Studies of the large intestine of sheep

2. Kinetics of liquid and solid phase markers in the caecum and proximal colon

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1. Experiments were undertaken to examine the errors associated with the use of indigestible markers, the ^{51}Cr-labelled complex of chromium ethylenediaminetetra-acetic acid (^{51}Cr-EDTA) and ^{103}Ru-labelled tris-(1,10-phenanthroline)-ruthenium(II)chloride (^{103}Ru-P), to measure liquid- and solid-phase digesta kinetics in the caecum and proximal colon of sheep.

2. First-order kinetics of markers were observed following either single injection or termination of continuous infusion. There were no differences between the half-times ($T_{\frac{1}{2}}$) of marker in the caecum plus proximal colon whether calculated from marker concentration in caecal digesta or in faeces. There were also no differences in the $T_{\frac{1}{2}}$ values calculated for the liquid- and solid-phase markers. When pool sizes calculated from the marker kinetics were compared with the volume of digesta present in the caecum and proximal colon at slaughter, it appeared that the ^{51}Cr-EDTA and ^{103}Ru-P caecal pools described the digesta contained in the entire caecum and proximal colon.

3. The flow-rates of dry matter (DM) through the caecum of sheep given 694 g lucerne (*Medicago sativa*) DM/d were similar whether estimated from total collection of faeces, by single injection of marker, or by the ratio, marker concentration: DM in either caecal digesta or faeces during continuous infusion of marker into either the rumen or the caecum.

4. In sheep given 553 g brome grass (*Bromus inermus*) DM/d the coefficient of variation of estimates of the plateau of ^{51}Cr-EDTA marker during continuous infusion into the caecum was greater when 130 ml infusate/d were administered than with 1000 ml/d.

5. In the sheep given brome grass the lines of best fit of decline in ln ^{51}Cr-EDTA marker concentration *v.* time following termination of the continuous infusions described previously and following single injection of marker in 20 or 2 ml into the caecum were examined. The variation was least when 1000 ml infusate/d had been administered and was unacceptably large following a single injection of 2 ml.

6. These experiments showed that tracer techniques could provide unbiased estimates of trace kinetics in the caecum and proximal colon.

A number of workers have used isotope-dilution techniques to measure production of volatile fatty acids (Faichney, 1969; Ulyatt *et al.* 1973; Glinsky *et al.* 1976), carbon dioxide (MacRae *et al.* 1978) and ammonia (Nolan *et al.* 1976; Glinsky & Tyler, 1977) in the caecum of sheep or horses. However, the validity of these techniques depends on adequacy of mixing of the isotope tracers with tracee and on maintenance of steady-state conditions (i.e. constant pool size and constant rates of inflow and outflow) in the caecum. There is some uncertainty whether these conditions are approached sufficiently closely in the caecum for tracer dilution techniques to be validly used (Ulyatt *et al.* 1975).

The following experiments were undertaken to provide information on marker kinetics in the caecum and proximal colon following single injection and continuous infusion of markers into the caecum. Expt 1 involved single injection into the caecum to compare liquid- and solid-phase markers and caecal and faecal sampling sites. Expt 2 entailed continuous

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infusions of ^{51}Cr-EDTA into the caecum. In Expt 3 kinetics of ^{51}Cr-EDTA following either single injection or termination of continuous infusion of marker were compared. Information from subsequent slaughter of sheep and from total collection of faeces was also used for direct comparison with measurements made with the markers.

MATERIALS AND METHODS

Expt 1

Merino wethers (2–3 years) of similar genetic origin and body-weight (29–35 kg) were prepared with cannulas in the rumen and the caecum as described previously (Dixon & Nolan, 1982).

The sheep were housed indoors in metabolism crates under continuous lighting and were accustomed to the sampling procedures before experiments were commenced. Anthelmintic (Thibenzole; Merke Sharpe & Dhome, Sydney, Australia) was administered regularly. The 800 g air-dry chopped lucerne (*Medicago sativa*)/d diet was fed for 60 d before the experiment and was fed in equal hourly portions for 7 d before and also during the experiment. Water was available at all times. Single injections (4 μCi; 10 ml) of an aqueous solution of ^{103}Ru-labelled tris – (1,10-phenanthroline)-ruthenium (II) chloride (^{103}Ru-P) (Tan *et al.* 1971) and the ^{51}Cr complex with ethylenediaminetetra-acetic acid (^{51}Cr-EDTA) (Downes & McDonald, 1964) (20–40 μCi with carrier) were made into the caecum of each of the six sheep via the caecal cannula. Plastic tubing attached to the injection syringe was used to direct the marker solution towards the pole of the caecum.

Caecal digesta was sampled by removing the plug of the caecal cannula and scraping out and discarding the digesta in the barrel of the cannula. A container was then attached to the cannula and positioned to collect 5–20 g digesta as it flowed from the cannula. Twelve samples of caecal digesta were obtained at intervals for approximately 850 min after each marker injection, while faeces were sampled at intervals for approximately 2000 min. Faecal samples were obtained from the rectum if defaecation had not occurred in the previous 60–100 min.

Expt 2

Merino wethers similar to those used in Expt 1 were prepared with caecal and rumen cannulas as described previously. An infusion line (Silastic; Dow Corning, Michigan, USA; internal diameter 1·02 mm) into the caecum was securely attached to the caecal wall either opposite the ileo-caecal junction for four sheep for one infusion, or midway between the cannula and the pole of the caecum for all other infusions. No differences due to the site of attachment of the infusion line were observed. Chopped lucerne hay was fed as described for Expt 1.

Twenty-seven continuous infusions of ^{51}Cr-EDTA (5–100 μCi/d with carrier) were made in ten sheep, the marker being infused in aqueous solution at 1·1–1·2 l/d for one group of four sheep and at the rate of 0·72–0·78 l/d in other infusions. Infusions were continued for 1100–2200 min, but only the 5–14 caecal digesta samples obtained more than 250 min after commencement of infusions were considered in calculations. Infusions of ^{15}N and ^{14}C tracers (to provide information to be reported later) were also made into the jugular vein or into the caecum. Caecal digesta was sampled as described previously, except that samples of 30–40 g were obtained.

Expt 3

Four mature Finnish Landrace–Suffolk wethers (40–46 kg body-weight) were surgically prepared and maintained as described for Expt 1. The diet (650 g air-dry pelleted brome grass (*Bromis inermis*)/d) was fed to the sheep for 54 d before the experiment and was fed in equal hourly portions for 14 d before and also during the experiment. A 4 × 4 Latin-Square design was used with periods of 7 d. In one period, one sheep did not consume all of the

offered food, and this treatment was repeated with the same sheep during a fifth period.

The four treatments consisted of administration via the caecal infusion line of ^{51}Cr-EDTA as a single injection (100 μCi) in either 2 ml or 20 ml warm (37°) saline (0·15 M-sodium chloride) (treatments 1 and 2 respectively) or as a continuous infusion (100 μCi/d) at the rate of 130 ml/d (treatment 3) or at 1000 ml/d (treatment 4) in saline. Continuous infusions of marker were commenced at 16.00–17.00 hours on day 1 and seven samples of caecal digesta (5–10 g) were obtained between 08.00 hours and 18.00 hours on day 2. A sample was obtained on day 3 at 08.00 hours and another was taken shortly before terminating the infusion of marker at 09.30–10.00 hours. Saline was infused into the caecum during the sampling period following termination of the marker infusion. Eleven caecal digesta samples were obtained between 10.00 hours and 21.00 hours on day 3, and five samples on day 4. Single injection of marker into the caecum (treatments 1 and 2) was performed at the same time as termination of the continuous infusions, and caecal digesta was sampled as described for treatments 3 and 4.

Following the marker experiments total faecal output was collected for 7 d.

Laboratory analysis

Samples of caecal digesta or of faeces (2–3 g) were placed into tared gamma counting vials, and the DM was determined by drying to a constant weight at 70°.

The radioactivity of ^{51}Cr-EDTA and ^{103}Ru-P in digesta and faeces was determined using a gamma spectrometer (Model 3002; Packard Instrument Co., Illinois, USA, for Expts 1 and 2; Biogamma; Beckman Instruments Inc., Fullerton, California, USA for Expt 3). When both isotopes were present in a sample the method of Tan *et al.* (1971) was used to calculate the proportion of each isotope appearing in each counting channel, with appropriate corrections for the effect of sample height on efficiency of counting.

Calculations

Half-time ($T_{\frac{1}{2}}$), pool size and flow from the caecal pool of water and DM were calculated by applying first-order kinetics (Shipley & Clark, 1972).

Statistical analysis

The $T_{\frac{1}{2}}$ of markers in the caecum of the six sheep in Expt 1 were compared by the homogeneity of the linear regressions of ln marker decline with time (Snedecor & Cochran, 1967).

Analysis of variance was used to compare the mean DM content of digesta, $T_{\frac{1}{2}}$, pool size and flow-rates for each of the treatments used in Expt 3. The Studentized Range (Snedecor & Cochran, 1967) was used to separate means into their respective classes.

The means of pool size measured by ^{51}Cr-EDTA kinetics or by slaughter were compared using a *t* test.

RESULTS

Mixing of the marker(s) with digesta in the caecum and proximal colon

The time required for mixing of marker through the caecal digesta pool following a single injection of marker was estimated from the decrease in variation about the fitted regression of ln marker concentration with time after injection upon omission of early samples from the relationship. It appeared that in all sheep where 10 or 20 ml of infusate was injected mixing was completed within 50–100 min of the injection time. In Expt 3 where 2 ml of infusate was injected, mixing was comparatively slow (200–500 min) in three of the four sheep.

Table 1. *Expts 1 and 2. Half-time (min) of dry matter in the caecal pool of six sheep given lucerne* (Medicago sativa) *calculated from marker concentration following single injection of* ^{51}Cr *complexed with ethylenediaminetetra-acetic acid* (^{51}Cr-*EDTA*) *and* ^{103}Ru-*labelled tris-(1,10-phenanthroline) – ruthenium (II) chloride* (^{103}Ru-*P*) *into the caecum, and sampling of caecal digesta and faeces*

(Mean values with their standard errors, ranges given in parenthesis)

Sampling site	Marker			
	^{51}Cr-EDTA		^{103}Ru-P	
Caecum	283 ± 41	(203– 462)	285 ± 38	(211–438)
Faeces	455 ± 160	(230–1242)	414 ± 118	(221–976)

Results for markers and sampling sites did not differ significantly.

Expt 1. Appearance of marker in faeces

The time required for the first appearance of marker in faeces following single injection into the caecum in Expt 1 was somewhat variable, with values of 300–600 min obtained for five sheep, and 1450 min for one sheep. Following the appearance of marker, the maximum marker concentration in faeces was reached within 100 min in three sheep but in the other three sheep marker concentration increased gradually over 150–400 min. The variation of the ln (marker concentration) about the fitted linear regression with time differed widely among sheep; the correlation coefficients for the ^{51}Cr-EDTA ranged from 0·86–0·99 (mean 0·98).

Expts 1 and 2. Kinetics in the caecum of sheep given lucerne

Mean values of $T_{\frac{1}{2}}$ calculated from the single injection of markers in Expt 1 showed no significant differences between the ^{51}Cr-EDTA and ^{103}Ru-P markers, or between the caecal and faecal sampling sites, although significant differences ($P < 0·05$) were found between sheep (Table 1).

Water flow-rates (mean ± SE) were not significantly different when estimated from single injection (2·72 ± 0·80 l/d; Expt 1) or continuous infusion (2·69 ± 0·97 l/d; Expt 2).

In Expt 1 the mean estimate of pool size of digesta water (562 ± 167 ml) was not significantly different from the weight of digesta water present in the entire caecum and proximal colon (460 ± 26 ml water) measured by the slaughter of similar sheep given the same diet (Dixon & Nolan, 1982).

Faecal DM flow calculated from the marker concentration in faeces following caecal ^{51}Cr-EDTA infusions (293 ± 12 g/d) or rumen ^{51}Cr-EDTA infusions (Dixon & Nolan, 1982) (289 ± 12 g/d) was similar to that measured by total collection of faeces (301 ± 4 g/d) or calculated from the concentration of ^{51}Cr-EDTA marker in digesta from the caecum, proximal colon or rectum at slaughter (282–297 g/d) (Table 2).

Expt 3. Kinetics in the caecum of sheep given brome grass

The estimation of digesta flow from the plateau marker concentration during continuous infusion was more variable when 130 ml marker solution/d (treatment 3) than when 1000 ml/d (treatment 4) were infused. The pooled coefficients of variation of plateau marker concentration were 38 and 26% respectively.

Regressions of the decline in ln marker concentration *v.* time following single injection or termination of continuous infusion in one sheep are shown in Fig. 1. For all sheep the

Table 2. *Expts 1 and 2. Flow of digesta (g dry matter/d) through the caecum or rectum of sheep given lucerne* (Medicago sativa) *measured by three methods*

(Mean values with their standard errors)

	Mean	SE
Total collection of faeces (four sheep for 7 d)	301	4
Infusion of ^{51}Cr-EDTA into the caecum and sampling of faeces on 'plateau' (nine infusions in three sheep)*	293	12
Flow measured by concentration of ^{51}Cr-EDTA in digesta at slaughter†		
Caecum	297	9
Upper proximal colon	297	11
Lower proximal colon	297	12
Rectum	289	12

* Expt 2.
† Data from Dixon & Nolan (1982).

variation about the regression line for the 2 ml single injection was larger (pooled R^2 0·70) than that associated with the 20 ml single injection or the continuous infusion of 130 ml/d (pooled R^2 0·87 and 0·89 respectively). The best fit among the methods considered was that following infusion of 1000 ml infusate/d (pooled R^2 0·97).

There were no significant differences between the treatments for the measurements of $T_{\frac{1}{2}}$, pool size or flow-rate in Expt 3 (Table 3). The large variation in the estimated DM flow with the single injection of 2 ml ^{51}Cr-EDTA solution was associated with large variation in both $T_{\frac{1}{2}}$ and pool size. The flows of DM through the caecal pool calculated from the marker kinetics were not significantly different from the flow of DM determined by total collection of faeces. The pool size of digesta water (458 ± 153 ml) estimated by ^{51}Cr-EDTA kinetics was not significantly different from the amount of digesta water in the entire caecum and proximal colon (592 ± 80 ml) subsequently measured by slaughter of the same sheep given the same diet (R. M. Dixon, unpublished results). The DM content of digesta in the caecum (Table 3) during infusion of 1000 ml/d (116 g/kg) was significantly less than that with the other treatments (128–133 g/kg).

DISCUSSION

General

In biological situations some deviation usually occurs from ideal steady-state conditions (Shipley & Clark, 1972). However, it has become generally accepted that providing precautions are taken such as ensuring that food is consumed in many small portions throughout the experimental period, acceptable quantitative measurements of transformations of metabolites can be made in the rumen and blood.

Observations of digesta flow through the terminal ileum have suggested that the flow entering the caecum tends to occur as pulses rather than as a continuous flow (Hogan & Phillipson, 1960; Ash & Kay, 1963; Goodall & Kay, 1965). Furthermore, motility of the caecum is such that partial or complete emptying of the digesta from the caecum tends to occur at intervals of 0·5–4 h (MacRae *et al.* 1973). These irregularities in inflow, outflow and pool size of the caecum may result in considerable deviation from ideal steady-state conditions over a period of only a few hours. However, these observations also suggest that net changes over longer periods (e.g. 12–36 h) are unlikely.

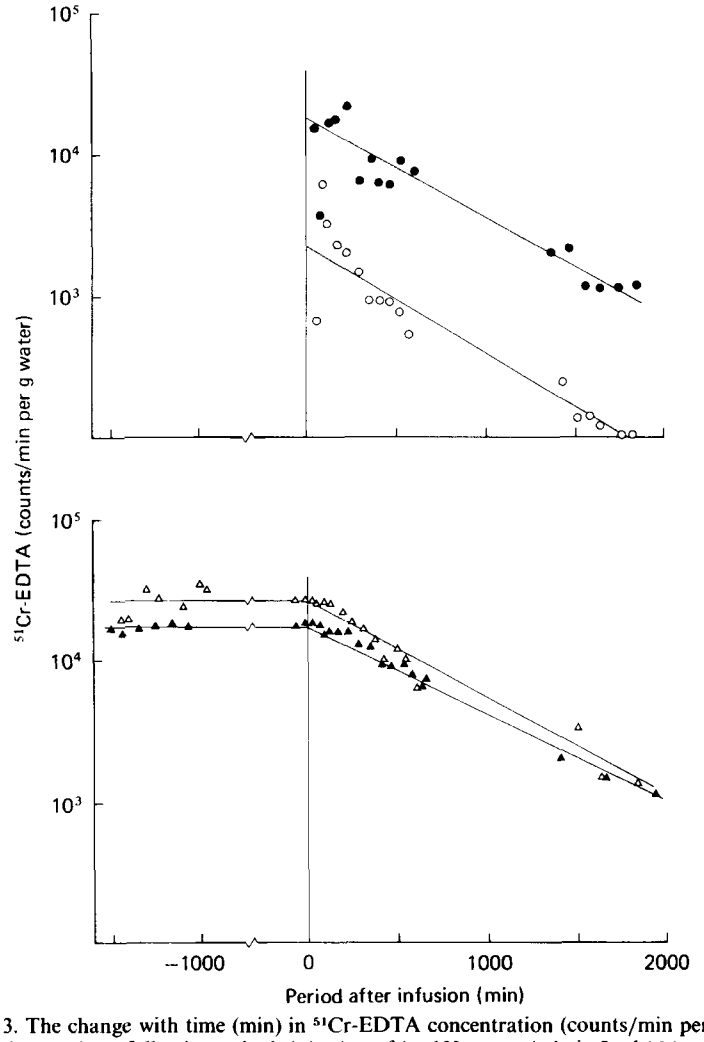

Fig. 1. Expt. 3. The change with time (min) in ^{51}Cr-EDTA concentration (counts/min per g water) in caecal digesta in one sheep following a single injection of 1×10^6 counts/min in 2 ml (○) and 20 ml (●) saline (0·15 M-sodium chloride) or during and following continuous infusions of 5×10^6 counts/min per d in 130 ml (△) and 1000 ml (▲) saline/d.

During a continuous infusion of tracer into the caecum, providing that samples are obtained at random over a period equivalent to many fluctuations in tracer concentration in the kinetic pool in the caecum, it is to be expected that the mean of the samples obtained on 'plateau' will be an unbiased estimate of the tracer concentration 'plateau'. This has been confirmed by computer simulation of tracer infusions with cyclical changes in pool size and flow of tracee into the pool (Rowe, 1978) and is compatible with the theoretical model discussed by Shipley & Clark (1972). For single injection experiments the effects of irregular flows through the caecum should be similar to these effects during continuous infusion experiments. If a large number of samples are obtained at random over a period equivalent to many fluctuations of flow through the pool, an unbiased estimate of the tracer concentration *v.* time curve should be obtained. Since in the single injection experiments sampling of caecal digesta was over a period equivalent to many fluctuations of flow through

Table 3. *Expt 3. Digesta kinetics of the caecal pool of four sheep given lucerne* (Medicago sativa) *measured by four methods of administration of* ^{51}*Cr-EDTA, faecal* DM *flow measured by total collection of faeces, and volume of water in the caecum and proximal colon measured at slaughter*

(Values given for treatments 1–4 are the means obtained in a 4 × 4 Latin Square design experiment)

	Single injection		Continuous infusion			
Treatment	1 2 ml	2 20 ml	3 130 ml/d	4 1000 ml/d	SEM	Over-all mean
DM content (g/kg)	132	133	128	116	1·7	127
$T_{\frac{1}{2}}$ (min)	523	388	370	423	214	426
Pool size (ml water)	614	374	299	543	217	458
Flow (ml water/d)	1516	963	1129	1840	669	1362
Flow (g DM/d)	233	148	180	241	95·5	201
Flow (g DM/d) measured by total collection of faeces (*n* 4)					6	194
Volume (ml water) of caecum and proximal colon (*n* 4)					80	592

DM, dry matter.

the caecum, negligible bias of this origin was probably introduced into measurement of digesta kinetics in the present studies, or those of Hecker (1971).

Considerable error may occur in single injection experiments where the tracer concentration *v.* time curve must be accurately estimated over a period which is short relative to the fluctuations of flow through the caecal pool. This may be necessary when tracers are used which have a short $T_{\frac{1}{2}}$ (e.g. [^{14}C]acetate or $H^{14}CO_3^-$). In addition, if tracers which are rapidly and extensively recycled to the primary pool in the caecum are used (e.g. $^{15}NH_4^+$), the tracer concentration *v.* time curve may not be defined sufficiently accurately for multi-compartmental models to be fitted to the data. Bias also would occur in the tracer concentration *v.* time curve following single injection if the injection of marker or handling of the animal during injection caused an emptying of digesta from the caecum, an event which tends to occur when cold solutions or when large volumes (20–100 ml) are injected into the caecum (D. W. Dellow, unpublished results). Furthermore, if the caecum remains in a quiescent phase for some time after such an emptying (MacRae *et al.* 1973), the rate of flow of digesta (and hence of tracee and tracer) from the caecal pool would also be influenced.

Separation of digesta phases

The similarity of values for $T_{\frac{1}{2}}$ calculated using both ^{51}Cr-EDTA and ^{103}Ru-P content of caecal digesta following single injection in Expt 1 indicated that there was no appreciable separation in movement of the solid and liquid digesta fractions. This conclusion is in accord with results from previous experiments using roughage diets (Coombe & Kay, 1965; MacRae *et al.* 1973; Grovum & Williams, 1973) and in subsequent experiments ^{51}Cr-EDTA alone was used to measure rates of digesta flow through the caecum and colon. In addition, the similarity of the DM content of caecal digesta samples whether obtained through a cannula into the caecum or by total collection at slaughter indicated that both phases of caecal digesta were representatively sampled from the cannula.

Marker kinetics

The linearity of the decline of ln (marker concentration) *v.* time in both caecal digesta and faeces following single injection or termination of continuous infusion (Expts 1 and 3) provides strong evidence for the existence of a single kinetic pool of ^{51}Cr-EDTA and ^{103}Ru-P markers in the large intestine. This agrees with previous studies (Hecker, 1971; Grovum & Williams, 1973) where first-order kinetics of liquid and solid markers were observed in the caecum. Furthermore, the quantity of digesta contained in the caecum and proximal colon in the same or similar sheep at slaughter suggested that the kinetic pool of marker described the digesta contained in the entire caecum and proximal colon.

The estimates of the rates of flow of digesta with each of the diets were similar with all the methods used, i.e. by in vivo dilution of markers in the caecum with either single injection or continuous-infusion techniques, by recovery of marker in faeces during continuous infusion, by concentration of marker in digesta at slaughter, or by total collection of faeces. These findings show that the marker techniques used provided unbiased estimates of the rate of flow of digesta through the caecum.

In Expt 3 the large variability in all measurements associated with the single injection of 2 ml infusate indicated that this method of administration was not satisfactory. Single injection of 20 ml infusate and continuous infusion of 130 ml/d were more precise, but the most satisfactory kinetics were obtained with the high rate of infusion of 1000 ml/d. However, the improved tracer kinetics were associated with a reduced DM content of caecal digesta. The final choice of infusion rate for a particular experiment may depend on the parameter being measured, but it appears necessary to accept some alteration from normal physiological conditions to obtain acceptable mathematical errors associated with the kinetic analysis of tracer concentration.

In conclusion, continuous infusion of tracers in a large volume of infusate provided the most reliable technique for estimating tracee kinetics in the caecum with an acceptable error.

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