J. Biosci., Vol. 1, Number 1, March 1979, pp. 99–107. © Printed in India.

Removal of radioactive strontium from the rat by feeding stable strontium

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MS received 23 August 1978; revised 23 October 1978

Abstract The effect of administering the stable isotope of strontium (as phosphate) at different dietary levels to adult rats (fed on a cereal and pulse-based diet containing 0·4% Ca) on the retention of radiostrontium (^{89}Sr) and radiocalcium (^{45}Ca) in the femur and the whole skeleton was studied for a period up to 6 weeks after an intraperitoneal injection of the two radioisotopes. The ability of strontium to remove ^{89}Sr under the above dietary conditions was examined. Feeding Sr at 0·5% or 1% levels for 6 weeks had no effect on the skeletal content of ^{89}Sr or ^{45}Ca while a dietary regimen of 2% Sr (2000 times the normal content), significantly lowered the ^{89}Sr and ^{45}Ca content by about 30% in the femur but not in the whole skeleton. At this Sr level, the urinary excretion of the isotopes increased with a concomitant decrease in their excretion in the faeces. This study underscores the limitations of dietary Sr to mobilise ^{89}Sr from the bones after it is incorporated in the bone mineral.

Keywords. ^{89}Sr removal; Sr feeding; rat ; cereal and pulse diet.

Introduction

Radioactive strontium (^{90}Sr), a fission-product, constitutes a potential health hazard in view of its localisation in the bones. This necessitates the development of suitable remedial measures and assessment of their adequacy for the specific removal of radio Sr from the body. To this end, several compounds have been tested for therapeutic use in experimental animals but only a few of them have been effective. Among such compounds, is the stable isotope of strontium (Sr), which as an isotopic diluent would be an obvious choice for removal of the isotope from the bones. Besides other modes of therapeutic administration, Sr has also been fed before and/or after the administration of ^{89}Sr, along with diets, mostly non-cereal, containing 0·5% to 2% Ca. While some workers have observed that feeding stable Sr reduced the radio Sr content by 20–80% (Cohn *et al.*, 1961; Teree *et al.*, 1965; Kriegel *et al.*, 1963), others did not notice any significant change in the radio Sr retention (Gross *et al.*, 1954; Harrison *et al.*, 1957; Mraz, 1960) These studies suffered from the following drawbacks: (*a*) the duration of feeding

was short and (*b*) the effect of Sr at different dietary levels as well as the effect on Ca retention was not studied using cereal diets. While there are many similarities between Sr and Ca, the body is known to discriminate between these two metal ions (Comar *et al*., 1956; Kshirsagar *et al*., 1966). Dietary levels of Ca influence the Sr metabolism (Wasserman *et al*., 1957; Thompson and Palmer, 1960) The bulk of Indian diets is made up of cereals and pulses. These two major constituents provide a considerable amount of the dietary intake of Ca which is lower than that in the Western countries where the main sources of Ca are milk and dairy products (Gopalan *et al*., 1969; Bronner, 1964) In addition, the presence of phytate in cereals and pulses may render the Ca and Sr in such diets not readily available since phytate is known to affect the intestinal absorption of Ca (Harris, 1955) and Sr (MacDonald *et al*., 1952). This investigation was, therefore, undertaken to study the effect of feeding Sr at different levels, on the retention up to 6 weeks of ^{89}Sr and radio-Ca (^{45}Ca) in the femur and the whole skeleton of rats fed on cereal and pulse-based diet.

Materials and methods

Animals

Adult male Wistar rats (6 months old) from the Bhabha Atomic Research Centre animal colony were used for the experiment.

Materials

^{89}Strontium (carrier-free, as $^{89}Sr\ (NO_3)_2$) and ^{45}calcium (high-specific activity 45mCi/g Ca, as $^{45}CaCl_2$) were obtained from the Isotope Division, Bhabha Atomic Research Centre, Trombay, Bombay, and were used after suitable dilution Strontium phosphate (CP grade) was purchased from Thomas Tyrer & Company, London, UK.

Experimental protocol

Rats were injected intraperitoneally with 5 μCi of ^{89}Sr and ^{45}Ca at the same time They were offered *ad libitum* water and stock diet based mainly on cereal and pulse with the following composition (%) : whole wheat 70, Bengal gram (*Cicer arietinum*) 20, fish meal 5, yeast powder 4, and oil mixture (including shark liver oil) 1 Details of this diet have been described (Kshirsagar, 1976a) While the Ca and total P content of the diet is 0·4%, the phytin P content is 0·2% (Aykroyd *et al*., 1963) Since the diet was offered without any prior treatment like cooking or heating, no reduction in the phytin content would be expected in the diet consumed by the rats. The control group was given the stock diet alone, while the strontium group was offered stock diet supplemented with strontium phosphate to provide 0·5%, 1% and 2% Sr in the diet for periods up to 6 weeks after the injection. The rats were placed individually in metabolic cages and had access to the diet immediately after the isotope administration. Urine and faeces were collected separately for each animal. Rats were killed at the interval of 2, 4 and 6 weeks after the injection. Both the femora and the rest of the skeleton were

removed, cleaned carefully of the soft tissues, weighed and dried. The skeletal tissues as well as the faeces were ashed at 600° C; the weighed ash was dissolved in and made up with 2N HNO_3. Urine was boiled with HNO_3 (17 N) till clear and made up with 2N HNO_3. Aliquots were taken for the metal analyses and radioactivity determination.

Methods

^{89}Sr and ^{45}Ca radioactivities were determined by the differential beta particle absorption counting technique (Comar, 1955) and corrected for self-absorption and decay. Standards for comparison were prepared from the original solutions used for the injections. The reproducibility of the determination of ^{89}Sr and ^{45}Ca radioactivities by this technique was about ± 5% Stable Ca was estimated by precipitation as oxalate and subsequent titration with $KMNO_4$.

Results

General

Retardation of growth was noticeable in the rats fed 2% Sr diet but none in those of 1% or 0·5% Sr groups as compared to the controls as seen earlier (Kshirsagar, 1976a). The ash and calcium (as determined by oxalate precipitation and titration with $KMNO_4$) contents of the skeleton did not change by feeding stable Sr.

^{89}Sr content of the skeleton

The effect of stable Sr on the ^{89}Sr content of the skeleton is described in table 1 It may be clarified here that results for the 0·5% and 1% Sr groups have been given in this and the subsequent table only for the 6-week period. There was no difference in the ^{89}Sr content between these and the control group ^{89}Sr (expressed as per cent injected dose, %ID) was eliminated from the skeleton of the control rats in the course of normal metabolic clearance over the period of study; thus in femur the ^{89}Sr radioactivity which was 1% at 2 weeks was reduced to 0·86% and 0·78% at 4 and 6 weeks respectively whereas in the whole skeleton it was lowered from 18·5% to 15% and 12% at the corresponding periods Feeding of 2% Sr diet for a period up to 6 weeks reduced the skeletal ^{89}Sr retention (%ID) as compared to that in the respective controls. While the loss of 15% in the ^{89}Sr radioactivity in the femur at 4 weeks was not statistically significant, those of 27% ($p < 0·05$) and 51% ($p < 0·01$) at 2 and 6 weeks respectively were significant The reduction in the whole skeleton which increased from 8% at 2 weeks to 15% and 27% at 4 and 6 weeks was, however, not significant at any period. Alimentation with 1 % or 0·5 % Sr diets for 6 weeks had no appreciable effect on the ^{89}Sr retention in the femur or whole skeleton.

^{45}Ca content of the skeleton

As a result of the skeletal metabolic turnover ^{45}Ca content (%ID) of the control rats was gradually lost over 6-week period as compared to that at 2 weeks (table 2);

Table 1. Effect of stable Sr on the ^{89}Sr content of the femur and the skeleton of the rat

Group	Femur			Whole skeleton		
	ID[a]	ID/g ash	ID/g Ca	ID	ID/g ash	ID /g Ca
2 weeks						
Control	1·00 ±0·111	1·31 ±0·176	3·20 ±0·444	18·50 ±3·430	1·21 ±0·144	4·84 ±0·840
2% Sr	0·73[b] ±0·229	0·96 ±0·279	2·60 ±0·743	16·93 ±4·228	1·46 ±0·404	4·90 ±1·340
4 weeks						
Control	0·86 ±0·091	1·16 ±0·144	3·00 ±0·363	14·66 ±2·800	1·21 ±0·118	3·97 ±0·568
2% Sr	0·73 ±0·165	0·90 ±0·228	2·30 ±0·606	12·43 ±3·449	0·95 ±0·330	3·29 ±1·094
6 weeks						
Control	0·78 ±0·063	0·96 ±0·101	2·35 ±0·256	11·66 ±1·980	0·96 ±0·083	3·16 ±0·485
2% Sr	0·38[c] ±0·132	0·45 ±0·161	1·19 ±0·429	8·48 ±2·440	0·66 ±0·233	2·24 ±0·774
1% Sr	0·71 ±0·047	0·79 ±0·055	2·04 ±0·094	10·81 ±0·560	0·84 ±0·047	2·79 ±0·099
0·5% Sr	0·87 ±0·080	0·95 ±0·096	2·41 ±0·162	11·58 ±0·970	0·98 ±0·081	3·12 ±0·172

[a] ID = % injected dose.
[b] Significantly different from control rats $p < 0·05$
[c] Significantly different from control rats $p < 0·01$
Values are means ± SD of 6 animals (except 2% Sr group at 6 weeks for which only 4 animals were available).

in femur it was 0·81% and 0·67% at 4 and 6 weeks respectively as against 1·26% at 2 weeks whereas in the whole skeleton it was 14·3% and 11·6% as compared to 18·6% in the corresponding periods. The decrease in ^{45}Ca concentration is higher than that in ^{89}Sr in femur but comparable in the whole skeleton. A regimen up to 6 weeks of 2% Sr diet significantly lowered the ^{45}Ca radioactivity (%ID) in the femur by 42% ($p < 0·01$) and 33% ($p < 0·05$) at 2 and 6 weeks respectively but the decrement of 15% and 23% in the ^{45}Ca content of the whole skeleton seen only at 4 and 6 weeks respectively was not significant ^{45}Ca retention in the whole skeleton or femur of rats fed 1% or 0·5% Sr diets for 6 weeks remained practically unaffected.

Table 2. Effect of stable Sr on the ^{45}Ca content of the femur and the skeleton of the rat

Group	Femur			Whole skeleton		
	ID	ID/g ash	ID/g Ca	ID	ID/g ash	ID/g Ca
2 weeks						
Control	1·26 ±0·310	1·64 ±0·415	4·00 ±1·060	18·63 ±3·408	1·60 ±0·371	5·37 ±1·118
2% Sr	0·73[a] ±0·141	0·96 ±0·205	2·66 ±0·632	18·31 ±4·760	1·52 ±0·447	4·97 ±1·426
4 weeks						
Control	0·81 ±0·269	1·09 ±0·387	2·80 ±0·918	14·26 ±2·952	0·94 ±0·322	3·74 ±0·968
2% Sr	0·62 ±0·115	0·74 ±0·168	1·95 ±0·624	12·12 ±3·888	0·94 ±0·357	3·21 ±1·255
6 weeks						
Control	0·67 ±0·179	0·75 ±0·240	1·93 ±0·612	11·58 ±1·232	0·90 ±0·214	2·94 ±0·645
2% Sr	0·45[b] ±0·081	0·55 ±0·118	1·40 ±0·365	8·95 ±2·748	0·68 ±0·258	2·37 ±0·823
1% Sr	0·58 ±0·033	0·73 ±0·069	1·79 ±0·141	11·23 ±1·209	0·87 ±0·151	2·70 ±0·489
0·5% Sr	0·63 ±0·058	0·88 ±0·12	2·10 ±0·245	12·63 ±2·094	1·09 ±0·261	3·48 ±0·847

[a] Significantly different from control rats $p < 0{\cdot}01$.

[b] Significantly different from control rats $p < 0{\cdot}05$.

Values are means ± SD of 6 animals (except 2% Sr group at 6 weeks for which only 4 animals were available).

Similar trend, as noted above, was discernible when ^{89}Sr or ^{45}Ca radioactivity was expressed as per cent injected dose per g of ash or Ca. ^{89}Sr or ^{45}Ca when expressed as %ID/g Ca was always higher in the whole skeleton than in the femur reflecting lowered uptake and removal of the two isotopes in the femur as compared to whole skeleton. Similar observations were made in the rabbit (Kshirsagar *et al.*, 1966) Comparison of the retention of ^{89}Sr and ^{45}Ca (^{89}Sr/^{45}Ca) in either the femur or the whole skeleton shows no significant change between the control and Sr-fed groups (table 3). The constant ratio reflects the metabolism of Ca and Sr as equal amounts (radioactivity) of the two nuclides were administered.

Table 3. Effect of stable Sr on the ^{89}Sr and ^{45}Ca excretion in urine and faeces

Group	^{89}Strontium			^{45}Calcium		$^{89}Sr/^{45}Ca$		
	Urine ID	Faeces ID	Urine / Faeces	Urine ID	Faeces ID	Urine / Faeces	Urine	Faeces
2 weeks								
Control	42·0 ± 6·10	36·8 ± 1·64	1·14	6·1 ± 1·62	67·3 ± 4·17	0·09	6·89 ± 1·48	0·55 ± 0·06
2% Sr	51·0 ± 4·60	20·6 ± 1·26	2·50	26·3 ± 2·59	48·2 ± 3·92	0·55	1·94 ± 0·18	0·43 ± 0·04
4 weeks								
Control	36·2 ± 3·60	38·8 ± 2·33	0·93	16·8 ± 4·00	62·8 ± 3·06	0·27	2·26 ± 0·60	0·62 ± 0·08
2% Sr	65·7 ± 7·97	22·6 ± 1·78	2·91	23·8 ± 1·89	60·9 ± 6·78	0·39	2·76 ± 0·45	0·37 ± 0·03
6 weeks								
Control	43·5 ± 8·70	33·5 ± 0·95	1·30	9·7 ± 2·81	73·4 ± 5·29	0·13	4·48 ± 1·04	0·46 ± 0·05
2% Sr	63·7 ± 6·29	18·7 ± 0·73	3·41	30·3 ± 3·28	53·4 ± 5·35	0·57	2·10 ± 0·31	0·35 ± 0·02

Values are means ± SD of 6 animals (except 2% Sr group at 6 weeks for which only 4 animals were available).

^{89}Sr and ^{45}Ca excretion in urine and faeces

The excretion of ^{89}Sr and ^{45}Ca in urine and faeces was studied at 2, 4 and 6 weeks only in the control and 2% Sr groups because the skeletal ^{89}Sr and ^{45}Ca contents of the 0·5% and 1% Sr group rats was found to be unaltered as compared to the controls. As seen from table 3, about 35–40% of the intraperitoneally injected ^{89}Sr was excreted in urine and faeces by the control rats over the period 2–6 weeks The ^{45}Ca excretion was lower in the urine (6–17% ID) but higher in the faeces (63–73% ID). On feeding Sr at 2% level, the faecal excretion of ^{89}Sr and ^{45}Ca decreased by about 40% and 3–28%, respectively. The urinary excretion increased by about 20–80% (^{89}Sr) and 40–300% (^{45}Ca). The shift in the excretion pattern is reflected in the increased ratio of ^{45}Ca radioactivity in urine to faeces in the 2% Sr-fed rats as compared to the controls. The $^{89}Sr/^{45}Ca$ ratio was on the contrary lower in the 2% Sr-fed rats than in the control group both in urine and faeces,

Discussion

Skeletal deposition of ^{89}Sr can be minimised by isotopic dilution which reduces the effective concentration of ^{89}Sr by raising the ratio of stable/^{89}Sr as high as possible. Since stable Sr is present in trace amounts in tissues (Bowen, 1966; Kshirsagar, 1976b) it provides inadequate diluent effect. Enhancement of this ratio can be achieved only by means of the administration of extraneous stable Sr (dietary or otherwise). This possibly raises the level of stable Sr in tissues, as Sr, unlike Ca, is not under homeostatic control (Comar and Wasserman, 1964; Kshirsagar, 1976b) Some of the earlier workers observed no reduction in the radio Sr retention in the whole skeleton of rabbits (Kidman *et al.*, 1950), or of rats due to oral administration of stable Sr (Gross *et al*., 1954; Harrison *et al*., 1957; Rubanovskaya and Ushakova, 1959) or tibial retention in chicks by prefeedmg Sr up to 1% level (Mraz, 1960). Others have, however, noted the removal of radio Sr by oral doses of stable Sr in growing rats (Lang and Schmidt, 1969) or in rabbits when fed Sr-enriched diet (Witkowska, 1976). Kawin (1959) found reducetion in the femoral retention of ^{89}Sr when this isotope as well as the stable Sr was injected intraperitoneally. Intragastric administration, however, had the opposite effect. Prior feeding for 2–10 days with about 1·8% stable Sr to rats reduced skeletal radio-Sr retention as observed by whole body counting (Cohn *et al*., 1961; Teree *et al* 1965; Depczyk *et al*., 1967) or by femoral analysis as seen by Kriegel *et al.* (1963) who also observed reduction in the femoral radio-Sr on feeding 2·5% Sr diet for 21 days after the radio-Sr injection. However, prefeeding would not be possible in the treatment of accidental ^{90}Sr poisoning. This emphasises the need for a suitable and convenient mode of therapy for such poisoning.

The present study has shown that whereas feeding 0·5% or 1% stable Sr for a period of 6 weeks had no effect on the ^{89}Sr or ^{45}Ca content of either the femur or the whole skeleton, a dietary regimen of 2% Sr significantly lowered the ^{89}Sr and ^{45}Ca radioactivity in the femur but not in the whole skeleton. The diverse response of single bone as against that of the entire skeleton arising mainly from the different metabolic turnover rates of the various bones as shown earlier by many workers (Kulp and Schulert, 1962; Bryant and Loutit, 1964; Kshirsagar *et al.*, 1966) stresses the need for caution while extrapolating the results obtained on single bones to the whole skeleton. Stable Sr content of the stock diet used in this study is 0·001% (Kshirsagar, 1976a). It is evident, therefore, that since even a dietary level of 2% Sr (2000 times the normal content) does not have any signi ficant influence on the ^{89}Sr or ^{45}Ca content of the whole skeleton, much higher levels of dietary Sr might be required to achieve any meaningful reduction in the body burden of radio-Sr. It is known that high levels of stable Sr may not be tolerated by the body and lead to toxic effects like paralysis, rickets in young (21 days old) rats (Kshirsagar, 1976a) and in pigs (Bartley and Reber, 1961). They also affect activities of enzymes like acid and alkaline phosphatases of rat tissues (Kshirsagar, 1975; 1976a).

The changes observed in the ^{89}Sr and ^{45}Ca retention in femur as well as excretion in the 2% Sr group would seem to suggest that the removal of ^{89}Sr from the skeletal tissue is possibly due to the diminished rate of bone mineral formation. This is evidenced by the comparable reduction in the ^{45}Ca retention in the femur. It is interesting to note that the increase in urinary excretion is balanced by a

somewhat proportionate decrease in the faecal output of both ^{89}Sr and ^{45}Ca Spencer *et al.* (1972) observed that administration of stable Sr (1500 times the normal dietary intake) increased the urinary excretion of ^{90}Sr. This excretion was more when Sr was given intravenously than when orally administered; the faecal excretion was unchanged.

Since Sr was fed as phosphate (a form apparently more suitable to counteract the effect of phytate), the increasing amount of non-phytin P accompanying the rise in Sr level would be expected to mitigate any adverse effect due to phytate; the phytin-P concentration decreased from 50% in the stock diet to about 25% in the 2% Sr diet which contained 0·85% P (total). While the latter P concentration is within the normal range of intake, it is pertinent to note that higher amounts of P in diet are known to reduce the deposition of radio-Sr in the skeleton (MacDonald *et al*., 1955).

In view of these considerations, the absence of any significant decrement in the skeletal radio-Sr content, as seen in this study, underscores the limitations of dietary stable strontium to mobilise the radio-Sr from the bone when once it gets fixed in the bone mineral.

Acknowledgements

The author thanks Dr K. Sundaram, Director, Division of Life Sciences, International Atomic Energy Agency, Vienna, Austria and Shri N. S. Rao, Head, Biology Group, Bhabha Atomic Research Centre, Trombay, Bombay, for their interest in the work

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