Experimental study of the influence of fluorine administration on tuberculous lesions in rabbits (Second Report)

Experiments using bovine tubercle bacilli

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I. Introduction

The biochemical significance of the fluorine ion on the animal body is one of the least known areas in experimental and clinical medicine. Since 1916 a dental disease called "mottled teeth" is assumed to be the result of chronic fluorine intoxication. Recently, because of evidence that mottled teeth did not easily become carious the protection of carious teeth is being attempted by investigators by the administration of flnorine ion in small amounts. On the other hand fluorine intoxications other than dental diseases in men and animals have attracted investigators' attentions.

In Japan M. Hirata[1] and Y. Hirata[2] found many patients in Takarazuka who suffered from mottled teeth and other more general conditions —— bone deformations, anaemia, apparent leucopenia etc. Since drinking water in Takarazuka has a high content of fluorine ion, Hirata postulated that these diseases, including mottled teeth, were the results of chronic fluorine intoxication. Recently Hamamoto[3] found many very heavy patients suffering from severe bone deformations in a village of Okayama prefecture and found a very high content of fluorine ion in the drinking water they used. So as Roholm[4] and others have already reported, there is no doubt that chronic fluorine intoxication can induce a certain disturbance in calcium metabolism especially in ossification.

Tuberculosis is a disease related pathogenically to calcium metabolism in the hosts. Caseous substances are known to have high contents of calcium salts, and

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in old encapsulated caseous lesions one may often find calcification or ossification. Calcifications of caseous lesions are thought to be the indicators of healed lesions. But the real significance of calcification of tuberculous lesions is not entirely clear.

Fluorine ion has a certain disturbing effect on calcium metabolism in animal bodies. When we administer intoxicating doses of fluoride salts to tuberculous animals, what is their influence on the tuberculous lesions of these animals? Perhaps the calcium metabolism in tuberculous lesions would be disturbed, and the histological and chemical aspects of these tuberculous lesions would change when compared to control animals.

II. Materials and Methods

We divided the animals into two groups —— experiments of acute and chronic fluorine intoxication. We divided the animals of each group into four subgroups.

(1) normal control rabbits
(2) rabbits fed NaF
(3) rabbits inoculated intravenously with 0.01 mg RM strain of bovine tubercle bacilli
(4) rabbits fed NaF after intravenous inoculations of 0.01 mg RM strain of bovine tubercle bacilli

NaF administration was begun five weeks after the inoculation of tubercle bacilli. In the experiments of acute intoxication, rabbits were fed daily 200 mg NaF for 10 days and in the experiments of chronic intoxication 40 mg NaF daily for 30-78 days.

All the animals which were killed or which died spontaneously were examined for pathological changes by the naked eye and part af each of several organs (lung, liver, kidney, spleen and heart) was fixed with formaline solution for the preparation of histological specimens; a part of each organ was also dried and ashed as described in the previous report in order to determine the fluorine and calcium content. Fluorine determinations were performed as described in the previous report. Calcium determinations were performed as described by Yanagizawa.[5]

III. Results

A. Group with acute intoxication:

Rabbits fed daily 200 mg NaF suffered a short time after the adminstration from severe anorrhexia and acute general debility. 10 days after the NaF administration tuberculous animals were dead. At the same time we killed the animals of the other groups.

1) The fluorine content of these animal tissues is presented in Table 1.

Of course the fluorine content of tissues of animals to which NaF has been administered is obviously larger than of those not receiving NaF. The increase

Table 1 Fluorine contents of various organs in acute intoxication

Rabbit	tuberculous fed NaF		non tuberculous fed NaF		tuberculous without NaF	
No.	4	5	6	7	1	3
lung (PPM)	27	17	19	12	10	8.6
liver (〃)	42	5.2	20	7.0		9.5
heart (〃)		50	59	10	12	19
kidney (〃)	25	12	7.6	10	19	13
bone (〃)	560	690	475	658	200	118
Duration of feeding (day)	10	10	10	10		
Intake (g)	1.6	1.6	1.6	1.6		

of fluorine in the bone tissues is greater than in any other tissue. The increase of fluorine content seemed to be greater in tuberculous animals than in non-tuberculous.

2) Pathological changes

a) Findings by the naked eye:

Tuberculous rabbits without fluorine administration:

General debility was severe.

Lungs: The pleura adhered over the whole sulface. The weight of the lungs increased to half the weight of the liver. Many gray yellow nodules of various sizes occupy almost tne whole lung tissue, partly congulomerated, partly separated.

In the liver a few miliary tubercles are seen.

The kidney contains a few miliary tubercles.

The spleens swelled noticeably, and blood congestions and numerous tubercles are seen.

Non-tuberculous rabbits fed sodium fluoride:

Slight general debility was recognized. There were almost no phathological changes in the findings of various organs by the naked eye.

Tuberculous rabbits fed sodium fluoride:

The findings were almost the same as those described above for tuberculous rabbits without fluorine administration.

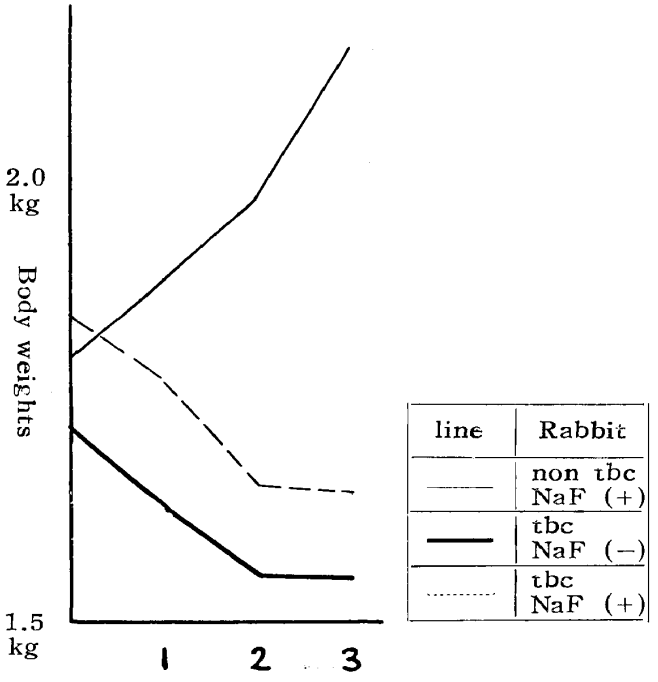
b) Histological findings:

The histological findings in various organs of acutely intoxicated rabbits with and without tuberculosis were almost the same as those chronically intoxicated rabbits. So we shall describe the findings in detail in the following section on chronic intoxication.

B. Group of chronic intoxication :

The general condition of animals fed 40 mg NaF daily was disturbed, but not so much as that of the cases of acute intoxication.

Chart 1 Body weights of rabbits in chronic intoxication



Curves of body weights are presented in chart 1. Animals, including tuberculous animals, remained alive, and we killed animals 30 days and 78 days after the NaF administrations.

1) Fluorine content

The fluorine content of these animals' tissues is shown in Table 2.

Table 2 Fluorine contents of various organs in chronic intoxication

Rabbit	tuberculous fed NaF					non tuberculous fed NaF					tuberculous without NaF				non tuberculous without NaF
No.	8	9	15	16	18	13	14	20	22	23	1	3	12	19	2
lung (PPM)	10	9.4	50	480	700	11	17	30	13	36	10	8.6	10	9.8	8.6
liver (〃)	4.0	6.2	14	5.9	18	7.5	9.0	10	18	36		9.5	10	0.6	9.5
heart (〃)		15	20	20		32	20	29	20	24	12	19	16	5.8	19
kidney (〃)	10	11	26	10	36	4.5	2.4	6.2	15	18	19	13	6.4	2.4	13
bone (〃)	480	790		440	2200	670	490	1600	1600	1700	200	100	80	320	230
Duration of feeding (day)	30	35	50	68	78	40	40	83	83	83					
Intake of NaF (g)	1.4	1.6	2.5	3.6	3.6	1.8	1.8	3.8	3.8	3.8					

The fluorine content of bones and lungs of fluorine fed animals was apparently larger than those not fed NaF. But the fluorine content of other organs was almost equal in both groups and differences in these values are almost negligible. The fluorine content of bones increased proportionately to the quantity of fluorine administered in both tuberculous and non-tuberculous animals. In tuberculous animals, the fluorine content of the lungs increased proportionately to the quantity of fluorine administered. However, these was no such increase in the lungs of non-tuberculous rabbits.

In tuberculous lungs of animals, which were not fed NaF, the fluorine content did not increase. As described below, in the experiments in which highly virulent bovine bacilli were injected intraveuously, tuberculous lesions were more severe in the lungs than in other organs. These data seem to indicate that administered fluoride may tend to adhere to tuberculous lesions.

2) Pathological changes

a) Findings by the naked eye:

Tuberculous rabbits without fluoride administration:

Lymph glands were swollen and tubercles were seen.

In the lung there were slight individual differences in the extent of tuberculous changes, but generally changes were very marked. Changes of the rabbits killed at 30 days after the administration were almost the same as those in rabbits killed at 78 days after the administrations.

The liver contained a few miliary tubercles.

The kidney contained many miliary tubercles.

The spleen was swollen and contained many tubercules.

The heart showed no changes.

Non-tuberculous rabbits fed fluoride:

Body weights increased as shown in chart 1. Subcutaneous fat was thicker than in tuberculous rabbits. Chronic intoxication with 40 mg fluoride daily did not induce noticeable disturbances in the general condition of the animals. But we found at autopsy that the bones, especially femurs, of these rabbits were fragile and we could break them easily. Blood coagulation of these rabbits may have been disturbed, and we always found non-coagulated blood in the hearts.

Tuberbulous rabbits fed fluoride:

The pathological changes in this group were almost the same as those described above in tuberculous rabbits not fed NaF. Blood coagulation was disturbed.

b) Histological findings:

Non-tuberculous rabbits fed fluuoride:

The greatest changes were seen in the kidneys. Degeneration (vacuoles) in urinary tubules were marked. There was marked dilatation and congestion of capillary vessels in the glomeruli.

In the liver there was dilatation and congestion of capillary vessels and slight degeneration of liver cells.

In the lung and heart there was slight congestion of capillary vessels, and no other changes.

Tuberculous rabbits without fluoride:

The lungs showed more change than any other organ. There was broad caseous necrosis with peripheral infitrations of mononuclears. In the alveoli near the tubercles, exudate many desquamated cells and emigrated leucocytes as well as marked dilatation and congestion of capillary vessels were seen. In some of the bronchi, desquamation of bronchial epithelium and exudates were seen.

In the liver there were a few tubercles with mononulear infiltration.

In the kidney there were a few tubercles with mononuclear infiltration.

There were many tubercles in the spleen with cellular infiltrations and slight caseation.

Tuberculous rabbits fed fluoride:

Tuberculous changes were very marked in the lungs ann almost the same as those of rabbits not fed fluoride.

There were a few tubercles in the liver with mononuclear infiltration and slight caseation.

The kidney showed a few tubercles with cellular infiltration and caseation and marked degeneration of tubules and codgestion of capillary vessels of tubules and glomeruli were seen.

The spleen was almost the same as described above.

The heart showed capillary congestion and partial degeneration of muscle cells.

Tubercle bacilli were seen in the peripheral areas of the tubercles.

3) Calcium content

Calcium determinations in the cases in the acute intoxication were not performed. In cases of chronic fluoride intoxication the calcium content of various

Table 3 Calcium contents of various organs in chronic intoxication

Rabbit	tuberculous fed NaF					tuberculous without NaF				non tuberculous			
										fed NaF			without
No.	8	9	15	16	18	3	12	17	19	13	22	23	2
lung (mg%)	35	72	105	500	115	45	60	60	90	62	55	35	54
liver (mg%)	22	41	105	50	33	22	67	48	35	55	37	37	50
heart (mg%)	33	57	90	55	65	50	57	46	52		25	40	52
kidney (mg%)	32	46	36	55	50	32		55		42	50	45	32
intake of NaF (g)	1.4	1.6	2.5	3.6	3.6					1.8	3.8	3.8	

organs was determined by the method described by Yanagizawa. The data are presented in Table 3.

We may say from this Table that (1) the calcium content of various organs of tuberculous rabbits is higher than that of non-tuberculous rabbits, and (2) the calcium content of lungs is generally higher than that of other organs and seems to increase proportionately to the quantity of fluorine administered.

So the highest values were seen in the lungs of tuberculous rabbits fed with 3.6 g NaF. In these lungs we found the highest contents of fluorine too. This may suggest the particular relationship between calcium and fluorine ion in tuberculous lesions.

IV. Discussion

Acute fluorine intoxication in rabbits fed daily 200 mg NaF led to heavy disturbances of various organs especially of the kidney. Animals inoculated with 0.01 mg bovine tubercle bacilli intravenously died spontaneously shortly after the fluorine administration. We could not observe how fluorine administration influenced the tuberculous process because of early death of the animals. Tuberculosis in rabbits poisoned with fluorine was almost the same as that rabbits receiving no fluorine.

In cases of chronic fluorine intoxication animals, including tuberculous ones, remained alive for a long time. 40 mg of NaF daily gave only slight disturbances to animals, and non-tuberculous animals fed fluorine were even able to increase their body weights. But we failed to find a definite differences in tuberculous changes between rabbits fed fluorine and those not fed fluorine. Macro-and microscopic findings were almost the same. In both groups tuberculous changes in lungs were extremely heavy. As we inoculated very highly virulent bovine tubercle bacilli, it is natural that tuberculous processes of these animals were always very active and progressive, and five weeks after the inoculations of bacilli —— at this period we began the fluorine administrations —— the tuberculous lesions in the lungs had developed already to a high grade and after that time pathological changes remained far advanced without regard to whether fluorine was administred or not. Therefore, for the purpose of observing the influence of fluorine ions which were assumed to be not so strong as those of recent chemotherapeutics such as streptomycin, isoniazide etc. upon tuberculous processes our present designations of experiments were inadequate. Now we are performing a new experiment by using a lower virulent human type tubercle bacilli than bovine RM strain. We shall describe these results in a subsequent report.

Concerning the contents of fluorine and calcium ions in tissues we gained an interesting result. Fluorine has been known to be an ion which combines easily with bone tissue. We too found high contents of fluorine in the bones. And we found relative high contents of fluorine in tuberculous lung tissues of fluorine fed rabbits. On the other hand the calcium content was also high in tuberculous lung

tissues. So in tuberculous lung tissue calcium and fluorine contents increase together. These results are assumed to indicate that calcium and fluorine ions are caught and combined together in animal tissues. As described above tuberculous changes were the heaviest in the lungs, so it may be certain that the fluorine ion has a particular affinity for tuberculous lesions in relationship to the calcium ion.

V. Summary

We performed experiments in which rabbits inoculated with bovine tubercle bacilli were fed fluoride in large (200 mg NaF daily) and in small (40 mg NaF daily) amounts. In cases of large amounts of fluoride administration animals suffered from heavy intoxication, and tuberculous rabbits thus treated died early. We could not observe any influence of fluorine administration on pathological changes or fluorine content of various organs of tuberculous rabbits.

Animals receiving a small amount of fluoride remained alive. But tuberculous lesions provoked by intravenous inoculation of highly virulent bovine tubercle bacilli had already developed to the maximal grade before the start of fluorine administration. Therefore we could not observe apparent signs of influences of fluorine administration.

However we found that fluorine ion combined with calcium ion was caught particularily in tuberculous lesions. This may suggest that fluorine administration will be able to influence a more chronically developing process of tuberculosis.

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