Biochem. J. (1960) **76**, 572

Fission Products and the Dairy Cow

SOME ASPECTS OF THE METABOLISM OF THE ALKALINE-EARTH ELEMENTS CALCIUM, STRONTIUM AND BARIUM*

BY R. J. GARNER, H. G. JONES† AND B. F. SANSOM

Radiobiological Laboratory, Agricultural Research Council, Field Station, Compton, Berks

(*Received* 21 *December* 1959)

Radioactive isotopes of strontium (^{89}Sr and ^{90}Sr) are generally regarded as the most dangerous of the products of nuclear fission. The passage of radiostrontium deposited on soil and vegetation into the human diet has therefore received considerable attention. Since strontium is similar to calcium in its metabolic behaviour it has become common practice to study the simultaneous behaviour of the two elements in the various physiological processes in plants and animals which are involved in these transfer processes. In this way it has been demonstrated that discrimination against strontium in favour of calcium occurs, for example, in their passage from the diet of a dairy cow to its milk (Comar & Wasserman, 1958).

^{140}Ba is also a constituent of mixed fission products and is present in significant amounts in tropospheric fall-out but, because of its relatively shorter half-life (12·8 days as compared with 54 days for ^{89}Sr and over 20 years for ^{90}Sr), it is considered to present a smaller hazard to the human and animal population than the strontium isotopes. Distribution studies in human tissues (Sowden, 1958) suggest that the behaviour of ^{140}Ba in the animal body differs from that of strontium and calcium and, in fact, may be more closely related to that of radium.

The present paper describes studies on the comparative behaviour of ^{45}Ca, ^{89}Sr and ^{140}Ba in the dairy cow. These experiments were designed particularly to yield information on the physiological processes involved in the discrimination between the three elements during their transfer from diet to milk.

* Part 1: Garner, Jones & Ekman (1960).

† On leave from M.R.C. Radiobiological Research Unit, Harwell, Berks.

METHODS

Animals

Mature, lactating Redpoll × Ayrshire cows have been used throughout these experiments. The animals received approx. 30 lb. of hay and commercial dairy nuts (2½ lb./gal. of milk) daily, divided into two equal feeds. Water was freely available. Complete separation of urine and faeces has been achieved by means of the metabolism harness described by Balch, Bartlett & Johnson (1951), modified to facilitate decontamination. Collection of urine and faeces was made at 6 hr. intervals (05.00, 11.00, 17.00 and 23.00 hr. daily) over an experimental period of 8 days. Milking was carried out with a standard machine at 06.00 and 16.00 hr. daily. Blood samples were obtained, by venepuncture, from the mammary veins.

Plan of experiments

Pairs of animals received ^{45}Ca and ^{89}Sr, ^{89}Sr and ^{140}Ba or ^{140}Ba and ^{45}Ca first orally and, 1–2 months later, the same isotopes intravenously. The approximate amounts of activity received by the animals are set out in Table 1.

Administration of radioactivity

In both oral and intravenous experiments, pile-produced, carrier-free ^{89}Sr and an equilibrium mixture of ^{140}Ba and its radioactive daughter ^{140}La in HCl solution neutralized to about pH 6 were used. For oral administration, ^{45}Ca in the form of neutron-irradiated $CaCO_3$ (specific activity approx. 200 μc of ^{45}Ca/g. of Ca) dissolved in the minimum amount of about 5N-HCl was employed. Material of higher specific activity (5–10 mc of ^{45}Ca/g. of Ca) in the form of a solution of $CaCl_2$ was used for intravenous injection.

The large volume of the $CaCl_2$ solution with relatively low specific activity necessitated feeding the material in a standard sheep feed (a mixture of molasses, flaked maize and oats) used on this Station. This procedure was therefore adopted in all the oral experiments. The animals were trained to eat this mixture by being offered small quantities

Table 1. *Plan of experiments*

For details see text.

Animal pair	Date	Treatment
1	16. v. 58	7 mc of ^{45}Ca; 1·5 mc of ^{89}Sr orally
	28. vii. 58	2 mc of ^{45}Ca; 1 mc of ^{89}Sr intravenously
2	6. xi. 58	1·5 mc of ^{89}Sr; 16 mc of ^{140}Ba orally
	3. xii. 58	1·5 mc of ^{89}Sr; 4 mc of ^{140}Ba intravenously
3	12. ii. 59	2 mc of ^{140}Ba; 6 mc of ^{45}Ca orally
	12. iii. 59	1 mc of ^{140}Ba; 3 mc of ^{45}Ca intravenously

for a few days before the experimental period. The large amount of stable Ca inevitably administered had no apparent effect on the blood Ca level. Comar & Wasserman (1956) have also observed that short-term changes in the dietary intake have no effect on Ca metabolism. Intravenous injections were made through an indwelling plastic catheter previously inserted into a mammary vein. A transient increase in blood Ca was observed after these injections. However, the highest value seen in any animal was 18 mg./100 ml. of plasma and values within the normal range were always seen after 1 hr.

The relevant mixture of isotopes was given to each animal immediately after either the morning or afternoon milking.

Counting methods

^{45}Ca *and* ^{89}Sr *experiments.* The ^{45}Ca and ^{89}Sr content of faecal samples was estimated by ashing samples (25 g.) of the thoroughly mixed, total daily output of fresh faeces at 650°, placing the ash in aluminium cups of diameter 2·5 cm. and counting under a thin end-window Geiger–Müller counter, type EHM 2 (20th Century Electronics Ltd.) The efficiency of this counting method was approx. 1% for ^{45}Ca and 6% for ^{89}Sr.

Milk from each collection was prepared for counting by pipetting 100 ml. samples into steel dishes of diameter 11·4 cm., adding a few drops of perchloric acid and drying under an infrared lamp. The samples were counted by a β-scintillation counter with a plastic phosphor of diameter 4·5 in., giving an efficiency of 2% for ^{45}Ca and 27% for ^{89}Sr.

Plasma and urine samples (2 and 10 ml. respectively) were assayed by precipitating calcium oxalate and strontium oxalate directly on to steel planchets of diameter 2·4 cm. (Comar, Hansard, Hood, Plumlee & Barrentine, 1951). With urine, carrier Ca (sufficient to give a total precipitate of about 50 mg.) was added to ensure complete precipitation of the radioactivity. Precipitates from both urine and plasma were counted under the end-window Geiger–Müller counter, with efficiencies of approx. 5% for ^{45}Ca and 11% for ^{89}Sr.

The ^{45}Ca and ^{89}Sr were distinguished by counting all samples with and without a polyvinyl chloride filter which absorbed all the ^{45}Ca β-particles but decreased the count rate of ^{89}Sr by 47·7%. The polyvinyl chloride filter was obtained from Velbex sheeting of thickness 0·020 in. and weight 61 mg./cm.^{2}, supplied by BX Plastics Ltd., London, E. 4.

^{89}Sr *and* ^{140}Ba–^{140}La *experiments.* Milk and urine were assayed by pipetting samples (100 ml.) into polyethylene bags (6 in. × 5 in.) subsequently sealed by heat. Samples (100 g.) of faeces were weighed into similar bags and the contents flattened, after sealing, into a uniform layer. The bags were counted by placing them flat in a containing tray, situated under the plastic phosphor of the β-scintillation counter. The efficiency of this method was approx. 3% for ^{89}Sr and 4% for ^{140}Ba–^{140}La. Differential counting was accomplished by using a $\frac{1}{8}$ in. Perspex filter, of weight 375 mg./cm.^{2}, which absorbed all the ^{89}Sr β-particles but decreased the count rate due to ^{140}Ba–^{140}La by 66·9%.

Plasma samples were assayed as liquids by means of a Veall-type Geiger-Müller counter. Counts due to ^{140}Ba–^{140}La were determined by counting also in a NaI (T1) well-type γ-scintillation counter, and surrounding the samples with a thin lead shield to absorb β-particles from ^{89}Sr. The efficiencies of counting were approx. 6·5% for ^{89}Sr and 20% for ^{140}Ba–^{140}La.

^{45}Ca *and* ^{140}Ba–^{140}La *experiments.* Faeces samples were prepared for assay by ashing at 650° and dissolving the ash in 20 ml. of 6N-HCl. Calcium oxalate and barium oxalate were precipitated from 2 ml. of this solution on to steel planchets of diameter 2·4 cm. Urine samples (200 ml.) were dried and ashed, the ash was dissolved in 40 ml. of 6N-HCl and the oxalates were precipitated from 5 ml. of this solution. The dried precipitates were counted in the β-scintillation counter. The supernatant solutions from the oxalate precipitations were retained and counted in the Veall-type Geiger–Müller counter as, in some cases, incomplete precipitation of barium oxalate occurred. The proportion of barium unprecipitated was never more than 20%. The efficiencies of counting ^{45}Ca and ^{140}Ba–^{140}La in this way were approx. 8 and 22% respectively.

Plasma and skimmed milk were assayed by pipetting 10 ml. samples into steel dishes of diameter 11·4 cm., drying under an infrared lamp and counting in the β-scintillation counter. (Skimmed milk was used in the later experiments because it could be dried more easily. A correction was made for the cream content of each sample when converting the results into an equivalent concentration in whole milk.) The efficiencies of counting ^{45}Ca and ^{140}Ba–^{140}La in this way were approx. 5·5 and 21% respectively in milk, and 5·7 and 23% respectively in plasma.

The ^{45}Ca and ^{140}Ba–^{140}La were distinguished by means of the polyvinyl chloride filter of weight 61 mg./cm.^{2} This decreased the count from ^{45}Ca to zero but decreased that due to ^{140}Ba–^{140}La by only 63·3%.

In all experiments in which ^{140}Ba–^{140}La was used the samples were set aside for 14 days before counting to allow equilibration between ^{140}Ba and its daughter ^{140}La.

In all the experiments suitable standards were prepared by adding known amounts of the appropriate isotopes to faeces, urine, milk and plasma obtained from the animals before administration of radioactivity. These were treated and counted by the methods already described.

In all experiments sufficient counts were obtained to give a probable error of less than 2% in the determination of each isotope in all samples. Corrections were made for background radiation, the dead-time of the counting instruments used, radioactive decay and, where applicable, self-absorption.

RESULTS

Recovery of radioactivity from faeces, urine and milk

The overall recoveries in the 8 days following administration are given in Table 2.

If it be assumed that the fraction of an orally administered isotope which is absorbed from the gut behaves similarly in every way to the same isotope introduced directly into the blood stream, then

$$\text{Percentage of material absorbed} = 100 \times \frac{\text{Percentage of orally administered material excreted in urine or milk}}{\text{Percentage of intravenously administered material excreted in urine or milk}}.$$

Table 2. *Percentage recovery of alkaline-earth elements from faeces, urine and milk in the 8 days after administration*

Figures given are the mean results (range in parentheses) from four animals.

	Oral administration			Intravenous administration		
	Faeces	Urine	Milk	Faeces	Urine	Milk
^{45}Ca	71·1 (57·2–81·5)	0·39 (0·10–0·64)	16·1 (11·2–23·2)	16·4 12·1–23·8)	1·17 (0·42–2·01)	31·9 (21·2–56·4)
^{89}Sr	89·3 (85·3–94·2)	1·34 (0·76–1·93)	1·94 (1·52–2·61)	18·0 (16·3–20·3)	21·1 (16·4–28·2)	16·4 (10·7–27·0)
^{140}Ba	98·1 (95·4–110·9)	1·08 (0·90–1·34)	0·56 (0·32–0·75)	36·5 (32·1–46·9)	34·2 (20·5–48·4)	10·2 (8·7–13·4)

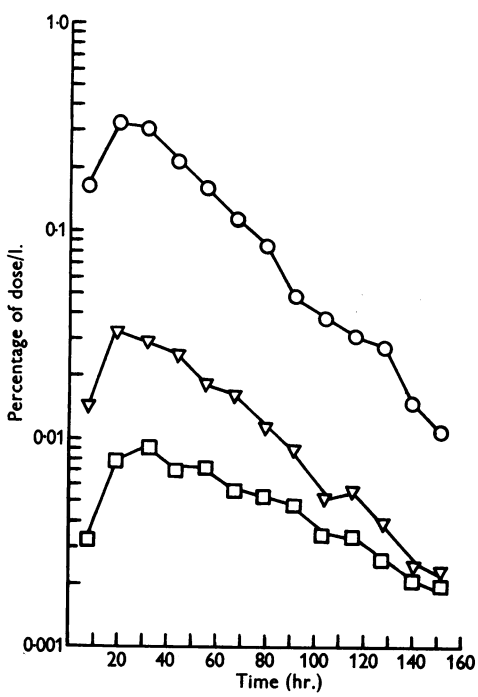

Fig. 1. Change in concentration with time of radioactive alkaline earths in milk after oral administration. ○, ^{45}Ca; ▽, ^{89}Sr; □, ^{140}Ba. Each point has been obtained by averaging the figures for four animals. Correction has been made for physical decay.

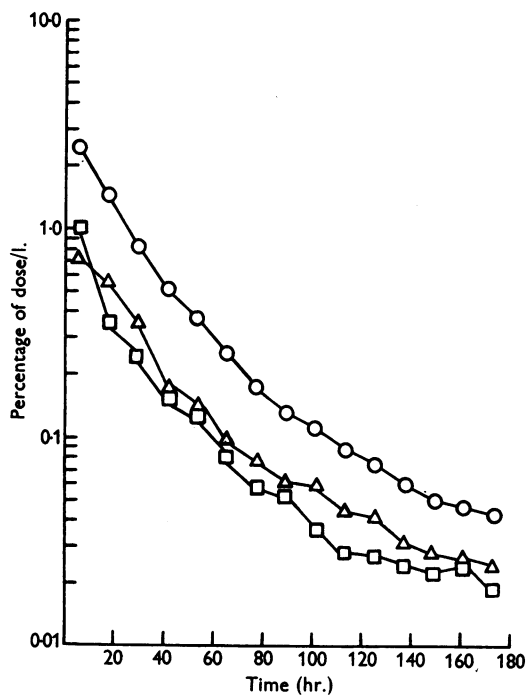

Fig. 2. Change in concentration with time in milk after intravenous administration. ○, ^{45}Ca; △, ^{89}Sr; □, ^{140}Ba. Each point has been obtained by averaging the figures for four animals. Correction has been made for physical decay.

With the results obtained in individual animals after oral administration 34–52 % (mean 40 %) of ^{45}Ca, 8–12 % (mean 11 %) of ^{89}Sr and 4–7 % (mean 6 %) of ^{140}Ba was absorbed from the gut.

Elimination of ^{45}Ca, ^{89}Sr and ^{140}Ba in milk

The change of concentration of these elements with time in milk after oral and intravenous administration is shown in Figs. 1 and 2. Ultimately the rate becomes exponential for each of the isotopes. The biological half-lives, calculated from regression lines fitted through the experimental points on the exponential parts of the curves, after oral administration, can be estimated to be 25, 28 and 53 hr. for ^{45}Ca, ^{89}Sr and ^{140}Ba respectively.

Discrimination between calcium, strontium and barium in their passage from diet to milk

Comar, Wasserman & Nold (1956) introduced the term 'strontium–calcium observed ratio' (OR) to denote the overall discrimination observed in the movement of the two elements from one phase to another under steady-state conditions. The 'observed ratio' can be defined as the product of

a number of 'discrimination factors' (DF), each of which is a measure of the extent to which the physiological process to which it refers contributes to the overall discrimination. Formulae for the derivation of DF's have been given (Comar *et al.* 1956; Wasserman, Lengemann & Comar, 1958), but with no explanation of their bases. An examination of these formulae indicates that, in order that the results of oral experiments may be used for the calculation of DF's, it has been necessary to ignore endogenous secretion into the alimentary canal. As is evident from Table 2, over 30 % of intravenously administered ^{140}Ba is eliminated from the body via this route. It has therefore been considered necessary to derive new formulae which require information from intravenous as well as oral experiments; these expressions are set out in Table 3. They can be shown to reduce to Comar's formulae if endogenous secretion is neglected.

The strontium–calcium $DF_{absorptive}$, i.e. that part of the overall discrimination due to differentiation during intestinal absorption, is defined as the percentage of orally administered strontium absorbed, divided by the percentage of orally administered calcium absorbed. The $DF_{urinary}$ measures the effect urinary excretion has on the relative amounts of strontium and calcium remaining in the body. Similarly, the DF_{faecal} represents the effect of endogenous secretion. The $DF_{lactational\text{-}body}$ is a measure of the effect the mammary gland has in discriminating between the amounts of strontium and calcium eliminated in the milk. The $DF_{lactational\text{-}milk}$ represents the effect that loss of strontium and calcium in the milk has on the amount remaining in the body. The $DF_{lactational\text{-}milk}$ and the $DF_{lactational\text{-}body}$ are obtained from the appropriate OR values (see Comar *et al.* 1956; Wasserman *et al.* 1958). Barium–strontium and barium–calcium discrimination factors are obtained similarly.

Observed ratios and discrimination factors are concepts which are strictly applicable only under steady-state conditions. In order to use the results of single dose experiments for their calculation it is therefore necessary to assume that these results can be used to predict the concentrations which would be found in the excreta under steady-state conditions after continuous administration. Experiments with ^{89}Sr and ^{45}Ca in cattle (Comar & Wasserman, 1956) and goats (Wasserman *et al.* 1958) and with ^{131}I in cattle (R. J. Garner & H. G. Jones, unpublished work) indicate that this assumption is justified. Since the time–concentration curve for repeated administration at equal time intervals is constructed simply by summation from that obtained from single-dose experiments it is apparent that the time to reach a constant concentration in the excreta after continuous administration is essentially the same as the time required for the amount of a single dose appearing in the excreta to fall to a small value. A period of 8 days has been found convenient. After 8 days the concentration in faeces, for example, had fallen to below 2·5 % of the peak value.

Values for OR and DF computed from the formulae in Table 3 and with this method of prediction are given in Table 4.

Table 3. *Expressions used in the calculation of strontium–calcium observed ratios and discrimination factors*

Observed ratios are used to measure overall discrimination between strontium and calcium in their transfer from one phase to another. Discrimination factors measure the contribution of individual physiological processes to this overall discrimination (Comar, Wasserman & Nold, 1956).

$$OR_{sample\text{-}precursor} = \frac{^{89}Sr/^{45}Ca \text{ of sample}}{^{89}Sr/^{45}Ca \text{ of precursor}}$$

$$DF_{absorptive} = \frac{\% \text{ of orally administered } ^{89}Sr \text{ absorbed}}{\% \text{ of orally administered } ^{45}Ca \text{ absorbed}}$$

$$DF_{urinary} = \frac{100 - \% \text{ of intravenously administered } ^{89}Sr \text{ excreted in urine}}{100 - \% \text{ of intravenously administered } ^{45}Ca \text{ excreted in urine}}$$

$$DF_{faecal} = \frac{100 - \% \text{ of intravenously administered } ^{89}Sr \text{ excreted in faeces}}{100 - \% \text{ of intravenously administered } ^{45}Ca \text{ excreted in faeces}}$$

$$DF_{lactational\text{-}milk} = \frac{OR_{milk\text{-}diet}}{DF_{absorptive} \times DF_{urinary} \times DF_{faecal}}$$

$$OR_{body\text{-}diet} = DF_{absorptive} \times \frac{\% \text{ of intravenously administered } ^{89}Sr \text{ retained in body}}{\% \text{ of intravenously administered } ^{45}Ca \text{ retained in body}}$$

$$DF_{lactational\text{-}body} = \frac{OR_{body\text{-}diet}}{DF_{absorptive} \times DF_{urinary} \times DF_{faecal}}$$

Table 4. *Strontium–calcium, barium–strontium and barium–calcium observed ratios and discrimination factors in lactating cows*

Intravenous administration	Sr–Ca	Ba–Sr	Ba–Ca
$OR_{faeces\text{-}injection}$	1·2; 1·3	1·8; 1·9	2·5; 1·9
$OR_{urine\text{-}injection}$	18; 11	1·8; 2·1	33; 91
$OR_{milk\text{-}injection}$	0·46; 0·48	0·81; 0·92	0·41; 0·47
Oral administration			
$OR_{faeces\text{-}diet}$	1·5; 1·2	1·1; 1·1	1·4; 1·1
$OR_{urine\text{-}diet}$	4·6; 2·5	0·75; 1·7	2·2; 13
$OR_{milk\text{-}diet}$	0·11; 0·11	0·33; 0·30	0·05; 0·06
$DF_{absorptive}$	0·2; 0·3	0·3; 0·5	0·1; 0·2
$DF_{urinary}$	0·7; 0·8	0·8; 0·8	0·8; 0·6
DF_{faecal}	1·0; 1·0	0·8; 0·8	0·8; 0·70
$DF_{lactational\text{-}milk}$	0·6; 0·5	1·4; 1·1	0·7; 0·7
$OR_{body\text{-}diet}$	0·17; 0·34	0·18; 0·13	0·09; 0·02
$DF_{lactational\text{-}body}$	1·0; 1·5	0·8; 0·5	1·2; 0·3

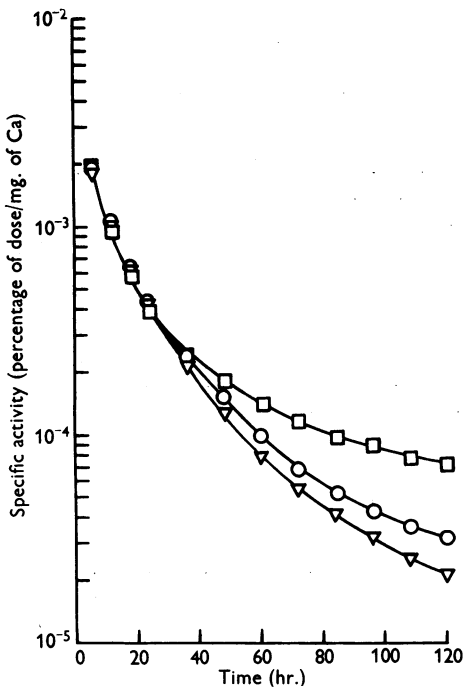

Fig. 3. Change in specific radioactivity of blood plasma (expressed as percentage of dose/mg. of Ca) after intravenous administration. ○, ^{45}Ca; ▽, ^{89}Sr; □, ^{140}Ba. Each point has been obtained by averaging the figures for four animals. Correction has been made for physical decay.

Skeletal metabolism of ^{45}Ca, ^{89}Sr *and* ^{140}Ba

Bauer, Carlsson & Lindquist (1955*a*) have described methods for differentiating quantitatively between skeletal exchange reactions, accretion and resorption, and have applied these methods to a comparative study of the metabolism of ^{90}Sr and ^{45}Ca and of ^{140}Ba and ^{45}Ca in the young rat (Bauer, Carlsson & Lindquist, 1955*b*, 1956).

Table 5. *'Accretion rate' and 'exchangeable calcium' in the whole body of cows*

Calculated from	'Accretion rate' (g. of Ca/hr.)	'Exchangeable Ca' (g.)
^{45}Ca	1·4	41
^{89}Sr	1·3	39
^{140}Ba	0·9	33

Initially attempts were made to apply these methods with, as bone samples, caudal vertebrae removed at intervals after intravenous administration of the isotopes. It became apparent, however, that the distribution of ^{45}Ca and ^{89}Sr was not uniform throughout the length of the tail and the results reported here are therefore confined to 'accretion rates', etc. in the whole skeleton, the whole body being treated in the same manner as a calcified tissue (see Bauer *et al.* 1955*a*). The total percentage of activity remaining in the body at any time was obtained by subtracting from 100 the percentage of the dose eliminated at that time in the urine, faeces and milk.

The change in activity in the blood plasma after intravenous injection is shown in Fig. 3. To facilitate comparison of the behaviour of the three isotopes the 'specific activity' in each case has been expressed as the percentage of the dose/mg. of Ca.

The 'accretion rate' and 'exchangeable calcium' are given in Table 5. These have been calculated by the formulae given by Bauer *et al.* (1955*a*) together with the mean values from four animals for percentage dose retained ($^{45}Ca_{Obs}$, $^{89}Sr_{Obs}$, $^{140}Ba_{Obs}$) and specific activity 9 and 21 hr. after dosing.

With these constants, curves have been constructed showing the changes with time of the amount of 'exchangeable' ^{45}Ca, ^{89}Sr or ^{140}Ba ($^{45}Ca_E$ etc. in the notation of Bauer *et al.* 1955*a*),

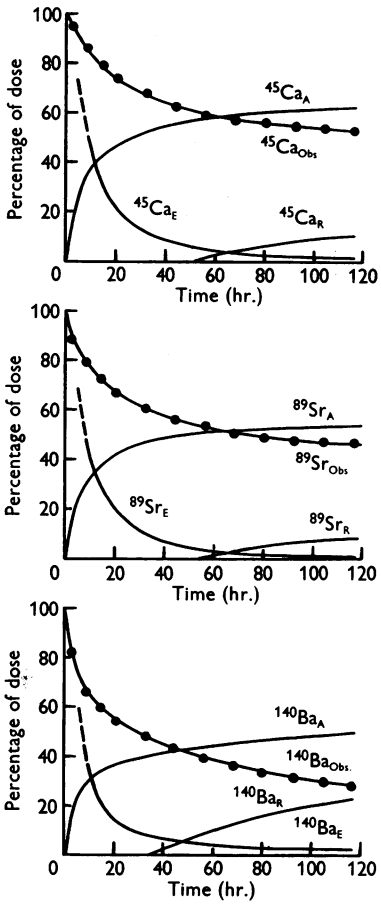

Fig. 4. Overall metabolism in the whole body (mean results for four animals). $^{45}Ca_{Obs}$, $^{89}Sr_{Obs}$, $^{140}Ba_{Obs}$: percentage of intravenously administered ^{45}Ca, ^{89}Sr or ^{140}Ba retained in the whole body. $^{45}Ca_E$, $^{89}Sr_E$, $^{140}Ba_E$: exchangeable fraction of ^{45}Ca, ^{89}Sr or ^{140}Ba in whole body. $^{45}Ca_A$, $^{89}Sr_A$, $^{140}Ba_A$: non-exchangeable fraction of ^{45}Ca, ^{89}Sr or ^{140}Ba in whole body. $^{45}Ca_R$, $^{89}Sr_R$, $^{140}Ba_R$: ^{45}Ca, ^{89}Sr or ^{140}Ba resorbed ($Ca_R = Ca_A + Ca_E - Ca_{Obs}$ etc.). The notation used is that of Bauer, Carlsson & Lindquist (1955*a*).

the amount incorporated into the 'non-exchangeable' fraction of the skeleton ($^{45}Ca_A$ etc.) and the amount removed as a result of resorption ($^{45}Ca_R$ etc.) (Fig. 4).

DISCUSSION

The most striking differences in the behaviour of ^{45}Ca, ^{89}Sr and ^{140}Ba are in the relative amounts eliminated from the body by the various routes after intravenous administration (Table 2). The ability of the ruminant kidney to differentiate between calcium, strontium and barium is particularly noteworthy and is mentioned below in connexion with individual discriminating processes. Comparison of the patterns of excretion of the three isotopes in the cow after intravenous administration with those in a typical non-ruminant animal such as the rat (Bauer *et al.* 1955*b*, 1956) indicates that the relative importance of the faecal as compared with the urinary route is greater in the ruminant than in the non-ruminant animal with calcium, approximately the same with strontium and considerably less with barium.

The time course of secretion into milk, after oral dosing, agrees closely with published results both for ^{45}Ca (Visek, Monroe, Swanson & Comar, 1953) and ^{89}Sr (Squire, Middleton, Sansom & Coid, 1958; Garner & Sansom, 1959). The 'tailing off' of the secretion curve after 6–7 days observed in other experiments is not apparent from Fig. 1, but presumably the biological half-lives of the concentration of the two isotopes in milk would ultimately become identical with those in bone. The longer half-life of ^{140}Ba in milk probably reflects the more rapid release of this isotope from the skeleton (see below).

It is seen from Fig. 1 that, during their transfer from diet to milk, there is considerable discrimination between calcium, strontium and barium in favour of calcium. In this series of experiments ^{45}Ca in the diet was preferentially transferred to the milk by a factor of 9 over strontium and of 18 over barium. The strontium–calcium $OR_{milk\text{-}diet}$ observed is in agreement with values reported by Comar & Wasserman (1958) and Cragle & Demott (1959).

It is apparent from Table 4 and by comparison of Figs. 1 and 2 that absorption from the gut plays the major part in this overall discrimination. Absorptive discrimination is particularly effective in differentiating between calcium and barium (mean $DF_{absorptive}$ 0·16). Mammary differentiation is second in importance with strontium and calcium but, with barium and calcium, the effects of urinary excretion, mammary secretion and endogenous secretion into the gut are almost equal.

Renal discrimination is apparently more complete in ruminants than in non-ruminants. The strontium–calcium $OR_{urine\text{-}blood}$ has been found to be 5 in the rat, for example (Comar *et al.* 1956), 12–20 in the goat (Wasserman *et al.* 1958), and 11–18 in the cow (present paper). The barium–calcium $OR_{urine\text{-}injection}$ after intraperitoneal administration of ^{45}Ca and ^{140}Ba to rats can be estimated to be about 10 (Bauer *et al.* 1956), as compared with a value of 30–90 after intravenous injection in the cow. Because the amount of calcium in ruminant urine is normally small, the effect of the kidneys on overall discrimination, as indicated by the $DF_{urinary}$ (Table 4), is only slightly greater than that in the non-ruminant animal.

Endogenous secretion into the gut is usually considered to play only a minor part in overall discrimination between strontium and calcium (Comar & Wasserman, 1958), although Cragle & Demott (1959) found an increase in the ^{89}Sr–^{45}Ca ratio of faeces with time, after simultaneous oral administration of the two isotopes, which they consider to be suggestive of some preferential secretion of strontium. The present results indicate that differential excretion via this route is small when strontium is considered but is of greater importance for barium. From the results of Bauer *et al.* (1956) a barium–calcium $OR_{faeces\text{-}injection}$ in the rat of about 3 can be inferred, indicating that 'faecal' discrimination is of the same general order in both ruminant and non-ruminant animals.

Despite the operation of a mechanism (mammary secretion) which tends to increase the strontium–calcium ratio in the body ($DF_{lactational\text{-}body} > 1$), the $OR_{body\text{-}diet}$ in the lactating cow (mean value 0·25) is of the same order as, or possibly slightly less than, that recorded in non-lactating, non-ruminant animals (0·16–0·54) (Comar & Wasserman, 1958).

The values for strontium–calcium OR's and DF's obtained in the present series of experiments are in general agreement with those published by Wasserman *et al.* (1958) for the goat.

The validity of the assumptions made by Bauer *et al.* (1955*a*) in deriving expressions for obtaining the accretion rate of calcium in bone and the exchangeable fraction of calcium in bone is open to question; it has, for example, been pointed out by Marshall, Rowland & Jowsey (1959) that there are long-term exchange reactions in bone, and these are not considered by Bauer *et al.* Nevertheless, these parameters are useful for comparing the behaviour of the different bone-seeking isotopes in any one species or of any one of them in different species. Unfortunately a direct comparison of the metabolism of ^{45}Ca, ^{89}Sr and ^{140}Ba in the young rats used by Bauer and his collaborators and the mature cows used in the present experiments is precluded by the difference in the ages of the two groups of animals.

The overall retention curve ($^{45}Ca_{Obs}$, $^{89}Sr_{Obs}$, $^{140}Ba_{Obs}$ of Fig. 4), if plotted on semi-logarithmic paper, can be resolved into three exponential components of half-life 5·5, 20·9 and 1085 hr., 5·8, 18·6 and 1083 hr., and 3·7, 21·6 and 199 hr. respectively for the three isotopes.

The rate of removal of ^{89}Sr and ^{45}Ca from the plasma of cows by exchange reactions ('exchangeable calcium') appears to be approximately the same; the rate of removal of ^{140}Ba may be smaller (Table 5). Bauer *et al.* (1955*b*, 1956) found that, in rats, there was little difference between the behaviour of the three isotopes in this respect. Whereas in rats the rate of incorporation of strontium into the non-exchangeable fraction of the bone-salt was the same as that of calcium (Bauer *et al.* 1955*b*) and that of barium twice that of calcium (Bauer *et al.* 1956), in cows the 'accretion rate' for strontium is 91 % and that for barium 61 %, of that for calcium. ^{140}Ba appears to be released (by resorption) from bone after a shorter time interval and more rapidly than either ^{45}Ca or ^{89}Sr (Fig. 4).

The important overall effect of the differences in metabolism of ^{45}Ca, ^{89}Sr and ^{140}Ba is that a considerably smaller proportion of dietary ^{89}Sr or ^{140}Ba than of dietary ^{45}Ca appears in the milk (Fig. 1) and is retained in the body (Fig. 4). Qualitatively, ^{140}Ba shows marked differences in its behaviour in the animal body from the other alkaline-earth elements studied.

SUMMARY

1. The isotopes ^{45}Ca and ^{89}Sr, ^{89}Sr and ^{140}Ba, or ^{45}Ca and ^{140}Ba have been administered simultaneously, first orally and, later, intravenously, to pairs of lactating cows.

2. The mean recoveries of ^{45}Ca, ^{89}Sr and ^{140}Ba, in the 8 days after oral administration, were (respectively): from faeces, 71, 89 and 98 %; from urine, 0·4, 1·3 and 1·1 %; from milk, 16, 1·9 and 0·6 % of the dose; and, in the 8 days after intravenous administration: from faeces, 16, 18 and 36 %; from urine, 1·2, 21 and 34 %; from milk, 32, 16 and 10 % of the dose.

3. Calculation of individual discrimination factors, by formulae which require information from intravenous, in addition to oral, experiments, indicates that absorptive discrimination made the most important contribution to overall discrimination between calcium, strontium and barium during their passage from diet to milk. Mammary secretion also played a considerable part with strontium; but, with barium, the effects of renal excretion, endogenous secretion into the gut and mammary secretion were approximately equal.

4. The comparative behaviour of ^{45}Ca, ^{89}Sr and ^{140}Ba in the whole body treated as a calcified tissue has been examined. The rate of removal of ^{45}Ca and ^{89}Sr from plasma by exchange reactions was approximately the same; the rate of removal of ^{140}Ba by this process was 80 % of that of ^{45}Ca. The rates of incorporation of ^{89}Sr and ^{140}Ba into the non-exchangeable fraction of the whole skeleton were 91 and 61 % respectively of that of ^{45}Ca. ^{140}Ba was released from the skeleton after a shorter time interval and at a greater rate than ^{45}Ca or ^{89}Sr.

5. The metabolism of ^{45}Ca, ^{89}Sr and ^{140}Ba in the cow is discussed in relation to published results on a typical non-ruminant animal, the rat.

We wish to thank Mr L. C. West, Miss N. Vincent, Miss J. A. N. L. Mackenzie and Mr M. Vagg for their technical assistance, and Mr E. Maggs and Mr N. H. Martin for their care of the experimental animals.

REFERENCES

Balch, C. C., Bartlett, S. & Johnson, V. W. (1951). *J. agric. Sci.* **41**, 98.

Bauer, G. C. H., Carlsson, A. & Lindquist, B. (1955*a*). *K. fysiogr. Sällsk. Lund Förh.* **25**, no. 1.

Bauer, G. C. H., Carlsson, A. & Lindquist, B. (1955*b*). *Acta physiol. scand.* **35**, 56.

Bauer, G. C. H., Carlsson, A. & Lindquist, B. (1956). *Biochem. J.* **63**, 535.

Comar, C. L., Hansard, S. L., Hood, S. L., Plumlee, H. P. & Barrentine, B. F. (1951). *Nucleonics*, **8**, 19.

Comar, C. L. & Wasserman, R. H. (1956). In *Progress in Nuclear Energy. Series VI. Biological Sciences*, vol. 1. London and New York: Pergamon Press Ltd.

Comar, C. L. & Wasserman, R. H. (1958). In *Radioisotopes in Scientific Research*, vol. 4. London, New York and Paris: Pergamon Press Ltd.

Comar, C. L., Wasserman, R. H. & Nold, M. M. (1956). *Proc. Soc. exp. Biol., N.Y.*, **92**, 859.

Cragle, R. G. & Demott, B. J. (1959). *J. Dairy Sci.* **42**, 1367.

Garner, R. J., Jones, H. G. & Ekman, L. (1960). *J. agric. Sci.* (in the Press).

Garner, R. J. & Sansom, B. F. (1959). *Vet. Rec.* **71**, 670.

Marshall, J. H., Rowland, R. E. & Jowsey, J. (1959). *Radiat. Res.* **10**, 258.

Sowden, E. M. (1958). *Biochem. J.* **70**, 712.

Squire, H. M., Middleton, L. J., Sansom, B. F. & Coid, C. R. (1958). In *Radioisotopes in Scientific Research*, vol. 4. London, New York and Paris: Pergamon Press Ltd.

Visek, W. J., Monroe, R. A., Swanson, E. W. & Comar, C. L. (1953). *J. Dairy Sci.* **36**, 373.

Wasserman, R. H., Lengemann, F. W. & Comar, C. L. (1958). *J. Dairy Sci.* **41**, 812.

Biochem. J. (1960) **76**, 579

Uncoupling Reagents and Metabolism

1. EFFECTS OF SALICYLATE AND 2:4-DINITROPHENOL ON THE INCORPORATION OF ^{14}C FROM LABELLED GLUCOSE AND ACETATE INTO THE SOLUBLE INTERMEDIATES OF ISOLATED RAT TISSUES

BY M. J. H. SMITH AND V. MOSES*
Department of Chemical Pathology, King's College Hospital Medical School, Denmark Hill, London, S.E. 5

(*Received* 29 *January* 1960)

Salicylate uncouples oxidative-phosphorylation reactions in respiring mitochondrial preparations (Brody, 1956; Penniall, Kalnitsky & Routh, 1956; Jeffrey & Smith, 1959; Packer, Austen & Knoblock, 1959). The increased oxygen consumption and diminished amounts of adenosine triphosphate and creatine phosphate observed in the isolated rat diaphragm incubated with salicylate (Smith & Jeffrey, 1956) are directly explicable in terms of this uncoupling action. However, several other reported effects of salicylate on the metabolism of isolated tissues, e.g. the decreased glycogen and protein synthesis produced in rat-liver slices and in diaphragm muscle (Smith, 1955; Manchester, Randle & Smith, 1958), bear a less obvious relationship to uncoupling and could be caused by salicylate acting on enzyme systems other than those involved in oxidative phosphorylation.

The present study is concerned with the effects of salicylate and 2:4-dinitrophenol on the incorporation of ^{14}C from [^{14}C]glucose and [2-^{14}C]acetate into the soluble metabolic intermediates of preparations of isolated rat tissues. The work was undertaken specifically to determine if salicylate produced any effects differing from those of the classical uncoupling agent and more generally to investigate the wider implications of an uncoupling action on tissue metabolism. A preliminary account of this work has been previously published (Smith & Moses, 1960).

* John Wyeth Fellow in Pharmacology of the Empire Rheumatism Council.

EXPERIMENTAL

Preparation of tissues. Male rats (wt. 200–250 g.) of the Wistar strain, maintained on M.R.C. cube diet no. 41, were starved for 24 hr. before being killed by stunning and decapitation. The required tissue (liver, kidney, brain, heart muscle or testis) was removed and placed in ice-cold medium. This medium [based on that used by Randle & Smith (1958) except that the K:Na ratio was altered according to the suggestion of Hastings, Teng, Nesbett & Sinex (1952)] contained (m-moles/l.): K_2HPO_4, 10·1; KCl, 123·0; NaCl, 4·5; Na_2SO_4, 0·3; $CaCl_2$, 1·35; $MgCl_2$, 1·3; glucose 1·0, dissolved in de-ionized water and adjusted to