

Kinetics of [^{103}Ru]phenanthroline and dysprosium particulate markers in the rumen of steers

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1. Two rumen cannulated steers consuming 5.5 kg air-dry lucerne (*Medicago sativa*) hay/d given at two-hourly intervals were used to study the kinetics in the rumen of the two particulate markers, ^{103}Ru -labelled Tris-(1,10-phenanthroline) ruthenium II chloride (^{103}Ru]P) and dysprosium (Dy). Provision of markers was achieved by allowing the steers to eat separated stems of the hay on to which had been sprayed solutions of the markers.
2. The intake of large-particle (retained by a 3.2 mm mesh screen) dry matter (DM) in boluses and the rumen large-particle pool size measured by emptying the rumen were used to calculate the turnover rate-constant of this pool (1.02 and 1.19/d for steers A and B respectively).
3. The decline with time of both ^{103}Ru]P and Dy associated with large-particle DM in raft digesta was best described by two-compartmental kinetics. The first compartment apparently reflected a combination of the processes of mixing of labelled particles throughout the rumen contents, physical migration of marker from the labelled hay, and physical breakdown of large particles to small particles.
4. The disappearance with time of ^{103}Ru]P and Dy associated with small particles from the raft, ventral digesta and faeces from 3 to 8 d was well described by a single kinetic compartment with a rate constant similar to that of the second compartment of the large particles from the raft.
5. ^{103}Ru]P was rapidly distributed through both the raft and ventral digesta of the rumen. This observation, taken together with measurements of migration of ^{103}Ru]P, suggested that on entering the rumen much of the ^{103}Ru]P did not remain associated with the original feed material.
6. The majority (63–64%) of Dy entered the raft digesta and mixed only slowly through the rumen contents. Some Dy (18–27%) apparently migrated rapidly from large particles and to small particles immediately after ingestion and there was also evidence for some slow migration from small to large particles.
7. When used under the conditions described for this experiment neither ^{103}Ru]P nor Dy was satisfactory as a marker to trace the passage through the rumen of a particular meal.

The rate of removal of dietary residues from the rumen determines the time during which dietary components are subjected to fermentation (Sutherland, 1976; Mertens, 1977; Bull *et al.* 1979), rumen fill and hence intake of low digestibility forages (Balch & Campling, 1962; Ellis, 1978), and efficiency of microbial synthesis (Harrison & McAllan, 1980). Markers such as the rare-earth cations and ^{103}Ru -labelled Tris-(1,10-phenanthroline) ruthenium II chloride (^{103}Ru]P), which adsorb to particulate material, may offer a simple and direct method of measuring the time for which particles are retained in the rumen (Ellis *et al.* 1979). In this regard Faichney & Griffiths (1978), Ellis (1978) and Ellis *et al.* (1979) have developed models to provide the best fit to data of the time course of marker concentrations in rumen digesta and faeces, and from these models derived rate-constants for various kinetic pools in the rumen.

The present experiment was intended to gain information on the behaviour and movement of the particulate markers ^{103}Ru]P and a rare earth, dysprosium (Dy), in the rumen of steers. The study was intended to determine whether the markers remained associated with the particulate material of a specific, labelled meal of hay, whether decline of the marker followed first-order kinetics in both large- and small-particle pools and if so whether the rate-constants of breakdown of large particles to small particles and passage of small particles from the rumen could be calculated from the decline of marker in each pool.

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MATERIALS AND METHODS

Expt 1

Two steers (333 and 352 kg body-weight, 15 months of age, predominantly Charolais) were each prepared with a 100 mm diameter cannula in the rumen (Hecker, 1969). A period of 21 d was allowed for adaptation to the diet of 5.5 kg air-dry lucerne (*Medicago sativa*) hay/d which contained 935 g dry matter (DM)/kg air-dry material, and 922 g organic matter, 19.2 g nitrogen, 597 g neutral-detergent fibre and 489 g acid-detergent fibre/kg DM. In vivo DM digestibility was 0.70. Commencing 10 d before the experiment, the steers were housed in metabolism crates under conditions of continuous lighting at room temperature and fed at two-hourly intervals. Cobalt-mineralized salt blocks (Canadian Salt Ltd., Montreal) and water were available at all times.

Predominantly stem material was separated from lucerne hay by vigorous manual shaking. [^{103}Ru]P (0.2 mCi in 30 ml of 0.005 M-RuP) prepared by the method of Tan *et al.* (1971) and $\text{DyCl}_3 \cdot 6\text{H}_2\text{O}$ (300 mg dissolved in the acid [^{103}Ru]P solution) was sprayed over 300 g of this stem material using a syringe and a 24 gauge needle, and the preparation was dried at 50° for 16 h.

On day 1 of the experiment, 120 g labelled stem was given to each steer at the time of a normal two-hourly meal. Unlabelled hay was then given to make up the normal meal.

Samples (80–100 g) were obtained from the rumen each 2 h for the first 24 h, each 4 h for the next 12 h, each 8 h until day 6, and then each 12 h up to 7.3 d so that thirty-two samples were obtained. Samples of raft digesta were obtained by opening the rumen cannula and bulking several small samples of digesta obtained from the vicinity of the cannula. Samples of ventral digesta were obtained using a 600 mm long sampling probe made from rigid plastic tubing (35 mm diameter) which could be occluded at the lower end by a rubber cone held in place by a metal rod passing through the length of tubing. The probe was introduced into the ventral region of the ventral sac of the rumen with the lower end of the tube occluded by the rubber cone, the rubber cone was opened briefly and then closed to trap digesta in the tube. Digesta (300–500 ml) obtained by this procedure were subsampled (100 ml) and the remainder was returned to the rumen. Voided faeces were subsampled at the same time that the rumen was sampled. Samples were stored at 5° pending analyses.

At 12 d after administration of the previously-described tracers, the rumen of each steer was emptied, this procedure commencing 1 h after the previous feed. The raft and ventral digesta were collected separately, the former being defined as that material which could be easily raked together in the rumen with the fingers and which was obviously of drier rather than fluid consistency. The division of raft and ventral material tended to be gradational, and this separation involved some subjective assessment.

Expt 2

Labelled hay stem (20 g) used in Expt 1 was chopped into 10–20 mm lengths with scissors. Digesta (5 kg) from the rumen of a steer fed on lucerne hay at intervals of 2 h were placed in a pail. Mercuric chloride (0.5 g in 50 ml water) was thoroughly mixed with the digesta before the labelled hay was added and also mixed thoroughly. Samples of digesta were obtained from the pail at 0, 1, 2, 4, 6, 9, 12, 26 and 31 h and were sieved immediately to isolate the large particles (those retained by a 3.2 mm mesh screen).

Expt 3

The rumen of each of three steers (one from Expt 1 and two similar steers) was emptied, and each steer was given approximately 1 kg lucerne hay or separated stem material or

separated leaf material prepared from lucerne hay as described previously. Quantitative collection of boluses of ingested feed material was made at the cardia.

Laboratory analyses

The DM content of rumen digesta was determined by drying 200–300 g subsamples at 70° for 3 d.

The large-particle fraction of digesta was isolated by a wet-sieving procedure. Samples of wet raft digesta (15 g) were sieved through a 45 mm diameter screen (3.2 mm mesh size) fixed in the bottom of a Perspex tube 240 mm in height. Distilled water (600 ml) was added to an outside close-fitting container of the sieving apparatus and the sample was stirred in a rotary fashion in the inner tube as it was slowly lifted within the outer container. This lifting and stirring cycle was carried out five times and the large particles retained on the screen were transferred quantitatively into vials for gamma counting and DM determination. The small-particle DM in a sample was calculated as the difference between the DM added to the sieving apparatus and the DM retained by the screen and would also have included soluble DM.

[¹⁰³Ru]P concentration in digesta fractions and faeces was determined using a gamma spectrophotometer (Model 8000, Beckman Instruments, Fullerton, California) with correction for small variations in counting efficiency with the height of sample in the counting vials. The concentration of [¹⁰³Ru]P and Dy (Kennelly, Aherne *et al.* 1980; Kennelly, Apps *et al.* 1980) associated with small particles of raft digesta was calculated by difference from the concentrations in mixed raft digesta and in large particles isolated from the raft. A portion of the labelled hay stem used in Expts 1 and 2 was macerated with water and subsamples of the resultant slurry analysed to determine the dose of [¹⁰³Ru]P and Dy given to the steers.

Calculations and statistical methods

In Expt 1, the regressions of ln (marker concentration/g DM) *v.* time for rumen samples were tested for curvilinearity by testing the significance of the second-degree polynomial in multiple regression (Snedecor & Cochran, 1967). When significant curvilinearity occurred in both steers with both markers attached to the large-particle DM, two exponential compartments were calculated using curve-peeling techniques (Shiple & Clark, 1972). The second compartment (k_2) was first calculated from the samples obtained over days 3–8 and then the first compartment (k_1) from the samples obtained from time zero until day 3. A single exponential compartment was fitted to the results for rumen digesta and for faeces for days 3–8.

The slopes of the k_2 compartment of the marker in large-particle DM, small-particle DM, ventral digesta and in faeces for days 3 to 8 were compared using the methods of Steel & Torrie (1960).

The proportions of marker in rumen raft large particles, raft small particles and in ventral digesta were calculated from the fitted equations of marker concentration/g DM and the amount of DM recovered when the rumen was emptied. The recovery of marker in the rumen at zero time was calculated from the amount ingested and the amount predicted from the fitted equations to have been present in the rumen at zero time.

The turnover rate-constant of the large-particle pool in the rumen (k_{LP}) was calculated by the method of Poppi *et al.* (1981) as:

$$k_{LP} = \frac{\text{Flow of large-particle DM entering the rumen (g/d)}}{\text{Pool size of large-particle DM in the rumen (g)}}$$

The amount of large particles entering the rumen was calculated from the total DM intake

Table 1. *Expt 1. Weights (kg) of digesta present when the rumen was emptied and the turnover rate-constant of the large-particle pool*

	Steer A	Steer B
Raft		
Large particles	1.81	1.67
Small particles	1.62	1.33
Ventral		
Large particles	1.13	0.85
Small particles	2.11	1.49
Turnover rate-constant of large-particle pool/d	1.02	1.19

and the proportion of bolus DM consisting of large particles in Expt 3. Pool size of large-particle DM in the rumen was calculated from the weight of rumen contents obtained by emptying the rumen and the proportion of large-particle DM in the rumen contents.

RESULTS

Expt 1. The weights of digesta present as large- and small-particle DM in the raft and as ventral digesta for each steer are given in Table 1. The DM content of raft digesta was 144 g/kg and that of ventral digesta was 67 g/kg. The raft comprised 51 and 56% of the total DM in the rumen, while large-particle DM comprised 53 and 56% of the raft and 44 and 47% of the total rumen digesta, in steers A and B respectively. The turnover rate-constants of the large-particle pool (k_{LP}) were 1.02 and 1.19/d for steers A and B respectively when it was assumed that 60.1% (from Expt 3) of the hay entered the large-particle pool.

The change with time of \ln (^{103}Ru]P concentration/g DM) in large and in small particles from the raft and in mixed ventral digesta for steer A are shown in Fig. 1. The decline for large-particle ^{103}Ru]P in the raft was curvilinear ($P < 0.05$ for steer A, $P < 0.10$ for steer B). Decline of ^{103}Ru]P associated with the small particles of rumen raft digesta and mixed ventral digesta was significantly curvilinear in only one case (steer A, raft small particles, $P < 0.01$). A single exponential compartment was fitted to all of the results since this appeared to provide the most consistent description. Following first appearance in faeces at 11–13 h, \ln (^{103}Ru]P concentration) increased to a maximum at 1.6–1.8 d. However, the decline in \ln (^{103}Ru]P faecal concentration) between 3 and 8 d was linear.

Table 2 gives the rate-constants of each of the ^{103}Ru]P kinetic pools in the rumen. The rate-constants of the second compartment of large-particle DM in the raft (k_2), of small-particle DM in the raft (k_s) and of the mixed ventral digesta (k_v) were similar within each steer (means 0.56 and 0.69/d for steers A and B respectively). The rate-constant derived from faecal samples (k_F) between day 3 and day 8 was not different ($P > 0.05$) from these values. The rate-constant of the first compartment of large-particle DM in the raft (k_1 ; 1.82 and 1.69/d for steers A and B respectively) was greater than that of the other sampled sites in the rumen described previously.

The concentration of ^{103}Ru]P/g DM associated with large-particle DM in the raft was much lower than for small-particle DM in the raft throughout the 8-d sampling period (Fig. 1a and b). There was no significant trend with time in the ratio, concentration of ^{103}Ru]P on large particles in the raft: concentration on small particles; on average it was 0.32 and 0.30 for steer A and B respectively. The recovery of ^{103}Ru]P (106 and 104% for steers A and

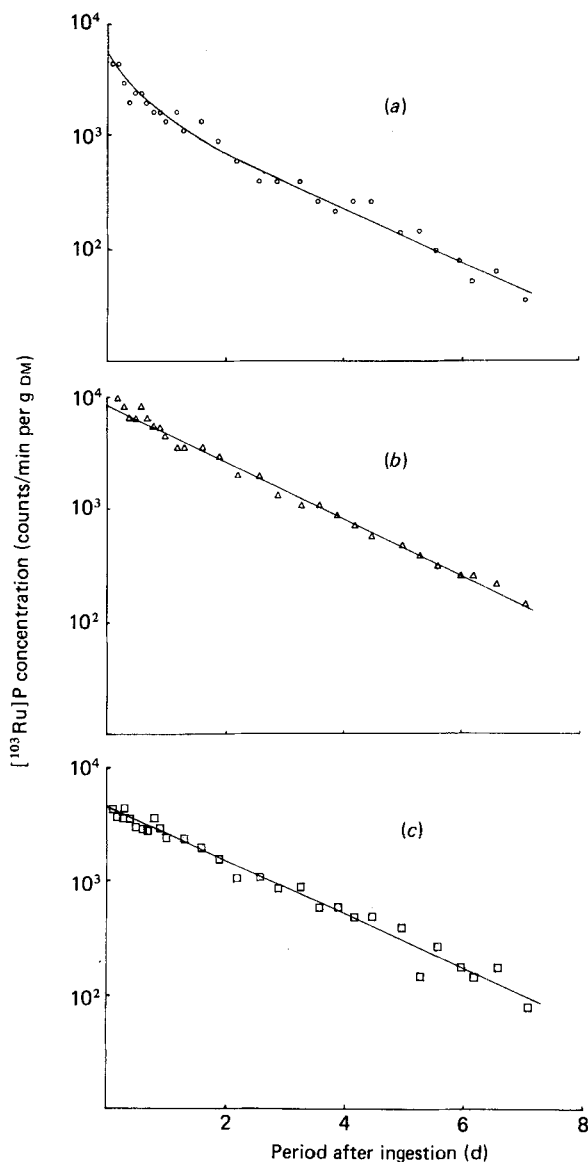


Fig. 1. Concentration (counts/min per g dry matter (DM)) of ^{103}Ru -labelled Tris-(1,10-phenanthroline) ruthenium II chloride (^{103}Ru]P) in (a) large particles of raft digesta, (b) small particles of raft digesta and (c) mixed digesta obtained from the ventral sac after ingestion by steer A of hay-stem labelled with ^{103}Ru]P.

B respectively) approached unity. The percentage of ^{103}Ru]P in the rumen that was present in the raft did not change significantly and was 40 and 60% for steers A and B respectively.

The change with time in Dy concentration/g DM in large- and in small-particle DM from the raft and in mixed ventral digesta for steer A are shown in Fig. 2. The decline of ln (large-particle Dy) in the raft with time was curvilinear ($P < 0.05$ for steer A, $P < 0.001$ for steer B), but was not curvilinear for Dy on the small-particle DM in the raft, in mixed ventral digesta and in faeces sampled from 3 to 8 d.

Table 2. The rate-constants ($/d$) of ^{103}Ru -labelled Tris-(1,10-phenanthroline) ruthenium II chloride ($[^{103}\text{Ru}]\text{P}$) and dysprosium (Dy) markers measured in digesta from the raft and the ventral sac

	Rate constant	Steer A	Steer B
$[^{103}\text{Ru}]\text{P}$ in:			
Raft large-particle DM	k_1	1.82	1.69
	k_2	0.592	0.687
Raft small-particle DM	k_s	0.570	0.697
Ventral digesta DM	k_v	0.525	0.682
Faeces	k_f	0.593	0.687
SE		0.027	0.034
Dy in:			
Raft large-particle DM	k_1	1.00	1.82
	k_2	0.505	0.491
Raft small-particle DM	k_s	0.503	0.627
Ventral small-particle DM	k_v	0.429	0.587
Faeces	k_f	0.530	0.647
SE		0.053	0.045

DM, dry matter.

k_1 and k_2 rate-constants of the first and second compartments of marker decline in the raft large-particle DM pool; k_s , k_v and k_f , rate-constants of single compartments of marker decline in raft small-particle DM.

SE, standard error of k_2 , k_s , k_v , k_f .

Table 2 gives the rate-constants of each of the Dy kinetic pools in the rumen. The rate-constants of the second compartment of the large-particle DM in the raft (k_2), the small-particle DM in the raft (k_s), and in mixed ventral digesta (k_v) were similar (means 0.48 and 0.57/d for steers A and B respectively). The rate-constants derived from faecal samples (k_f) were not significantly different from these values. The rate-constants of the first compartment of large-particle Dy in the raft (k_1 ; 1.00 and 1.82/d for steers A and B respectively) were greater than those of the other sampled sites in the rumen.

The concentration of Dy/g DM of large particles was 46 and 53% of that associated with small particles in the raft throughout the 8 d sampling period, for steers A and B respectively.

The apparent recovery of the Dy dosed into the rumen (124 and 165% for steers A and B respectively) was greater than the amount ingested. Consequently, the pool size of DM in the rumen calculated from the concentrations of marker at zero time and the dose consumed was greater than the DM measured by emptying the rumen.

There was significant change with time in the ratio, Dy present in the raft digesta:Dy present in the ventral digesta. The linear regression equations were:

Steer A: ratio = $1.72 - 0.133$ (time) (r 0.640, n 29, residual SD 0.356)

Steer B: ratio = $1.76 - 0.031$ (time) (r 0.383, n 30, residual SD 0.380)

indicating that on ingestion of the labelled hay, 63 and 64% of the Dy entered the raft digesta of steers A and B respectively, and that the Dy marker mixed slowly with the digesta in the ventral region of the rumen.

Expt 2. The change with time in concentration of $[^{103}\text{Ru}]\text{P}$ associated with the large-particle DM isolated from digesta in the pail is shown in Fig. 3. The concentration of the $[^{103}\text{Ru}]\text{P}$ decreased rapidly within 2 h and then reached a plateau so that $29 \pm 3\%$ (mean \pm SE) of the $[^{103}\text{Ru}]\text{P}$ was associated with the large-particle DM for the remainder of the sampling period. That 76% of the $[^{103}\text{Ru}]\text{P}$ was associated with large particles after 1 h indicated that

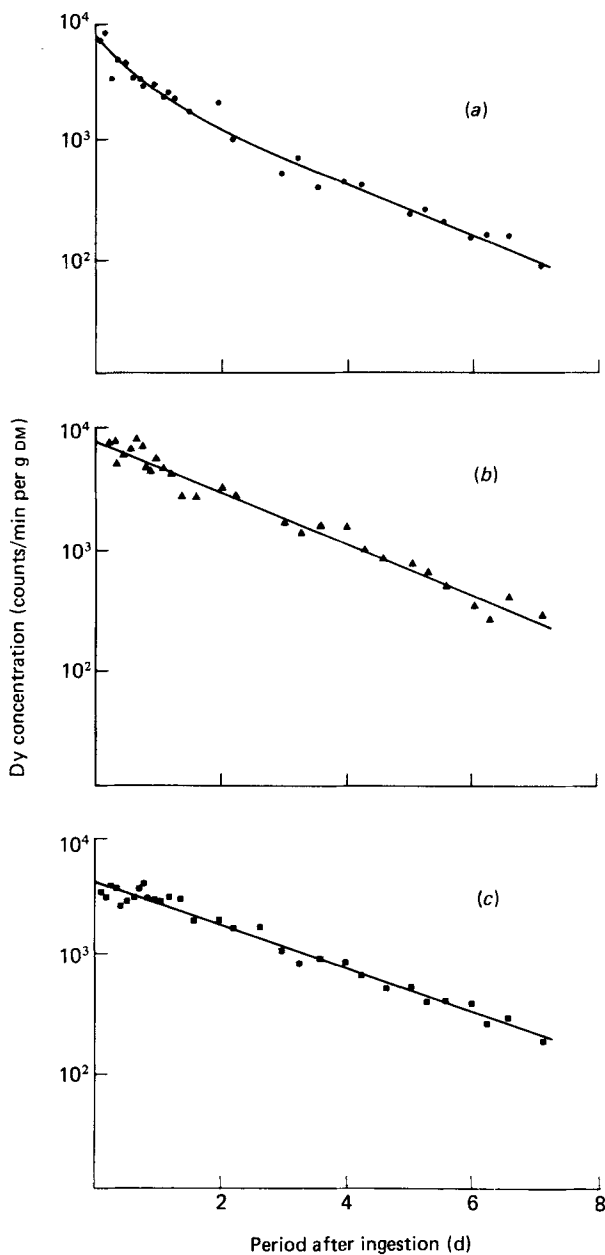


Fig. 2. Concentration (counts/min per g dry matter (DM)) of dysprosium (Dy) in (a) large particles of raft digesta, (b) small particles of raft digesta and (c) mixed digesta obtained from the ventral sac after ingestion by steer A of hay-stem labelled with Dy.

the transfer of $[^{103}\text{Ru}]P$ from the large-particle DM occurred in the digesta, and not during the sieving procedure.

Expt 3. When feed boluses were collected for sieving, 0.601 ± 0.033 hay, 0.689 ± 0.012 hay stem and 0.457 ± 0.054 hay leaf remained as large-particle DM following mastication during ingestion.

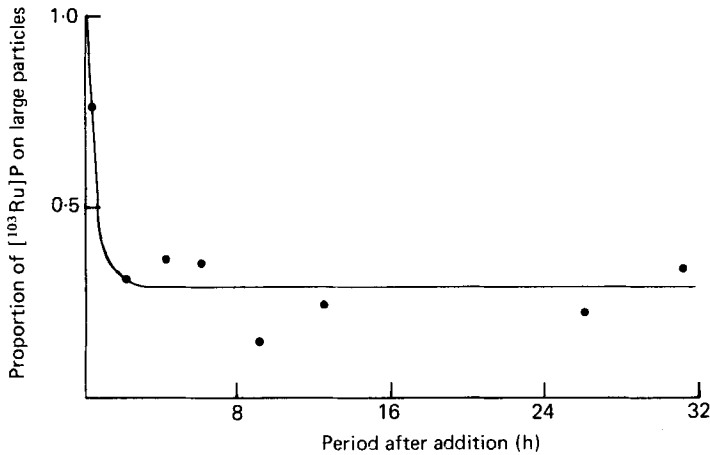


Fig. 3. Proportion of ^{103}Ru -labelled Tris-(1,10-phenanthroline)ruthenium II chloride ($[^{103}\text{Ru}]\text{P}$) associated with large particles in rumen digesta under in vitro conditions following addition of the $[^{103}\text{Ru}]\text{P}$ attached to large-particle hay stem.

DISCUSSION

The representation of rumen DM as consisting of pools of large particles ineligible to leave the rumen and small particles which may pass from the rumen is undoubtedly a simplistic approach (Poppi *et al.* 1980, 1981). Nevertheless, this model is likely to represent events in the rumen in more realistic terms than that achieved by considering the rumen DM as a single kinetic pool (Ellis, 1978; Faichney, 1980), while avoiding the complexities of considering a large number of rumen pools. The DM retained by a 3.2 mm mesh screen using the wet-sieving techniques described for this study was of a physical size too large to pass in significant quantity from the rumen of steers (R. M. Dixon & L. P. Milligan, unpublished observations). Consequently, in the present experiment the material retained by this and larger screens was considered to represent the large-particle pool.

Large-particle pool

The turnover rate-constant of the large-particle pool (k_{LP}) calculated from intake and rumen pool size of large particles represents the sum of the rate-constants for digestion of large particles to absorbable products and of physical breakdown of large particles to small particles. If the $[^{103}\text{Ru}]\text{P}$ and Dy markers remained attached to the large-particle DM and were only removed from the large-particle pool by physical breakdown or by digestion of the large particles, and if the large particles in the rumen behaved as a single kinetic pool, then the first compartment (k_1) of the two-compartmental decline of marker in the large-particle pool would measure the turnover rate-constant of the large-particle pool in the rumen. Hence the rate-constant k_1 should be equal to the rate-constant measured by emptying of the rumen (k_{LP}).

The finding that the k_1 of $[^{103}\text{Ru}]\text{P}$ in raft large-particle DM was 78 and 42% greater than k_{LP} for steers A and B respectively, suggested that the k_1 of $[^{103}\text{Ru}]\text{P}$ reflected mixing of $[^{103}\text{Ru}]\text{P}$ -labelled particles through the rumen and loss of $[^{103}\text{Ru}]\text{P}$ from the large-particle pool by physical migration as well as by loss of labelled large-particles from the pool. The observation in Expt 2 that 71% of the $[^{103}\text{Ru}]\text{P}$ readily migrated from the large-particle feed material under in vitro conditions agrees with other observations of migration of $[^{103}\text{Ru}]\text{P}$ from feed material (Faichney & Griffiths, 1978) and suggests that migration of $[^{103}\text{Ru}]\text{P}$ is likely to be the most important reason for the difference between k_1 and k_{LP} . Some error

was inherent in the calculation of k_{LP} , first because the proportion of large-particle DM in boluses was estimated subsequently and without steer B, and second because rumen digesta pool-size may have varied between the two-hourly meals. However, the proportion of large-particle DM in boluses was consistent for all three steers and error from the second source would introduce a directly proportional error in the estimation of k_{LP} . These errors are not likely to provide an explanation for the observed differences between k_1 and k_{LP} .

The results for Dy were less conclusive since, for steer A, k_1 was approximately equal to the k_{LP} and for steer B, k_1 was 53% greater than k_{LP} . The discrepancy with this marker may be primarily associated with slow mixing of the Dy-labelled large particles throughout the rumen contents, since the majority of the Dy dose entered the raft and appeared to be only slowly mixed through the ventral digesta of the rumen. That there was poor mixing of the ingested Dy-labelled hay throughout the rumen contents is also indicated by the observation that the predicted recovery of Dy in the rumen was much greater than the amount of Dy ingested, presumably because the labelled hay tended to be present at the sampling sites in the rumen. Given slow mixing of Dy-labelled large particles through the rumen, the k_1 of Dy on raft large particles would have reflected the process of mixing of Dy-labelled large particles throughout the rumen contents as well as the digestion and breakdown of large particles. Slow mixing of Dy-labelled particles is in agreement with the development of models by Matis (1972) and Ellis *et al.* (1979) where appearance of ingested marker at a sampling site in the rumen was best described by complex models that incorporated a time delay for mixing to occur in the rumen. The apparent slow mixing of Dy-labelled large particles in the present experiment may have been associated with the sampling of digesta from only two sites in the rumen, as opposed to bulking digesta obtained from many sites in the rumen, and also it may have been accentuated by the small quantity of Dy-labelled hay given to the steers (2% of the rumen DM pool).

The observation that at zero time after dosing, 64–68% [^{103}Ru]P and 58–49% Dy in raft digesta were associated with small-particle DM was not consistent with the measurement in Expt 3 that 31% hay stem was broken down to small particles by mastication during ingestion. This suggests that 33–37% [^{103}Ru]P and 18–27% Dy readily migrated from the labelled hay stem immediately after ingestion. That there was at least some slow transfer of both [^{103}Ru]P and Dy markers from the small-particle pool to the large-particle pool was indicated by the similarity of the k_2 rate-constant of the large-particle pool to the rate-constants derived for small particles, ventral digesta and faeces for both markers. If there were no migration of marker to unlabelled large-particles entering the rumen in feed, then the decline of marker associated with large-particle DM should have been described by first-order kinetics. If Dy is as strongly adsorbed to particulate material as some workers have argued (Ellis & Huston, 1968; Huston & Ellis, 1968; Ellis *et al.* 1979), perhaps the Dy being transferred to the large-particle pool is released from particulate material as it is digested to absorbable end-products or is transferred in association with movement of microbes to large particles.

In conclusion, it appeared that neither the k_1 nor the k_2 for either [^{103}Ru]P or Dy was an adequate measure of the turnover rate of the large-particle pool in the rumen. At least for animals in steady-state conditions the measurement of the rate-constant of turnover of large particles by emptying the rumen appears to offer a simpler and more direct approach.

Small-particle pool

The similar rate-constants measured with both [^{103}Ru]P and Dy for small particles in the raft (k_s) and for ventral digesta (k_v) indicated that the turnover rate-constant for the large particles present in ventral digesta did not greatly influence the k_v and that the small particles in the raft and ventral digesta formed a homogenous pool. Furthermore, this

rate-constant should have reflected the rate of removal of small particles from the rumen since this is the only route by which the marker could leave the rumen. However, since at any time some of the marker was not available to leave the rumen because it was associated with the large-particle pool, as noted previously, then the rate-constant of marker associated with small particles would have underestimated the actual rate-constant for the passage of the small-particle pool from the rumen to the small intestine.

The similar rate-constant of each marker, whether measured in faeces, ventral digesta, small particles from the raft, or by fitting a single compartment to the results obtained for mixed raft digesta or raft large particles, indicates that the sampling site in the rumen is not of importance in measuring the rate-constant of [^{103}Ru]P or Dy marker in the rumen. This observation is consistent with results obtained by Faichney & Griffiths (1978) using [^{103}Ru]P, where in three of four sheep similar rate-constants were calculated for the larger and smaller particulate fractions of abomasal digesta. However, the lower concentration of marker associated with large-particle DM than with small-particle DM in the present experiment does mean that it will be important to obtain samples with a consistent distribution of particle sizes.

The greater rate-constant of passage from the rumen of [^{103}Ru]P than Dy may have been due to differences in distribution of the markers both between the large- and small-particle pools (more Dy than [^{103}Ru]P was associated with large particles) and amongst particles of various sizes within the small-particle pool. Since the ease with which particles constituting the small-particle pool pass from the rumen increases with decreasing particle size (Poppi *et al.* 1980), the greater rate-constant for [^{103}Ru]P than for Dy could have been because a greater proportion of the former marker was associated with the extremely small particles (e.g. free-floating bacteria). Alternatively, the smaller rate-constant of Dy may be due to the formation of insoluble hydroxides or salts (Ellis, 1968; Kennelly *et al.* 1981 *a, b*) that may not leave the rumen as readily as small particles of digesta.

Retention time of particulate material in the rumen

The rate-constant of [^{103}Ru]P (Kennedy & Milligan, 1978) and of rare earth cations (Grovmum & Williams, 1973; Hartnell & Satter, 1979; Ellis *et al.* 1979) measured in mixed rumen digesta or faeces has been used as an estimate of the time that feed residues remain in the rumen. The present study suggests that at least with cattle fed on long hay diets, the single rate-constant of disappearance of a particulate marker from the rumen will reflect, but underestimate, the rate of removal of the small particles from the rumen.

In conclusion, since only two steers were used and differences were observed between the steers, some reservations must be expressed with regard to the accuracy of estimation of the rate-constants. Nevertheless, the present study does show that, with the methods of attachment of markers to feed materials used, difficulties were experienced with the use of both [^{103}Ru]P and Dy as particulate markers of a specific meal. Consequently, using the rate-constant of disappearance from the rumen of these markers as direct, absolute measures of the average retention time of particulate material in the rumen may result in considerable error.

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