THE DEPOSITION OF Sr^{89} IN RABBIT BONES FOLLOWING INTRAVENOUS INJECTION.*

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FACTORS affecting the retention of radioactive strontium in the skeleton of rabbits following a single intravenous injection have already been reported (Kidman, Tutt and Vaughan, 1950, 1951). The following investigation makes a detailed study of (i) the relation of total skeletal retention to blood levels of strontium at different times and different ages after a single injection; (ii) the relative retention in different bones of the rabbit at different ages; (iii) the relative retention in different parts of the same long bone at varying periods up to three weeks after injection.

EXPERIMENTAL PROCEDURE.

Injection solution.—Sr^{89} was prepared by irradiation of strontium carbonate in the pile at Chalk River. It was given into the marginal vein of the ear as strontium chloride made up in solution to be as nearly as possible isotonic and neutral. Carrier was inevitably present; the amount given varied since the specific activity of strontium changed as the Sr^{89} decayed and it was desired to give a relatively constant amount of Sr^{89}.

Rabbits.—Dutch rabbits from the A.R.C. station, Compton, were used (Kidman *et al.*, 1950, 1951). They were kept on a medium calcium diet composed of oats, cabbage and hay.

I. In the first series of experiments on the relation of total skeletal uptake to blood levels two groups of animals were used:

(*a*) Forty weanlings aged 5–7 weeks. They were killed in groups of 1–4 at intervals up to 9 days after an injection of between 3–18 μc. Sr^{89} per kg. The solution contained amounts of carrier which varied between 10–15 mg. $SrCl_2$ per kg. body weight, in a volume less than 1 ml.

(*b*) Thirty-five animals aged 10–11 months. They were killed in groups of 1–3 at intervals up to 9 days after an injection of between 4–17 μc. Sr^{89} per kg. The solution contained 12 mg. $SrCl_2$ per kg. body weight given in a volume of 2–3 ml.

Blood samples were taken from the marginal vein of the ear and the total blood volume calculated from the body weight (Courtice, 1943). Complete faecal and urinary estimations of Sr^{89} were carried out on the excreta of five weanling rabbits killed 5, 7 or 9 days after the injection, and that of 2 adult

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rabbits killed 9 days after the injection. Samples of urine from the bladder were also examined in the case of 2 weanling rabbits killed 10 minutes after the injection and of 10 adults killed at different times up to 24 hours after the injection.

II. In the second group of experiments, planned to determine the retention in different bones of the same animal at different ages and at different times after a single injection, use was made of the bones of animals in Group I.

III. In the third group of experiments planned to study the relative retention in different parts of the same bone the rabbits were 3–4 months old. The bones of younger rabbits proved so difficult to separate into constituent parts that the results obtained are not included. The quantities of Sr^{89} per kg. given to each rabbit varied from 50–190 μc. in a volume of from 5–10 ml. containing 90–160 mg. $SrCl_2$ per kg. It is theoretically possible that this amount of carrier may have affected retention, but evidence from other work suggests it did not (Schubert and Wallace, 1950; Vaughan, Kidman and Tutt, 1952). Animals were killed 10 minutes, 24 hours, 9 days and 3 weeks after injection. Fourteen bones were analysed in the first three groups and ten in the fourth group.

Preparation of the bones.

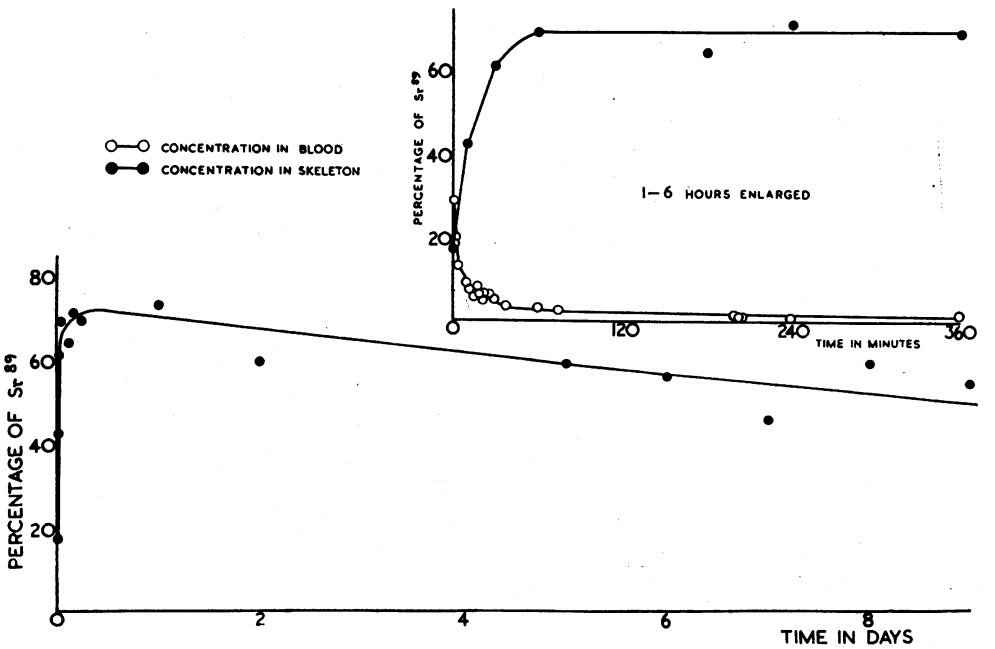
In experiments I and II the bones were removed as previously described (Kidman *et al.*, 1950) and analysed for retention separately, except that the four feet were analysed as one; the ribs and vertebrae which were difficult to clean were also analysed together, and the skull bones were regarded as one unit. Each figure therefore represents the average value for a pair of bones, and not that of a single bone. The complete skeletal retention figures were obtained by adding the values for each pair or group of bones. The humerus, radius, femur, tibia and fibula were grouped as long bones, the scapula and pelvis as flat bones. The skull was not included when the average value for flat bones was estimated, since it proved difficult to clean and also contained the teeth, which have been shown to concentrate injected strontium to a greater extent than does the skull (Pecher, 1941; Scott *et al.*, 1947).

In experiment III the results recorded were obtained on the humerus and the tibia-fibula. Immediately after removal from the carcass, which was not boiled, the bones were coated with paraffin wax, split lengthwise and sawn in half across the centre of the shaft. The bone marrow was washed out with a fine jet of water. The bones were fixed in alcohol for 3–7 days, the alcohol being changed 3 times. This was done as autoradiographic studies were being made in parallel. Insignificant amounts of Sr^{89} were found in the alcohol. The periosteal and endosteal surfaces of the bones were scraped to remove any adherent tissue. The scrapings were analysed for their Sr^{89} content in the earlier experiments, but the weight and consistency of tissue obtained was so variable that subsequently the scrapings were not included in the analysis. The sections of bone were then divided by a fret-saw into epiphysis, epiphyseal plate, metaphysis and diaphysis, and the strontium content of each part estimated separately.

Estimation of radioactive strontium.

Estimation of Sr^{89} in the blood, urine, faeces and bones was carried out as previously described (Kidman *et al.*, 1950, 1951). In the case of the portions of

bone used in experiment II the alcohol was first evaporated over a water-bath and the pieces weighed before dissolving in nitric acid. The results were expressed as the percentage of the injected Sr^{89} per gramme of dried bone or as the percentage of the total Sr^{89} retained in the skeleton per gramme of bone. The faeces were ashed, in the first instance over a bunsen burner and finally in a muffle furnace, and the strontium extracted from the ash by boiling with tartaric acid solution. The mixture was allowed to cool and the supernatant liquor decanted. The process was repeated until the ash was no longer active; the Sr^{89} content of the total liquor was then estimated. Corrections were made in all cases for radioactive decay.



Concentration of Sr^{89} in blood and skeleton of 5–6 weeks old rabbits at different times after intravenous injection.

RESULTS.

The Relation of Total Skeletal Retention to Blood Levels of Strontium at Different Time Intervals after a Single Injection.

Rabbits aged 10–11 *months.*—The concentration of Sr^{89} in the circulating blood fell and that in the skeleton rose after injection. Animals killed within 30 seconds of the injection had 50 per cent of the injected dose in the circulating blood; after 30 minutes this had fallen to 13 per cent, and in 6 hours it had reached 7 per cent. The skeletal figures at the same time were 11·7 per cent, 21·5 per cent, and 26·2 per cent respectively. Since large quantities of blood were not available, later samples had an extremely low counting rate; the results did not justify the length of time required to obtain an accurate figure.

The maximum skeletal concentration was reached 3–6 hours after the injection, the figure being between 28 and 30 per cent of the injected dose (Table I). The

value decreased sharply from the maximum to 16·4 per cent in 24 hours. After 48 hours it was 18·8 per cent, and then fell slowly to 8·5 per cent at the end of nine days. The rapid fall in concentration between 6 and 24 hours must be regarded as significant since the standard deviations are so small (Table I), but the rise between 24 hours and 48 hours is not significant.

TABLE I.—*Mean Values for Sr^{89} Retained in the Skeleton of* 10–11 *Months Old Rabbits at Different Times after Injection Expressed as Percentage of the Injected Dose.*

Time after injection.	Number of rabbits.	Mean percentage retained.	Standard deviation.
20–30 seconds	3	11·7	1·9
6–7 minutes	1	13·8	—
10 ,,	2	15·7	—
30 ,,	3	21·5	4·9
1 hour	3	24·0	3·2
3 hours	3	27·1	5·5
4 ,,	3	29·9	3·5
6 ,,	3	26·2	2·1
1 day	3	16·4	2·9
2 days	3	18·8	1·7
5 ,,	3	13·6	3·7
7 ,,	1	17·2	—
9 ,,	3	8·5	1·0

Rabbits aged 5–6 *weeks.*—The general shape of the curves obtained when skeletal and blood concentrations were plotted against the time after injection was similar to that for the adult rabbits, but the blood concentration fell and the skeletal concentration rose more rapidly (see Fig.). The blood concentration 6 hours after the injection reached a lower level than in the adults (Table II).

TABLE II.—*Mean Values for Sr^{89} Retained in the Skeleton of* 5–6 *Weeks Old Rabbits at Different Times after Injection, Expressed as Percentage of the Injected Dose.*

Time after injection.	Number of rabbits.	Mean percentage retained.	Standard deviation.
25–30 seconds	3	17·4	2·13
10 minutes	4	42·6	3·47
30 ,,	2	61·3	—
1 hour	3	69·6	6·90
3 hours	4	64·1	10·49
4 ,,	2	71·5	—
6 ,,	3	69·8	5·66
1 day	3	73·0	7·56
2 days	3	60·0	9·94
5 ,,	3	59·8	3·44
6 ,,	1	56·9	—
7 ,,	4	46·6	5·32
8 ,,	2	60·0	—
9 ,,	3	55·1	4·54

Animals killed 30 seconds after injection had 30 per cent of the injected strontium in the circulating blood ; within half an hour this value had fallen to 5·5 per cent, and at 6 hours it was less than 1 per cent. The corresponding figures for the skeleton were 17, 61 and 70 per cent respectively. The maximum skeletal concentration of 70 per cent was reached between 3 and 6 hours after the injection and the value decreased more gradually and more evenly than did that for the adults, reaching 55 per cent at the end of 9 days There was considerable variation in the skeletal retention of individual animals at any one time as shown by the high standard deviation (Table II), which ranged from 2·13 to 10·49.

Excretion.—The figures obtained for the Sr^{89} excreted in the urine and faeces did not differ significantly from those previously recorded (Kidman *et al.*, 1950), and are therefore not given here. Total recovery of the injected dose was satisfactory, being greater than 90 per cent in each case. Less than 10 per cent of injected Sr^{89} was recovered from the urine in the bladder of rabbits killed up to 6 hours after the injection, except in one case, when 12 per cent was recovered from the bladder urine of a rabbit killed 1 hour after the injection.

The Relative Retention in Different Bones of the Rabbit at Different Ages.

The percentage of the injected dose retained in any one bone in either group of animals varied with the time after injection in the same way as did the total percentage in the whole skeleton.

If the amount of Sr^{89} found in any one bone was expressed as a percentage per gramme weight of the amount retained in the whole skeleton at that time, all the results for one bone could be examined together, regardless of the time after injection when the estimation was made (Table III). In the case of the

TABLE III.—*Mean Values for Sr^{89} per g. of Bone in Individual Bones Expressed as a Percentage of the Dose Retained in the Whole Skeleton at Different Times after Injection in Rabbits of Different Ages.*

	5–7 weeks (40 rabbits).	11 months (35 rabbits).
Femur	1·56	0·46
Tibia and fibula	1·58	0·45
Radius and ulna	1·56	0·48
Humerus	1·56	0·53
Scapula	1·51	0·69
Pelvic girdle	1·55	0·65
Standard error	±0·034	±0·014

old animals the figure for the femur, tibia and radius and ulna is approximately the same, 0·46, 0·45 and 0·48 per cent per gramme of bone respectively, while that for the humerus is somewhat higher—0·53 per cent. This difference is possibly significant. The difference between the long and the flat bones is clearly significant, the percentage for the scapula being 0·69 and for the pelvic girdle 0·65. Such differences are not apparent between the flat and the long bones in the case of the young animals (Table III). The Sr^{89} content of the flat bones is 1·51 and 1·55 per cent and that of the long bones 1·53 per cent.

The Relative Retention in Different Parts of the same Long Bone at Varying Times up to 3 weeks after Injection.

In none of the bones examined 10 minutes, 24 hours, 9 days or 21 days after the injection was the Sr^{89} evenly distributed between the epiphysis, epiphyseal plate, metaphysis and diaphysis (Table IV). An analysis of variance showed

TABLE IV.—*Mean Values for Retention of Sr^{89} per g. of Bone in Different Parts of the Bone (Tibia and Humerus) at Varying Times after Injection Expressed as Percentage of Injected Dose. (Age of Rabbits at Time of Injection 3–4 Months.)*

	10 minutes (14 bones).	24 hours (14 bones).	9 days (14 bones).	21 days (10 bones).
Epiphysis	0·35	0·59	0·46	0·23
Epiphyseal plate	0·58	1·45	0·65	0·26
Metaphysis	0·42	1·26	0·84	0·42
Diaphysis	0·17	0·55	0·52	0·31
Standard error	0·029	0·083	0·033	0·012

that although the scatter of figures in any one group was high, the differences between the means were in the majority of cases significant.

Ten minutes after the injection there was a significantly greater concentration of Sr^{89} in the plate than in any other region, while the diaphysis contained the least concentration. The difference between the epiphysis and the metaphysis was barely significant.

Twenty-four hours after the injection the difference between the concentration in the plate and in the metaphysis was hardly significant, although both contained a significantly higher concentration than the epiphysis and the diaphysis. The difference between the latter was not significant.

Nine days after the injection the metaphysis contained the greatest concentration, followed by the epiphyseal plate. The difference between the epiphysis and the diaphysis, which here had the direction opposite from that at 10 minutes and 24 hours, was barely significant.

TABLE V.—*Differences between Mean Values of Sr^{89} per g. of Separate Portions of Bone at Different Times.*

	24 hr.–10 min.	9 days–24 hr.	21 days–9 days.
Epiphysis	0·244 ±0·063	−0·132 ±0·090	−0·226 ±0·071
Epiphyseal plate	0·873 ±0·167	−0·797 ±0·173	−0·390 ±0·078
Metaphysis	0·849 ±0·200	−0·423 ±0·212	−0·422 ±0·086
Diaphysis	0·380 ±0·082	−0·025 ±0·108	−0·214 ±0·086

Twenty-one days after the injection the metaphysis contained the highest concentration, followed by the diaphysis. The difference between the epiphyseal plate and the epiphysis, both of which contained a lower concentration than the other parts of the bone, was scarcely significant.

In Table V are shown the differences in the means of various bone portions at different times with their standard errors. Taking as significant any difference greater than 2·2 times, and as non-significant any of less than 2·0 times its standard error, differences, except for the plate, between 9 days and 1 day are non-significant and all others are very clearly significant.

DISCUSSION.

The results in the first group of experiments show that there is a rapid uptake of Sr^{89} by the skeleton. In weanlings 42 per cent of the injected Sr^{89} is found in the skeleton in 10 minutes, and a maximum figure in the neighbourhood of 70 per cent is present within 1 hour. In older rabbits in which the epiphyseal plate has closed and where growth is almost absent, the uptake within 10 minutes is 15 per cent of the injected dose and the maximum figure of 30 per cent is reached in about 4 hours. At the end of 9 days the skeletal content of the young rabbits has fallen to 55 per cent and of the older rabbits to 8·5 per cent. Analysis of the Sr^{89} content of different parts of the same long bone at different times shows that initially the highest activity is found in the region of the epiphyseal plate, i.e., the most actively growing part of the bone; 9 days after injection it is in the metaphysis, and 3 weeks after injection, though it still remains in absolute terms highest in the metaphysis, there is relatively an increase in the diaphysis. This movement of radioactive strontium within the bone is apparent only, and is due to displacement of bone formed when the uptake of radioactive strontium was high by even younger bone formed when the blood no longer contains radioactive material, and finally by removal of the radioactive trabeculae in the metaphysis by the normal process of resorption. The selective localization of radioactive strontium in areas of active bone growth and the apparent changes in the site of deposition due to formation of fresh bone containing no radioactive isotope are well illustrated by autoradiographic studies of long bones following a single injection of Sr^{89} (Kidman, Rayner, Tutt and Vaughan, to be published).

Somewhat similar studies of the apparent movement of P^{32} within the bone have been recorded by Leblond, Wilkinson, Bélanger and Robichon (1950). Pecher (1941) has also noted that Sr^{89} is concentrated in spongy rather than in compact bone.

The results of analysis of different bones show that the uptake in old animals is greater in the flat bones than in the long bones, but the differences between the long and flat bones in the young is not significant. This is possibly associated with the fact that in young animals the long bones contain active red marrow throughout, while later the flat bones contain the greater quantity of marrow and therefore a greater area of bone surface is exposed to the circulating isotope. To obtain an accurate estimate of the amount of an isotope retained in the skeleton soon after injection it is therefore essential to analyse the entire skeleton. Any figure obtained by calculation based on the analysis of one or two bones (Swift, Prosser and Mika, 1946; Scott *et al.*, 1947; Anthony, Lathrop and Finkle, 1947; Swift and Prosser, 1947; Jones and Copp, 1951) is likely to be approximate

only. The fact that Hodges *et al.* (1950) found no significant differences in strontium content between the different bones in any one human individual is accounted for presumably by the fact that they were studying long-term retention only. In the present observations differences between retention in the long and flat bones became less with increasing time after injection. Anthony, Lathrop and Finkle (1947) have presented some data on different bones in rats, mice and rabbits, and conclude that the average distribution of radioactive strontium from bone to bone is essentially uniform in the three species. Taking the concentration in the femur as 100 the average concentration in all the bones measured was in the range 95 ± 16. For any given bone the maximum individual variation was usually about $\pm$ 50 per cent of the average value for all animals, and for a given animal the maximum variation in concentration from one bone to another was about 2–3-fold. No attention appears to have been paid to the relative ages of any of the animals.

The combination of autoradiographic (Kidman *et al.*, to be published) and chemical studies suggests that two mechanisms at least are involved in the deposition and in the removal of Sr^{89} from the skeleton :

(i) Sr^{89} is incorporated in sites of active calcification probably in the form of a strontium phosphate carbonate complex ; from such areas it is removed by the normal process of resorption. Uptake and retention by this mechanism is greater in young than in old animals since the process of bone formation is more active.

(ii) Sr^{89} is deposited by ionic exchange on the surface of bone crystals throughout the bone, and may penetrate from the surface into the crystal by a process of diffusion. In their studies of strontium uptake by bone Jones and Copp (1951) appear to have ignored the importance of this surface phenomenon.

Taken as a whole the figures given here for retention of Sr^{89} in the long bones confirm certain of the findings obtained by the autoradiographic studies already mentioned. It is clear that Sr^{89} is rapidly taken up from the circulating blood by the skeleton, and that deposition within the skeleton is more active in certain areas than others. The areas of most active bone growth are those in which the radioactive isotope is most concentrated. Such areas are therefore more exposed to radiation damage than areas in which deposition is less. In any assessment of the possible hazard of radioactive strontium this selective deposition in certain parts of any one bone must be considered.

SUMMARY.

The uptake by the skeleton of a single dose of Sr^{89} given by intravenous injection is extremely rapid : in young animals 70 per cent of the injected Sr^{89} is present in the bone within 4 hours, with a consequent rapid lowering of the blood level. The fall in skeletal Sr^{89} follows a smooth curve for 9 days, reaching a figure of 55 per cent. In older animals the uptake is equally rapid but reaches a lower maximum, and the curve for retention falls unevenly and to a greater extent in the first 9 days.

In older animals retention is greater in the flat bones than in the long bones. This may be associated with the fact that in young animals there is red marrow throughout the shaft of the long bones, while in older animals there is more red marrow in the flat bones.

There is no significant difference in retention in different long bones or different flat bones.

In rabbits of 3–4 months the concentration of Sr^{89} is at first greater in the epiphyseal plate and later in the metaphysis; this displacement is evidently due to subsequent bone growth.

The localized concentration of radioactive strontium within the bone must be remembered in considering toxic hazards.

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