Intestinal radiocalcium absorption in the goat: measurement by a double-isotope technique

BY KNUT HOVE

Department of Animal Nutrition, Agricultural University of Norway, 1432 Ås-NLH, Norway

(*Received 9 February 1983 – Accepted 19 July 1983*)

1. Intestinal radiocalcium absorption was measured in goats by a double-isotope technique involving injection of $^{45}CaCl_2$ intravenously and $^{47}CaCl_2$ into the abomasum. Cumulative absorption of radiocalcium was calculated by deconvolution analysis form curves of plasma radioactivity.

2. Repeated measurements at 2 d intervals gave highly reproducible results (r 0·94, $P < 0·001$). No systematic difference between two consecutive measurements was observed. A good agreement between absorption of radiocalcium from simultaneously administered $^{47}CaCl_2$ and ^{46}Ca-labelled hay (r 0·93, $P < 0·001$) seems to justify the use of inorganic ^{47}Ca as a tracer for Ca in ruminant diets.

3. Two- to three-fold increases in radiocalcium absorption 48 h after oral treatment with 1,25-dihydroxycholecalciferol or leaves of *Solanum malacoxylon* showed the usefulness of the method in situations of rapidly changing Ca absorption.

4. Endogenous adaptations in intestinal radiocalcium absorption from 20 to 43% were observed in lactating goats when Ca intakes decreased from 12 to 4 g/d.

5. It is concluded that the double-isotope technique is a suitable method for studies of Ca absorption in ruminants when tracer is introduced into the abomasum. The test is completed in 3–4 h and may therefore be used in situations where the absorption of Ca undergoes rapid changes.

In ruminant species the large capacity of the rumen and the continuous passage of rumen contents through the abomasum to the absorptive sites for calcium in the small intestine represent methodological problems for the use of oral Ca isotopes when short-term measurements of Ca absorption are required. A major part of the measurements of Ca absorption in ruminants so far has thus utilized balance techniques combined with intravenous administration of Ca isotope (Braithwaite *et al.* 1969; Ramberg *et al.* 1970, 1975). More direct methods involving the Thiry–Vella loop preparation and estimations of the disappearance of radiocalcium from intestinal contents after enteral radiocalcium infusions have also been reported (Van't Klooster, 1976; Abdel-Hafeez *et al.* 1982). The availability of two radiocalcium isotopes (^{45}Ca and ^{47}Ca) has stimulated the use of a double-isotope technique involving simultaneous administration of different radiocalcium tracers intravenously and into the gastrointestinal tract. In man and single-stomached animals this technique has been accepted both as a research tool and in diagnostic work, and a similar technique has recently been applied to sheep fed on a low-phosphate diet (Abdel-Hafeez *et al.* 1982).

The aim of the present work was to study more extensively the potential use of the double-isotope technique in ruminants and, especially, to evaluate the possibilities of measuring the rapid changes in Ca absorption which follow treatment with active metabolites of cholecalciferol. The results obtained with abomasally fistulated goats indicate that the double-isotope technique provides a rapid and reproducible method for estimation of radiocalcium absorption in ruminants.

EXPERIMENTAL

Mature female goats of a local dairy breed were used for the experiments. Surgery was performed under general anaesthesia at least 1 month before measurements were started. A plastic cannula with a central bore of 9 mm was placed in the abomasum about 100 mm

Table 1. *Daily intakes (g/d) of air-dry feed and calcium in the basal diet given to non-lactating and lactating goats*

Ingredient	Non-lactating goats		Lactating goats	
	Intake	Ca	Intake	Ca
Hay	200	0·46	500	1·08
Barley	300	0·11	400	0·14
Extracted soya-bean meal	25	0·08	150	0·48
Total	525	0·65	1050	1·70

proximal to the pylorus. The cannula was taken out through a stab wound approximately 20 mm ventral to the caudal angle of the rib cage.

A basal, low-Ca diet including barley (whole or ground and pelleted), mature hay and extracted soya-bean meal was given in all experiments. The diet was formulated to provide an adequate intake of nutrients for maintenance and lactation, except for Ca. The intake of Ca in non-lactating goats was 0·65 g/d from the basal diet and in lactating goats 1·7 g/d (Table 1). The actual intakes of Ca were regulated to required levels either by the addition of calcium acetate to the drinking-water or by infusions of calcium acetate solutions into the abomasum. The goats tolerated the taste of calcium acetate well and water intakes were normal. Ca administration with the drinking-water was discontinued 2 d before absorption measurements took place. The same daily amount of calcium acetate was given as a continuous abomasal infusion of approximately 0·6 l/24 h. This infusion ended on the day of measurement 3–4 h before radiocalcium was given, so as to allow time for the passage of the calcium acetate-containing digesta from abomasum and small intestine.

Measurements of radiocalcium absorption

The animals were fed and milked as usual in the morning on the days of the experiments. When the goats had finished eating, an indwelling catheter was placed in a jugular vein under local anaesthesia. To estimate the rate of removal of Ca from the plasma compartment, 0·7–0·9 MBq $^{45}CaCl_2$ (0·37–1·45 GBq/mg Ca; Amersham International, Amersham, Bucks) in 3–5 ml saline (9 g sodium chloride/l) was given intravenously in the other jugular vein. The intestinal tracer was given through the cannula directly into the abomasum. The $^{47}CaCl_2$ (74–184 kBq, 9–18 MBq/mg Ca; Amersham International) was injected in 50–75 ml water containing $CaCl_2$ (10 mg Ca) as a carrier. Both tracers were given in 1–2 min. The ^{45}Ca was measured in 0·5 ml plasma by liquid-scintillation counting (Packard Tricarb 3000; Packard Instrument Co. Inc., Illinois). Samples were counted to an accuracy of 10^4 counts and corrected for quenching by external standardization. Solutions of ^{45}Ca used for injection were diluted with water and 0·5 ml of a mixture of goat plasma and ^{45}Ca solution (4:1, v/v) was counted to determine the dose given. Plasma radioactivity was expressed as a percentage of the dose in 1 l plasma. The radioactivity from ^{47}Ca in 5 ml plasma was measured in a counter with a 50 × 50 mm sodium iodide crystal (LKB 1280 Ultrogamma; LKB Instruments, Bromma, Sweden). Samples were counted for 30 min or up to 10^4 counts. Dilutions of the dose infused were counted under identical conditions and the radioactivity of the plasma expressed as a percentage of the infused dose in 1 l plasma.

The best smooth curves for the appearance in plasma of abomasally infused tracer and the clearance of intravenously administered tracer were drawn by hand. Values for the concentration of radioactivity with time after the administration of tracer were read from

both curves at 10 min intervals from 10 to 180 or 240 min. In preliminary experiments intervals of 5 and 20 min were also tested, but only small differences in calculated absorption could be detected. Thus the rates of absorption for 10 min intervals were calculated by a deconvolution technique according to Hart & Spencer (1967) and Marshall (1976). The cumulative absorption of abomasal tracer was the sum of the individual absorption rates from the 10 min periods.

An asymptote (β_0) for the cumulative absorption was estimated by fitting the calculated values (cum) to the exponential:

$$\text{cum} = \beta_0 - e(\beta_1 - \beta_2 \times \text{time})$$

by a non-linear least-squares iterative procedure (Gauss–Newton method). A 95% confidence interval for the asymptote was estimated. The values obtained for the cumulative absorption toward the end of the experiment were usually situated close to the middle of the confidence interval and deviated in a major part of the experiments only by 2–3% from the asymptote. However, in some experiments absorption seemed delayed, resulting in large (10–20%) discrepancies between cumulative absorption values at 180–240 min and β_0. Values from these experiments were not included.

Verification of the technique

Series 1. Reproducibility of radiocalcium absorption. Eight goats were submitted to two consecutive absorption tests with a 2 d interval. The test was made twice in two goats, thus giving a total of ten pairs of absorption measurements. Non-lactating, pregnant and lactating goats were included to give a wide range of absorptions.

Series 2. Effects of varying amounts of cold Ca in the intestine during measurements of radiocalcium absorption. Five goats were maintained for 4 weeks on a high-Ca diet (4·65 g/d) by supplementation of the basal diet (Table 1) with 16 g calcium acetate in order to obtain a low efficiency of absorption. One set of measurements was performed during continuous abomasal infusion of calcium acetate (4 g Ca/d). After 2 d another set of measurements was started 4 h after the infusion had stopped.

Several weeks later, a high efficiency of Ca absorption was induced in three goats by oral administration of 5 μg 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) in 10 ml ethanol (500 ml/l) daily for 3 d. Measurements were done without infusion of Ca on the following day and repeated 1 d later during continuous abomasal infusion of Ca (4 g/d). This infusion started 1 h before radiocalcium was given.

Series 3. Radiocalcium absorption from an inorganic ($^{47}CaCl_2$) and an organic (^{45}Ca-labelled hay) Ca source. Hay labelled with ^{45}Ca was obtained by growing Rye grass (*Lolium multiflorum* var. *westerwoldicum*) on an artificial substrate. The grass was grown to heading and cut about 20 mm above the substrate. $^{45}CaCl_2$ (222 MBq) was then added to the culture. Grass containing radiocalcium was harvested at the next heading after a growth period of 27 d, and dried to hay at 105°. Analysis of ashed hay showed a Ca content of 6·0 mg/g dry matter (DM) and radioactive ^{45}Ca content of approximately 4 MBq/g DM.

In preparation for absorption experiments, 2·5 g hay was finely ground in a mortar and incubated anaerobically with 50 ml of freshly drawn rumen fluid for 16 h in a shaking water-bath at 38°. The incubation mixture of hay and rumen fluid was injected into the abomasum through the cannula followed immediately by injection of an ordinary dose of $^{47}CaCl_2$ in 50 ml distilled water. Blood samples for measurements of plasma radioactivity were taken at the usual intervals for 3 h after administration of radiocalcium. Plasma disappearance of intravenously administered ^{45}Ca was measured 2–4 d earlier when the goats were assumed to have a comparable efficiency of Ca absorption.

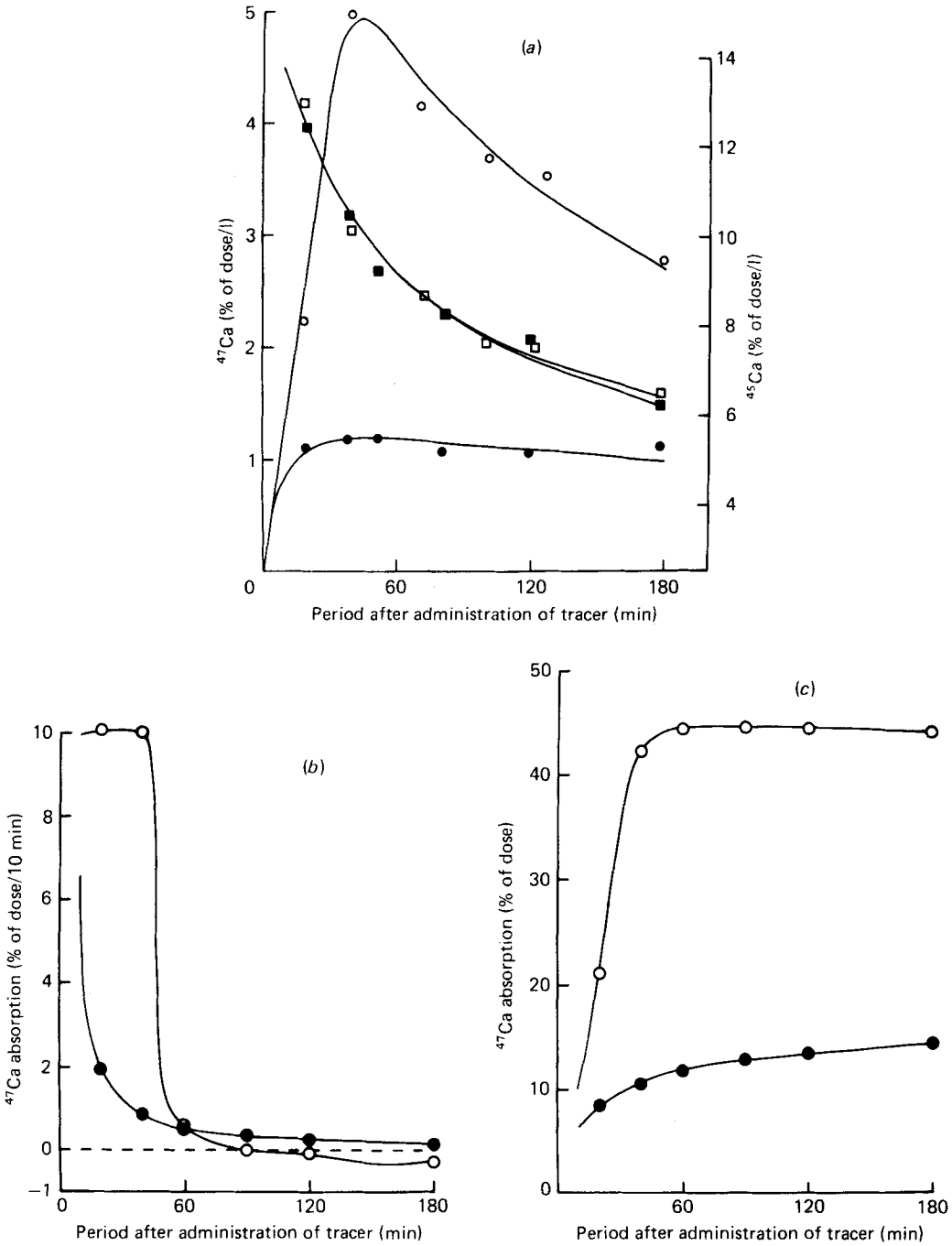

Fig. 1. (*a*) Plasma radioactivity expressed as a percentage of the administered dose/l, (*b*) absorption (% of dose/10 min) and (*c*) cumulative absorption (% of dose) of orally administered ^{47}Ca with time in a non-lactating goat before (●, ■) and 2 d after (○, □) an oral dose of 5 μg 1,25-dihydroxycholecalciferol. (●, ○) ^{47}Ca administered orally, (■, □) ^{45}Ca given intravenously. The rates of absorption given in (*b*) and (*c*) were calculated for 10 min intervals from 0 to 180 min after tracer administration. For clarity, only values corresponding to the times of blood sampling are plotted.

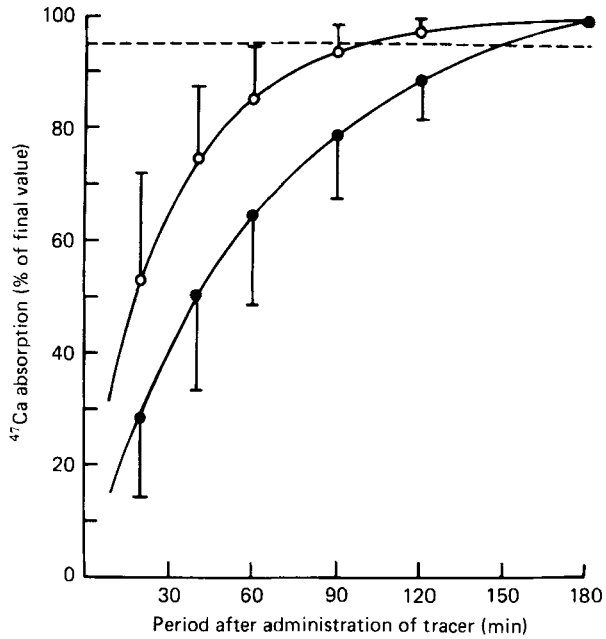

Fig. 2. Absorption of radiocalcium with time from 0 to 180 min after tracer administration, expressed as a percentage of the absorption after 180 min. Values given were obtained for 10 min intervals after deconvolution analyses of initial observations of plasma radioactivity. Only points corresponding to the times of blood sampling are plotted. (○) Values from twenty-two experiments with five lactating goats, (●) values from twenty-four experiments in six non-lactating goats. Points are mean values; standard deviations are represented by vertical bars. (---) Represents 95% of the final value.

Radiocalcium absorption from the two sources was compared in four goats with unstimulated Ca absorption and in three goats with stimulated Ca absorption following an oral dose of 25 μg $1,25(OH)_2D_3$.

Applications of the technique

Series 4. Rapid changes in radiocalcium absorption. The ability of the radiocalcium absorption test to respond to rapidly changing efficiencies of Ca absorption was tested 2 d after administration of oral doses of either 5 μg $1,25(OH)_2D_3$ in 10 ml ethanol or 5 g of dried leaves of the South American calcinogenic plant *Solanum malacoxylon*. Intakes of Ca were not controlled.

Series 5. Changes in radiocalcium absorption with changes in dietary Ca intake in lactating goats. Five goats in the second half of lactation were used for the experiment, which included three periods of 4 weeks duration. The basal diet supplied 1·70 g Ca/d (Table 1) and the intake of Ca was regulated independently by supplementation with calcium acetate through abomasal infusion or drinking-water. Thus daily intakes of Ca were 3·6–3·9, 6·4–6·6 and 11·4–12·2 g in periods 1, 2 and 3 respectively. Radiocalcium absorption was measured at the end of each period, starting 4 h after the ending of the Ca infusion. Because of repeated technical problems with the infusion, only three goats completed period 1 and four goats completed period 3.

RESULTS

Plasma radiocalcium concentrations and the time-course of tracer absorption. Typical examples of concentrations of radiocalcium in plasma are given in Fig. 1 (*a*). Usually, 10–14% of the dose/l plasma was recovered 20 min after intravenous tracer injection. The curves

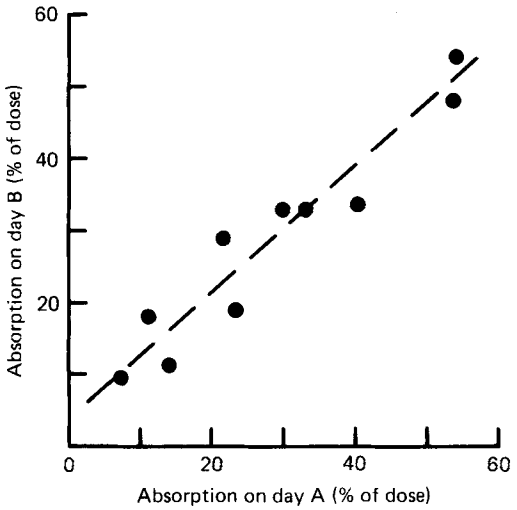

Fig. 3. Reproducibility of repeated measurements of radiocalcium absorption (% of dose) when carried out twice (days A and B) with 2 or 2 d intervals in eight goats. Non-lactating goats had low rates of absorption while higher rates were obtained in pregnant or lactating goats (r 0·94, $P < 0·01$).

of radiocalcium disappearance were not markedly influenced by changes in absorption efficiency as illustrated by Fig. 1(*a*). The ^{47}Ca-radioactivity could always be detected in plasma 20 min after abomasal injection of $^{47}CaCl_2$. A peak in ^{47}Ca concentration was usually reached between 40 and 90 min after tracer administration, followed by a decline which with time became increasingly parallel to the intravenous disappearance curve. Values of about 1% of the injected dose/l plasma were measured when the final radiocalcium absorption was 5–15%, as compared with 4–5% of the dose/l plasma within the higher range of absorption efficiencies (40–60%). An example of calculated curves for radiocalcium absorption and cumulative absorption is given in Fig. 1(*b*, *c*). A rapid initial rate of tracer absorption (10–40 min) was followed by a more or less gradual return to zero absorption as final values for cumulative absorption were approached.

The shapes of the curves for absorption *v.* time after tracer administration were examined in a set of twenty-two determinations in six non-lactating goats. Absorption values at 180 min were taken as final values and the preceding values are expressed relative to the final values in Fig. 2. A rapid increase was observed within 20–40 min, followed by a more gradual increase to the maximal values. Half-maximal absorption was on average seen after 18 min in the lactating goats and after about 40 min in the non-lactating goats, and 95% of the maximal values were reached after 100 and 135 min respectively.

Verification of the technique

Series. 1. Reproducibility of radiocalcium absorption. Repeated measurements at 2 d intervals in different goats gave good agreement irrespective of the absolute rate of absorption (r 0·94, Fig. 3). The value of the regression coefficient (0·88) was not significantly different from 1·0 ($P > 0·20$, t test).

Series 2. Effects of varying amounts of Ca in the intestines during measurements of Ca absorption. Results from determinations of radiocalcium absorption conducted during abomasal calcium acetate infusions were compared with results obtained when only Ca from the basal diet was present in the intestines. Differences in radiocalcium absorption were not detected when measurements were carried out in goats with unstimulated absorption

Table 2. *Effect of intestinal load of calcium on measured radiocalcium absorption (percentage of the dose absorbed in 240 min)*

(Mean values (ranges given in parentheses) for cumulative absorptions obtained in four goats with unstimulated absorption of Ca and three goats given 15 μg 1,25-dihydroxycholecalciferol (5 μg/d for 3 d) to stimulate Ca absorption)

	Intestinal load of Ca (g/d)	
Mode of absorption	0·65	4·65
Unstimulated	18·3 (9·2–26·6)	17·8 (14·4–21·1)
Stimulated	69·2 (65·7–71·8)	60·0 (58·9–60·6)

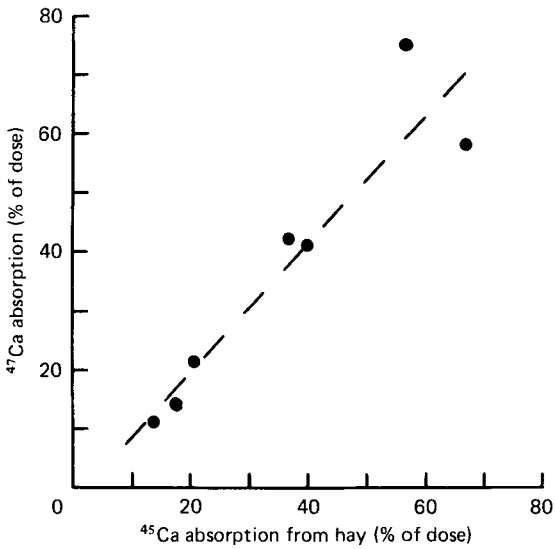

Fig. 4. Relationship between radiocalcium absorption (% of dose) from ^{45}Ca in hay (x) and $^{47}CaCl_2$ (y) in four goats. Three experiments were carried out with unstimulated absorption (10–20%) and four experiments after stimulation of Ca absorption with 1,25-dihydroxycholecalciferol (absorption of 40–70% of the tracer dose). Both tracers were injected simultaneously into the abomasum. For details of cultivation and preparation of grass containing ^{45}Ca, see p. 147. ($y = 0{\cdot}02 + 1{\cdot}09\ x$, r 0·93, $P < 0{\cdot}01$.)

(Table 2). Radiocalcium absorption was, however, reduced from an average of 69·2 to 60·0% during the abomasal infusion in three goats with stimulated intestinal Ca transport (Table 2). Reductions were seen in each of the three goats tested (6·9, 9·7 and 11·2% respectively) but the small number of animals precluded tests of statistical significance.

Series 3. Absorption of radiocalcium from $^{47}CaCl_2$ and from ^{45}Ca-labelled hay. The rates of absorption of the two Ca-isotopes paralleled each other throughout the period of observation. Good agreement between the cumulative absorptions of the individual isotopes was seen irrespective of the radiocalcium source tested (Fig. 4, r 0·93, $P < 0{\cdot}001$). The relationship was consistent throughout the range of activities of absorption studied. The regression coefficient (1·09, Fig. 4) was not significantly different from 1·0 ($P > 0{\cdot}10$, t test).

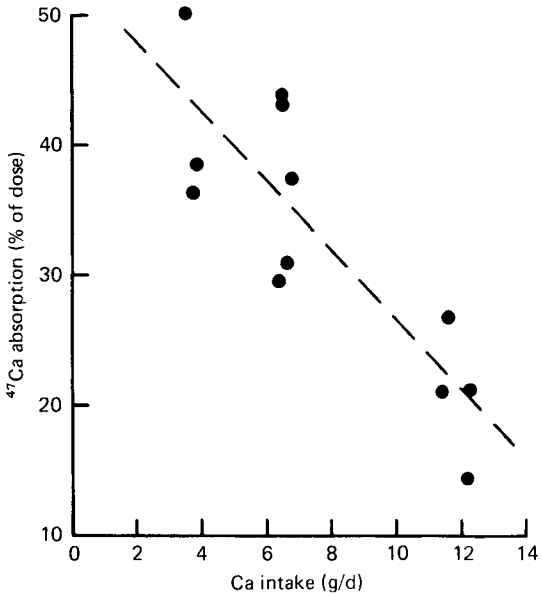

Fig. 5. Relationship between radiocalcium absorption (% of dose; y) and calcium intake (g/d; x) in lactating goats given approximately 4, 7 and 12 g Ca/d. Measurements were carried out after 4 weeks of adaptation to each level of Ca intake. ($y = 53{\cdot}5 - 2{\cdot}7\,x$, r 0·84, $P < 0{\cdot}01$).

Application of the technique

Series 4. Changes in radiocalcium absorption after administration of 1,25$(OH)_2D_3$ and dried leaves of Solanum malacoxylon. Without changes in Ca intake, cumulative absorption of radiocalcium increased from 27 to 54% 2 d after feeding *Solanum malacoxylon* leaves. Similarly, radiocalcium absorption was increased from 15 to 44% 2 d after administration of 5 μg of 1,25$(OH)_2D_3$ (Fig. 1(*a*, *b*, *c*).

Series 5. Changes in radiocalcium absorption with changes in Ca intake. A progressive decline in the cumulative absorption of radiocalcium was observed when the intake of Ca in the diet was increased from about 3·6 to 12 g/d (Fig. 5, r −0·84, $P < 0{\cdot}01$). The goats produced 0·5–0·8 l milk, equivalent to a daily loss of 0·6–1·0 g Ca. Endogenous losses of Ca were not measured. By using a value of 10–15 mg Ca/kg body-weight obtained in sheep (Braithwaite, 1975), total losses of about 1·5 g/d could, however, be expected. In the present study, values for the absorption of Ca on intakes of 4·0, 6·5 and 12·0 g/d were, according to the regression line, 1·7, 2·4 and 2·5 g/d respectively.

DISCUSSION

The most commonly used method for the estimation of Ca absorption in intact ruminants has hitherto been a combination of balance measurements and radiocalcium kinetics (Visek *et al.* 1952, 1953). This procedure, combined with more sophisticated multi-compartmental modelling, has yielded estimates of the various pathways of Ca metabolism both in cows (Mayer *et al.* 1967; Ramberg *et al.* 1970, 1975) and sheep (Braithwaite *et al.* 1969; Braithwaite, 1975, 1978). The method requires a steady-state with regard to Ca fluxes in the animal throughout a period of observation which usually lasts for 7–10 d in ruminants. In contrast, the double-isotope technique,utilizing simultaneous intravenous and abomasal administration of ^{45}Ca and ^{47}Ca, provided values for the calculation of intestinal Ca absorption in goats from observations of plasma radioactivity for 3–4 h following tracer

administration. The short time required for completion of the observations gave an opportunity for repeated measurements after 1–2 d. Tests of the reproducibility of the procedure (series 1) gave a highly significant correlation between values observed on two consecutive measurements covering the whole range of cumulative absorptions from 10 to 55% of the doses of tracer given. The double-isotope technique therefore seems well suited for studies where rapid changes in Ca absorption are likely to take place. This would especially apply to changes in Ca absorption around parturition in dairy ruminants, to studies of the time-course of adaptation after changes in Ca intake and in studies of the actions of metabolites of cholecalciferol with effects on Ca absorption. In the present study the effects of $1,25(OH)_2D_3$ and leaves of *Solanum malacoxylon*, both potent stimulators of Ca absorption in other species (Omdahl *et al.* 1971; Norman *et al.* 1971; Corradino & Wasserman, 1974; Fox & Care, 1979), were tested within 1–2 d after administration (series 4). Radiocalcium absorption was greatly enhanced after oral treatment with both substances. More detailed studies of the effects of several 1α-hydroxylated metabolites of cholecalciferol on radiocalcium absorption in the goat are given in a separate paper (Hove, 1984).

In pigs given sedatives to allow oral dosing of tracer, Harmeyer *et al.* (1976) observed a lag period of about 2 h before the concentration of radiocalcium increased in plasma. In the present study, peak plasma radioactivity was usually observed 40–100 min after tracer injection, with the shortest time interval between dose administration and peak in goats with an activated absorption of Ca. This is consistent with peak radioactivity values 1–2 h after administration in man (Avioli *et al.* 1965; Marshall, 1976). The present experiments were carried out about 1 h after morning feeding. The rapid emptying of the stomach at this time of day and the fact that the tracer was given in the pyloric part of the abomasum can probably explain the rapid absorption which was found in the first two or three 10 min periods after tracer administration. A further indication of an effect of the rate of abomasal emptying on the absorption of tracer is given in Fig. 2. The lactating goats, fed at about twice the maintenance level, reached 50% of their maximal cumulative absorption in about half the time required by the non-lactating goats.

The double-isotope technique measured only intestinal absorption of radiocalcium. The importance of the ruminant forestomachs in Ca absorption has been subject to considerable controversy. Most direct studies have concluded that there is a net secretion and only negligible absorption of Ca in the stomachs (Phillipson & Storry, 1965; Kemp *et al.* 1973; Van't Klooster, 1976). Results indicating absorption of Ca from the stomachs have also appeared (Grace *et al.* 1974). Ramberg (1972), estimated Ca absorption in a mature cow by deconvolution analysis of results obtained by simultaneous rumen and intravenous administration of the two Ca tracers. Due to a large delay in the rumen, absorption of oral tracer took place over a period of 24–48 h. A similar delay was also seen in goats (Visek *et al.* 1952) and in sheep (Jones & Luthman, 1978). This protracted period of absorption is consistent with a delayed transport of radiocalcium to the absorptive sites. Thus the bulk of evidence indicates that forestomach absorption of Ca is without quantitative importance when compared with the intestinal absorption of Ca. The abomasal injection of radiocalcium consequently seems justified when the Ca-absorbing capacity of the whole digestive tract is to be estimated. Abomasal tracer administration is advantageous both with regard to the time required for completion of measurements and in minimizing the amount of tracer required.

Transit of digesta from the pylorus to the terminal ileum takes 2–3 h in ruminants. The very rapid increase in cumulative absorption of tracer (Fig. 1(*c*)) may allow the conclusion that the duodenum and the early parts of the jejunum are the most important parts of the intestine for Ca absorption in the goat.

Intestinal absorption of Ca seems to be brought about by two independent processes in

studied avian and mammalian species. The active component is saturable and in rat, dog, man and pig maximal transport occurs at an intestinal calcium ion concentration of 2–5 mmol/l (Wasserman & Taylor, 1976; Fox *et al.* 1978). Starting at a lumen Ca concentration of about 4–7 mmol/l, Ca is absorbed also by a passive, non-saturable diffusion process. Unfortunately, direct experimental evidence supporting this two-component model for Ca absorption seems to be lacking in ruminants (Wasserman & Taylor 1976; Abdel-Hafeez *et al.* 1982). Since saturation of the active transport system occurs at about 2–5 mmol/l in studied species, it seems likely that a similar maximum could be present in the ruminant. In the present study total Ca concentrations in the abomasal supernatant fraction were 4–5·5 mmol/l when the goats were given the basal diet, and 10–25 (average 18) mmol/l when calcium acetate (4 g Ca/d) was given with the drinking-water. In sheep, approximately 50–70% of the water leaves the small intestine between the pylorus and the terminal ileum (Grovum & Williams, 1973). With the slow rate of absorption (10–20% of the dose) observed in the unstimulated goats of the present study, most of the Ca would stay in the gut and the total Ca concentration would increase considerably towards the ileum. Absorption by diffusion is, however, governed by the free Ca concentration. The plant material which is always present in gut contents of ruminants binds Ca^{2+} efficiently (Van't Klooster, 1967; Branch *et al.* 1975). Storry (1961*a*, *b*) showed that this binding of Ca was strongly dependent on pH. About 90% of the total Ca was ultrafiltrable in abomasal contents, while about 50% was ultrafiltrable in the first quarter of the small intestine (pH 5·5) and from 10 to 5% in the more distal parts (pH 6·5 and above). Most of the radiocalcium absorption in the present study would probably occur by active absorption, since the total Ca concentration of the abomasal contents was at or below the concentration where net absorption by passive diffusion becomes noticeable. When Ca was supplemented, sufficiently high Ca concentrations were reached to allow Ca absorption by diffusion in the proximal parts of the intestine. Information on the extent to which this occurred must await further characterization of the mechanisms of Ca absorption in ruminants.

Measurements of radiocalcium absorption in monogastric species are carried out in the fasting state and with a small load of carrier (20 mg Ca). High amounts of carrier Ca given with the oral tracer reduced and delayed the absorption of radiocalcium in man (Marshall, 1976). The absorptive sites in the intestine of ruminants are continuously receiving digesta, and the content of Ca in the diet is a major determinant of the load of Ca in which the radiocalcium is diluted during absorption. Ca concentrations of abomasal and probably also of proximal intestinal contents were low in the present study. This was true also when the goats were given high-Ca diets before the tests, since Ca infusions were discontinued 3–4 h before tracer administration.

Since lumen Ca concentrations would be close to those required for saturation and the unidirectional flux of tracer from lumen to blood would be low, it seems probable that the methods used in the present study yielded estimates of the maximal capacity of the active intestinal Ca transport.

The adaptation in Ca absorption measured in lactating goats in series 5, when Ca intakes were varied from approximately 4 to 12 g/d, showed that the double-isotope method was sensitive to changes in Ca absorption as regulated by the animal itself. The roughage content of the diet in series 5 differed somewhat from that used in the non-lactating goats (Table 1) and this may have influenced the Ca availability. However, calculations of total Ca absorption based on the cumulative absorption of radiocalcium yielded reasonable results: 1·7 g Ca was, on average, absorbed at a Ca intake of 4 g/d and 2·4 g at a Ca intake of 12 g/d. This would match endogenous losses and milk secretion and would, at the highest Ca intake, allow for a reasonable rate of net bone accretion.

Certain assumptions must be made in order to convert results from measurements of

radiocalcium absorption to rates of absorption of Ca from ordinary diets. First, oral Ca tracer is given as the chloride, while dietary Ca is largely of plant origin. A very high correlation (r 0·93) was obtained in the simultaneous tests of radiocalcium absorption from $^{47}CaCl_2$ and ^{45}Ca in ryegrass (series 3). This result strongly supports the validity of using inorganic radiocalcium as a tracer for the Ca in regular ruminant diets.

A further problem arises from the influence of the load of dietary Ca in the intestine on the fate of the oral tracer as demonstrated in man by Marshall (1976). The results obtained in series 2 indicated, however, that this problem would be of minor importance in ruminants given natural diets or diets enriched with Ca to the level used in the present study (about 9 g Ca/kg DM). A reduced rate of radiocalcium absorption can probably be expected when substantially higher concentrations of Ca are present. Although the assumptions for conversion of radiocalcium absorption measurements stated previously seem to be met on ordinary ruminant diets, it should again be emphasized that a more precise interpretation of results obtained from the double-isotope technique in a nutritional context should await a better understanding of the mechanisms involved in Ca absorption in the ruminant. The results obtained with the double-isotope technique in the present study and by Hove (1984) demonstrate, however, that the technique is a promising alternative to measurements of Ca absorption by traditional Ca-kinetic methods in ruminant species.

The work was financed by a grant from the Norwegian Agricultural Research Council.

REFERENCES

Abdel-Hafeez, H. M., Manas-Almendros, M., Ross, R., Care, A. D. & Marshall, D. H. (1982). *British Journal of Nutrition* **47**, 69–77.
Avioli, L. V., McDonald, J. E., Singer, R. A. & Henneman, P. M. (1965). *Journal of Clinical Investigation* **44**, 128–139.
Braithwaite, G. D. (1975). *British Journal of Nutrition* **34**, 311–324.
Braithwaite, G. D. (1978). *British Journal of Nutrition* **39**, 213–218.
Braithwaite, G. D., Glascock, R. F. & Raizuddin, Sh. (1969). *British Journal of Nutrition* **23**, 827–834.
Branch, W. J., Southgate, D. A. T. & James, W. P. T. (1975). *Proceedings of the Nutrition Society* **34**, 120A.
Corradino, R. A. & Wasserman, R. H. (1974). *Nature* **252**, 716–718.
Fox, J. & Care, A. D. (1979). *Journal of Endocrinology* **82**, 417–424.
Fox, J., Care, A. D. & Swaminathan, R. (1978). *British Journal of Nutrition* **39**, 431–439.
Grace, N. D., Ulyatt, M. J. & MacRae, I. C. (1974). *Journal of Agricultural Science* **82**, 321–330.
Grovum, W. L. & Williams, V. J. (1973). *British Journal of Nutrition* **29**, 13–21.
Harmeyer, J., Vogelsang, G. & von Grabe, C. (1976). In *Nuclear Techniques in Animal Production and Health*, pp. 243–252 [C. von Grabe, editor]. Vienna: International Atomic Energy Commission.
Hart, H. & Spencer, H. (1967). *Proceedings of the Society for Experimental Biology* **126**, 365–371.
Hove, K. (1984). *British Journal of Nutrition* **51**, 157–164.
Jones, B. & Luthman, J. (1978). *Acta Veterinaria Scandinavica* **19**, 204–214.
Kemp, A., Van't Klooster, A. T., Rogers, P. A. M. & Geurink, J. H. (1973). *Netherlands Journal of Agricultural Science* **21**, 44–55.
Marshall, D. H. (1976). In *Calcium, Phosphate and Magnesium Metabolism*, pp. 257–297 [B. E. C. Nordin, editor]. London: Churchill Livingstone.
Mayer, G. P., Ramberg, C. F. Jr. & Kronfeld, D. S. (1967). *Journal of Nutrition* **92**, 253–260.
Norman, A. W., Myrtic, J. F., Midgett, R. J., Nowickz, H. G., Williams, V. & Popjak, G. (1971). *Science* **173**, 51–54.
Omdahl, J. L., Holick, M., Suda, T., Tanaka, Y. & DeLuca, H. F. (1971). *Biochemistry* **10**, 2935–2940.
Phillipson, A. T. & Storry, J. E. (1965). *Journal of Physiology* **181**, 130–150.
Ramberg, C. F. Jr. (1972). In *Production Diseases in Farm Animals*, pp. 195–200 [J. M. Payne, K. G. Hibbit and B. F. Sansom, editors]. London: Ballière Tindall.
Ramberg, C. F. Jr., Kronfeld, D.S. & Wilson, G. D. A. (1975). In *Digestion and Metabolism in the Ruminant*, pp. 231–242. [I. W. McDonald and A. C. I. Warner, editors]. Armidale, Australia: University of New England Publishing Unit.
Ramberg, C. F. Jr., Mayer, G. P., Kronfeld, D. S., Phang, I. M. & Berman, M. (1970). *American Journal of Physiology* **219**, 1166–1176.
Storry, J. E. (1961*a*). *Journal of Agricultural Science* **57**, 97–102.
Storry, J. E. (1961*b*). *Journal of Agricultural Science* **57**, 103–109.

Van't Klooster, A. Th. (1967). *Mededelingen Landbouwhogeschool Wageningen*, 65–67.
Van't Klooster, A. Th. (1976). *Zeitschrift für Tierphysiologie, Tierernährung und Futtermittelkunde* **37**, 169–182.
Visek, W. J., Barnes, L. L. & Loosli, J. K. (1952). *Journal of Dairy Science* **35**, 783–792.
Visek, W. J., Monroe, R. A., Swanson, E. W. & Comar, C. L. (1953). *Journal of Dairy Science* **36**, 373–384.
Wasserman, R. H. & Taylor, A. N. (1976). In *Handbook of Physiology*, section 7, vol. VII, pp. 137–155 [R. O. Greep and E. B. Astwood, editors]. Washington, D.C.: American Physiological Society.

Printed in Great Britain