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Original article

Passage of internal and external markers of particulate matter through the rumen of sheep

G.J. Faichney *, C. Poncet [1] and R.C. Boston [2]
With the technical assistance of L. Bernard, S. Pochet, M.-T. Beaufort, E. Delval, M. Fabre, P. Pichon and J. Flechet

[1] *Laboratoire de la digestion des ruminants, INRA de Theix, 63122 Ceyrat, France*
[2] *School of Mathematical and Physical Sciences, Murdoch University, Murdoch, WA 6150, Australia*

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Summary — Four Texel wethers fistulated at the rumen and the duodenum, and three fistulated at the œsophagus, were used to compare the passage through the rumen of 1 internal and 2 external markers of a population of particles specified by a wet-sieving procedure as those particles retained on a 0.4 mm sieve after passing through a 0.8 mm sieve (fraction 3). The internal marker was indigestible acid-detergent lignin (IADL); Yb and Cr were the external markers, each bound to the particles by a method intended to ensure that they did not exchange with unlabelled particles.

Chewing during eating accounted for 9.8% of the comminution of dietary particles between the mouth and the pylorus. Whereas 85.3% of the feed particles were greater than 11.3 mm long (4.0 mm sieve), 93.6% of the particles in duodenal digesta were less than 2.83 mm long (1.0 mm sieve).

The results indicated that the methods used, bound the external markers irreversibly to the particles, at least during their passage through the rumen. There were no significant differences between any of the markers in the mean retention time of the specified particles, either in the pool of fraction 3 particles within the rumen or of the rumen itself. It was concluded that the assumptions which were used in calculating IADL mean retention times, to account for the entrapment of fine particles in the digesta matrix and for random comminution, gave a reasonable representation of these processes; mean retention times of specifically labelled particles apply to the small particles entrained with them. When estimates of particle mean retention time are to be made, external markers should only be applied to particles in a relatively narrowly defined range of sizes.

sheep — rumen — passage rate — particulate matter — external and internal particulate markers

Résumé — Le transit des marqueurs internes et externes des parois végétales à travers le rumen du mouton. *Quatre moutons de race Texel munis d'une canule du rumen et d'une canule simple duodénale, et 3 moutons munis d'une canule de l'œsophage ont été utilisés pour comparer la vitesse de passage dans le rumen d'un marqueur interne et de deux marqueurs externes; ces derniers ont été fixés sur un ensemble de particules (fraction 3) retenu par un tamis de 0,4 mm de maille après avoir traversé un tamis de 0,8 mm. Le marqueur interne a été la fraction indigestible du résidu ADL (IADL); les marqueurs externes ont été l'ytterbium et le chrome, chacun fixé*

* *On sabbatical leave from:* Division of Animal Production, CSIRO, P.O. Box 239, Blacktown, NSW, 2148, Australia.

séparément sur les particules par une méthode destinée à éviter leur migration sur des particules non marquées.

La mastication durant l'ingestion est responsable de 9,8% de la comminution des particules alimentaires entre la bouche et le pylore. Alors que 85,3% des particules de l'aliment ont une taille supérieure à 11,3 mm de long (tamis 4,0 mm), 93,6% des particules du contenu duodénal ont une longueur inférieure à 2,83 mm (tamis 1,0 mm).

Les résultats montrent que les méthodes de marquage utilisées fixent les marqueurs externes sur les particules de façon irréversible, au moins dans le rumen. Le temps de rétention moyen des particules marquées, dans le compartiment des particules de même taille du rumen et dans le rumen lui-même n'a pas été significativement différent selon le marqueur utilisé. Il en résulte que les hypothèses émises pour le calcul des temps de rétention moyens de l'IADL afin de prendre en compte le fait que 1) les petites particules sont emprisonnées dans la masse du contenu ruminal, 2) la diminution de taille des particules s'effectue à la fois progressivement, par étapes successives, et rapidement, en courcircuitant les étapes intermédiaires, conduisent à une représentation correcte de ces processus; les temps de rétention moyens des particules marquées spécifiquement sont aussi ceux des petites particules qui transitent avec elles. Quant on veut mesurer les temps de rétention moyens des particules, les marqueurs externes doivent être utilisés uniquement en étant fixés sur une population de particules de taille peu variable.

mouton — rumen — vitesse de passage — particules alimentaires — marqueurs internes et externes

Introduction

The extent to which feed constituents are digested in the rumen depends on both the rate of degradation and the time available for degradation to occur, *i.e.*, the mean retention time (MRT). The measurement of solute MRT is relatively straightforward (Faichney, 1975b, 1986), but that for particle MRT, is not so easy. Although there are methodological problems with lignin (Fahey and Jung, 1983), it can be used to determine the net MRT of particulate matter (Faichney, 1980, 1986). However, the procedures are laborious and time consuming.

External markers such as the rare earth elements (Ellis and Huston, 1968) and the phenanthroline complex of ruthenium (Tan *et al.*, 1971) have been used, but give low MRT values because of rapid exchange between binding sites (Poncet, 1976; Faichney and Griffiths, 1978; Faichney, 1986). The Cr-mordant procedure (Martz *et al.*, 1974; Udén *et al.*, 1980) produces marked particles which are stable, but their MRT is often less than expected (*e.g.* Ushida *et al.*, 1986). This may be due to the effect of the Cr on specific gravity, either directly (Ehle *et al.*, 1984; Hsaio *et al.*, 1987), or by reducing particle degradation (Martz *et al.*, 1974) and hence, the gas production which reduces functional specific gravity (Hooper and Welch, 1984), or the fact that the particle size distribution of the maker differs from that of ingesta (Faichney, 1986), or both.

A method has recently been proposed (Ellis and Beever 1984; Beever and Ellis, 1985) by which the rare earth element Yb can be bound to rumen particles strongly enough to prevent exchange. This paper reports an experiment in which the MRT of Yb, so bound to a fraction of rumen particles specified by a wet-sieving procedure, was compared with that of Cr bound to the same fraction by the procedure of Udén *et al.* (1980). The values obtained using these external markers were then compared with those obtained by following the passage of

indigestible acid-detergent lignin through the rumen. In calculating the latter values, it was found that allowance had to be made, not only for the entrapment of fine particles within the digesta matrix (Faichney, 1986), but also for irregular reduction in particle length (random communication). A preliminary report of some of the data has been published (Faichney and Poncet, 1987).

Materials and Methods

Animals and diet

Four mature Texel wethers were used, each fitted with a permanent cannula in the rumen and a permanent simple cannula in the proximal duodenum. They were maintained in metabolism cages under continous lighting in an air-conditioned room in which the mean (± SD) maximum and minimum temperatures during the experiment were, respectively, 20.0 ± 2.2 °C and 17.8 ± 2.4 °C. Three additional similar sheep, fistulated at the œsophagus, were maintained in pens in an animal house. All sheep were given a diet of chopped rye-grass hay prepared by passage through a screenless hammer mill followed by passage over an oscillating mesh tray to remove dust. The hay was given at the rate of 863 g dry matter (DM)/d in equal meals at 3 h intervals and contained (g/kg DM) : organic matter 910, nitrogen 20.3, acid-detergent fibre 341, acid-detergent lignin (ADL) 26. The sheep were accustomed to the diet during a 14 d preliminary period.

Particle markers

A specific fraction of particles was isolated by a wet-sieving procedure from rumen digesta taken from a donor sheep given the same diet. The apparatus used was an Analysette 3 (Fritsch GMBH, Idar-Oberstein, FDR) with 7 sieves, numbered 1 to 7 from smallest to largest pore size, *viz.*, 0.16, 0.25, 0.40, 0.80, 1.0, 2.0 and 4.0 mm. It vibrated at 3 000 oscillations/min with an amplitude of 0.9 mm and a water flow of 1.3 L /min. Rumen digesta, dispersed in about 1 L of water, were washed onto the top sieve (#7) and the apparatus was run for 5 min. The sieves were then inspected so that any clumps of particles could be dispersed and the appartus was run for another 5 min. Particles that passed sieve number 4 (0.8 mm), but were retained on sieve number 3 (0.4 mm), were extracted with neutral detergent (Ellis and Beever, 1984). After thorough washing, the particles were again wet-sieved and those retained on sieve number 3, designated fraction 3, were squeezed dry (DM ≈ 0.2) and taken for labelling.

One batch of particles, 100 g fresh weight, was labelled with ^{169}Yb using a competitive binding technique (Ellis and Beever, 1984; Beever and Ellis, 1985). The competitive ligand used was citric acid. The particles were maintained in suspension by magnetic stirring for 24 h in 400 ml of an acid solution containing $YbCl_3$ and citrate in equimolar proportions : 4 mg Yb, 370 kBq ^{169}Yb and 4.855 mg citric acid per g particle DM; the pH, initially adjusted to 2.5 using 0.1 N HCl, was 3.2 at the end of the incubation. The labelled particles were washed copiously with tap water and squeezed dry. Using this procedure, 0.38 of the ^{169}Yb was bound to the particles so that a dose of 20 g fresh weight contained 555 kBq of bound ^{169}Yb.

A second 100 g batch of fraction 3 particles was labelled with Cr as described by Udén *et al.* (1980). Sufficient ^{51}Cr (as chromate) was included to recover a dose of 3.7 mBq in 20 g fresh weight of particles; 0.30 of the ^{51}Cr was recovered in the particles.

Period 1

Following an intraruminal dose of fraction 3 particles labelled with ^{169}Yb, rumen digesta samples were taken at increasing intervals for 96 h. Samples were taken using a concentric tube probe (30 mm o.d.), the outer tube of which was sealed at the bottom, both tubes having a side hole 110 mm long and occupying one third of the circumference near the bottom. The probe was inserted into the rumen through the fistula, the inner tube was rotated until the two holes coincided and then counter-rotated to enclose the sample. For each sample, a subsample was taken for DM determination and the remainder was strained through polyester fibre cloth of 160 µm pore size. Both filtrate and filtrand were sampled for DM and radioactivity determinations; the remainder of

the filtrand was separated by the wet-sieving procedure and fractions 1—5 were sampled to determine DM and radioactivity. One sheep failed to adapt to the conditions of the experiment; it would not feed during this period and was replaced.

Period 2

Seven days after the ^{169}Yb-labelled dose, the sheep were given an intra-ruminal dose of fraction 3 particles labelled with ^{51}Cr. Samples were taken and prepared as in Period 1.

Period 3

Seven days after the ^{51}Cr-labelled dose was given, a 7-d collection of total faecal output was begun. After 3 d, primed continuous infusions of [^{51}Cr] EDTA (65 kBq in 5 ml/h), which remains in solution (Downes and McDonald, 1964), and of $^{169}YbCl_3$ (15 kBq in 6 ml/h of solution at pH 2.5), which associates with particulate matter (Siddons *et al.*, 1985), were begun and maintained simultaneously for 6 d. On the fourth d of infusion, urine was collected for 24 h so that corrections could be made for [^{51}Cr] EDTA absorption (Faichney, 1975a; 1980; 1986). During the fifth and sixth d, a total of 9 samples of digesta were taken from the rumen and duodenum; for both rumen and duodenal digesta, taken from each sheep, composite samples were prepared of digesta, and of filtrate and filtrand obtained from the digesta, by straining through polyester fibre cloth. After the last sample, the infusions were terminated; 3 samples of rumen digesta were taken at 3-h intervals on the seventh d, and at the same times on the eighth d, for the measurement of marker disappearance rates. All samples were stored at –15 °C until analysed.

Ingesta collection

Prior to feeding on 2 successive d, each of the oesophageally fistulated sheep were offered about 300 g of the chopped hay. Boli were diverted through the fistula, combined for each sheep and stored at –15 °C until analysed. The 3 sheep ate at 14.8, 17.2 and 22.1 g DM/min; 38, 40 and 64% respectively, of the DM eaten, was collected.

Analyses

Subsamples of rumen ingesta and of rumen and duodenal digesta (3— 6 g DM), were separated by the wet-sieving procedure; the fractions were quantitatively transferred to tared aluminium dishes and dried at 80 °C for 24 h. The remainder of each sample was subsampled and wet-sieved; the fractions (1 + 2), 3, 4 and (5 + 6 + 7) were combined, freeze-dried, ground through a 1 mm screen and analysed for ADL. Samples of digesta, their fractions, faeces and urine were assayed for ^{51}Cr and/or ^{169}Yb (simultaneously) in duplicate tared tubes using a model 5260 Auto Gamma Spectrometer (Packard Instrument Co., Illinois). The chemical methods used were as described by Faichney and White (1983).

Calculations

For periods 1 and 2, marker disappearance from the particles in fraction 3 was described by the model $y = A\exp^{(-t/MRT)}$, where y = marker concentration (fraction of the dose/kg DM) at time t (h) after the dose, the constant A = marker concentration at zero time and MRT = mean retention time (h) of the marker in the pool of fraction 3 particles within the rumen. Rumen MRT's of the markers were calculated from the concentration/time curves of the markers in rumen digesta by a numerical integration procedure using the CONSAM/SAAM computer package (Boston *et al.*, 1981).

For period 3, the quantity and composition of rumen and duodenal true digesta were calculated by the double-marker technique (Faichney, 1975b; 1980). The particle size distribution in true digesta was calculated as described by Faichney (1984). Corrections for the 'filter-bed' effect, (*i.e.*, entrapment of fine particles by larger particles), were applied to the ADL fractions (pools) and flows isolated by the wet-sieving procedure, as described by Faichney (1986). Rumen MRT's of solutes (*i.e.*, MRT of [^{51}Cr] EDTA corrected for absorption) (Faichney, 1986) and ^{169}Yb were calculated from the marker disappearance rates (Faichney, 1975b), and indigestible (I) ADL as [rumen ADL content (g)/faecal ADL output (g/h)]. MRT's of the ADL pools, defined by wet-sieving, were calculated as [pool ADL content (g)/pool ADL loss (g/h)], where loss equals outflow, plus comminution.

Results

Organic matter and nitrogen intakes and the partition of their digestion between the stomach and intestines are shown in Table I. Digestion of the diet, which contained 127 g crude protein/kg DM, provided 174 g apparently digested crude protein/kg digestible OM. The amount of OM in the rumen was closely related to liveweight, increasing by 15.5 g per kg increase in liveweight (r = 0.984). The mean apparent digestibility of ADL was 0.271 ± 0.009.

The liveweights of the sheep and the rumen MRT's of solutes, ^{169} Yb and IADL (*i.e.*, the net value for particulate matter), measured during period 3, are shown in Table II. There was a close relationship between MRT and liveweight which accounted for 0.954, 0.910 and 0.992 of the variance in, respectively, solute, ^{169}Yb and IADL MRT's. There were close relationships between the MRT's of ^{169}Yb

Table I. Partition of organic matter and nitrogen digestion between the stomach and intestines in four sheep given a chopped rye-grass hay (mean ± SEM).

	Organic matter	*Nitrogen*	*Non-ammonia nitrogen*
Intake (g/d)	786	17.5	
Digestibility	0.596 ± 0.013	0.658 ± 0.010	
Rumen pool (g)	554 ± 58		13.3 ± 1.6
Duodenal flow (g/d)	459 ± 30		19.0 ± 0.9
Digested in stomach (fraction of total digested)	0.696 ± 0.049		
Digested in intestines (g/d)			13.0 ± 0.8
(fraction of duodenal flow)			0.683 ± 0.009
(g CP/kg DOMI)*			174 ± 14

* CP = 6.25 x non-ammonia N; DOMI = digestible organic matter intake.

Table II. Live weights and rumen mean retention times of solutes, ^{169}Yb (infused in solution) and indigestible acid-detergent lignin (IADL) for sheep given a chopped rye-grass hay.

Sheep	*Liveweight*	*Rumen mean retention time (hours)*		
	(kg)	*Solutes*	*^{169} Yb*	*IADL*
1	45.8	11.2	12.6	26.4
2	61.8	15.9	20.6	42.2
3	58.5	14.6	18.9	39.6
4	50.5	11.5	12.2	29.9
Mean	54.2	13.3	16.1	34.5
SEM	3.7	1.2	2.2	3.8

(r = 0.994) and IADL (r = 0.987) and the MRT of solutes; they increased by, respectively, 1.85 and 3.23 h per unit increase in solute MRT.

The size distribution of the DM in the feed, ingesta, rumen contents and digesta flowing through the duodenum, is shown in Figure 1. Of the particles retained (DM) by the sieves, those passing the 1 mm sieve (# 5, maximum particle length 2.83 mm — see Fig. 5) made up, respectively, 4.2, 10.2, 57.9 and 93.6% of the particles of feed, ingesta, rumen contents and duodenal flow (Fig. 2); 85.3% of the feed particles were greater than 11.3 mm long (4 mm sieve). Chewing during eating reduced the modulus of fineness (Poppi *et al.*, 1980) by 6.2%; for duodenal digesta, that was 63.1% less than the feed. Thus, chewing during eating accounted for 9.8%, and processes associated with rumination and microbial activity for 90.2%, of the particles size reduction that occurred between the mouth and the pylorus.

External markers

The curves describing the disappearance of the markers from the pool of fraction 3 particles are shown in Figure 3. The curves were described well by a single exponential model (r^2 > 0.99) and were similar for the 2 markers. The curves of marker disappearance from the rumen, which cannot be described by a single exponential model, are shown in Figure 4 and confirm the similarity in behaviour of the 2 markers. The MRT's of the markers, calculated from the curves shown in Figures 3 and 4, are shown in Table III. There was no significant difference between the MRT for ^{169}Yb-labelled particles and that for ^{51}Cr-labelled particles in either fraction 3 or the rumen. The differences between sheeps were due to their different liveweights, which accounted for, between 0.963 and 0.992, of the variance in MRT.

Internal markers

When MRT's were calculated from the ADL pools, assuming either sequential or random comminution, and that small particles, (*i.e.*, ADL passing sieve # 1), should behave like solutes (Hangate, 1966; Hogan and Weston, 1967), they

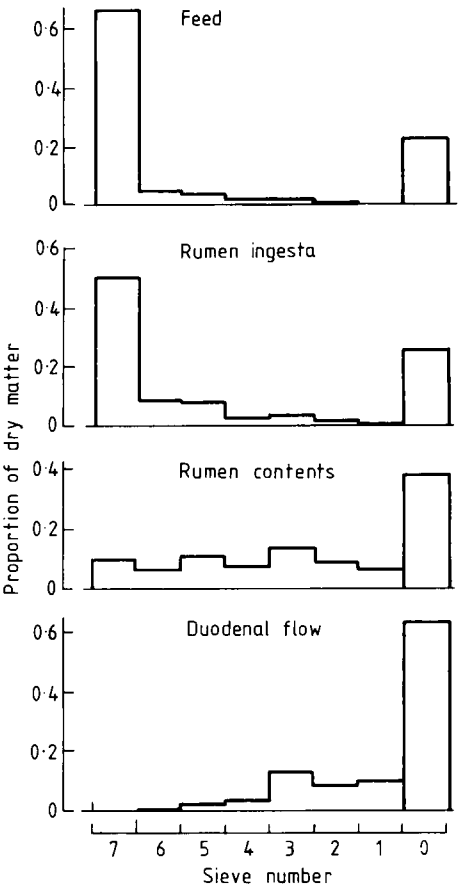

Fig. 1. Particle size distribution of the dry matter of feed, rumen ingesta, rumen contents and digesta flowing through the duodenum of sheep given a chopped rye-grass hay.

Table III. Mean retention times within the pool of fraction 3 particles and in the rumen of ^{169}Yb and ^{51}Cr irreversibly bound to fraction 3 for sheep given a chopped rye-grass hay.

Sheep	*Mean retention time (h)*			
	^{169}Yb-fraction 3		*^{51}Cr-fraction 3*	
	Pool	*Rumen*	*Pool*	*Rumen*
1	10.2	15.1	10.9	14.5
2			22.2	29.7
3	21.7	30.0	20.8	28.2
4	15.8	19.4	15.3	21.7
Mean ($n = 3$) ± SEM	15.9 ± 3.3	21.5 ± 4.4	15.7 ± 2.9	21.5 ± 4.0
Mean ($n = 4$) ± SEM			17.3 ± 2.6	23.6 ± 3.5

were overestimated by 63% and 56%, respectively. When the "filter-bed" correction (Faichney, 1986) was applied, but assuming sequential comminution, the MRT of fraction 3 was 41% less than that obtained using the external markers. Therefore, the MRT's for the particle fractions were calculated using both the "filter-bed" correction and random comminution, shown diagrammatically in Figure 5. Random comminution was represented by assuming that ADL leaving a fraction, and passing to fractions of smaller particle size, did so in proportion to the amount of ADL in those fractions. The MRT's obtained are shown in Table IV; also in Table IV are values for a 3-pool model in which ADL passing the

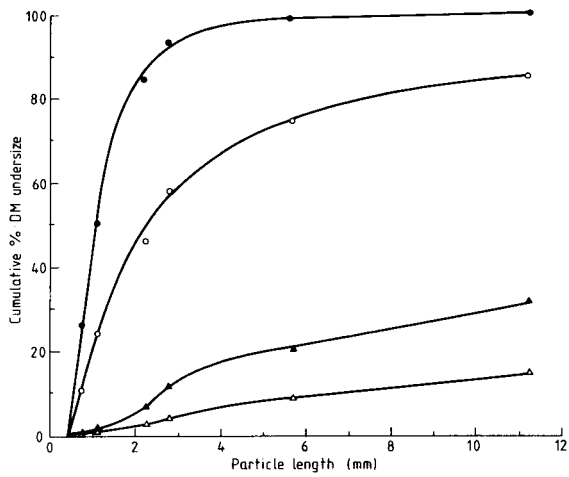

Fig. 2. Relationship between cumulative distribution of particles and particle length in feed (Δ), rumen digesta (▲), rumen contents (○) and digesta flowing through the duodenum (●) of sheep given a chopped rye-grass hay.

Table IV. Mean retention times within pools of size-defined particle fractions and in the rumen of indigestible acid-detergent lignin entering those fractions, calculated assuming fine particle entrapment and random comminution (see text), for 4 sheep given a chopped rye-grass hay.

	Mean retention time (h)			
	5-pool model		*3-pool model*	
Fraction (sieve #)	*Pool*	*Rumen*	*Pool*	*Rumen*
5 + 6 + 7	14.3 ± 2.1	35.5 ± 4.0	14.3 ± 2.1	35.7 ± 4.0
4	21.4 ± 2.0	33.3 ± 2.7		
3	19.4 ± 1.9	23.2 ± 2.2	22.7 ± 2.2	23.6 ± 2.0
1 + 2	17.7 ± 1.7	18.6 ± 1.5		

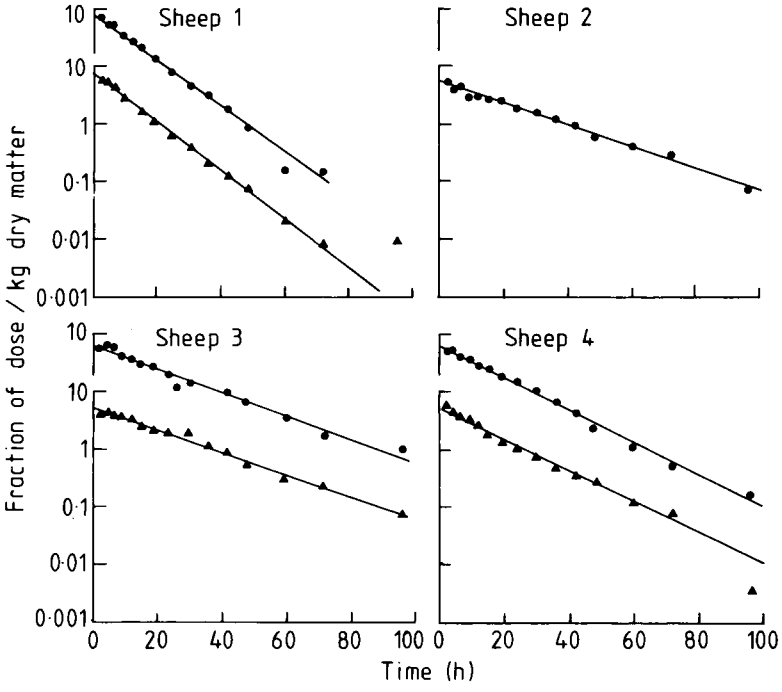

Fig. 3. Disappearance from the pool of fraction 3 particles in the rumen of ^{169}Yb (▲) and ^{51}Cr (●) irreversibly bound to fraction 3 particles in 4 sheep given a chopped rye-grass hay.

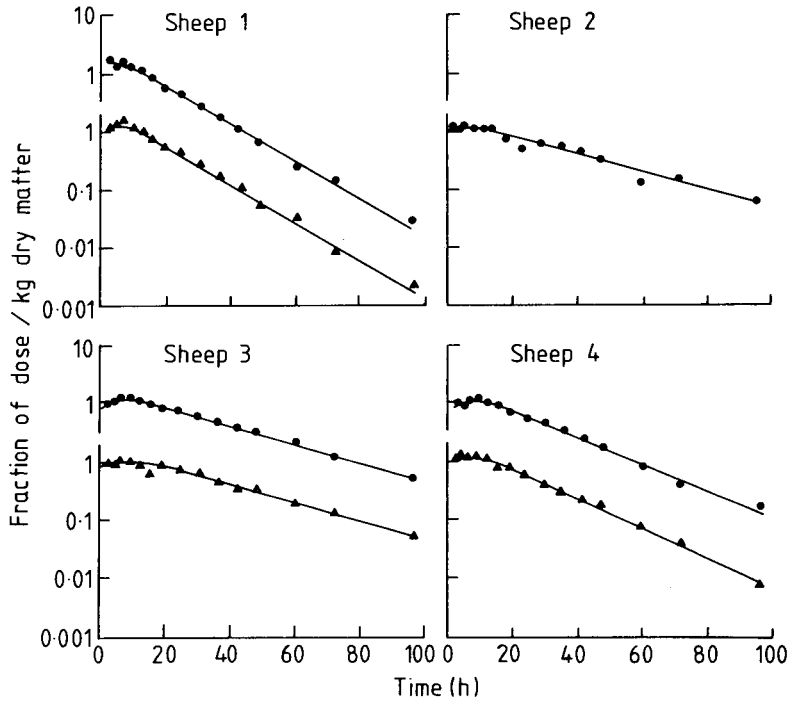

Fig. 4. Disappearance from the rumen of ^{169}Yb (▲) and ^{51}Cr (●) irreversibly bound to fraction 3 particles in 4 sheep given a chopped rye-grass hay.

1.0 mm sieve and retained on sieves 1, 2, 3 and 4, were combined to constitute medium-sized particles (Faichney, 1986). The values obtained using the 5-pool model for the MRT of fraction 3 particles within their pool and in the rumen were not significantly different from those obtained with the external markers ($P > 0.05$); the means were within one SD of each other. For the 3-pool model, values calculated using the simpler sequential model, under-estimated MRT in the medium particle pool by only 6% and of medium particles in the rumen by only 3% when compared with the values in Table IV which were obtained using the assumption of random comminution.

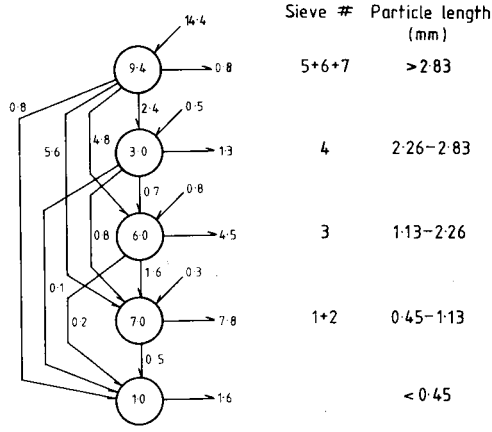

Fig. 5. The 5-pool model of the passage of indigestible acid-detergent lignin through the rumen showing the theoretical range of particle length for each pool calculated from sieve pore size (Vaage *et al.*, 1984), together with ADL pools (g) and flows (g/d) for sheep 3.

Discussion

The results obtained in the experiment show that, used appropriately, external and internal markers can provide consistent estimates of particle kinetics in the rumen. They also show that particle size analyses of feed and digesta, although allowing insights into the role of chewing during eating and ruminating, do not fully define kinetic entities in the rumen. The results shown in Figure 4, confirm that when markers are irreversibly bound to particles, their disappearance following their introduction into the rumen cannot be described by a single exponential model (Faichney, 1986).

The close relationships between the MRT's of the particulate markers and that of solutes (Table II), are consistent with the results of Faichney and White (1988), and emphasize the importance of water as the vehicle for transport out of the rumen (Faichney *et al.*, 1980-1981). The MRT of infused ^{169}Yb was 1.2 times greater than that for solutes, which is consistent with the values expected for markers that exchange between particles (Faichney, 1986). By contrast, the MRT of IADL was 2.6 times that for solutes. This is consistent with a factor of 2.8 for chopped lucerne hay (Faichney, 1986), but less than the factors of 3.2 found for ground and pelleted lucerne (Faichney, 1986) and 3.4— 4.5 for ground and pelleted mixed diets (Faichney, 1980; Faichney and Barry, 1986; Faichney and White, 1988). These results suggest that differential retention of solutes and particles is enhanced by grinding and pelleting (Faichney, 1986). The close relationship between MRT and liveweight was the consequence of relationships between liveweight, DM intake and rumen OM content. Thus, the heavier sheep had relatively lower intakes, more OM in the rumen and, hence, longer MRT's.

Following the doses of ^{169}Yb- and ^{51}Cr-labelled fraction 3 particles, some radioactivity was detected in fractions 4 and 5. It was concluded that this was an artifact due to incomplete separation during wet-sieving because, first, the levels of radioactivity in fractions 4 and 5 were variable, second, there was no evidence for a second component in the radioactivity/time curves for fraction 3 (Fig. 3) and, third, transfer of ^{51}Cr to other particles from those labelled would not have been expected because the mordanted complex is stable (Uden *et al.*, 1980). It can be concluded that the competitive ligand procedure used, based on that of Ellis and Beever (1984), appeared to irreversibly bind ^{169}Yb to the particles, at least during their passage through the rumen.

It might have been expected that ^{51}Cr-fraction 3 particles would have a higher specific gravity and thus a shorter MRT (Campling and Feer, 1962) than ^{169}Yb-fraction 3 particles. The Cr-mordanting procedure increases particles specific gravity (Ehle *et al.*, 1984; Hsaio *et al.*, 1987) and subtantially inhibits degradation (Martz *et al.*, 1974) and, hence, the gas production that would cause a decrease in functional specific gravity (Hooper and Welch, 1984). On the other hand, although bound Yb decreases degradation (Teeter *et al.*, 1984), it does so to a lesser extent than Cr treatment and the amount of Yb bound in the present experiment was considerably less than reported by these workers. The observation that the pool and rumen MRT's for ^{51}Cr-fraction 3 and ^{169}Yb-fraction 3 were virtually identical, indicates that such mechanisms were not sufficient to cause differences between these 2 external markers in the present experiment.

The MRT of the small particles (*i.e.* those passing sieve # 1), which would be expected to behave as if they were

solutes (Hungate, 1966; Hogan and Weston, 1967), could not be calculated directly from the particle size distribution of ADL. When allowances were made for the entrapment of small particles within the mass of larger particles using the "filter-bed" correction of Faichney (1986), the simple assumption of sequential comminution did not provide realistic estimates of MRT for fraction 3. When random comminution was assumed, allowing ADL to by-pass one or more of the sieves in the series, the pool and rumen MRT's for fraction 3 were not significantly different from those determined using the external markers. Although there were large errors in MRT's when sequential communition was assumed, considering the particles on sieves # 5-7 as large particles having a low probability of passage from the rumen, and combining those on sieves # 1-4 as medium particles having a high probability of passage (see Kennedy, 1984) resulted in MRT values which were only marginally underestimated. Thus the MRT's for large and medium particles, reported earlier (Table IV, Faichney, 1986), were underestimated by 2—7% whereas those reported for a 7-pool model (Table V, Faichney, 1986) would have been substantially underestimated.

The tendency for the pool MRT, determined using IADL, to be a little longer than for the external marker, if real, could have been due to one or a combination of three factors. First, the comminution of particles by chewing during eating may have been greater in the experimental sheep than in the oesophageally-fistulated sheep. In the latter sheep, chewing accounted for only about 10% of the comminution that occurred, substantially less than the value of 35% that can be calculated for sheep given lucerne hay (Faichney G.J., unpublished data). Ulyatt *et al.* (1986) reported a reduction, due to chewing, of 34.6% in the proportion of large particles in meadow hay given to sheep, whereas, a value of 10.3% was found in this experiment. This difference that could be related to the higher intake rate recorded here (mean 18.0 g DM/min *cf.* 10.7 g DM/min). Thus, if ADL entering fraction (5 + 6 + 7), as shown in Figure 5, is reduced on the assumption that the extent of reduction due to chewing was the same as reported by Ulyatt *et al.* (1986), and ADL entering the other fractions is increased *pro rata*, the pool MRT of fraction 3 would be reduced by 11%; its mean value in Table IV would need to be reduced by 11% to equal that for ^{51}Cr-fraction 3 in Table III. In addition, if the external markers did inhibit particle degradation to some degree, a lower functional specific gravity and, hence, a longer mean retention time might be expected for IADL-fraction 3, than for the external markers. Finally, as external markers bind to the surface of the particles (Faichney, 1986), and there was a 4-fold range of surface area in fraction 3 particles, their concentrations would be greater in the smaller particles within the range so that their MRT's would be biased towards those of the smaller particles. For this reason, external markers should only be applied to particles in a relatively narrowly defined range of sizes when estimates of particle MRT are to be made.

Whether or not any of these factors were involved, the 3 markers gave remarkably similar estimates of the passage of fraction 3 particles through the rumen. It can be concluded that the assumptions used to account for the entrapment of fine particles in the digesta matrix, and for random comminution, gave a reasonable representation of the processes occurring in the rumen. These aspects of particle dynamics must be taken into account in the interpretation of

data obtained by wet-sieving. Finally, it is clear that the MRT's determined by the external markers apply, not only to the specified particles, but also to the small particles that are entrained with them.

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