The Metabolism of Radium in Dairy Cows

By B. F. SANSOM AND R. J. GARNER*

*Agricultural Research Council Institute for Research on Animal Diseases,
Compton, Newbury, Berks.*

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1. The isotopes ^{45}Ca and ^{224}Ra were administered simultaneously, both orally and intravenously, to two pairs of cows. 2. The first pair of animals received large doses of ^{224}Ra and ^{45}Ca , which produced clinical and metabolic disturbances. 3. The second pair of animals received much smaller doses and showed no clinical disturbances. The mean recoveries in these animals of ^{45}Ca and ^{224}Ra in the 8 days after the oral administration were respectively: from faeces, 63.2 and 103.8%; from urine, 0.26 and 0.44%; from milk, 15.8 and 0.35% of the dose; and in the 8 days after intravenous administration: from faeces, 20.5 and 36.5%; from urine, 1.56 and 17.6%; from milk, 49.3 and 11.8% of the dose. 4. Calculation of discrimination factors shows that absorptive discrimination played the most important part in the overall discrimination between the calcium and radium in their passage from diet to milk, but that mammary discrimination plays a more important role than in the overall discrimination between calcium and strontium or barium. 5. In its treatment by the kidney radium is more like strontium than barium, but radium is actively secreted by the gut in the same way as barium. 6. In its skeletal metabolism radium is indistinguishable from calcium and strontium except in its rate of resorption, which is similar to that of barium.

In a previous paper (Garner, Jones & Sansom, 1960) the metabolism of strontium and barium in dairy cows was compared with that of calcium as part of a programme of work on fission products. Radium has now been studied to provide a complete survey of the relative behaviour in the cow of the series of alkaline-earth elements calcium, strontium, barium and radium. Moreover, although it is not a fission product, radium might as a result of accidental circumstances enter food chains and constitute a radiological hazard by its transfer to milk. Its transfer from the diet of cows to their milk was studied particularly.

It was expected that the absorption of radium from the gastrointestinal tract would be very low and therefore a large oral dose of ^{224}Ra was administered to two cows together with a moderate dose of ^{45}Ca . Four days after dosing both cows went off their feed and their milk yield fell sharply. The subsequent clinical observations on these cows have been published (Sansom, Garner & West, 1964). Owing to these radiation effects the metabolic results of this experiment (referred to below as Expt. B) are of doubtful value and the experiment was repeated with small amounts of ^{224}Ra and ^{45}Ca .

* Present address: Colorado State University—Public Health Service, Collaborative Radiological Health Animal Research Laboratory, Fort Collins, Colorado, U.S.A.

No adverse clinical symptoms were apparent during the latter experiment (referred to below as Expt. A), and it is mainly the results of this experiment that are reported in the present paper. Some of the results of Expt. B are included for comparison.

METHODS

The methods used in both Expts. A and B were identical with those reported by Garner *et al.* (1960) except in the following respects.

Plan of experiments. Expt. A. Each of two cows received approx. 2mc of ^{224}Ra and 2mc of ^{45}Ca intravenously and 11 weeks later approx. 5mc of ^{224}Ra and 5mc of ^{45}Ca orally. The ^{224}Ra (thorium-X) was separated from its parent ^{228}Th (radio-thorium) by The Radiochemical Centre, Amersham, Bucks.

Expt. B. Two cows received approx. 24mc of ^{224}Ra and 5mc of ^{45}Ca orally, and 56 days later 4mc of ^{224}Ra and 2mc of ^{45}Ca intravenously.

Counting methods. ^{224}Ra . Samples (10ml.) of plasma, milk and urine and duplicate 5g. samples of thoroughly mixed faeces were counted directly in a NaI (Tl) well-type scintillation counter. All samples were kept for at least 3 days after collection to allow equilibration between ^{224}Ra and its daughter products. The efficiency of counting was approx. 65%.

^{45}Ca . The ^{45}Ca content of plasma and skimmed milk was assayed by pipetting 10ml. samples into steel dishes of diam. 11.4cm., drying under an infrared lamp and counting in a β -scintillation counter. A correction was

Table 1. *Percentage recovery of ^{45}Ca and ^{224}Ra from faeces, urine and milk, and milk yield in the 8 days after administration*

Values given are the results from the two animals. The yield of milk in litres is given in parentheses.

Yield (l.)	Oral administration						Intravenous administration					
	Faeces		Urine		Milk		Faeces		Urine		Milk	
	1	2	1	2	1	2	1	2	1	2	1	2
					(173)	(174)					(165)	(156)
^{45}Ca	61.9	64.6	0.44	0.071	14.1	17.5	20.3	20.7	0.82	2.3	50.0	48.7
^{224}Ra	106.1	101.6	0.42	0.46	0.41	0.29	40.8	32.2	11.3	23.9	14.5	9.2

made for the cream content of each sample when converting the results into the equivalent concentration in whole milk. The efficiency of counting ^{45}Ca in plasma and skimmed milk in this way was approx. 5.6%.

The ^{45}Ca content of faeces was estimated by ashing 25g. samples of the thoroughly mixed total daily output of fresh faeces at 650° and dissolving the ash in 20ml. of 6N-HCl. Calcium oxalate was precipitated from 2ml. of this solution on to steel planchets of diam. 2.4cm. Urine samples (200ml.) were dried and ashed, the ash was dissolved in 40ml. of 6N-HCl and calcium oxalate precipitated from 5ml. of this solution. The dried precipitates were counted under an end-window Geiger-Müller tube (type EHM2; The General Electric Co. Ltd., Kingsway, London, W.C. 2. The efficiency was approx. 5%.

^{45}Ca was assayed in all samples at least 1 month after collection to allow the ^{224}Ra (half-life 3.64 days) to decay.

Definitions. The terms 'observed ratio' and 'discrimination factor' are used to describe the relative behaviour of ^{224}Ra and ^{45}Ca . The observed ratio (OR) may be defined as the overall discrimination observed in the movement of the two elements from one phase to another, i.e.:

$$\text{OR}(\text{sample-precursor}) = \frac{^{224}\text{Ra}/^{45}\text{Ca of sample}}{^{224}\text{Ra}/^{45}\text{Ca of precursor}}$$

This observed ratio can be derived from the product of a number of 'discrimination factors' (DF), each measuring the extent to which a particular physiological process to which it refers contributes to the overall discrimination. Thus the discrimination factor for the process of intestinal absorption may be defined as:

$$\begin{aligned} \text{DF}(\text{absorptive}) \\ = \frac{\% \text{ of orally administered } ^{224}\text{Ra} \text{ absorbed}}{\% \text{ of orally administered } ^{45}\text{Ca} \text{ absorbed}} \end{aligned}$$

For further details and discussion see Garner *et al.* (1960).

RESULTS

The overall recoveries of ^{224}Ra and ^{45}Ca in Expt. A in the 8 days after administration are given in Table 1. Table 2 shows the percentage secretion of ^{224}Ra and ^{45}Ca in the milk in Expt. B.

If it is assumed that the proportion of an oral

Table 2. *Percentage secretion of ^{45}Ca and ^{224}Ra in milk, and the milk yield in the 8 days after administration*

Values given are the results from the animals clinically affected by the dose.

Yield (l.).....	Oral administration		Intravenous administration	
	Milk		Milk	
	1	2	1	2
	(81)	(103)	(80)	(75)
^{45}Ca	7.3	7.2	50.0	43.8
^{224}Ra	0.27	0.20	3.5	4.1

Fig. 1. Change in concentration with time of ^{45}Ca and ^{224}Ra in milk after oral administration. $\circ$, ^{45}Ca ; $\square$, ^{224}Ra . Each point was obtained by averaging the values for two animals. The peak concentrations of ^{45}Ca and ^{224}Ra in the milk of individual animals were 0.37 and 0.54, and 0.0051 and 0.0065% of dose/l. respectively. Correction was made for physical decay.

dose absorbed from the gastrointestinal tract is given by the ratio:

$$\frac{\% \text{ of orally administered material excreted in milk}}{\% \text{ of intravenously administered material excreted in milk}}$$

then the percentage absorption of ⁴⁵Ca and ²²⁴Ra is approx. 32 and 3% respectively in the healthy

animals but approx. 15 and 6% respectively in the animals suffering from radiation effects.

Elimination of ⁴⁵Ca and ²²⁴Ra in milk. The change of concentration of these elements with time in milk after the oral and intravenous administrations of Expt. A is shown in Figs. 1 and 2. After oral administration the rate ultimately becomes exponential for both isotopes and the biological half-lives, calculated from regression lines fitted through the experimental points on the exponential part of the curve are 24 and 50hr. for ⁴⁵Ca and ²²⁴Ra respectively.

Discrimination between calcium and radium. The behaviour of calcium in Expt. A is within the range of that observed previously. The behaviour of radium relative to that of calcium is shown in Table 3 by comparing observed ratios and discrimination factors as defined above. For ease of comparison the values for barium and strontium are also given. The values for OR (milk-injection) and OR (milk-diet) derived from Expt. B are also included.

Skeletal metabolism of ⁴⁵Ca and ²²⁴Ra. The change in activity of ⁴⁵Ca and ²²⁴Ra in the blood plasma after the intravenous injections of Expt. A is shown in Fig. 3. In both cases the results are expressed as the percentage of the dose/mg. of calcium and are the mean results from the two cows. The 'accretion rate' and 'exchangeable calcium', given in Table 4, were calculated by means of the formulae given by Bauer, Carlsson & Lindquist (1955) from the specific activity 11 and 23hr. after dosing. The values derived from the results of experiments with ⁸⁹Sr and ¹⁴⁰Ba are also included (see Garner *et al.* 1960). By using these constants curves have been drawn showing the change with time in the amount of 'exchangeable'

Fig. 2. Change in concentration with time in milk after intravenous administration. ○, ⁴⁵Ca; □, ²²⁴Ra. Each point was obtained by averaging the values for two animals. The peak concentrations of ⁴⁵Ca and ²²⁴Ra in the milk of individual animals were 3.81 and 2.42, and 0.41 and 0.43% of dose/l. respectively. Correction was made for physical decay.

Table 3. *Strontium/calcium, barium/calcium and radium/calcium observed ratios and discrimination factors in lactating cows*

Each value is the result for an individual cow.

Intravenous administration	Sr/Ca	Ba/Ca	Ra/Ca (A)	Ra/Ca (B)
OR(faeces-injection)	1.2; 1.3	2.5; 1.9	2.1; 1.6	
OR(urine-injection)	18; 11	33; 91	13.8; 12.8	
OR(milk-injection)	0.46; 0.48	0.41; 0.47	0.29; 0.19	0.07; 0.09
Oral administration				
OR(faeces-diet)	1.5; 1.2	1.4; 1.1	1.7; 1.6	
OR(urine-diet)	4.6; 2.5	2.2; 1.3	1.0; 6.5	
OR(milk-diet)	0.11; 0.11	0.05; 0.06	0.029; 0.017	0.037; 0.028
DF(absorptive)	0.2; 0.3	0.1; 0.2	0.1; 0.09	0.52; 0.30
DF(urinary)	0.7; 0.8	0.8; 0.6	0.9; 0.7	
DF(faecal)	1.0; 1.0	0.8; 0.7	0.75; 0.85	
DF(lactational-milk)	0.6; 0.5	0.7; 0.7	0.43; 0.32	
OR(body-diet)	0.17; 0.34	0.09; 0.02	0.11; 0.09	
DF(lactational-body)	1.0; 1.5	1.2; 0.3	1.8; 1.7

Fig. 3. Change in specific radioactivity of blood plasma (expressed as percentage of dose/mg. of Ca) after intravenous administration. $\circ$, ^{45}Ca ; $\square$, ^{224}Ra . Each point was obtained by averaging the values for two animals. The concentrations of ^{45}Ca and ^{224}Ra in the plasma of individual animals at 8 hr. after dosing were 0.00105 and 0.00105, and 0.00113 and 0.00114% of dose/mg. of Ca respectively. Correction was made for physical decay.

Fig. 4. Overall metabolism in the whole body (mean results for two animals). $^{45}\text{Ca}_{\text{obs}}$, $^{224}\text{Ra}_{\text{obs}}$: percentage of intravenously administered ^{45}Ca or ^{224}Ra retained in the whole body. $^{45}\text{Ca}_E$, $^{224}\text{Ra}_E$: exchangeable fraction of ^{45}Ca or ^{224}Ra in whole body. $^{45}\text{Ca}_A$, $^{224}\text{Ra}_A$: non-exchangeable fraction of ^{45}Ca or ^{224}Ra in whole body. $^{45}\text{Ca}_R$, $^{224}\text{Ra}_R$: ^{45}Ca or ^{224}Ra resorbed ($\text{Ca}_R = \text{Ca}_A + \text{Ca}_E - \text{Ca}_{\text{obs}}$ etc.). The notation used is that of Bauer *et al.* (1955).

Table 4. 'Accretion rate' and 'exchangeable calcium' in the whole body of cows

Calc. from	'Accretion rate' (g./hr.)	'Exchangeable Ca' (g.)
^{45}Ca	1.3	43
^{89}Sr	1.3	39
^{140}Ba	0.9	33
^{224}Ra	1.3	40

^{45}Ca and ^{224}Ra ($^{45}\text{Ca}_E$ and $^{224}\text{Ra}_E$ etc.), the amount of ^{45}Ca and ^{224}Ra incorporated into the 'non-exchangeable' fraction of the skeleton ($^{45}\text{Ca}_A$ and $^{224}\text{Ra}_A$ etc.) and the amount removed as a result of resorption ($^{45}\text{Ca}_R$ and $^{224}\text{Ra}_R$ etc.) (Fig. 4).

DISCUSSION

In several respects the alkaline-earth elements calcium, strontium, barium and radium form a consistent series; the behaviour of each is related

to that of its predecessor in the series in a consistent way. Thus the proportion of an oral dose of each element absorbed from the gut decreases in the order Ca, Sr, Ba, Ra. This is shown both by the fact that the faecal excretion of an oral dose of the elements increases in this order and also that the values of the DF (absorptive) for the pairs of elements decrease in the order Sr/Ca, Ba/Ca, Ra/Ca.

The secretion of an oral dose of the elements into milk also falls into a similar series. This is due partly to the effect of the gradual decline in the DF (absorptive) but also to a decline in the value of the OR (milk-injection). Radium, however, does appear to be discriminated against by the udder to a greater extent than might have been expected. Thus the mean OR (milk-injection) values for Sr/Ca, Ba/Ca and Ra/Ca are 0.47, 0.44 and 0.24 respectively.

In other respects radium appears sometimes to behave like strontium and sometimes more like barium. Radium seems to be actively secreted into the gut like barium; the amount of intravenously administered radium excreted in the faeces is nearly double the amount of calcium and the DF (faecal) for Ra/Ca is similar to that for Ba/Ca. It has also been observed (Mays & Taylor, 1963) that both elements are concentrated in pigmented tissue such as occurs in pigmented melanomata. On the other hand, the kidney treats radium more like strontium than barium; the OR (urine-injection) for Ra/Ca is not, as might have been expected, higher than that for Ba/Ca but virtually the same as for Sr/Ca. The skeletal metabolism of radium appears in certain respects to be more akin to that of calcium and strontium and in others to that of barium. Thus the values for the bone accretion rate and the size of the exchangeable pool of calcium in the whole body of cows derived from calcium, strontium and radium data are similar and differ from the values derived from the barium data (Table 4). The similarity of radium to calcium is also shown by the similarity of the curves of Ca_A and Ca_B , and Ra_A and Ra_B in this experiment. There do, however, appear to be significant differences between the calcium curves shown here and those given by Garner *et al.* (1960). ^{45}Ca is lost from the body more rapidly ($^{45}Ca_{obs}$ falls more steeply) and resorption of ^{45}Ca from bone appears to occur earlier and to a greater extent than in the earlier experiments. The first difference is probably attributable to the very high secretion of intravenously administered ^{45}Ca into the milk in these experiments, but the reason for the second is obscure. The cows used in all the experiments were of comparable age and milk yield. However, in its resorption from bone ($^{224}Ra_R$) radium behaves more like barium, in that resorption starts earlier and occurs to a greater extent than that of calcium. This presumably is one reason for the slower decline in the plasma specific radioactivity of radium than that of calcium after a single intravenous dose.

The results obtained in Expt. B, in which radiation injury was inflicted on the cows, are presented only in part. So far as the secretion of radium into milk is concerned they reinforce the

conclusion that the udder plays a large part in the overall discrimination against this element in its passage from diet to milk. However, the fact that the OR (milk-diet) values from both Expts. A and B are very similar arises from large differences in both the OR (milk-injection) and the DF (absorptive) values. The latter are so different, both from those of Expt. A, in which no clinical effects were observed, and from what might have been predicted from the known behaviour of strontium and barium, that there must be a strong presumption that they are not normal. There were signs of acute gastrointestinal disturbance and constipation in these animals, although the calculated radiation doses were far below those observed by Nold, Hayes & Comar (1960) to produce pathological changes or clinical effects in the goat (for discussion see Sansom *et al.* 1964). Thus the relation between either the clinical effects, or the metabolic disturbance of the animals' relative absorption of radium and calcium, and the calculated radiation doses administered in Expt. B remain unexplained.

The most important conclusion from these studies of the differences in metabolism of ^{45}Ca , ^{89}Sr , ^{140}Ba and ^{224}Ra is that they form a series in which after either an intravenous or an oral dose a smaller proportion of the dose of each element is secreted in the milk as the series is descended: ^{45}Ca , ^{89}Sr , ^{140}Ba , ^{224}Ra . Qualitatively both ^{140}Ba and ^{224}Ra show marked differences of behaviour in the animal body from either ^{45}Ca or ^{89}Sr .

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