SOME EFFECTS OF FLUORIDE ON CALCIUM METABOLISM IN THE BONES OF YOUNG RATS

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[*Manuscript received April* 27, 1959]

Summary

Radioautography has indicated that in rats which were fed fluoride in the food at a rate of 400 p.p.m. (as sodium fluoride), and then injected with 17·6 μc ^{45}Ca, a process occurred which tended to cause a wider distribution of ^{45}Ca activity throughout the epiphyseal and trabecular regions of the femora and humeri. It also appeared that the labelled calcium was retained in these regions to a greater extent than in the control animals.

Radiochemical analyses showed that there was a tendency for the bones of fluoride-treated animals to have a greater overall ^{45}Ca activity, with significant increases in the lumbar and cervical vertebrae.

Few significant differences between the calcium content of the bones of fluoride-treated and control animals have been demonstrated, but there was some evidence to indicate that calcium is deposited and resorbed at a slower rate in fluoride-treated animals.

There appeared to be a tendency for the bones of fluoride-treated animals to have an ash content higher than that of the corresponding bones of the control animals, especially in the group of animals killed 10 days after the ^{45}Ca injection. There was also some evidence to indicate that ash-forming materials are deposited and resorbed at a slower rate in fluoride-treated animals.

Fluoride feeding has been shown to cause large increases in the bone fluoride level, with larger increases in the bones of animals exposed to fluoride feeding for the longer periods of time.

X-ray studies have shown no marked abnormalities in bone formation in fluoride-treated animals.

The humerus was the bone least affected by fluoride in all the experimental work.

I. INTRODUCTION

In Queensland, the high fluoride level of many waters is responsible for the occurrence of chronic endemic fluorosis in Merino sheep. An extensive study of this condition has been made by Harvey (1952, 1953*a*, 1953*b*), but the mechanisms by which fluoride exerts its deleterious effects are still relatively obscure.

Comar, Lotz, and Boyd (1952) and Comar *et al.* (1953) have studied the deposition and removal of ^{45}Ca in normal and fluoride-treated pigs using radioautographic techniques. They have reported that fluoride fed in the diet at concentrations of 200 and 1000 p.p.m. (as fluorine) caused a decrease in bone and body growth, and that a process occurs which tends to remove the ^{45}Ca originally deposited in the epiphyseal regions. They therefore suggested that fluoride caused

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increased bone resorption in these regions, the ^{45}Ca liberated in this process being utilized locally in the trabecular bone.

The bones of rats fed sodium fluoride at a rate of 4 mg per day for 10 weeks have been shown by Wadwhani (1954) to contain less nitrogen, more calcium, phosphate, carbonate, and sodium than those of control animals. The greatest differences between control and experimental animals were observed in the scapulae and the epiphyses. This author has suggested that fluoride affects the formation of collagen. No similar significant differences were observed when the experimental animals were fed sodium fluoride at a rate of 2 mg per day.

Kono (1954) has demonstrated a more active resorption in trabecular bone with rats fed fluoride at greater than 50 p.p.m. and a diminished resorption if the fluoride is fed at less than 10 p.p.m.

It has been reported by Arizono and Ichikawa (1955) as a result of histochemical studies that in rat fluorosis there is an increase in the calcification of dental pulp, bone shafts, trabeculae, and epiphyseal plates, the extent of the change being proportional to the dose and the period of administration of the fluoride.

Bélanger *et al.* (1958) have shown that in fluoride-treated pigs (1000 p.p.m. NaF), a rachitic condition similar to that caused by vitamin D deficiency occurs. Accompanying this condition they found decreased growth, imperfect mineralization, increased cartilage at the heads of the long bones, overproduction of osteoid, and increased acid-resistant ash which was only partly calcium fluoride.

The present study was commenced in order to investigate the effects of fluoride intake on the bones of young rats. X-ray, radioautographic, radiochemical, and purely analytical techniques were used in the hope that the results obtained would help in the understanding of the mechanism of fluoride toxicity and in the development of suitable methods for the further study of endemic fluorosis in Merino sheep.

II. Material and Methods

Thirty female rats, 7 weeks old and born on the same day and weighing 70–90 g, were paired by weight and placed on a basal diet of sucrose (70 per cent.), casein (18 per cent.), fat (5 per cent.) vitamin powder (Cuthbertson 1957) (1 per cent.) salt mixture (5 per cent.) and "Vetemul" (Nicholas Pty. Ltd.) (1 per cent.). This diet contained 0·9 per cent. calcium and 0·6 per cent. phosphorus. The composition of the vitamin powder and salt mixture is detailed in Table 1.

One member of each pair was fed sodium fluoride (400 p.p.m.) mixed with the diet; thereafter, until killing, the animals were pair-fed so as to ensure equal intakes, except, of course, for fluoride. On the 30th and 31st days of feeding the animals (wt. 100–130 g) were each injected intraperitoneally with 2 ml of a solution of $^{45}CaCl_2$ in isotonic $CaCl_2$ at pH 7·4, containing 4·4 μc ^{45}Ca (3·7 mg calcium) per ml. Groups of animals consisting of five randomly selected pairs were killed 18 hr (group A), 10 days (group B), and 30 days (group C) after the final injection. Immediately after death each animal was X-rayed from the dorsal and lateral positions. "Kodirex" no-screen X-ray film was used and processed by the methods recommended by the manufacturer.

The bulk of the adhering flesh was removed from all bones, care being taken to avoid damage. The bones were then autoclaved at a pressure of 20 lb/sq. in. for 10 min. After this treatment the remaining flesh was easily removed without damaging the bone.

The bones for radioautography were first dried at 80°C and then placed in activated acrylic monomer (Imperial Chemical Industries Ltd.) to which had been added a small quantity of benzoyl peroxide. The specimens were then placed in a vacuum desiccator which was then evacuated. This treatment enabled the embedding material to permeate the bone pores and marrow cavities. After about 30 min, the specimens were removed and cured at 60°C for 48 hr, after which time

TABLE 1

COMPOSITION OF THE VITAMIN POWDER AND SALT MIXTURE MOIETY OF THE BASAL DIET

Vitamin Powder	Content (mg)	Salt Mixture	Content (g)
Thiamine hydrochloride	100	$MgSO_4.7H_2O$	32·9
Riboflavin	100	NaCl	69
Pyridoxine	40	KCl	112
Calcium pantothenate	240	KH_2PO_4	212
Nicotinic acid	200	$MgCO_3$	25
Vitamin B_{12}	0·6	Ferric citrate	31
Choline chloride	20,000	$CaCO_3$	542·5
α-Tocopherol	1,000	KI	0·08
		$CuSO_4.5H_2O$	1·4
Made up to 200 g with sucrose		$MnSO_4.4H_2O$	0·4
		$CoSO_4.7H_2O$	0·03
		$NaH_2PO_4.2H_2O$	300

the embedding material had set hard. Approximately mid-sagittal ground hemi-sections were prepared by sectioning on a lathe and polishing flat. These sections were then pressed on to strips of "Kodirex" no-screen X-ray film attached to glass slides, wrapped in black paper, and set aside at 4°C for 24 hr. The radioautographs were then developed using the standard techniques recommended by the manufacturer for the particular film used.

Bones required for chemical and radiochemical analysis were dried at 80°C, ground finely, rendered fat-free by Soxhlet extraction with petroleum ether (B.P. 60–80°C), and again dried at 80°C. These materials were then dry-ashed at 700–800°C, dissolved in 2N HCl, and diluted to 10 ml with distilled water.

Samples were prepared for radioactive counting by transferring 0·3-ml portions of these solutions to 24-mm planchets, neutralizing the excess acid with 15N NH_4OH, adding one drop of 10 per cent. (w/v) kaolin in alcohol to assist spreading, and drying under an infra-red lamp. The radioactive counting was carried out using

an end-window Geiger–Müller tube in association with a standard recording and scaling unit. All samples were prepared in triplicate and, provided a sufficient number of counts were recorded (approx. 5000 for each sample) and corrections were made for self-absorption, the results were reproducible to within 4 per cent. of each other.

Calcium was determined in these solutions by taking suitable portions and titrating with ethylenediaminetetra-acetic acid, using murexide as indicator (Dunstone 1957).

Fluoride was determined in these solutions by the method described in a report of a Sub-Committee of the Analytical Methods Committee (Society of Public Analysts (Great Britain) 1944) as modified by Harvey (1952).

III. Results

The mean sodium fluoride intake for all experimental animals was $3 \cdot 7 \pm 0 \cdot 1$ mg per animal per day.

After the injection of ^{45}Ca, the animals showed no increases in body weight for about 2 weeks. Some animals actually lost weight immediately after the injection. This may have been caused by the relatively large amount of calcium that had to be injected because of the low specific activity of the ^{45}Ca available. A second reason may have been the large amount of ^{45}Ca administered ($17 \cdot 6$ μc). However, at all times during the experiment, the physical condition of control and experimental animal in each pair did not differ significantly.

Radioautographs have been made of one femur and one humerus from each animal; however, in the interest of economy of space, only selected radioautographs of the femora and humeri are presented. Similar radioautographs were obtained for corresponding animals of each group.

Figures 1 and 2 (Plate 1) represent typical radioautographs of the femora from a pair of animals from group A, while Figures 3 and 4 (Plate 1) and Figures 5 and 6 (Plate 1) represent the radioautographs of the femora from pairs of animals from groups B and C respectively.

Plate 1, Figure 1, the radioautograph of the femur of a control animal, shows a distinct line of deposition of the ^{45}Ca in the epiphyseal region. Plate 1, Figure 2, the corresponding radioautograph for a fluoride-treated animal is similar to Plate 1, Figure 1, but in this case the deposition of ^{45}Ca appears to be more diffuse and there seems to be greater deposition in the trabecular bone. In both treated and untreated animals there is considerable ^{45}Ca activity in the "funnel" region of the diaphysis and in the periosteal bone.

Figures 3 and 4 (Plate 1) respectively show the radioautographs of the femora of control and fluoride-treated animals killed 10 days after ^{45}Ca injection. Figure 4 differs from Figure 3 in that there appears to be a wider distribution of ^{45}Ca activity throughout the epiphyseal and trabecular regions with greater overall activity. The areas of activity in the funnel region of the diaphysis and the periosteal bone are still present in both treated and untreated animals.

TABLE 2

EFFECT OF FLUORIDE FEEDING ON ^{45}Ca CONTENT OF BONES

Mean values ± S.E. for the specific activity of the ^{45}Ca are given for the results, which are expressed as a percentage of the administered dose per milligram of calcium

Bone	Group	No. of Animals	Control (% × 10^3)	Fluoride-treated (% × 10^3)
Femur	A	4	54 ± 6	55 ± 6
	B	4	53 ± 2	51 ± 3
	C	4	43 ± 2	47 ± 2
Humerus	A	4	58 ± 4	60 ± 4
	B	4	52 ± 6	59 ± 1
	C	5	41 ± 1	41 ± 2
Lumbar vertebra	A	4	63 ± 3	63 ± 5
	B	5	68 ± 4	73 ± 2
	C	5	46 ± 3	55 ± 1
Cervical vertebra	A	4	51 ± 4	63 ± 4
	B	5	68 ± 4	76 ± 5
	C	5	57 ± 3	60 ± 4
Mandible	C	5	58 ± 3	62 ± 3

Analysis of Variance

Bone	Source of Variation	Degrees of Freedom	Sum of Squares (× 10^6)	Mean Square (× 10^6)	Variance Ratio
Femur	Mean response	1	48	48	1·37
	Between groups	2	152	76	2·18
	Within groups	9	312	35	—
Humerus	Mean response	1	105	105	1·01
	Between groups	2	187	93·5	0·90
	Within groups	10	1035	103·5	—
Lumbar vertebra	Mean response	1	293	293	8·9*
	Between groups	2	487	244	7·4**
	Within groups	11	360	33	—
Cervical vertebra	Mean response	1	729	729	6·18*
	Between groups	2	871	435	3·69
	Within groups	11	1299	118	—

Test of Significance of Difference between Groups

Bone		Mean Difference Required for Significance at:		Groups	Mean Difference between Groups
		5% Level	1% Level		
Lumbar vertebra	Means of 4 and 5	0·0085	0·0121	A and B	0·0056
	Means of 5 and 5	0·0080	0·0113	B and C	0·0038
				A and C	0·0094

* $P < 0·05$. ** $P < 0·01$.

Little ^{45}Ca activity is apparent in the epiphyseal regions of the femora from animals of the third group (Plate 1, Fig. 5 (control) and Plate 1, Fig. 6 (fluoride-treated)). What little activity there is appears to be greater in the case of fluoride-treated animals especially in the region of trabecular bone. The characteristic areas of deposition in the funnel region of the diaphysis and in the periosteal bone are still present, and practically identical with those of the radioautographs from the animals of the first two groups.

Similar patterns were observed with the humeri, but differences between control and fluoride-treated animals were not so apparent (Plate 2, Figs. 7–12). Here, the radioautographs of the humeri show considerable ^{45}Ca deposition in the posterior periosteal and in the anterior endosteal regions of the bone shafts, whereas the radioautographs of the femora show only periosteal deposition in the bone shafts. Similar observations have been made by Tomlin, Henry, and Kon (1953).

The results of the radioactivity measurements on the femora, humeri, vertebrae, and mandibles (group C only) are given in Table 2. An appropriate statistical treatment of the experimental data has been carried out and included in the table. The specific activity of the ^{45}Ca is expressed as a percentage of the original dose administered per milligram of calcium present in the bone.

No significant differences between the ^{45}Ca activities of the femora and humeri of fluoride-treated and control animals were observed, but the activities of the lumbar and cervical vertebrae of fluoride-treated animals were significantly higher than those of the control animals (lumbar vertebrae, mean response $P < 0{\cdot}05$; cervical vertebrae, mean response $P < 0{\cdot}05$). A significant difference between groups was also found for the lumbar vertebrae ($P < 0{\cdot}01$), the differences of activity between fluoride-treated and control animals being greatest in group C and least in group A; however, only the difference between groups A and C was statistically significant at the 5 per cent. level. An interesting observation with respect to the metabolism of ^{45}Ca is that while the femur and humerus retain an approximately constant specific activity for a period of 10 days followed by a decrease, the vertebrae show a considerable increase in specific activity over the initial 10 days followed by a decrease similar to that observed for the femur and humerus.

Table 3 summarizes the results of the calcium analyses of the various bones. No significant differences between the values for control and fluoride-treated animals could be demonstrated, except in the case of the cervical vertebrae where the fluoride-treated animals showed a significantly lower calcium content (mean response $P < 0{\cdot}05$). Significant differences between groups were obtained for the femora ($P < 0{\cdot}05$), and the cervical vertebrae ($P < 0{\cdot}05$). Mean differences required for significance between any two of the groups show that the differential effect between groups A and B and groups A and C of the femora are significant at the 1 per cent. and 5 per cent. levels respectively, while the differential effect between groups A and B and groups B and C of the cervical vertebrae are significant at the 5 per cent. level. In the 9-day period between the killing of groups A and B, there appears to be a decrease in the calcium content of bones of all animals, followed by an increase in the following 20 days. Both the initial decreases and subsequent increases are

TABLE 3

EFFECT OF FLUORIDE FEEDING ON THE CALCIUM CONTENT OF BONES

Mean values ± S.E. are given for the percentage calcium in the water- and fat-free bones

Bone	Group	No. of Animals	Control (%)	Fluoride-treated (%)
Femur	A	4	24·0 ± 0·4	23·2 ± 0·4
	B	4	23·1 ± 0·4	23·9 ± 0·2
	C	4	24·3 ± 0·1	24·6 ± 0·4
Humerus	A	5	24·3 ± 0·1	24·2 ± 0·1
	B	4	24·4 ± 0·8	24·7 ± 0·4
	C	5	25·3 ± 0·5	25·4 ± 0·5
Lumbar vertebra	A	4	22·1 ± 0·4	22·9 ± 0·6
	B	5	21·0 ± 0·3	21·6 ± 0·4
	C	5	23·0 ± 0·4	22·8 ± 0·2
Cervical vertebra	A	5	23·7 ± 0·3	23·1 ± 0·3
	B	5	22·3 ± 0·3	22·5 ± 0·2
	C	5	23·4 ± 0·3	22·7 ± 0·1
Mandible	C	5	26·6 ± 0·3	26·1 ± 0·3

Analysis of Variance

Bone	Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio
Femur	Mean response	1	0·163	0·163	0·31
	Between groups	2	4·77	2·39	4·6*
	Within groups	9	4·69	0·52	—
Humerus	Mean response	1	0·121	0·121	0·13
	Between groups	2	0·462	0·231	0·26
	Within groups	11	9·887	0·90	—
Lumbar vertebra	Mean response	1	2·49	2·49	3·61
	Between groups	2	5·20	2·60	3·77
	Within groups	11	7·56	0·69	—
Cervical vertebra	Mean response	1	1·73	1·73	6·65*
	Between groups	2	3·93	1·96	7·54**
	Within groups	12	3·09	0·26	—

Test of Significance of Differences between Groups

Bone		Mean Difference Required for Significance at:		Groups	Mean Difference between Groups
		5% Level	1% Level		
Cervical vertebra	Means of 5 and 5	0·70	0·99	A and B	0·78
				B and C	0·84
				A and C	0·06
Femur	Means of 4 and 4	1·05	1·48	A and B	1·48
				B and C	0·42
				A and C	1·06

*$P < 0·05$. **$P < 0·01$.

somewhat smaller in the fluoride-treated animals, indicating a slower resorption and uptake of calcium in these animals. The humerus is the only bone that does not follow this pattern.

The percentage ash of the water- and fat-free bones of fluoride-treated and control animals is shown in Table 4. Only the lumbar vertebrae show a significant difference ($P < 0{\cdot}01$) between the percentage ash of fluoride-treated and control animals, the treated animals having the higher values. Significance between groups was obtained for lumbar vertebrae ($P<0{\cdot}01$), and the cervical vertebrae ($P<0{\cdot}05$).

In the case of the lumbar vertebrae, the differences between the percentage ash of control and fluoride-treated animals was greatest in group A and least in group C, the difference between groups A and C being significant at the 5 per cent. level. With the cervical vertebrae, the greatest differences in percentage ash between fluoride-treated and control animals occurred in group B, the difference between groups B and C being significant at the 5 per cent. level. In the 9-day period between the killing of groups A and B, there appears to be a decrease in the ash content of bones of all animals, followed by an increase in the subsequent 20 days. Both the initial decreases and subsequent increases are somewhat smaller in the fluoride-treated animals, indicating a slower release and uptake of ash-forming materials in the fluoride-treated animals. Again the only bone that does not follow this pattern is the humerus.

The fluoride content of the water- and fat-free bones is shown in Table 5. The bones from all animals were not examined, but the results indicate that the bones of fluoride-fed animals contain 15–20 times more fluoride than those of the control animals. The difference in fluoride content between the bones of fluoride-treated and control animals is greatest in group C, and least in group A. The observed differences were of such magnitude that statistical analyses were not carried out.

No gross abnormalities in bone structure, such as the formation of exostoses, bone thickening, etc. could be detected in the X-rays of fluoride-treated animals when compared with those of the control animals.

IV. Discussion

The radioautographs obtained from the femora of the animals of group A (killed 18 hr after final injection) were similar to those obtained by Comar *et al.* (1953) for pigs killed 5 hr after ^{45}Ca injection. This would then verify the fact observed by these authors that in both fluoride-treated and untreated animals there is initial deposition of ^{45}Ca in the epiphyseal region with the fluoride-treated animals showing more diffuse deposition in this and in the trabecular regions.

In the radioautographs of the femora and humeri from the second group of animals, there appeared to be a wider distribution of ^{45}Ca in the area immediately below the epiphysis in the fluoride-fed animals. It would also appear that the labelled calcium in the bones of fluoride-treated animals is retained in these regions to a greater extent than in the control animals.

The radioautographs obtained from control and fluoride-treated animals of the third group show that the line of initial epiphyseal deposition present in the

TABLE 4

EFFECT OF FLUORIDE FEEDING ON THE ASH CONTENT OF BONES

Mean values ± S.E. are given for the percentage ash of the water- and fat-free bones

Bone	Group	No. of Animals	Control (%)	Fluoride-treated (%)
Femur	A	4	64·4 ± 0·5	63·1 ± 0·7
	B	4	60·0 ± 0·3	61·9 ± 0·2
	C	4	62·1 ± 0·3	62·9 ± 0·3
Humerus	A	5	57·3 ± 0·5	56·5 ± 0·7
	B	4	63·4 ± 0·8	64·3 ± 0·1
	C	5	63·2 ± 0·4	63·5 ± 0·4
Lumbar vertebra	A	4	57·5 ± 0·3	59·6 ± 0·2
	B	5	54·7 ± 0·7	56·2 ± 0·4
	C	5	59·9 ± 0·4	60·5 ± 0·4
Cervical vertebra	A	5	60·4 ± 0·5	60·7 ± 0·4
	B	5	56·5 ± 0·6	58·2 ± 0·5
	C	5	61·1 ± 1·0	60·8 ± 0·4
Mandible	C	5	68·9 ± 0·8	69·5 ± 0·7

Analysis of Variance

Bone	Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio
Femur	Mean response	1	2·43	2·43	0·74
	Between groups	2	23·22	11·61	3·56
	Within groups	9	29·39	3·26	—
Humerus	Mean response	1	0·4	0·4	0·072
	Between groups	2	4·59	2·30	0·41
	Within groups	11	61·26	5·57	—
Lumbar vertebra	Mean response	1	23·92	23·92	32·8**
	Between groups	2	28·86	14·43	19·8**
	Within groups	11	8·07	0·73	—
Cervical vertebra	Mean response	1	0·55	0·55	0·34
	Between groups	2	16·6	8·3	5·2*
	Within groups	12	19·32	1·60	—

Test of Significance of Difference between Groups

Bone		Mean Difference Required for Significance at:		Groups	Mean Difference between Groups
		5% Level	1% Level		
Lumbar vertebrae	Means of 4 and 5	1·26	1·69	A and B	0·60
	Means of 5 and 5	1·19	1·67	B and C	0·87
				A and C	1·47
Cervical vertebrae	Means of 5 and 5	1·75	2·26	A and B	1·40
				B and C	2·06
				A and C	0·66

*$P < 0·05$. **$P < 0·01$.

radioautographs of the bones from animals of the first two groups is absent in both control and fluoride-treated animals. This indicates a removal of the ^{45}Ca from these regions. Although only small differences between the radioautographs of the bones of control and fluoride-treated animals were observed, it appears that in the case of the treated animals more ^{45}Ca activity was present in the trabecular regions. Thus it is suggested that resorption of ^{45}Ca originally deposited in the epiphyseal region occurs in both control and fluoride-treated animals with a greater utilization of the removed calcium in the trabecular bone of the fluoride-treated animals.

This retention of ^{45}Ca activity, as visualized by the radioautographs, is supported to some extent by the radiochemical analyses which indicate that there is a tendency towards greater overall ^{45}Ca activity in the bones of fluoride-treated animals. The most pronounced increases in ^{45}Ca activity were observed in the vertebrae. This is not surprising, as it is a well-known fact that these bones are often seriously affected in fluorotic conditions (Harvey 1952). Significant differences between groups have only been demonstrated for the lumbar vertebrae and in this case the increase in ^{45}Ca activity for fluoride-treated animals is greatest in group C and least in group A (no difference between control and fluoride-treated animals in this group). It is therefore suggested that with these bones, and possibly with others, the rate of initial deposition of ^{45}Ca is almost the same in both control and fluoride-treated animals, but the ^{45}Ca activity is retained to a greater extent in the fluoride-treated animals. Increased ^{45}Ca retention is contrary to the observations of Comar, Lotz, and Boyd (1952) and Comar *et al.* (1953) and it is difficult to find an explanation of how it might occur. One of a number of possibilities is that the retention might be the result of the formation in the matrix of calcium fluoride or an organic salt of calcium which is less easily removed than the usual hydroxylapatite crystal. Bélanger *et al.* (1958) have reported the occurrence of such compounds in the matrix between the cartilage cells in the bones of fluoride-treated pigs, the bones showing an increased acid-resistant ash. The tendency towards increased ash in our experiments also lends some support to this explanation.

Fluoride appeared to exert no significant overall effect on the calcium content of bones, except in the case of the cervical vertebrae where fluoride-treated animals showed a slightly decreased calcium content. These results do not agree with those obtained by Wadwhani (1954) who found an increased calcium content in the bones of rats fed 4 mg sodium fluoride per day for 10 weeks. His experiments would correspond to the group C experiments of this communication. The diet fed by Wadwhani contained 0·27 per cent. phosphorus and 0·38 per cent. calcium, whereas the diet used in these experiments contained 0·6 per cent. phosphorus and 0·9 per cent. calcium, and it is possible that the high level of dietary calcium may have influenced the effect of the fluoride. Several workers (Ranganathan 1944; Smith and Shaner 1944; Greenwood *et al.* 1946; Wadwhani 1954) report that high calcium levels in the diet decrease the effects of fluoride. On the other hand, Harvey (1952) and Suttie, Phillips, and Miller (1958) have found that high dietary calcium levels do not influence the effect of fluoride. In view of the fluoride content of the bones of the experimental rats of this study, it appears that the dietary calcium level has had little influence on the uptake of fluoride by the bones. The significant differential effect observed between groups for the calcium content of the femora

and cervical vertebrae indicates that a slower resorption and subsequent uptake of calcium has occurred in the fluoride-treated animals. Although no significance could be demonstrated, the lumbar vertebrae showed a similar pattern, while the humerus showed little difference between fluoride-treated and control animals. This lends some support to the explanation that the greater retention of ^{45}Ca in bones of fluoride-treated animals is due to the formation of calcium fluoride or a calcium salt in the matrix, this compound being less easily resorbed than the usual hydroxylapatite crystal.

TABLE 5

EFFECT OF FLUORIDE FEEDING ON THE FLUORIDE CONTENT OF BONES

The results are expressed in p.p.m. of fluorine in water- and fat-free bone

Bone	Group	Control (p.p.m.)	Fluoride-treated (p.p.m.)
Femur	A	154	3090
	B	225	3780
	C	374	4810
Humerus	A	210	3460
	B	310	3950
	C	310	4210
Lumbar vertebra	A	155	3560
	B	317	3930
	C	299	4990
Cervical vertebra	A	148	3450
	B	309	3530
	C	291	4700
Mandible	C	385	4100

There appeared to be an overall tendency for the bones of fluoride-treated animals to have a greater ash content than those of the control animals, but this is by no means statistically significant. Smith and Lantz (1933) and Munoz (1936) have found that fluoride decreases the percentage ash of rat bones. Kick *et al.* (1935) have found that fluoride exerts no apparent effect on the ash content of pig bones, while McClure and Mitchell (1931) have reported that fluoride increases the ash content of rat bones. Peirce (1938) has shown slight increases in the ash content of the bones of fluoride-treated sheep but the values were still within the normal range. It appears that the increase in ash content of the bones of the fluoride-treated rats of these experiments follows a pattern similar to that observed by Peirce (1938) for sheep.

The significant differential effect observed between groups for the ash content of the lumbar and cervical vertebrae, with the effect for the femora being almost significant at the 5 per cent. level, indicates a slower resorption and subsequent uptake of ash-producing materials in fluoride-treated animals. This gives some support to the explanation previously given for calcium retention in fluoride-treated animals, if the ash-producing materials are compounds such as those suggested by Bélanger *et al.* (1958).

The decrease in the ash and calcium content of bones that occurred in the 9-day period between the killing of groups A and B was not expected and an explanation of its occurrence is not possible without further investigation.

No marked differences in the bones of control and fluoride-treated animals were observed by X-ray examination. Exostoses and other malformations of the bone, which occur in animals fed fluoride for a considerable period of time, did not appear in these experiments, presumably because of the shorter period of administration.

The results of the radiochemical and chemical analyses indicate that fluoride exerts a greater effect on the vertebrae, particularly the lumbar vertebrae, than on the long bones. No explanation of how these effects might occur is offered, but it is hoped that in the near future a more detailed study of these effects on the vertebrae will be commenced.

V. Acknowledgments

The authors are indebted to Professor H. J. G. Hines, Dr. J. M. Harvey, Dr. C. C. Kratzing, and Dr. O. Budtz-Olsen for their advice and helpful criticism. Thanks are due to Dr. J. A. Sagar for assistance with the X-rays, Mr. K. P. Haydock for advice on statistical treatment, and Mr. E. Hollywood and Miss N. Bowyer for preparing the photographs. One of us (E.P.) wishes to thank C.S.I.R.O. the for the award of a Junior Post-Graduate Studentship.

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Explanation of Plates 1 and 2

Plate 1

Figs. 1 and 2.—Radioautographs of the femora of control (Fig. 1) and fluoride-fed (Fig. 2) animals killed 18 hr after ^{45}Ca injection. Note the distinct line of deposition of ^{45}Ca in the epiphyseal region and the slightly greater ^{45}Ca activity in the trabecular region of the femur of the fluoride-treated animal.

Figs. 3 and 4.—Radioautographs of the femora of control (Fig. 3) and fluoride-fed (Fig. 4) animals killed 10 days after ^{45}Ca injection. Note the wider distribution of ^{45}Ca activity in the trabecular region of the femur of the fluoride-treated animal.

Figs. 5 and 6.—Radioautographs of the femora of control (Fig. 5) and fluoride-fed (Fig. 6) animals killed 30 days after ^{45}Ca injection. Note the absence of the line of original deposition of ^{45}Ca in both control and fluoride-treated animals. There appears to be slightly more ^{45}Ca activity in the trabecular bone of the fluoride-treated animal.

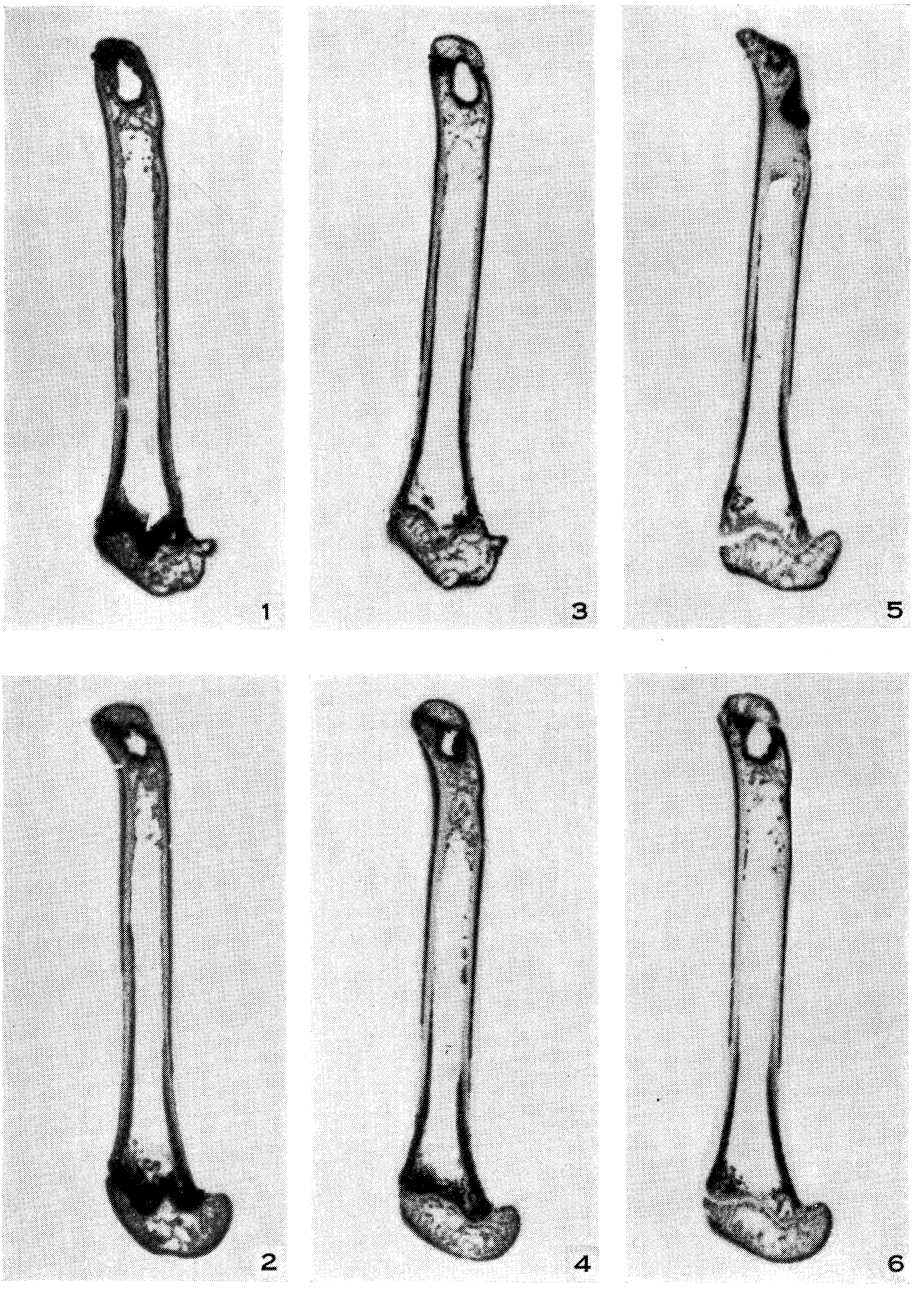
Plate 2

The radioautographs of the humeri of control and fluoride-fed animals do not show such marked differences as do those of the femora, but they exhibit the same essential features. Note the areas of endosteal and periosteal deposition of the ^{45}Ca

Figs. 7, 9, and 11.—Radioautographs of the humeri of control animals killed respectively 18 hr, 10 days, and 30 days after ^{45}Ca injection.

Figs. 8, 10, and 12.—Corresponding radioautographs of the humeri of fluoride-treated animals.

EFFECT OF FLUORIDE ON BONE METABOLISM



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