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THE EFFECT OF CARRIER STRONTIUM ON THE ABSORPTION OF ORAL DOSES OF RADIOACTIVE STRONTIUM IN RATS

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Carrier strontium had relatively little effect on the retention of an oral dose of radioactive strontium by the rat when it was administered immediately after the radioactive dose. The proportion of the radioactive dose which was excreted in the urine, on the other hand, increased progressively with the carrier dose. There was a decreased uptake of radioactive strontium in rats fed on a special low strontium diet. The effects of dietary strontium are discussed. Evidence was found of a discrimination by the rat against strontium in favour of calcium which was accounted for, at least in part, by a preferential urinary excretion of strontium.

When an oral dose of radioactive strontium is given to experimental animals as a liquid, an appreciable fraction is deposited in the skeleton from which its removal is slow (Hamilton, 1944, 1947; Lisco, Finkel, and Brues, 1947; Kidman, Tutt and Vaughan, 1950). In the form of ^{90}Sr, with a physical half-life of 28 years, radioactive strontium presents a considerable hazard to health and it is not surprising that several attempts have been made to diminish the intestinal absorption and consequent bone retention in experimental animals. Thus, doses of chemical agents or non-radioactive strontium (carrier) have been used (Hamilton, 1944; MacDonald, Nusbaum, Ezmirlian, Barbera, Alexander, Spain and Rounds, 1952; Gross, Taylor, Lee and Watson, 1953), but results from such experiments have not been encouraging. A systematic study of the effect of doses of carrier on the retention of orally administered radioactive strontium, however, has not been attempted.

In the present experiments, the skeletal retention and excretion of a single oral dose of radioactive strontium, ^{89}Sr, has been measured in rats killed 24 hr. afterwards. Different groups of rats received varying amounts of carrier, up to 120 mg./animal, by the same route immediately after the radioactive dose. It has been shown that the absorption of a single dose of strontium from the alimentary canal is approximately proportional to the dose, so that simultaneous administration of oral doses of radioactive and carrier strontium is without any appreciable effect on % of the radioactive dose retained. This result was unaffected by a prolonged experimental period between giving the radioactive dose and the sacrifice of the animals. In addition, the effect of a low strontium diet on the absorption of the radioactive dose has been studied.

METHOD

Male albino rats of a highly in-bred stock were used. These rats were 12 weeks old and weighed about 300 g. They were fed on a stock diet of commercial rat nuts together with water. The calcium, strontium, and phosphorus content of the diet was assayed. Calcium was estimated volumetrically by the permanganate method (Kolthoff and Sandell, 1952), phosphorus colorimetrically by the method of Briggs as modified by Martland and Robison (1926), and strontium by the radioactivation method of Harrison and Raymond (1955). The normal turnover of dietary calcium and strontium was determined for six animals which were kept in metabolism cages on the stock diet with water *ad lib.* Thus the calcium and strontium intake as well as their urinary and faecal excretions were assayed. At sacrifice, the skeletal contents of calcium and strontium were also determined.

Strontium, both radioactive and carrier, was administered by stomach tube under light amylobarbitone anaesthesia. The radioactive dose was 0.1 ml., or about 0.5 μC., from a carrier-free solution of ^{89}Sr. In animals which did not receive carrier, this dose was washed into the stomach with 1 ml. of water while, for the other animals, the wash was provided by 1 ml. of an aqueous solution of strontium chloride. These animals were killed 24 hr. after the administration of the radioactive strontium.

Urine, including the cage washings, was assayed for radioactivity on an M6H Geiger counter (Veall type) after acidifying with nitric acid. Faeces including the contents of the large bowel were dried and ashed at 650° overnight. The ash was dissolved in nitric acid for counting. Whole skeletons were dissected as free as possible from soft tissue and dissolved in nitric acid. The radioactivity of each sample was corrected for background, paralysis time of the counter and radioactive decay. The error in counting for any specimen was less than 2%. In a few animals sacrificed at the beginning of the experiment, the carcass as well as the alimentary canal down to the caecum was assayed for radioactivity. The activity of both was found to be less than 2% of the total so that these activities were neglected in the later animals. The mean recovery of activity in urine, faeces, and skeleton in 62 animals was 95.3% of the dose with a standard error of ±1.1. The absorbed fraction was regarded as the ratio of the combined urinary and skeletal activities to the total dose. This assessment of absorption appeared to be reasonable since the faecal excretion of parenterally administered strontium is small (Comar, Wasserman, and Nold, 1956; Jones and Coid, 1956).

In order to assess the effect of prolonged excretion on the retention of the radioactive dose with and without carrier, twelve rats were kept in metabolism cages for 12 days after an oral dose of radioactive strontium. Six rats were given the radioactive dose without carrier and six the radioactive dose followed immediately by an oral dose of about 120 mg. of carrier, the administration of the dose and the feeding being as in the shorter-term experiments. Urine and faeces were collected separately and skeletal assays were made at sacrifice of each animal. The effect of a diminished dietary intake of strontium on the absorption of the radioactive dose was investigated following the preparation of a special diet in which the essential nutritive constituents contained little or no natural calcium with its inevitable content of strontium. To this was added repurified calcium carbonate (Analar) to restore the calcium content of this diet to that of the stock diet. The strontium content of calcium carbonate (Analar) which is normally of the order of 200 p.p.m. by weight was reduced some 20-fold by the precipitation of strontium nitrate in concentrated nitric acid followed by the reconversion of the nitrate to calcium carbonate by the addition of ammonium carbonate (Analar). The special diet was analysed for calcium, strontium, and phosphorus by the same methods as those employed for the stock diet.

Five animals were maintained for 21 days on the special diet, by which time it was confirmed that they were in calcium and strontium balance. Another five animals were maintained over the same period on the special diet, to which strontium had been added, to restore the content to that of the stock diet. In this way, any effect of the diet other than the strontium content was eliminated. A carrier-free radioactive dose of strontium was given to both groups and the animals sacrificed 24 hr. later.

RESULTS

Table I gives results of analysis of the stock and special diets for calcium, strontium and phosphorus together with the other main constituents. It will be seen that the diets were similar in essential composition. The stock diet was in the form of extruded, dried nuts while the special diet was fed as a stiff paste, in weighed amounts. No difficulty was experienced in getting the rats to take the special diet.

TABLE I

COMPARISON OF THE STOCK RAT DIET WITH THE SPECIAL DIET

The amount of each constituent is expressed as % by weight. The stock diet was Rat Cake 14% made up by the North Eastern Agricultural Co-operative Society Limited, Aberdeen. For mineral contents of the special diet other than Ca and P see Hansard, Comar, and Plumlee (1951).

Constituent	Diet: Stock	Diet: Special
Protein	19·2	25·0
Fat	4·9	4·0
Carbohydrate ..	52·6	59·0
Calcium	1·3	1·0
Phosphorus ..	1·0	0·8
Strontium	$2{\cdot}6\times10^{-3}$	$1{\cdot}6\times10^{-4}$
Crude fibre	4·8	—
Water	12·5	8·0
Total ash	6·0	5·4

The results for the strontium and calcium intake and the skeletal content and excretion of these elements for rats fed on the standard diet are given in Table II. Each figure is a mean for six animals maintained on the diet for at least four weeks before the assays were made. It will be seen that these rats were approximately in strontium and calcium balance. Further, it is to be noted that the skeletal ratio of strontium to calcium was appreciably smaller than the dietary ratio.

TABLE II

SKELETAL RETENTION AND EXCRETION OF DIETARY STRONTIUM AND CALCIUM/RAT

Mean of six results.

Mineral	Daily Dietary Intake (mg.)	Skeletal Content (mg.)	Excretion: Urine (mg./day)	Excretion: Faeces (mg./day)
Sr ..	0·85	2·66±0·17	0·042±0·014	0·86±0·11
Ca ..	395	3,230±130	14·10±1·97	374±58
$Sr/Ca\times10^3$	2·2	0·82	3·0	2·3

Table III shows the effect of doses of carrier strontium from 0 to 120 mg. on the skeletal retention and urinary excretion of a simultaneous oral dose of carrier-free radioactive strontium. The lowest carrier dose was so small that it was not surprising that the mean retention and excretion obtained for these animals was about the same as for those receiving the carrier-free dose. When the carrier dose was increased, the proportion of the radioactive dose which was retained in the skeleton was approximately constant although there appeared to be a slight increase in the value for 5 μg. strontium/g. body weight. The urinary excretion, on the other hand, showed a progressive increase for carrier doses greater than 2 μg./g. body weight.

TABLE III

EFFECT OF SIMULTANEOUS DOSES OF CARRIER STRONTIUM ON THE ABSORPTION OF RADIOACTIVE STRONTIUM

In the last column, % of dose absorbed = % of dose in urine + % in skeleton.

Carrier Sr Added (μg./g. Body Wt.)	No. of Rats	% of Administered Dose (±S.E.)			% of Dose Absorbed (±S.E.)
		Skeleton	Urine	Faeces	
None	9	11·8±1·5	2·5±1·0	80·9±5·3	14·3±2·2
1	5	10·3±2·2	2·0±0·2	82·4±2·7	12·3±2·2
2	5	12·7±3·5	2·4±0·8	78·0±5·9	15·1±4·1
5	6	19·0±3·4	3·4±1·1	70·3±6·1	22·3±3·9
20	5	14·3±4·1	3·6±1·0	74·6±6·6	17·9±5·6
80	5	13·1±2·8	4·6±1·4	77·4±3·1	17·7±3·7
400	5	11·0±1·4	7·1±1·9	71·7±4·9	18·1±2·6

Table IV shows that the skeletal retention of the radioactive dose was unchanged by a simultaneous dose of carrier strontium. In this experiment, it was observed that the urinary excretion was negligible after the first 24 hr. whether carrier was given or not.

TABLE IV

ABSORPTION OF RADIOACTIVE STRONTIUM BY RATS ON THE STOCK DIET SACRIFICED 12 DAYS AFTER AN ORAL DOSE

Mean of six results in each group. See Table III for an explanation of % of dose absorbed.

Oral Carrier Strontium (μg./g.)	% of Administered Dose (±S.E.)			% of Dose Absorbed (±S.E.)
	Skeleton	Urine	Faeces	
None	6·1±0·7	3·4±0·4	89·8±3·3	9·5±1·5
400	7·0±1·0	10·3±0·8	82·8±1·4	17·3±1·8

TABLE V

EFFECT OF A LOW STRONTIUM DIET ON THE ABSORPTION OF AN ORAL DOSE OF RADIOACTIVE STRONTIUM

Mean of 5 results in each group. See Table III for an explanation of % of dose absorbed.

Dietary Sr (mg./Rat/Day)	% of Administered Dose (±S.E.)			% of Dose Absorbed (±S.E.)
	Skeleton	Urine	Faeces	
0·4	9·0 (±3·0)	2·1 (±0·5)	87·1 (±6·6)	11·1 (±3·4)
0·024	5·5 (±3·2)	2·0 (±1·2)	90·0 (±3·7)	7·5 (±4·2)

The results obtained for rats fed on the special diet are shown in Table V. It will be seen that, for the animals fed on the low strontium diet, the skeletal retention, and consequently the percentage of the radioactive dose absorbed, were decreased as compared with litter mates fed on the same diet, to which strontium had been added, to bring the mean dietary intake up to 0.4 mg./rat/day.

DISCUSSION

If the strontium absorbed from the alimentary canal is independent of the amount present, an increase in the amount of carrier strontium would act as an isotopic diluent in the gut and so decrease the absorption of a simultaneously administered radioactive dose. The results given in Table III show that this was not the case. Since the skeletal retention of the radioactive dose is approximately independent of the amount of carrier given, except for the slight increase observed for a carrier dose of 5 μg./g. body weight, the significant increase in the urinary excretion of the radioactive dose with increased carrier must lead to an increased absorption. The small increase in the absorption observed (Table III, last column) is masked by the high skeletal retention at 5 μg./g. already mentioned. Despite a change of 200-fold in the carrier dose, the absorption of the radioactive dose only increased 4%. Over the experimental range studied, it is concluded that the amount of strontium absorbed from the alimentary canal is approximately proportional to the dose.

Since the blood concentrations of strontium following an oral dose depend upon the amount absorbed from the alimentary canal, blood strontium must be temporarily increased. If the renal clearance in the rat is constant, as in humans (Harrison, Raymond and Tretheway, 1955), the rate of urinary excretion should increase with the amount of carrier given. This is in accord with the results given in Table III.

The increased rate of urinary excretion of the radioactive dose compared with that for a carrier-free dose has been cited as evidence of the benefit of carrier as a therapeutic agent following an ingestion of radioactive strontium (Gross *et al.*, 1953). It is clear from the results given in Table III, however, that no such benefit is obtained. The present results were for a 24 hr. period. However, a longer period between the administration of the dose and death of the animals might have revealed a decreased retention of the radioactive dose with carrier due to this increased urinary excretion.

The results given in Table IV show that the skeletal retention of the radioactive dose was independent of the carrier level in the 12 day animals. As already mentioned, in this experiment it was observed that the urinary excretion was quite small 24 hr. or later after the dose whether carrier was given or not. It is quite unlikely, therefore, that prolonging the observation period would have appreciably changed the pattern of the results. The increased urinary excretion of radioactive strontium when carrier is given is thus no indication of a decreased retention.

In view of the fact that the present results show that the overall absorption of strontium from the alimentary canal increased with the amount of strontium given, the effect of diminishing the dietary intake of strontium was deemed to be of special interest. A decrease in the total strontium fed to the animal will produce a decrease in the body pools and, in particular, in the amount of carrier present in the gut. The decreased absorption observed in the animals (Table V) is, therefore, to be expected from the earlier experiments. Recent experiments by Alexander, Nusbaum and MacDonald (1956) have shown that the ratio of dietary strontium to calcium is reflected in the skeletal ratio, animals fed on the higher strontium diet having the higher skeletal ratio of strontium to calcium, a result in agreement with that of the present experiments.

It has been shown independently by Hansard and Plumlee (1954) in the case of calcium, and by Hamilton (1944) for strontium, that a low calcium diet, when fed to rats, leads to an appreciable increase in the absorption of an oral dose of radioactive calcium or strontium. Indeed, Hamilton obtained a 5- to 7-fold increase in the strontium absorbed on a low calcium diet (less than 0.2% Ca). It would appear, therefore, that a low calcium diet produces the opposite effect on strontium absorption from strontium starvation.

From Table II it is seen that the strontium to calcium ratio in the skeleton was 0.82×10^{-3}, whereas the corresponding ratio in the diet was 2.2×10^{-3}, and in the urine 3.0×10^{-3}. The ratio in the urine is thus 1.4 times higher than that in the diet, while that of the skeleton is 2.5 times lower. These figures are in good agreement with the strontium to calcium ratios for the diet, skeleton and urine of rats obtained by Comar *et al.* (1956). It is clear that the kidney of the rat excretes strontium in preference to calcium and that this accounts, at least in part, for the lower strontium to calcium ratio of the skeleton.

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