A deficient diet without fluoride supplementation.

No particular difference was observed between the group whose vitamin A deficient diet was supplemented by 5 ppm of sodium fluoride and the group whose vitamin A deficient diet contained no fluoride.

The histological changes and the ossification period of the epiphyseal cartilage were corroborated by measuring the width of the different layers of the epiphyseal cartilage and by counting the number of cells.

5) Osteoporosis was observed in the cortex and trabeculae of all bones. The number of bone marrow cells was decreased and hyperemia was present in the fluoride rats.

CHANGES OF THE HEART MUSCLE DUE TO CHRONIC FLUOROSIS

Part I

ELECTROCARDIOGRAM AND CARDIAC X-RAYS IN INHABITANTS OF A HIGH FLUORIDE ZONE

by

Iteshi Okushi
Tokushima, Japan

(Abstracted from Shikoku Acta Medica 5:159-165, June 1954)

Studies on the influence of fluoride upon the heart are sparse. In reviewing the literature, clinical data pertaining to the cardiovascular system in acute fluoride toxicosis were presented by Tappeiner (1, 2), Roholm (3, 4) and Larner (5), pharmacological data by DeNito (6), Gottdenker and Rothberger (7, 8) and Chenowith (9) and electrocardiographic studies by Chatel (10) and Lepeschkin (11), but no systematic studies on the involvement of the heart in fluoride toxicosis are available. Dental fluorosis occurs in chronic fluoride poisoning but not in acute fluoride toxicosis.

Sodium fluoride in drinking water has recently been advocated for the prevention of dental caries. A study of the effect of chronic fluoride toxicosis upon the heart was therefore of interest.

The author has conducted electrocardiographic and roentgenologic examinations in Shionoe village, Kagawa Prefecture, in 1952, and in Odani village, Sanyo town, Okayama Prefecture, in 1953. Many instances of myocardial damage were observed electrocardiographically; other cases, exhibiting hypertrophy and dilatation of the cardiac chambers, were recorded roentgenographically.

Methods and Procedures

In 1952, the author examined electrocardiograms and X-rays of 35 residents (25 children and 10 adults) of Shionoe village, Kagawa Prefecture, and of four adults of Odani village, Okayama Prefecture, in 1953. The electrocardiographic examinations were carried out with standard limb leads, and roentgenologic examinations were performed by 2m, 58KV, 30 m^A. The fluoride content of drinking water was determined by the aluminum hematoxylin method (12). The mottled enamel of the inhabitants who were examined was classified by Kawahara (13).

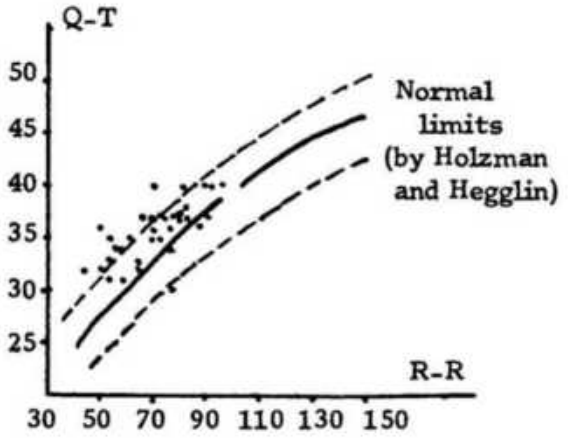
From the Department of Internal Medicine, School of Medicine, Tokushima University, Japan.

Results

Many of the inhabitants of the high fluoride zone had myocardial damage. A higher incidence of myocardial damage occurred in "mottled-enamel" children than in those not so affected. The Q-T interval was prolonged frequently in the mottled-teeth subjects (Fig. 1).

Fig. 1

Q-T Interval in 35 Residents with Mottled Teeth



Electrocardiogram and X-Ray Findings in High Fluoride Okayama

Four residents with mottled enamel and marked limitation in various joints of Odani village, Sanyo town, Okayama Prefecture, were selected for the roentgenological studies. The roentgenologic bone changes were identical with those seen in chronic fluoride poisoning. Patients whose movement had become restricted because of the skeletal changes were eventually unable to move about without aid. Usually they had no general complaints, except for periodic mild rheumatic pains. The fluoride content of their drinking water ranged from 6 to 13 ppm. The patients had been drinking this water daily for more than 10 years. The following are the cardiological findings in the four cases:

1) C. T., a 57 year old male had a blood pressure of 140/100. The fluoride content of his drinking water was 8.5 ppm.

Electrocardiogram: R-R= 0.95 second, R_1 = 0.6 mV, R_2 = 0.7 mV, R_3 = 0.2 mV, T_1 = 0.5 mV, T_2 = 0.7 mV, QT = 0.4 second, QRS = 0.14 second, and was characterized by a tall, slender R wave in lead I-II and the wide, slurred S wave, i.e. a pattern indicative of Wilson's right bundle branch block.

X-Ray picture: (Moritz' measurement) Lb (Lungenbasis) = 26.0 cm, Mr (Mediandistance right) = 5,8 cm, M1 (Mediandistance left) = 10.2 cm, therefore

Tr (Transverse diameter) = 16.0 cm, L (Longitudinal diameter) = 16.7 cm, therefore L-Tr = 0.7 cm, uQ (unterer Querabstand) = 5.9 cm, Br (Querdurchmesser) = 10.5 cm, therefore Tr-Br = 5.5 cm, Bh (height of arch) = 2.3 cm.

This X-ray established evidence of considerable dilatation of the right and left ventricles.

2) E. F., a 54 year old male with a blood pressure of 110/90. The fluoride content of the drinking water was 8.5 ppm.

Electrocardiogram: R-R = 0.75 second, R_1 = 0.5 mV, R_2 = 1.3 mV, R_3 = 0.8 mV, T_1 = 0.25 mV, T_2 = 0.4 mV, T_3 = 0.15 mV, and P wave showed notched, pointed elevation (0.3 mV) and broad (0.12 second) in lead II-III. This electrocardiogram showed a marked pulmonary P with prolonged P-R interval (0.23 second).

X-ray picture: Lb=2.65 cm, Mr=3.9 cm, M1=10.8 cm, Tr-14.7cm, L= 16.7 cm, L-Tr=2.0 cm, oQ=3.7 cm, uQ= 6.5 cm, Br= 10.2 cm, Bh=2.2 cm. This X-ray showed evidence of marked dilatation of the left ventricle figure.

3) K. F., a 46 year old male. Fluoride content of water was 6.0 ppm, blood pressure 130.

Electrocardiogram: R-R = 0.8 second, R_1 = 2.0 mV, R_2 = 1.2 mV, R_3 = 0.5 mV, T_1 = 0.5 mV, T_2 = 0.3 mV, T_3 = 0.3 mV, S_3 = 1.3 mV. This EKG showed a marked left axis deviation with elevated S-T in lead I and slight prolonged P-R interval (0.2 second).

X-Ray picture: Lb = 25.5 cm, Mr = 5.5 cm, M1 = 6.1 cm, therefore, Tr = 11.6 cm. This X-ray revealed evidence of dilatation of the right ventricle.

4) S.H., a 29 year old female. Fluoride content of water was 13.0 ppm. Her electrocardiogram: R_1 = 0.7 mV, R_2 = 1.4 mV, R_3 = 1.5 mV, T_1 = 0.2 mV, T_3 = 0, S_1 = 0.6 mV, P-R = 0.2 second, and P showed pointed elevation (0.3 mV) indicative of right ventricular strain with pulmonary P and prolonged P-R interval. The X-ray picture: Lb = 22.0, Mr = 3.9 cm, M1 = 6.6 cm, therefore, Tr = 10.5 cm, L = 11.8 cm, oQ = 5.3 cm, uQ = 3.7 cm, therefore, Br = 9.0 cm. Bh = 1.2 cm. This X-ray is normal.

(The original paper contains a tabulation of all EKG changes in 20 children with dental fluorosis and 16 without mottling. Editor)

Discussion

The electrocardiograms showed marked myocardial damage in the two cases residing in the high fluoride areas and the x-rays provided evidence of greater dilatation of heart in local inhabitants than in individuals elsewhere.

According to investigators at the Iowa Agricultural Experiment Station (15,16), studies on the effect of fluorides upon growth and reproduction of albino rats yielded the following conclusions: "The toxic effects produced by sodium fluoride vary directly with the amount of calcium salts in the ration"; "the toxic effects of calcium fluoride are much less marked than those of sodium fluoride". Own observations showed that the myocardial damage was proportional to the fluoride content of drinking water and to the extent of mottled enamel. Our data have been confirmed by laboratory experiments which will be published later.

Larner (5) observed that the heart and brains appear to be organs which are very susceptible to fluoride-containing compounds. The authors, in their experiments on chronic fluoride poisoning as well as in inhabitants of the high fluoride zone, observed myocardial damage, bundle branch block and pulmonary P and such pathological changes as myocarditis and myocardial degeneration.

In a survey of "Aso-Volcano" Disease, Hatano (18) quoted Okinaka (17) who had performed electrographic and X-ray examinations in inhabitants in Shikimi village, Kumamoto Prefecture and found right bundle branch block and dilatation of the ventricles. The author (19) surveyed the same district in 1953 and obtained the same results, but the myocardial damage of the inhabitants in this district was less severe than in the high fluoride region of Kagawa and Okayama Prefecture. The fluoride content (0.1 to 1.1 ppm) of the well water of the Aso-Volcano district was lower than in both high fluoride areas. Okinaka attributed the myocardial damage to an endocrine disorder associated with the parotis gland. However, the author considers the high fluoride content of drinking water the cause.

Summary

1) The author examined inhabitants of a high fluoride zone (Shionoe village, Kagawa Prefecture, and Odani village, Sanyo town, Okayama Prefecture). In residents of these zones, greater myocardial damage was detected electrocardiographically and dilatation of the cardiac sillouette roentgenographically than in nonfluoride areas.

2) The myocardial damage appears to be related to the high fluoride content of drinking water.

In concluding, the author wishes to express his deep gratitude to Prof. T. Takamori for his cordial guidance and for revising this paper.

Bibliography

1. Tappeiner, H.: Zur Kentniss der Wirkung des Fluornatrium. Arch. f. exper. Path. B. 25:203, 1889.
2. Tappeiner, H.: Mittheilung über die Wirkungen des Fluornatrium. Arch. f. exper. Path. B. 27:108, 1890.
3. Roholm, K.: Fluorschädigungen. Arbeitsmedizin in Abhandlungen über Berufskrankheiten und deren Verhütung. Heft 7, 1937.
4. Roholm, K.: Fluorvergiftung bei Kryolitharbeitern. Artiv. Für Gewerbepathologie und Gewerbehygiene B. 7:255, 1937.
5. Larner, J.: Toxicological and Metabolic Effects of Fluorine-Containing Compounds. Industrial Medicine and Surgery. 19:535, 1950.
6. DeNito, G.: Richerche Farmacologiche Sul Fluoruro Disidio. Riv. di. Path. Sper. 3:294, 1928.
7. Gottdenker, F. and Rothberger, C.J.: Uber die Wirking von Natriumfluorid auf das Froschherz. Arch. f. Exper. Path. u. Pharmakol. B. 179:24, 1935.
8. Gottdenker, F. and Rothberger, C.J.: Uber die Wirking von Natriumfluorid auf das Warmblüterherz. Arch. f. Exper. Path. u. Pharmakol. B. 179:38, 1935.
9. Chenowith, M.B. and Gilman, A.: Studies on the Pharmacology of Fluoroacetate. J. Pharmacol. Experimental Therap. 82:90, 1946.
10. Chatel, A. De: Versuch einer Analyse der Pathologischen Veränderungen des Zwischenstückes und der Endschwankung des Electrokardiogramms auf Grund von Lokalen Ableitungen. Ztschr. f.d. ges. Exper. Med. B. 98:389, 1936.
11. Lepeschkin, E.: Modern Electrocardiography V.I., 1951.
12. Okuno, H.: A New Colorimetric Method for Fluorine Determination. J. Chem. Soc. Japan 63:23, 1943.
13. Kawahara, H.: The New Method of Classifying Mottled Teeth Based on Fluorine Content in Drinking Water. A Monthly Record of Dental Science V. 52, No. 7, 1952.
14. Hamamono, E., Fujiwara, H.: On Bone Changes Observed in Residents of High Fluorine Zone. Proceeding of the Japan Academy 30:53, 1954.
15. Iowa Agric. Exper. Station Report for Year Ending June 30, 1928, p. 29.
16. Iowa Agric. Exper. Station Report for Year Ending June 30, 1929, P. 29.
17. Okinaka, S.: Aso-Volcano Disease. Japanese Medical Journal 1405:3, 1951.
18. Hatano, S.: The Study of Aso-Volcano Disease. Japanese Path. Journal. 33:332, 1944.
19. Takamori, T., Okushi, I.: Aso-Volcano Disease. Part III (in preparation).
20. Smith, M.C.: J. Dent. Res., 15:281, 1936.
21. Smith, M.C., Lantz, M.E. and Smith, H.V.: J.A.D.A. 22, 1935.
22. Churchill, H.V.: Occurrence of Fluorides in some Waters of the United States. Jour. Ind. and Eng. Chem. 23:996, 1931.
23. Brandl, J. and Tappeiner, H.: Ztschr f. Biol. 28, 1891.
24. Tamai, I.: The Journal of the Wakayama Medical Society 3:23, 1952.

Part II

EXPERIMENTAL STUDIES ON THE EFFECTS OF SODIUM FLUORIDE UPON THE HEART MUSCLE OF RABBITS

by

Iteshi Okushi
Tokushima, Japan

(Abstracted from Shikoku Acta Medica 5: 238-245, June 1954)

Fluoride is abundant in the mineral as well as in the plant and animal worlds. It is present in water in varying amounts. In the earth's crust it is found as the 20th element in abundance occurring as the minerals fluospar (CaF_2), cryolite (Na_3ALF_6), and sedimentary phosphate rock ($Ca_3(PO_4)_2$ CaF_2.

In the past, the toxicity hazard of fluoride compounds has been encountered in the smelting of cryolite. In 1932 Møller and Gudjonsson (1) detected toxic effects in 30 workers who had been employed for a long time at a factory crushing cryolite in Denmark. About the same time, Roholm described "Eine neue Krankheit" (2, 3) after conducting several detailed experiments on fluoride poisoning. Toxicity by fluoride, as severe as that from working in a cryolite factory, can be caused by a high content of fluoride in drinking water as shown by Smith (4), Smith, Lantz and Smith (5) in 1931, by Churchill (6) and others (7 to 13).

In Part I of this study (14) the author reported a higher incidence of myocardial damage electrocardiographically and cardiac dilatation roentgenographically, in inhabitants of a high-fluoride zone than where water contained little or no fluoride. The myocardial damage was related to the high content of fluoride in drinking water.

In this study, Part II, rabbits were given sodium fluoride orally to supplement our previous observations.

Experimental Material and Methods

Healthy mature white rabbits weighing about 2kg were divided into four groups according to the dosage of sodium fluoride:

A Group:	11 individuals		
	received	orally	10 mg/kg NaF daily
B Group:	10	"	30 mg/kg NaF
C Group:	13	"	50 mg/kg NaF
D Group:	3	"	100 mg/kg NaF
E Group:	16	"	Control

Nutritious food such as the "Tofukara" (the residue of bean-curd) and grass (standard diet) were given to rabbits, and their health was given constant attention.

Electrocardiography was performed in limb leads. Each limb was fixed on an insulated table. Since the rabbit heart had a tendency to move with the change of the body position in the photography, great care was taken to avoid an error due to this factor. Normal limits of PQ and QT interval were employed in accordance with Shimizu's (24) expression as follows:

$$QT = 2.83 \sqrt{RR} \pm 0.025$$
$$PQ = 2.32 \sqrt{RR} \pm 0.025$$

Rabbits were killed at various intervals and every heart was studied macroscopically and histologically.

Discussion

The author had previously described a rise in myocardial damage electrocardiographically and cardiac dilatation in the X-rays in residents of high-fluoride areas. These changes which appear to be due to the high content of fluoride in drinking water, were fully confirmed by the current laboratory experiments.

The degree of myocardial damage was proportionate to the dosage of sodium fluoride and the length of time of its administration. The pathological changes appeared to be more prominent in the papillary muscles and on the inside wall of the myocardium than at the exterior cardiac wall.

Patterns of myocardial damage such as depressed ST, inverted T, prolonged QT interval, bundle branch block, and pulmonary P, were observed in these laboratory experiments as well as in our field work (14).

In autopsied cows in Nango village, Kumamoto Prefecture, Kono and the author found hypertrophy of the myocardium associated with dental fluorosis and fluoride bone changes. In the heart muscle, we observed numerous small hemorrhages, slight infiltration of round cells, and marked regressive degeneration similar to that produced in the current laboratory experiment (Fig. 1 to 7). We also observed the same changes in rats recently (15). Takamori and Kawahara (16) noted marked cloudy swelling of the skeletal muscle of frogs suspended in a 25 mg% of NaF solution.

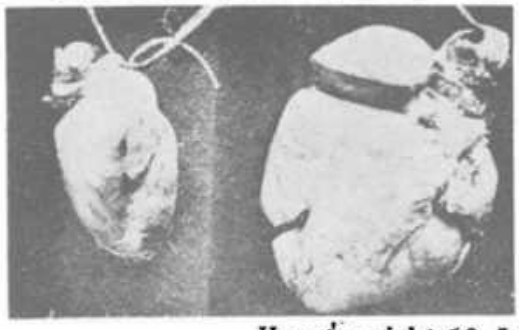
Among the available studies, Friedenthal (17) assumed that calcium precipitation is the principal cause of poisoning by sodium fluoride. Meyerhof (18) reported that a n/500 NaF solution completely inhibited fermentation. Lipine (19) noted that fluoride inhibited glycolysis in muscle.

Embden drew attention to the fact that fluoride decreased the amount

Fig. 1

Heart Size in Fluorodic Rabbit No. 301

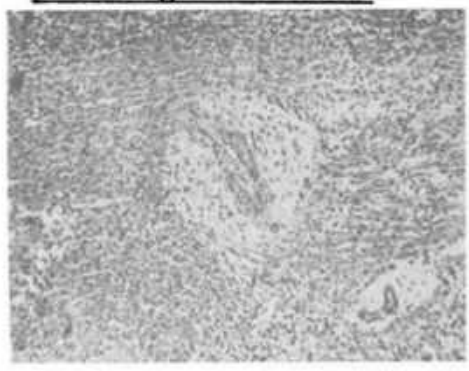
Control NaF 30 mg/kg (51 Days)



Heart weight 10.5 gr.

Fig. 2

Infiltration with Round Cells, Thickening of Adventitia



Rabbit No. 101. NaF 10 mg/kg (132 days)
Left ventricle (x 170)

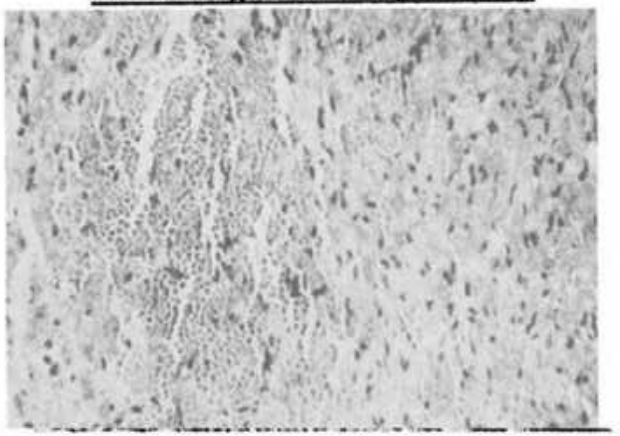
Fig. 3

Diffuse Hemorrhages, Infiltration of Round Cells and Cloudy Swelling

Rabbit No. 304. NaF 30 mg/kg (19 days).
Papillary muscle (x 130).

Fig. 4

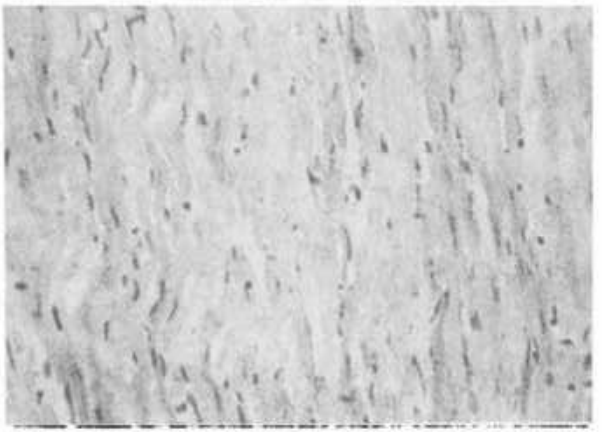
Hemorrhage and Cloudy Swelling



Rabbit No. 310. NaF 30 mg/kg (51 days)
Right ventricle (x 400).

Fig. 5

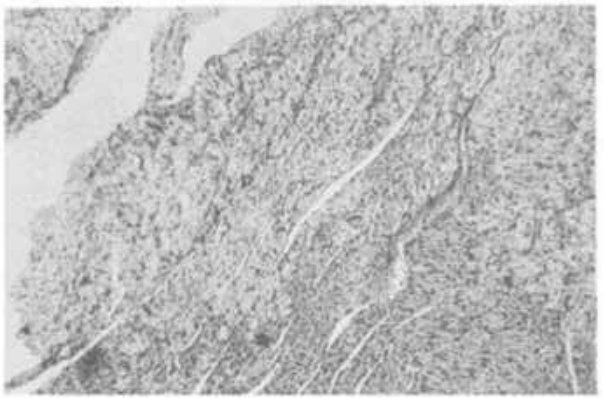
Cloudy Swelling



Rabbit No. 503. NaF 50 mg/kg (59 days).
Left ventricle (x 400).

Fig. 6

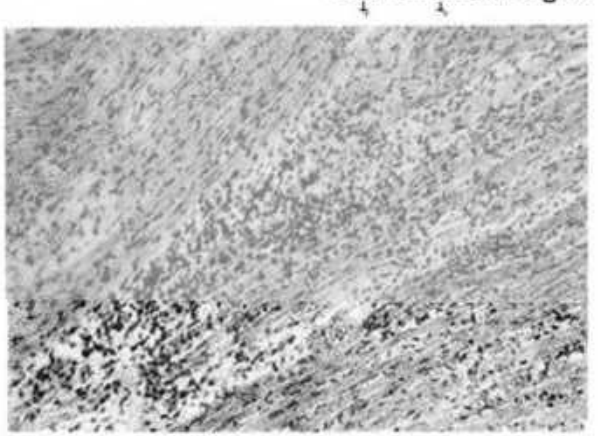
Cloudy Swelling, Vacuolar and Colloid Degeneration



Rabbit No. 502. NaF 50 mg/kg (60 days)
Left ventricle (x 130)

Fig. 7

Infiltration with Round Cells and Small Hemorrhages



Rabbit No. 507. 50 mg/kg (31 days)
Right auricle (x 170)

of free phosphoric acid formed in muscle suspended in butyric solutions. Extensive studies concerned with the action of fluoride on glycolysis and the influence of fluoride ions upon the chemical changes associated with muscle metabolism have been carried out by Embden and his school (20-23). Their experiments indicated that tartrate and fluoride inhibit or even reverse the glycogen breakdown in muscle. It is concluded that fluoride affects the muscular metabolism by depressing the enzymatic reaction and the intermediary metabolism (24).

Summary

In the electrocardiogram of rabbits given sodium fluoride orally, a pattern of myocardial damage was observed, namely depressed ST, inverted T, prolonged QT interval, multifocal ventricular premature contraction, bundle branch block, and pulmonary P. Histologically, regressive degeneration, cellular infiltration, hyperemia, hemorrhages and thickening of vessel wall were noted in the heart muscle.

Bibliography

1. Flemming Møller, P. and Gudjonsson, SK, V.: Massive Fluorosis of Bones and Ligaments. Acta Radiologica 13:269, 1932.
2. Roholm, K.: Fluorvergiftung. Eine Uebersicht über die Rolle des Fluors in der Pathologie und Physiologie. Erg. Inn. Med. 75:822, 1939.
3. Roholm, K.: Fluorvergiftung, "Eine neue Krankheit". Kl. Wschr. 15:1425, 1936.
4. Smith, M.C.: J. Dent. Res. 15:281, 1936.
5. Smith, M.C., Lantz, M.E. and Smith, H.V.: J.A.D.A. 22, 1935
6. Churchill, H.V.: Indust. Engin. Chem. 23:996, 1931.
7. Tappeiner, H.: Zur Kentniss der Wirkung des Fluornatrium. Arch. f. exper. Path. 25:203, 1889.
8. Schulz, H.: Ebenda 25:326, 1899.
9. Tappeiner, H.: Mittheilung uber die Wirkung des Fluornatrium. Arch. f. exper. Path. 27:108, 1890.
10. DeNito, G.: Richerche Farmacologiche sul Fluoruro di Sodio. Riv. di Path. Sper 3:294, 1928.
11. Gottdenker, F. and Rothberger, C.J.: Ueber die Wirkung von Natriumfluorid auf das Froschherz. Arch. f. exper. Path. u. Pharmakol. 179:24, 1935.
12. Gottdenker, F. and Rothberger, C.J.: Uber die Wirking von Natriumfluorid auf das Warmbluterherz. Arch. f. exper. Path. u. Pharmakol 179:38, 1935.
13. Chenowith, M.B., Gilman, A.: Studies on the Pharmacology of Fluoroacetate. J. Pharmacol. Expt'l. Therap. 82:90, 1946.
14. Okushi, I.: Changes of the Heart Muscle Due to Chronic Fluorosis. Part I. Electrocardiogram and Heart X-ray Picture made in Inhabitants of High-Fluoride Zone. Shikoku Acta Medica 5:159-245, 1954.
15. Kono, K. and Okushi, I.: Experimental Studies on the Effects of Sodium Fluoride Upon the Heart Muscle of a Rat. Shikoku Acta Medica (in preparation).
16. Kawahara, H. and Kawahara, K.: Influence of NaF solution upon the Fertilization, Egg-Division, Incubation and Early Development of Toad-Embryo, Fluoride 4:167-171, October, 1971.
17. Friedenthal, H.: Engelmanns Arch. S. 145, 1901.
18. Meyerhof, O.: Uber die Enzymatische Milchsäurebildung im Muskelextrakt. 178:462, 1926.

19. Lepine: Blutzucker (Bergmann), S. 156, 1913.
20. Embden, G. and E. Lehnartz: Zeitschr. f. Physiol. Chem. 134:243, 1924.
21. Embden, G. and C. Haymann: Zeitschr. f. Physiol. Chem. 137 , 1924.
22. Abraham, A. and P. Kahn: 141:161, 1924.
23. Lantz, H. and M. E. Mayer: Zeitschr. f. Physiol. Chem. 141:181, 1924.
24. Shimizu: N. J. A. C. 12, No. 5-6, 1948.

ELECTROCARDIOGRAPHIC STUDIES OF THE INHABITANTS IN HIGH FLUORIDE DISTRICTS

by

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(Reproduced from the Tokushima Journal of Experimental Medicine 3:50-53, 1956)

In the Aso volcano districts of Kumamoto Prefecture, Japan, a dental disease occurs which is known locally as "Yonaba", meaning "teeth affected by volcano ash". Undoubtedly, this condition is identical with mottled teeth, irrespective of the cause.

Since the reports of Smith, Lantz and Smith (1) and Churchill (2), we know that mottled enamel is usually caused by drinking water which contains fluoride in excess of 1 ppm.

No systematic studies on how fluoride affects the cardiovascular system had appeared previous to Okushi's report (3) from our department in 1954.

The authors examined the inhabitants of a high fluoride area where a high incidence of mottled enamel was encountered. In 1953, electrocardiographic and X-ray examinations in Shionoe, Kagawa Prefecture, and in Odani, Okayama Prefecture, revealed many instances of myocardial damage, cardiac hypertrophy and dilatation. In 1954 Okushi (4) published experimental data concerned with the effect of sodium fluoride upon the heart muscle of rabbits. This communication presents EKG observations on subjects residing in the Mt. Aso fluoride area.

Method and Procedure

In 1954 electrocardiograms were taken on 102 inhabitants (90 children and 12 adults) of the Aso volcano district and 59 children in Beppu city. The examina-

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

tions were carried out with standard limb and chest leads by means of the Sanborn electrocardiograph.

Fluoride content of drinking water was determined by the aluminum hematoxylin method (5). Kawahara's (6, 7) classification of mottled enamel of the inhabitants was adopted.

Results

A) Electrocardiograms of Residents of the Aso Volcano District:

1) Narikawa Village (Fluoride content of drinking water 0.6 to 4.4 ppm):

Among 12 adults the following results were obtained:

One case with a high degree of myocardial damage, with coronary insufficiency and mitral p wave; one case of multiple ventricular premature contractions with prolonged Q-T interval (Fig. 1); one case of nodal premature contractions, with nodal escape and coronary insufficiency (Fig. 2); one case of auricular fibrillation with multifocal ventricular premature contractions and coronary insufficiency (Fig. 3); one case of coronary insufficiency with prolonged Q-T interval; five cases with prolonged Q-T interval. In only two of the 12 cases with mottled teeth was a normal electrocardiogram obtained.

Among 22 children, one case showed evidence of right ventricular strain with a high degree of coronary insufficiency (Fig. 4); two showed ventricular strain; five prolonged Q-T interval. In 14 cases, the ECG was within normal limits.

2) Kuronagare Village (Fluoride content 1.6 to 1.9 ppm):

Of 8 children, one case showed right ventricular strain with coronary insufficiency and p pulmonale pattern; one patient had a right bundle branch block; one case, right ventricular strain. Five cases were within normal limits.

3) Imamachi Village (Fluoride content of drinking water 5.9 to 6.2 ppm):

Of five children, two cases showed a high degree of ventricular strain with coronary insufficiency; one a prolonged Q-T. Two cases were within normal limits.

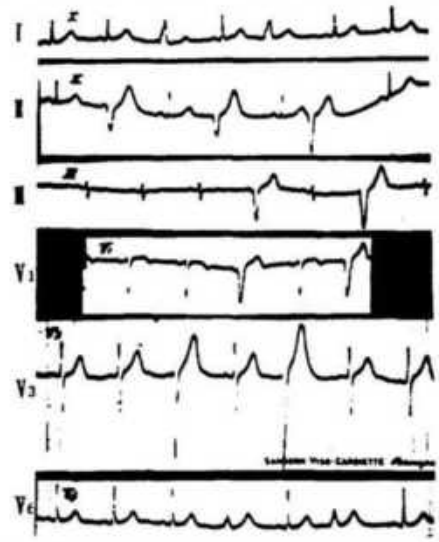
4) Onoda Village (Fluoride content 0.5 to 0.7 ppm):

Of three children, one case showed left ventricular extrasystoles with right ventricular strain and p pulmonale pattern, one case right ventricular strain with sinus tachycardia. In one case the EKG was within normal limits.

Samples of EKG Changes

Fig. 1

♂ 79 Yrs. Narikawa (F^- in Water 0.6 to 4.4 ppm). Mottling Enamel P_5 B_3.



Multiple ventricular premature contractions with prolonged Q-T interval.

Fig. 2

♂ 30 Yrs; Narikawa, Mottling P_3 B_3.

Depressed ST_2, ST_3. Nodal premature contraction with nodal escape and coronary insufficiency.

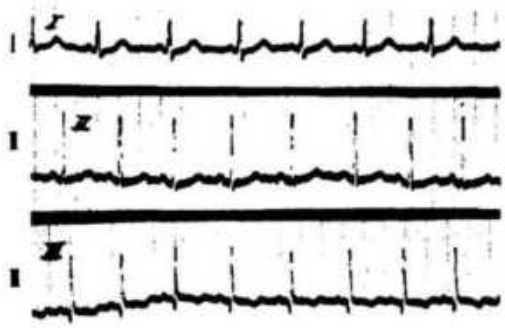
Fig. 3

♂ 62 Yrs.; Narikawa

Marked myocardial damage; auricular fibrillation, multifocal ventricular premature contraction; coronary insufficiency.

Fig. 4

♀ 16 Yrs; Narikawa. Mottling P_2B_3



Right ventricular strain; coronary insufficiency.

5) Nakadori Village (Fluoride content 0.6 to 0.7 ppm):

Of 4 children, one showed prolonged Q-T interval. The other three electrocardiograms were within normal limits.

6) Other villages of the Aso volcano district, Yunoura, Dabaru, Hosen, Tamachi, Yamada, Ogura, Kurokawa and Miyaji (Fluoride content 0.0 ppm):

Of 45 children, three showed a high degree of myocardial damage; five, slight myocardial damage. The remaining 37 were within normal limits.

In the inhabitants of Aso volcano district, of 56 subjects with mottled teeth, 30 showed myocardial damage but, of 46 without dental fluorosis, only six had myocardial damage.

B) Electrocardiograms in Beppu city with practically no fluoride in drinking water (0.0 to 0.1 ppm):

Of 59 children, two showed evidence of slight right ventricular strain; one exhibited a prolonged Q-T interval. The remaining 56 were within normal limits.

Discussion

The relation between the electrocardiographic findings and the fluoride content of drinking water in the respective villages is shown in Table 1.

Our studies indicate a higher incidence of myocardial damage in the high-fluoride districts, namely 10 among 12 in Narikawa village (0.6 to 4.4 ppm), and 0 among 11 adults in fluoride-free Yunoura village ($a > 0.01$). It is also noteworthy that a significantly higher incidence of myocardial damage in school children occurred in high fluoride districts, namely 8 among 22 children in Narikawa village, than in nonfluoride districts such as Yunoura village ($a > 0.05$).

A higher incidence of myocardial damage was also noted in inhabitants of the Aso volcano districts than in those of Beppu city (0.0 to 0.1 ppm). Of 102 inhabitants of the Aso volcano district, 36 cases had myocardial damage; whereas of the 59 school children examined in Beppu city, only three were thus affected.

The relation between myocardial damage and mottled enamel in the 102 inhabitants of the Aso volcano district is shown in Table 2.

Our results further indicate that the majority of residents with myocardial damage also have mottled teeth ($a > 0.01$). Conversely, among those without mottled teeth, the negative EKG findings outnumber those with myocardial damage ($a > 0.05$).

TABLE 1

The Relation between the Electrocardiographic Readings and the Fluoride Content of the Drinking Water

	Village	No. of cases	E. C. G. within normal limits	E. C. G. Degree of myocardial damage I	II	III	Fluoride in ppm
Adults	Narikawa	12	2	5	1	4	0.6 to 4.4
	Narikawa	22	14	5	2	1	
	Yunoura	11	11				0.0
	Dabaru	2	2				
	Hosen	1	1				
	Tamachi	1		1			
	Onoda	3	1		1	1	0.5 to 0.7
Children	Kuronagare	8	5		1	2	1.6 to 1.9
	Imamachi	5	2	1		2	5.9 to 6.2
	Yamada	16	12	3		1	0.0
	Ogura	2	1			1	0.0
	Nakadori	4	3	1			0.6 to 0.7
	Kurokawa	6	4	1		1	
	Miyaji	6	5	1			
	Beppu City	59	56	2	1		0.0 to 0.1

TABLE 2

The Relation between Myocardial Damage and Mottled Teeth

Myocardial damage \ Mottled teeth	(+)	(-)	Total
(+)	30	6	36
(-)	26	40	66
Total	56	46	102

Conclusion

1) In the Aso-volcano district, a higher incidence of myocardial damage based on electrocardiographic studies was found in inhabitants of high fluoride communities than in "nonfluoride" villages.

2) In subjects with mottled teeth, greater myocardial damage was encountered electrocardiographically than in persons without mottling in the Aso-volcano district.

3) The authors conclude that the myocardial damage observed in the inhabitants of the Aso-volcanic district is predominantly due to the excessive fluoride content of the drinking water.

Bibliography

1. Smith, M.C., Lantz, M.E. and Smith, H.V.: J.A.D.A. 22, 1953.
2. Churchill, H.V.: Indust. Engin. Chem, 23:996, 1931.
3. Okushi, I.: Shikoku Acta Medica, 5:55, 1954.
4. Okushi, I.: Shikoku Acta Medica 5: 52, 1954.
5. Okuno, H.: J. Chem. Soc. Japan, 63:23, 1943.
6. Kawahara, H.: A Monthly Record of Dental Science, 52:7, 1952.
7. Takamori, T.: The Tokushima Journal of Experimental Medicine, 2:25, 1955.

Corrections:

Volume 4, pages 5-15:
In the article by Drs. Buck and Reusmann "A New Semiautomatic Method for Fluoride Determination in Plant and Air Samples", the names of two co-authors, Dr. Ixfeld and Mr. Pallasch, who collaborated in the development of the apparatus illustrated, were inadvertently omitted. Herewith we wish to acknowledge their co-authorship.

Drs. M. Buck and G. Reusmann

Volume 4, page 105:
The editorial cited the article by G. A. Posen, J. R. Marier et al. as documentation for four deaths in Ottawa, Canada. The reference should have been "New Facts on Fluoridation" Saturday Review, March 1, 1969, page 54.

In the following sentence the word "probably" should be substituted for "considered" so that it reads: "Fluoridated water for hemodialysis was probably the cause of death."

SUBJECT INDEX

In the January 1972 issue, articles by the following collaborators of Prof. Takamori will appear:

Kasaburo Abe	Tadashi Iwase
Setsuo Tanaka	Mitsugi Hirao
Hitoshi Kawahara	Jiro Imura

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