137. THE RETENTION AND ELIMINATION OF FLUORINE IN BONES

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The occurrence and role of fluorine at toxic and non-toxic levels in animal nutrition is of increasing interest. Much of the older quantitative work is of little value because only recently have reliable methods been devised for the estimation of traces of this element.

The present work aimed at investigating (1) the F content of human bones at different ages, under conditions in which there were no obvious signs of fluorosis; (2) the uptake and elimination of F from the bones of rats which had received toxic amounts of NaF.

Assuming that minute non-toxic quantities of F are taken in, it would be reasonable to expect that the F content of calcified tissues would rise with increasing age, for several reasons. First, natural deposits of calcium phosphate have a special affinity for F, as a result of which rock phosphates contain significant amounts of this element, which are also to be found in fossil teeth and bones. This last fact was probably responsible for the idea that F is a constant and considerable constituent of dental tissues, in particular the enamel in which it occurs as fluorapatite. Secondly, the F ion is toxic; it forms insoluble Ca salts and so presumably one method of detoxication might be to store it as apatite in the teeth and bones.

From the work reviewed by Roholm [1937] the conclusion may be drawn that owing to faulty methods of determination, there are insufficient data on which to give a final answer as to the relation between age and F content in the absence of signs of F intoxication. Roholm [1937] made a set of determinations on adult human bones and found an average of 0·093 % F (lowest 0·059 %, highest 0·21 %). The average F content of land animal bones, including man, has also been determined recently by Klement [1938], who gave the value of 0·05 % of the inorganic substance of bone and suggested that there is probably an increase with age. This was also the opinion of Sharpless & McCollum [1933] working on rats. Marcovitch & Stanley [1938], who determined the F content of the whole eviscerated bodies of rats at birth, at 29 and at 90 days, came to the conclusion that the F content of the whole body decreased considerably with increasing age, but this has no direct bearing on the present work, which deals only with bones.

In the past much divergence of opinion has existed with regard to the role of F and its occurrence in the tissues because of the difficulty of estimating it unless careful attention is given to details. We have used the colorimetric method in which the F bleaches a zirconium-alizarin lake and have followed the details and design of apparatus for the distillation of the F as HF, employed at the laboratory of the Ministry of Agriculture and Fisheries. We are greatly indebted to Dr H. H. Green of that laboratory for his advice in this matter.

1. *The fluorine content of human rib bones at different ages*

The specimens of human bones used in this investigation (25 samples) were obtained from two of the large London hospitals. Rib bones were chosen because they were easy to obtain. The bones were collected from persons who had been resident in London, which up to the present has not been considered a fluorosis area. Certain sources of London water contain up to 0·5 p.p.m. but the tap water would contain less than this.

After post mortem examination the bones were placed in alcohol for a few days, after which they were treated for several days with acetone to free them from fat and water. They could then easily be ground to a fine powder on a rotating circular file. This powder was again extracted with acetone, passed through a fine wire mesh and dried. For the spectroscopic examination the powder was used straight away. Details of the spectrographic method have already been published [Lowater & Murray, 1937]. We took the opportunity of analysing the bone ash for Ca, P and Mg. For these determinations the powders were ashed directly and the usual standard methods employed. For the chemical determination of F the powders were ashed with magnesia ashing mixture, a suspension of 1 % MgO in 7 % Mg acetate, account being taken of the F in all reagents by blank determinations.

Spectrographic analyses. The spectrographic analyses were carried out before the chemical analyses so that a qualitative comparison could be made of the F contents of the bones. The bone specimens were handed over as a series of numbered samples. When the spectrograms were complete, the specimens were arranged in the order of increasing F content, as judged from the spectrograms. This order proved to be in general the order of the age of the subjects. The results are set out in Table 1 and plotted in Fig. 1.

Table 1

Cause of death	Age years	Samples in the order of increasing F content judged from spectrograms
Asphyxia	1	1
Hydrocephalus	½	2
Full-term foetus (stillborn)	0	3
Pneumonia	2	3
Cerebral haemorrhage	6	4
Uraemia	11½	5
Mastoid	14	6
Lymphatic leukaemia	15	7
Mitral stenosis	16	8
Accident	22	9
Acute uraemia, nephritis	14	10
Cardiac failure, nephritis	30	11
Cardiac failure	30	12
Staphylococcal septicaemia	21	13
Died in labour	25	14
Suppurative cholangitis and jaundice	37	15
Bacterial endocarditis	27	16
Bacterial endocarditis	29	17
Cause not noted	32	18
Carcinoma of stomach	59	19
Fractured vertebrae	60	20
Pulmonary embolism and tuberculosis	68	21
Heart failure	37	23

Having obtained a relationship between age and F content of the bones, we carried out a set of chemical determinations on the bones, the results of which are given in Table 2.

When these results are plotted side by side with the spectrographic results they show, on the whole, an increase of F content with age. From simple statistical tests it appears that with the exception of the value found at 22 years and the second of the values found at 37 years the results are not inconsistent

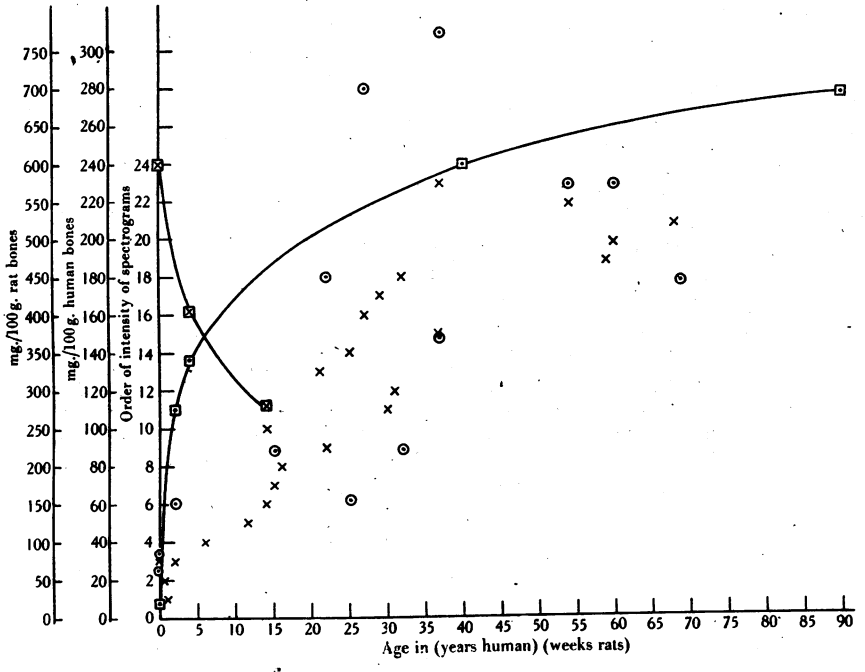

Fig. 1. Showing (1) Order of F content of human bones in relation to age taken from the spectrograms; denoted ×. (2) F content of human bones in relation to age; denoted ⊙. (3) Uptake of F by rat bones on a diet containing 0·05% NaF; denoted ⊡. (4) Elimination of F from rat bones; denoted ⊠.

Table 2. *Analyses on human bones at different ages*

Age years	Ca %	P %	Ca/P	Mg %	Ash %	F % in fat-free bone
	in ash of bones					
0	—	—	—	—	—	0·024
0	—	—	—	—	—	0·033
2	—	—	—	—	52·1	0·06
6	40·13	17·72	2·26	0·72	—	—
15	—	—	—	—	50·5	0·088
22	40·24	17·18	2·34	0·88	45·3	0·18
25	40·02	17·30	2·31	0·48	—	0·061
27	—	—	—	—	47·4	0·28
29	40·33	17·61	2·27	0·78	—	—
32	40·65	17·77	2·29	0·78	—	—
32	—	—	—	—	45·8	0·089
33	40·11	16·87	2·37	0·52	—	—
37	40·46	17·22	2·35	0·62	45·1	0·31
37	—	—	—	—	53·4	0·15
54	—	—	—	—	—	0·23
59	38·21	17·05	2·24	0·65	—	—
59	39·53	17·28	2·29	0·64	48·5	0·09
60	40·66	16·93	2·42	0·50	48·6	0·23
68	40·52	17·28	2·35	0·88	47·3	0·18

with a law that the F increases by 0·002 % for each year. The chemical results also show how remarkably constant the composition of the bone ash is. In two cases the Ca differed more than usual from the mean value. The Mg figures show fluctuations which appear to be without significance. This was somewhat surprising, since we had been led to expect, from the work of Day *et al.* [1935] on rats, that the Mg content would show a progressive increase with age. These rats were all given the same diet, whereas that of the human subjects must have varied widely.

2. *The uptake and elimination of fluorine by the bones of rats during and after fluorine feeding*

Rats were fed from the age of 6 weeks on the diet used in previous work [Murray, 1936]. The NaF, when included, was given as a constant proportion of the diet which was moistened by the addition of 1 ml. of 0·05 % NaF per g. of diet, with a maximum intake of 15 g. of diet per day. The actual diet itself contained 0·00047 % or 4·7 p.p.m. F. At different ages control rats and F-fed rats were killed and the long bones analysed for F. The bones of the F-fed rats were unusually white, thickened and very brittle. Table 3 gives the F content of the long bones of the rats at different ages. A set of determinations was also made of the F content of the bones of two groups of rats which had been on the diet for about 36 weeks but which had been taken off the diet for different periods before being killed. Table 3 also shows the fall in F content, i.e. the elimination from the bones in these two groups, which were of different stocks.

Table 3. A. *Uptake of F by the long bones of rats on a diet containing 0·05 % NaF*

Age weeks	Time on NaF weeks	% F in fat-free bones
6	0	0·019
90	0	0·028
98	0	0·040
8	2	0·273
10	4	0·343
14	8	0·428
16	10	0·644
46	40	0·600
80	74	0·617
90	84	0·705

B. *Elimination of F from the bones after withdrawal of the NaF from the diet*

Series (*a*): rats on the NaF diet 40 weeks

Age weeks	Time from the withdrawal of NaF till death weeks	% F in fat-free bones
46	0	0·600
50	4	0·407
60	14	0·287

Series (*b*): rats on the NaF diet 32 weeks

38	0	0·943
42	4	0·673
50	12	0·302

It appears that the uptake of F is rapid at first and then becomes more gradual, in fact the results when plotted fit a logarithmic curve. This might merely be due to the growth rate, but in view of the elimination rate, is not

necessarily so related. The highest concentration found was 0·943 %. In human cases of fluorosis resulting from the intake of cryolite dust, the highest concentration reported has been 1·3 % [Roholm, 1937]. Elimination seems to follow a course similar to that of the uptake. Unfortunately we have not carried out this process over a long enough period to secure complete elimination, but the results suggest that in human cases of fluorosis a fair degree of recovery might be expected when there was no further exposure to F. A similar experiment has been reported on a cow which had been exposed to a F-containing pasture [Bosworth & Green, 1941]. The animal was removed and grazed on a F-free pasture; the F content of the rib bones fell rapidly at first, but a good deal was still retained and being excreted at a time when the cow no longer showed clinical signs of fluorosis.

SUMMARY

1. The fluorine contents of human bones of different ages have been compared spectrographically and determined chemically. There is in general a rise of fluorine content with increase of age. The lowest value was 0·02 % and the highest recorded here, with no evidence of fluorosis, was 0·3 %.

2. Rats fed small amounts of NaF take up fluorine in the bones, the concentration of fluorine in relation to age following a logarithmic curve.

3. After withdrawal of the fluorine from the diet the elimination from the bones is the inverse of the uptake.

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