Rate of passage of digesta in sheep

4.* Passage of marker through the alimentary tract and the biological relevance of rate-constants derived from the changes in concentration of marker in faeces

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1. The rates of passage of markers of fluid and particulate digesta through the sheep alimentary tract have been described mathematically in single-injection experiments by analysing the concentration curves of marker excretion in faecal dry matter and then predicting these concentrations by means of an equation containing two exponential terms. Three components related to this equation are a transit time for digesta in the intestines, a half-time of marker in the reticulo-rumen and a half-time tentatively associated with the passage of marker through the caecum and proximal colon. With this approach no surgical interference with an animal is required to obtain meaningful information about gut function.

2. Abomasal cannulation did not affect the rate of passage of ^{51}Cr EDTA through the gut.

3. The mean half-times ($T_{\frac{1}{2}}$) for ^{51}Cr EDTA in the reticulo-rumen and hind-gut, respectively, were 646 and 236 min for a roughage diet and 890 and 607 min for a diet consisting mainly of wheat grain, both diets providing approximately the same amounts of digestible dry matter. These results indicated that the changes in half-times were proportionately much greater in the caecum and proximal colon than in the reticulo-rumen. The mean transit time of marker through the digestive tract also increased from 721 to 1345 min when the concentrate diet was given.

4. The complex of chromium-51 with EDTA was excreted faster in faeces than cerium-144–praseodymium-144 when both were given together as a single injection into the reticulo-rumen. This was caused mainly by differential rates of elimination of water and particulate matter from the reticulo-rumen.

Two rate-constants can be obtained from the cumulative excretion curve of a marker in the faeces of sheep after a single injection into the reticulo-rumen (Blaxter, Graham & Wainman, 1956). However, there is little experimental evidence as to the organs of the digestive tract to which these rate-constants apply.

Waldo, Miller, Okamoto & Moore (1965) and Putnam, Bond & Lehmann (1967) described the decreasing portion of the concentration curve of marker in the faeces of cattle by the average life and the half-life of marker respectively. The concept of half-life of marker in digesta has been used in the experiment now reported because it is simple and well defined and because it applies to passage of digesta through the abomasum, and caecum and proximal colon (Grovum & Williams, 1973) as well as the reticulo-rumen (Bullen, Scarisbrick & Maddock, 1953).

The purpose of this paper is to show the biological significance of half-times derived from concentration curves of marker in faeces and to use these values in mathematical equations to describe this pattern of marker excretion from sheep.

EXPERIMENTAL

Sheep

Mature merino wethers were used in Expt 2 (nos 1–3, 19–23), Expt 3 (nos 1–3, 13, 14), Expt 4 (nos 2, 6, 18) and Expt 5 (nos 1–3, 15–17).

Housing

The sheep were held in single pens in an animal house and were given their experimental rations for at least 3 weeks. They were then kept in metabolism cages for 5 d before experiments were begun and for the duration of the experimental periods. The experimental room was illuminated continuously.

Feeding techniques

Except for sheep no. 6, the animals in Expts 2, 3 and 4 were hand-fed with about one-eleventh of their daily food intake on the hour from 08.00 to 18.00 hours during the preliminary feeding period and while samples were collected. The automatic equipment used to feed sheep 6 and the sheep in Expt 5 (Minson & Cowper, 1966) delivered the ration in approximately equal amounts at hourly intervals throughout the preliminary and experimental periods. Water was available *ad lib*.

Surgery

All sheep except nos. 15–18 were fitted with abomasal fistulas (Jarrett, 1948). The sheep were allowed to recover from the surgery for a minimum of 14 d before any experimental work was started.

Radioactivity counting

Markers. The complex of chromium-51 with EDTA (supplied by Australian Atomic Energy Commission, Lucas Heights, NSW, Australia) (^{51}Cr EDTA) was used to mark the water phase of digesta (Downes & McDonald, 1964) and cerium-144-praseodymium-144 (Radiochemical Centre, Amersham, Bucks., England) was used to mark particulate matter (Miller, Perry, Chandler & Cragle, 1967; Ellis & Huston, 1968; Huston & Ellis, 1968). When Downes & McDonald (1964) injected ^{51}Cr EDTA into the rumen of sheep, from 3 to 5 % of the radiation was recovered in the urine within 7 d. Downes & McDonald also injected ^{51}Cr EDTA into the blood in other experiments but did not recover any of the radiation in either rumen fluid or faeces. This indicates that the marker is not recycled between plasma and the fluid fraction of digesta.

Equipment. A two-channel Packard Model 3002 Tricarb Scintillation Spectrometer (Packard Instrument Company Inc., Illinois 60515, USA) was used for counting the isotopes individually or together.

Correction for interference. Measurement of the mutual interference between ^{51}Cr and ^{144}Pr when counted together was described by Grovum & Williams (1973).
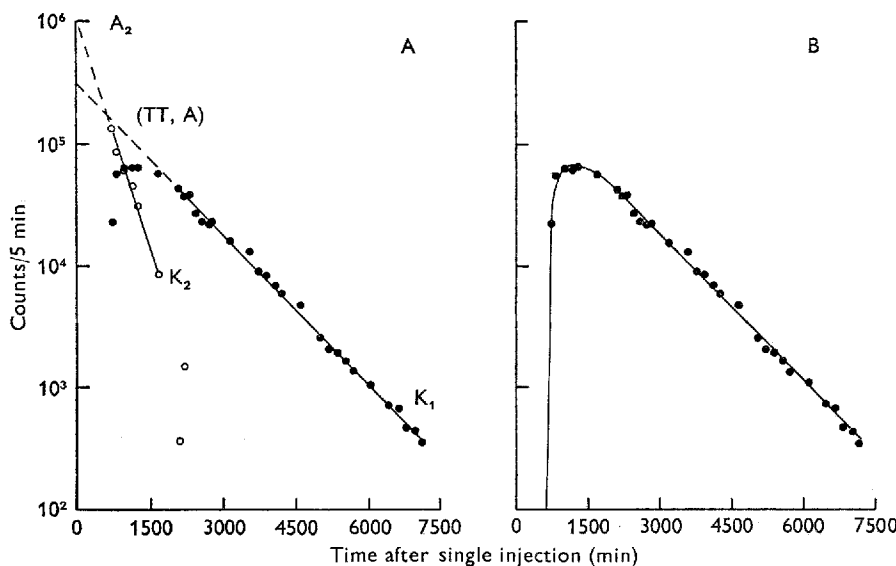
Fig. 1. (A) Diagrammatic representation of the analysis of a marker concentration curve in faeces after a single injection into the reticulo-rumen of a sheep given 800 g lucerne chaff/d. Values (●) are adjusted counts of ^{51}Cr EDTA/0·5 g faecal dry matter. Positive counts C (equation 2, p. 316) are indicated (○). A_1, k_1, A_2, k_2, A and TT have been defined on p. 316 and p. 317.

(B) The fit between ^{51}Cr EDTA concentrations in faecal dry matter (●) and calculated concentrations (solid line) obtained by solving the following equation for $t > 646$ min.

$$y = A\,e^{-k_1(t-TT)} - A\,e^{-k_2(t-TT)} = 175202\,e^{-0.00095(t-646)} - 175202\,e^{-0.00286(t-646)}$$

Preparation of samples

Reticulo-rumen. These samples were centrifuged at 2500 *g* for 30 min and 3 ml portions of the supernatant fraction were counted for ^{51}Cr.

Faecal samples. The three-pellet samples of fresh faeces, collected in duplicate in Expts 2–5, were dried in plastic scintillation tubes in a vacuum oven at 73°, transferred to a desiccator and weighed to the nearest mg before counting.

Adjustments to the counts of radioactivity in faeces

Each sample count was corrected for background, interference, counting time, weight and height for the pellet sample and for decay in the isotopes as measured by changes in the counting rates of standards.

Statistical methods

Sample times. The mid-points between successive times of sampling were used as the sample times for marker concentrations in faecal dry matter. When faeces were not available for collection, the times of sampling were still recorded.

Analysis of marker excretion results. ^{51}Cr EDTA was placed into the reticulo-rumen as a single injection by stomach tube and the changes in marker concentration in faecal dry matter were obtained over a 5–10 d period by counting three-pellet samples of faeces tha thad been defaecated by the sheep. Equation (1), which is similar to

Table 1. *Analysis of changes in concentration of marker in faeces after giving sheep a single injection of marker into the reticulo-rumen: an example of the method used to obtain sets of k_2, A_2, A and TT with k_1 and A_1 fixed*

Sample no.	Sample time (min)	Extra-polated* count (x)	Observed† corrected count (y)	Positive count (C) (x−y)	Set of C values used	$k_2 \times 10^3$	A_2	A	TT (min)
1	750	158734	23847	134887	—	—	—	—	—
2	850	144340	56152	88188	—	—	—	—	—
3	1000	125158	64092	61066	—	—	—	—	—
4	1155	108011	62151	45860	1–4	2·60	869431	183050	600
5	1275	96367	65532	30835	1–5	2·64	901550	181896	607
6	1680	65573	56962	8611	1–6	2·86	1109530	175202	646
7	2100	43988	43630	358	1–7	4·09	3925817	152192	794
8	2225	39060	37567	1493	1–8	3·62	2389281	158954	749

A_1, k_1, A_2, k_2, A and TT are defined below and on p. 317.
* Extrapolated count $= A_1 e^{-k_1 t} = 323817 e^{-0.00095t}$.
† Counts of ^{51}Cr EDTA/0·50 g faecal dry matter per 5 min.

equation no. 4 used by Blaxter *et al.* (1956), was used to describe the concentration curve of marker in faecal dry matter (Fig. 1 B).

$$y = A e^{-k_1(t-TT)} - A e^{-k_2(t-TT)} \quad \text{for} \quad t \geqslant TT,$$
$$y = 0 \quad \text{for} \quad t < TT, \tag{1}$$

where y and A are adjusted marker concentrations in faecal dry matter, k_1 and k_2 are rate-constants, TT is a calculated time for first appearance of marker in faeces and t is the sample time in min after the single injection. The meanings of k_1, k_2, A and TT are illustrated diagrammatically in Fig. 1 A.

The terminal portion of the marker concentration curve on semi-logarithmic graph paper was linear (Fig. 1 A). The absolute value of the regression coefficient obtained from the natural logarithms of the faecal counts in the linear portion of this curve was called k_1. The point at which to start this regression analysis was selected by eye from a plot of the marker concentrations so that values in the rising or peak portion of the curve did not appear to bias the regression coefficient. The point selected was as near the peak as possible. A_1 (Fig. 1 A) is the antilogarithm of the intercept calculated from the transformed values. A_1 and k_1 were then held constant and equation (2), described generally by Rescigno & Segre (1966), was used to calculate the positive counts (C) corresponding to each sample time t for the rising and peak portion of the curve (Table 1):

$$C = A_1 e^{-k_1 t} - \text{adjusted three-pellet count at time } t. \tag{2}$$

The rate-constant k_2 in the example given in Fig. 1 A was obtained from regression analysis involving the natural logarithms of the first six values of C given in Table 1, where sample 1 refers to the faecal sample in which marker was first detected. The absolute value of the regression coefficient (0·00286) was called k_2. The y intercept A_2 (1109530) shown in Fig. 1 A was obtained by taking the antilogarithms of that calculated in the regression analysis. The extrapolated terminal exponential intersected

Table 2. *Method of fitting a curve to concentration changes of marker in faecal dry matter* (DM) (*Fig.* 1) *of sheep.* A_1 (323 817) *and* k_1 (0·00095) *values were obtained with sample nos 7–31. The five columns of goodness of fit correspond with the five sets of* k_2, A_2, A *and TT values given in Table* 1

Sample no.	Sample time (min)	Observed count/ 0·5 g DM per 5 min	Observed count × 100 ÷ calculated count knowing k_1, k_2, A, TT and t in equation 1 (p. 316)				
			Sample nos for calculation of k_2, A_2, A and TT				
			1–7	1–8	1–6*	1–5	1–4
1	750	23 847	100	3870	84	70	69
2	850	56 152	242	164	121	116	115
3	1 000	64 092	108	105	104	106	106
4	1 155	62 151	85	87	93	95	96
5	1 275	65 532	87	90	97	101	101
6	1 680	56 962	93	95	101	104	105
7	2 100	43 630	101	102	106	108	108
8	2 225	37 567	97	98	101	103	103
9	2 345	38 839	112	113	116	118	118
10	2 460	27 119	87	88	90	91	91
11	2 590	23 924	87	87	89	90	90
12	2 720	22 641	93	93	95	95	96
13	2 830	23 062	105	105	107	107	108
14	3 180	15 789	100	100	101	102	102
15	3 570	13 403	123	123	124	124	124
16	3 750	9 056	99	99	99	99	99
17	3 915	8 415	107	107	108	108	108
18	4 095	6 756	102	102	102	103	103
19	4 260	5 900	105	105	105	105	105
20	4 620	4 773	119	119	119	119	119
21	5 010	2 588	94	94	94	94	94
22	5 200	2 051	89	89	89	89	89
23	5 372	1 921	98	98	98	98	98
24	5 552	1 639	99	99	99	99	99
25	5 710	1 359	96	96	96	96	96
26	6 070	1 031	102	102	102	102	102
27	6 460	688	99	99	99	99	99
28	6 645	657	112	112	112	113	113
29	6 810	467	94	94	94	94	94
30	6 975	428	100	100	100	100	100
31	7 140	341	94	94	94	94	94

* Column of best fit between observed and calculated values

the extrapolated regression line through the first six values of C at a count called A (175 202) and at a calculated time for first appearance of marker named TT (Fig. 1A). Thus:

$$A_1 e^{-k_1 TT} = A_2 e^{-k_2 TT} = 175\,202,$$

and the TT value of 646 was calculated with equation (3):

$$TT = \frac{\ln A_2 - \ln A_1}{k_2 - k_1}. \tag{3}$$

The value of y in equation (1) is 0·0 when $t = TT$.

These final values of k_2, A and TT were obtained as follows: separate k_2, A_2, A and

Table 3. *Comparisons of values of the rate constants k_1 and k_2 (equation 1, p. 316) and of the constant m (equation 4, below) used to describe the changes in concentration of ^{51}Cr EDTA in faecal dry matter following single injections into the reticulo-rumen of sheep*

Ration (g/d) ...	$k_1 \times 100$	$k_2 \times 100$	k_2/k_1	$m \times 100$	m/k_1
Lucerne chaff (LC)-800	0·0757	0·425	5·61	0·117	1·55
LC – 800	0·0825	0·297	3·60	0·141	1·71
LC – 800	0·1010	0·341	3·38	0·153	1·51
LC – 800	0·0867	0·286	3·30	0·127	1·46
LC – 800	0·0995	0·255	2·56	0·136	1·37
Wheat – 300, LC – 400	0·0957	0·210	2·19	0·108	1·13
LC – 800	0·133	0·244	1·83	0·164	1·23
Wheaten chaff – 800	0·118	0·213	1·81	0·147	1·25
Wheat – 500, LC – 100	0·0745	0·116	1·56	0·0860	1·15
Wheat – 100, LC – 650	0·0883	0·129	1·46	0·0980	1·11
Wheat – 500, LC – 100	0·0816	0·113	1·38	0·0920	1·13
LC – 800	0·151	0·205	1·36	0·160	1·06

TT values were calculated using values of *C* for sample nos 1–4, 1–5, ..., 1–x, where *x* is the number of the last sample having a positive value for *C* (Table 1). *X* is 8 for the example given in Table 1 and Fig. 1. For each set of k_2, A_2, *A* and *TT*, each observed concentration of marker ($n = 31$ in Fig. 1) was expressed as a percentage of a calculated concentration obtained by substituting numbers for k_2, *A*, *TT* and k_1 in equation (1) for the appropriate sample time *t* (Table 2). The values of k_2, *A* and *TT* for sample nos 1–6 were then selected because they gave the best fit by subjective assessment between observations and the calculated concentrations, the best fit being characterized by percentage values which were close to and alternated frequently about 100 from the first to the last sample number. This criterion of fit was used because, in each experiment, the range of concentrations of marker was large. Grovum (1971) reported six more examples of the curve-fitting procedure illustrated in Table 2. The independent selections by the authors of the best fits in these examples were in good agreement.

The half-time of marker in a pool 0·693/*k* has been notated as $T_{\frac{1}{2}}$. Also, in order that all measurements associated with marker excretion can be expressed in min, the half-times $T_{\frac{1}{2}k_1}$ (0·693/k_1) and $T_{\frac{1}{2}k_2}$ (0·693/k_2) have been used in place of their respective rate-constants.

Equation (1) cannot be used to describe the concentration curve of marker excretion in faecal dry matter when $k_1 = k_2$ because in this instance the predicted concentration of marker (*y*) is equal to zero for all sample times. However, such curves can be described with the equation:

$$y = mEv\, e^{-mv}, \qquad (4)$$

where *y* and *E* are marker concentrations, *v* is the time of sampling in min after the estimated time of first appearance of marker in faecal dry matter, and *m* is the absolute value of the regression coefficient obtained as follows. The observed concentrations of marker in dry matter (*y*) are divided by the respective values of *v*. The natural logarithms of the quotients (*y*/*v*) are related in simple regression analysis to the values of *v*. Thus $y/v = mE\, e^{-mv}$ and $\ln(y/v) = \ln(mE) - mv$, which is the equation for a straight

line, ln (mE) being the intercept on the y axis and m being the slope. The value m is not a rate-constant and should not be divided into 0·693 to obtain a half-time. Equation (4) may be integrated to obtain an equation which is similar in form to one reported by Blaxter *et al.* (1956, p. 76).

Values of m were obtained for concentration curves of ^{51}Cr EDTA in faecal dry matter, having $k_2:k_1$ ratios ranging from 5·61 to 1·36 (Table 3). The differences among the magnitudes of k_1, k_2 and m decreased as the ratio $k_2:k_1$ approached 1·36. Rescigno & Segre (1966) have shown that equation 4 can be derived from an equation similar to equation (1) when $k_1 = k_2 = m$. In this series of papers, the rate of passage of markers through the gut was described with equation 1 because reasonably good fits between the observed and calculated concentrations of markers in faecal dry matter were always obtained and because the rate-constants k_1 and k_2 appeared to be more meaningful biologically than the single value of m.

Experiment 1

The purpose of the experiment was to compare the magnitudes of k_1, k_2, TT, A, A_1 and A_2 for concentration curves of marker in faecal dry matter, obtained with the method described and with FLXNLR, a mathematical curve-fitting programme written by the late Mr L. R. Harris, Computer Centre, University of New England, Armidale, NSW 2351, Australia. In this programme, an iterative procedure was used whereby initial estimates of k_1, k_2, A_1 and A_2 were altered sequentially, to minimize a weighted variance about the curve, and refined until their magnitudes were altered by less than 1 % of their preceding values (White, Steel, Leng & Luick, 1969). The variance was calculated as

$$\Sigma \left(\frac{\text{observed value} - \text{expected value}}{\text{expected value}} \right)^2.$$

Eighty-seven different excretion curves resulting from single injections of ^{51}Cr EDTA or ^{144}Pr into the reticulo-rumen were analysed by (*a*) the method described and (*b*) FLXNLR. The results were accumulated over a period of 3 years from fifteen different sheep given various diets and various levels of lucerne chaff/d. Most of this information will be reported in this series of papers. The effects of sheep or diets were not studied. Paired estimates of $T_{\frac{1}{2}k_1}$, $T_{\frac{1}{2}k_2}$, TT, A, A_1 and A_2 were compared by Student's t test.

Experiment 2

The effect of abomasal cannulation on marker kinetics in the digestive tract was studied. ^{51}Cr EDTA was administered into the reticulo-rumen of eight sheep as a single injection before and after abomasal cannulation. On each occasion three-pellet samples of voided faeces were obtained periodically in duplicate and the rates of excretion of marker were determined. The animals were given 800 g lucerne chaff/d. They were allowed a minimum of 2 weeks to recover from surgery before being injected with marker.

Experiment 3

Part 1. The purpose was to compare the concentration changes of ^{51}Cr EDTA in the reticulo-rumen fluid with subsequent concentration changes of this marker in faecal dry matter over a 5 d period. A single-injection technique was used.

Sheep nos 1–3 and 13 were given 800 g lucerne chaff/d and sheep no. 14 received 600 g wheaten chaff/d. ^{51}Cr EDTA in 200 ml distilled water was placed in the reticulo-rumen of each sheep by stomach tube. Samples of reticulo-rumen contents were obtained daily for 5 d by means of a stomach tube attached to a hand vacuum pump. Voided faeces were sampled periodically in duplicate over 5 d. The three pellet samples were dried and counted and then the marker concentration changes were analysed.

Part 2. When faecal ^{51}Cr EDTA counts from part 1 decreased to negligible values a single injection of ^{51}Cr EDTA in 40 ml distilled water was given into the abomasum of all sheep except no. 14. Duplicate faecal samples for counting were obtained over a 3 d period. The half-times ($T_{\frac{1}{2}CPC}$) determined from these samples (Grovum & Williams, 1973) were compared with $T_{\frac{1}{2}k_2}$ values derived from the marker excretion curves produced in part 1.

Experiment 4

The purpose of this experiment was to test the response of $T_{\frac{1}{2}k_1}$, $T_{\frac{1}{2}k_2}$ and TT to changes in diet. No design was used. The marker excretion information was accumulated over a period of 3 years.

The three rations used consisted of 800 g lucerne chaff/d (1), 300 g wheat + 400 g lucerne chaff/d (2) and 500 g wheat + 100 g lucerne chaff/d (3). Each ration was given to sheep nos 2 and 18 on separate occasions. Sheep no. 6 received rations 1 and 2 but died before receiving ration 3. Digestibility was not measured, but the calculated digestible dry-matter intakes were similar for these three rations.

^{51}Cr EDTA in 200 ml distilled water was administered as a single injection by stomach tube into the reticulo-rumen, and duplicate three-pellet samples of faeces were obtained over a 5–9 d period.

Experiment 5

Part 1. This experiment was conducted to find whether the differential passage of ^{51}Cr EDTA and ^{144}Pr from the reticulo-rumen described by Grovum & Williams (1973) could also be detected in faecal dry matter.

The sheep were given 800 g lucerne chaff/d. ^{51}Cr EDTA and ^{144}Ce–^{144}Pr in 200 ml distilled water were administered by stomach tube as a single injection into the reticulo-rumen. The samples of voided faeces obtained in duplicate over a 5 d period were dried and counted to determine ^{51}Cr EDTA and ^{144}Pr concentrations in the faecal dry matter.

Part 2. A comparison was made of $T_{\frac{1}{2}k_2}$ values from part 1 and other half-times obtained by injecting markers into the abomasum and sampling faeces ($T_{\frac{1}{2}CPC}$).

^{51}Cr EDTA and ^{144}Ce–^{144}Pr in 40 ml distilled water were given as a single injection into the abomasum of sheep nos 1–3 when faecal marker concentrations from part 1 were negligible. Faecal samples were obtained over a 3 d period.

Table 4. *Comparison of two methods of analysing changes in concentration of marker in faecal dry matter after single injections of markers were placed into the reticulo-rumen of sheep*

	Mean result ($n = 87$)				
	This paper (1)	FLXNLR* (2)	(1)/(2)	SE of pair differences	Significance of Student's t test
$T_{\frac{1}{2}k_1}$	773·6	764·0	1·01	3·9	$P < 0·01$
$T_{\frac{1}{2}k_2}$	284·7	298·3	0·95	4·9	$P < 0·005$
TT	782·5	769·5	1·02	7·08	$P < 0·05$
A	171 480	193 746	0·89	7 025	$P < 0·005$
A_1	372 890	423 279	0·88	17 175	$P < 0·005$
A_2	3 381 942	3 807 974	0·89	373 616	NS

k_1, A_1, k_2, A_2, A and TT are defined on pages 316 and 317.
NS, not significant.
* A mathematical curve-fitting programme written by the late Mr L. R. Harris of the Computer Centre, University of New England, Armidale, NSW 2351, Australia.

RESULTS

Expt 1. Comparison of methods used to calculate $T_{\frac{1}{2}k_1}$, $T_{\frac{1}{2}k_2}$, TT, A, A_1 and A_2

The means of $T_{\frac{1}{2}k_1}$, $T_{\frac{1}{2}k_2}$, TT, A and A_1 for methods in this paper and FLXNLR were significantly different when Student's t test was used on pair differences (Table 4). However, the differences between the means of $T_{\frac{1}{2}k_1}$, $T_{\frac{1}{2}k_2}$ and TT were small.

Expt 2. Effect of abomasal cannulation

There were no significant changes in TT, $T_{\frac{1}{2}k_1}$ or $T_{\frac{1}{2}k_2}$ when the Student's t test was used on pair differences before and after surgery.

Expt 3. Biological significance of $T_{\frac{1}{2}k_1}$ and $T_{\frac{1}{2}k_2}$

There was no significant difference between the $T_{\frac{1}{2}}$ of ^{51}Cr EDTA in reticulo-rumen supernatant fraction and $T_{\frac{1}{2}k_1}$ for the marker in faecal dry matter when Student's t test was used on pair differences from the five sheep. The $T_{\frac{1}{2}}$ of marker in the rumen varied from 94 to 108 % of $T_{\frac{1}{2}k_1}$ in faeces. The mean half-times were 829 and 826 min respectively. The results for sheep no. 3, which were the most variable, are shown in Fig. 2. Regarding the reticulo-rumen, values of points 1 and 2 and similar subsequent pairs lie on opposite sides of the regression line. The $T_{\frac{1}{2}}$ of the marker in the rumen was smaller during the day than during the night.

The mean of $T_{\frac{1}{2}k_2}$ values obtained in part 1 for sheep eating 800 g lucerne chaff/d (264 min) was 66 % of the mean $T_{\frac{1}{2}}$CPC (400 min) obtained in part 2. The difference was significant ($P < 0·005$) by Student's t test on pair differences. The range of individual percentage figures was 60–76.

Similar results were obtained when FLXNLR was used to analyse the curves of marker concentrations.
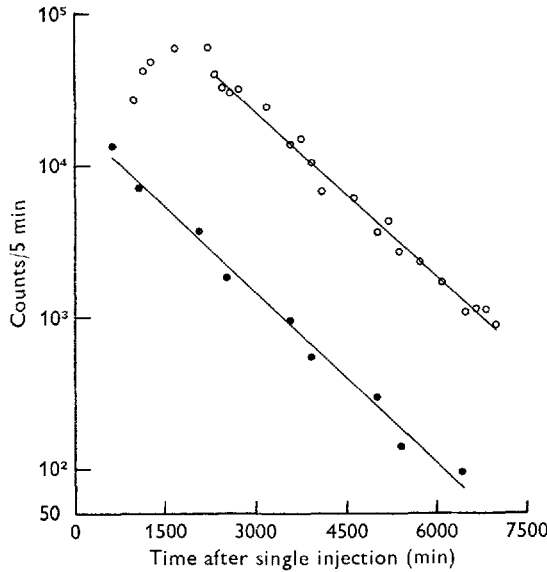
Fig. 2. Changes in concentration of ^{51}Cr EDTA in 3 ml samples of reticulo-rumen fluid (●) and the subsequent changes in concentration in 0·5 g faecal dry matter (○) of a sheep given 800 g lucerne chaff/d. Lines of best fit have been drawn through the terminal exponential in faeces and the values for the reticulo-rumen. $T_{\frac{1}{2}}$ for rumen sampling is 795 min; $T_{\frac{1}{2}k_1}$ for faecal sampling is 825 min.

Expt 4. Response of $T_{\frac{1}{2}k_1}$, $T_{\frac{1}{2}k_2}$ and TT to dietary treatments

The ^{51}Cr EDTA excretion values and the calculated curves for each sheep and dietary treatment are shown in Fig. 3. The magnitudes of TT, $T_{\frac{1}{2}k_1}$ and $T_{\frac{1}{2}k_2}$ were similar for sheep nos 2 and 18, given rations 1 and 2. There were large increases in TT, $T_{\frac{1}{2}k_2}$ and $T_{\frac{1}{2}k_1}$ between rations 1 or 2 and ration 3. The means for TT, $T_{\frac{1}{2}k_2}$ and $T_{\frac{1}{2}k_1}$ were 721, 236 and 646 min respectively for ration 1 and 1345, 607 and 890 min respectively for ration 3. The means of TT and $T_{\frac{1}{2}k_2}$ for sheep nos 2 and 18 increased by 87% and 157% respectively between rations 1 and 3, but $T_{\frac{1}{2}k_1}$ increased by only 38%.

TT, $T_{\frac{1}{2}k_2}$ and $T_{\frac{1}{2}k_1}$ for sheep no. 6 were all larger for ration 2 than for ration 1 (Fig. 3).

The curves calculated from equation (1) accounted for the large changes in marker excretion patterns in faecal dry matter between rations. However, some irregularities in marker concentration were not accounted for in the curve-fitting procedure.

The use of FLXNLR to analyse these marker concentration curves did not influence the interpretation of the results.

Expt 5. Differential rates of appearance of ^{51}Cr EDTA and ^{144}Pr in faeces

The $T_{\frac{1}{2}k_1}$ for ^{51}Cr EDTA in faecal dry matter was smaller in each sheep than that for ^{144}Pr (Fig. 4). The slopes were significantly different for the six sheep ($P < 0.05$) by an analysis of covariance. Similar results were obtained when FLXNLR was used to analyse the marker excretion values in part 1.

The mean $T_{\frac{1}{2}k_2}$ for ^{51}Cr EDTA and ^{144}Pr (264 min) was 65% of that for corresponding $T_{\frac{1}{2}}$CPG values (409 min). The difference was significant ($P < 0.005$).
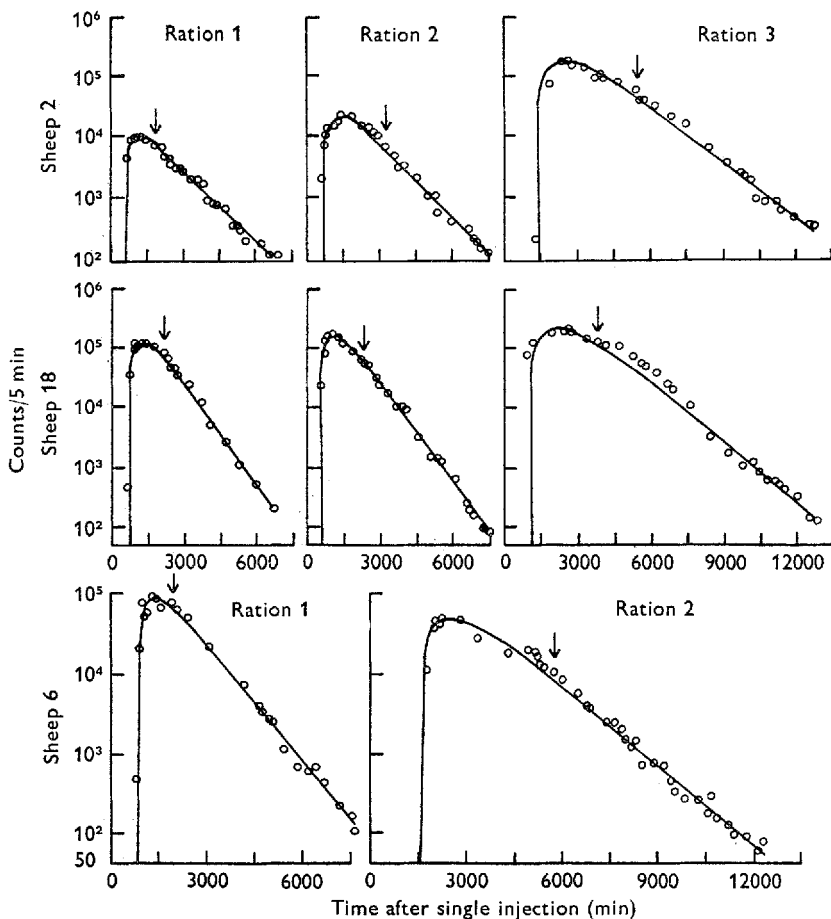
Fig. 3. Effect of ration composition on changes in ^{51}Cr EDTA concentration in 0·5 g faecal dry matter after single injections of marker into the reticulo-rumen of sheep; O, observed values; curve calculated from equation 1 (p. 316) (solid line). The arrows indicate the first value used in the regression analyses to calculate $T_{\frac{1}{2}k_1}$. The concentrations plotted for sheep no. 18 given ration 1 are ten times the values obtained. Ration 1, 800 g lucerne chaff; ration 2, 300 g wheat, 400 g lucerne chaff; ration 3, 500 g wheat, 100 g lucerne chaff.

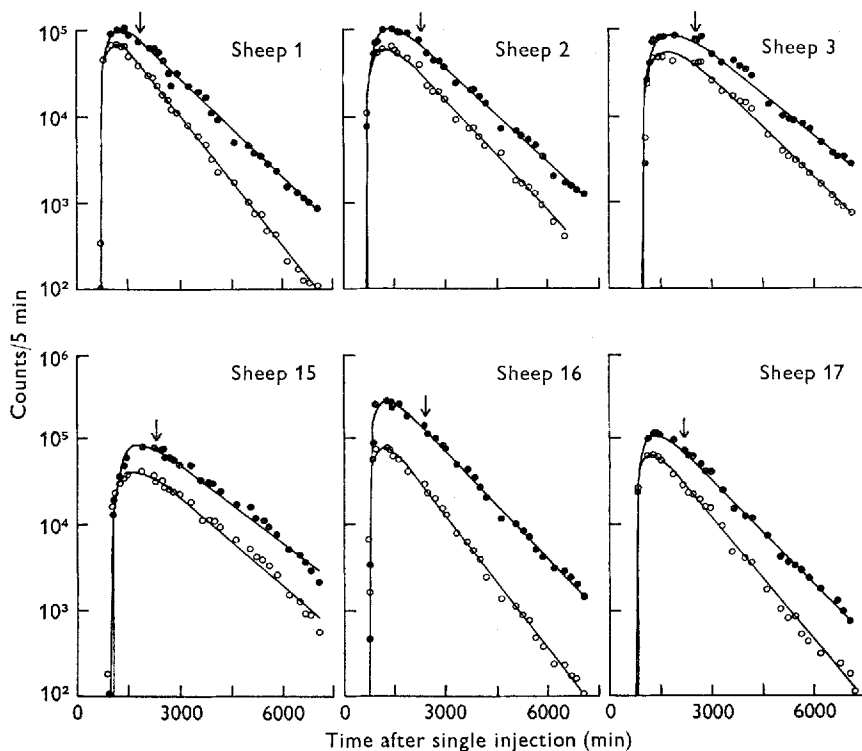| Ration	Sheep no.	TT	$T_{\frac{1}{2}k_2}$	$T_{\frac{1}{2}k_1}$
1	2	679	187	769
	18	763	284	522
	6	873	244	618
2	2	752	330	725
	18	561	245	549
	6	1 571	503	890
3	2	1 547	596	931
	18	1 143	617	849
	6	—	—	—

Fig. 4. Differential rates of appearance of ^{51}Cr EDTA (O) and ^{144}Pr (●) in 0·5 g faecal dry matter after a single injection of both markers into the reticulo-rumen of sheep given 800 g lucerne chaff/d. The solid lines are curves calculated from equation 1 (p. 316). The arrows indicate the first value used in the regression analyses to calculate $T_{\frac{1}{2}k_1}$. The ^{144}Pr concentrations for all sheep except no. 16 have been multiplied by 0·5 to facilitate plotting of the results.

Sheep no.	^{144}Pr			^{51}Cr EDTA		
	TT	$T_{\frac{1}{2}k_2}$	$T_{\frac{1}{2}k_1}$	TT	$T_{\frac{1}{2}k_2}$	$T_{\frac{1}{2}k_1}$
1	739	201	788	717	188	585
2	726	253	825	668	233	697
3	960	377	903	980	332	756
15	1019	293	981	917	303	863
16	743	195	728	715	179	580
17	806	205	743	763	170	627

DISCUSSION

The mathematical model

Single injections of ^{51}Cr EDTA and ^{144}Pr were eliminated exponentially from the reticulo-rumen, abomasum, and caecum and proximal colon in sheep with good appetite (Grovum & Williams, 1973). The half-times of markers placed in the abomasum were small compared with those for each of the other two organs. A two-compartment system (equation 1) has been used to describe the changes in concentration of marker in faecal dry matter because good fits of calculated curves to results were produced (Figs. 1B, 3, 4) and because it seemed meaningless to try to extract a third relatively small compartment from the results when the number of points in the initial

portions of the marker concentration curves were limited. Also marker concentrations were the most variable in samples collected just after first appearance (unpublished results). The influence of the abomasum on the marker excretion pattern has probably been incorporated into $T_{\frac{1}{2}k_2}$. However, the abomasal component in $T_{\frac{1}{2}k_2}$ should be small.

Subjective assessments were used to calculate $T_{\frac{1}{2}k_1}$ and $T_{\frac{1}{2}k_2}$ and TT from marker concentration curves in faecal dry matter by the method described in the text. The results obtained were similar in magnitude although significantly different from those obtained with FLXNLR. However, the differences between the values of $T_{\frac{1}{2}k_1}$, $T_{\frac{1}{2}k_2}$ and TT would probably be well within the repeatability for a sheep in a given physiological state and therefore either method is satisfactory. This is supported by the fact that the interpretation of results presented was the same with both forms of analyses. A computer programme written to analyse the marker concentration curves as described in this paper is at least ten times faster than FLXNLR. This economy in computer time is important in reducing the cost of each experiment. The biological significance of A, A_1 or A_2 is undefined. Therefore no meaningful comment can be made about the differences between the two forms of analysis.

TT is a measure of the transit time of marker through the omasum and the small and large intestines because the difference between thirty-one values of TT reported in this paper and corresponding times of first appearance of marker in faeces were not significantly different by Student's t test on pair differences. The means were 772 and 776 min respectively. The transit time of ^{51}Cr EDTA through the reticulo-rumen and abomasum should be close to zero because a large number of radioactive atoms were injected and mixing of the entire contents of the organs would cause some marker to leave almost immediately after entry. This argument may not apply to the caecum and proximal colon, since its mixing of digesta appears to be imperfect (Grovum & Williams, 1973). The gradient of digesta water content along the organ (Grovum & Hecker, 1973) also indicated a general distal movement of contents. The mixing of the entire volume of digesta was incomplete.

$T_{\frac{1}{2}k_1}$ reflects the rate of removal of marker from the reticulo-rumen of sheep (Fig. 2). Thus the kinetics of digesta in the reticulo-rumen can now be obtained without rumen cannulation and without disturbing the sheep by taking samples with a stomach tube.

The reason why $T_{\frac{1}{2}k_2}$ for ^{51}Cr EDTA was consistently less than $T_{\frac{1}{2}CPC}$ is difficult to explain. Single injections of markers were always given between 21.00 and 24.00 hours. If there was a diurnal pattern of digesta mixing in the caecum and proximal colon, $T_{\frac{1}{2}k_2}$ might be affected differently from the $T_{\frac{1}{2}CPC}$ values because $T_{\frac{1}{2}k_2}$ was determined over a shorter period of time. The effect of time of injection on $T_{\frac{1}{2}k_2}$ has not been evaluated. However, the good fits between the concentrations of ^{144}Pr and ^{51}Cr EDTA in faeces and the calculated curves indicate that $T_{\frac{1}{2}k_2}$ described the way digesta and marker passed through the hind-gut. Warner & Stacy (1968) stated that complete mixing of ^{51}Cr EDTA injected into the reticulo-rumen required about 1·5 h. Therefore the relatively broad peak of the marker concentration curve in faeces does not simply reflect the changes in concentration of the marker in the rumen. Since the half-times of ^{144}Pr and ^{51}Cr EDTA were relatively small in the abomasum compared

with those in the caecum and proximal colon (Grovum & Williams, 1973) there is reason to associate $T_{\frac{1}{2}k_2}$ with the kinetics of digesta flowing through the caecum and proximal colon. This argument is enhanced by the fact that $T_{\frac{1}{2}k_2}$ was positively correlated ($P < 0.005$) with $T_{\frac{1}{2}CPC}$ when sheep were given different amounts of lucerne chaff (Grovum & Williams, unpublished). The changes in $T_{\frac{1}{2}k_2}$ therefore probably reflect differences in the times that digesta spend in the caecum and proximal colon, but the values for average retention times $(1/k_2)$ may not be the true times that digesta spend in these organs.

Blaxter *et al.* (1956) gave a single injection of stained food into the reticulo-rumen of sheep and described the curve of cumulative stained particle excretion in faeces with an equation containing three constants k_1, k_2 and τ. There is no doubt that their constants and equation described the marker excretion values presented. The best-fitting curve for their results had a minimum for residual sum of squares and zero sum of residuals (K. L. Blaxter, private communication). A comparison of the fourth equation used by Blaxter *et al.* (1956) and equation 1 in this paper shows that the same basic equations have been used but that their k_2 and k_1 refer to our k_1 and k_2 respectively. Blaxter *et al.* (1956) stated that they had no direct biological evidence as to the meaning of k_1, k_2 and τ but they tentatively associated their k_1, the larger rate-constant (shorter retention time) with the reticulo-rumen. Because of the differences in terminology and the relative magnitudes of their rate-constants, it is clear that their k_2 and k_1 are more likely to be associated with the reticulo-rumen and caecum–proximal colon respectively. Our direct experimentation, with diets of lucerne chaff, has always shown that the markers ^{51}Cr EDTA and ^{144}Pr spent more time in the reticulo-rumen than in the caecum and proximal colon of each sheep studied. In our terminology k_1 ($0.693/T_{\frac{1}{2}k_1}$) has always been less than k_2 ($T_{\frac{1}{2}k_1} > T_{\frac{1}{2}k_2}$) and in their work k_2 was always less than k_1 except for the sheep given 600 g long, dried grass, in which the rate-constants were equal. The latter situation of having equal rate-constants is no doubt real for the marker excretion curve obtained but it could reflect the difficulties in the use of stained food particles as markers (Ellis & Huston, 1967) rather than the times available for digestion in different parts of the gut. It is unlikely that the elimination of stained hay particles from the reticulo-rumen could be described with an exponential equation of one term ($y = A\ e^{-kt}$) as their concentration in contents from the reticulo-rumen of cows (Balch, 1950) increased to a maximum from 5 to 30 h after injection and then decreased with time. This phenomenon would affect the shape of the cumulative excretion curve of stained hay particles in the faeces and hence the values of k_1 and k_2 obtained by Blaxter *et al.* (1956).

Brandt & Thacker (1958) re-analysed the marker excretion results of Balch, Balch, Bartlett, Johnson, Rowland & Turner (1954) to obtain half-times T_2 and T_1 for the reticulo-rumen and rest of the gut, respectively, in cows. Their T_2 values for each diet were greater than T_1. Shellenberger & Kesler (1961) also found that T_2 was greater than T_1 in cows. Other studies with adult cattle (Balch, 1950; King & Moore, 1957; Campling, Freer & Balch, 1961; Campling & Freer, 1962), sheep (Weston, 1968), and calves (Miller, Moss, Hall & Gorman, 1969) have shown that markers administered as single injections into the abomasum or duodenum were excreted in faeces faster than

similar markers placed into the reticulo-rumen. These results support the thesis that the half-time of marker in the reticulo-rumen is larger than that in the caecum and proximal colon.

Experiment 4. Effects of ration on excretion patterns

The rate of marker excretion from sheep was slower when diets composed mainly of wheat were given instead of all-roughage rations. This is generally supported by results for sheep (Eng, Riewe, Craig & Smith, 1964; Leaver, Campling & Holmes, 1969) and cattle (Balch *et al.*, 1954; Balch, Balch, Bartlett, Bartrum, Johnson, Rowland & Turner, 1955; Montgomery & Baumgardt, 1965). However, the results of Freer & Campling (1963) and Bines & Davey (1970) for cattle do not support this conclusion. Ruckebusch (1970) found that feeding sheep on a grain diet in place of a roughage diet decreased the rate of passage of marker through the gut distal to the duodenum and also decreased total gut activity, especially the peristaltic component. Our results indicate that the rate of propulsion of digesta in the intestines, as judged by increased values of TT, was decreased with the concentrate rations. This may result from a reduced flow of indigestible bulk through the intestines.

The changes in TT, $T_{\frac{1}{2}k_2}$ and $T_{\frac{1}{2}k_1}$ indicate that the hind-gut was more than 50% responsible for the extended pattern of marker excretion with the concentrate diet. The differences in $T_{\frac{1}{2}k_1}$ values between rations 1 and 3 were smaller quantitatively and relatively than the changes in $T_{\frac{1}{2}k_2}$.

Although the calculated curves did not account for irregularities in some of the marker excretion results (Fig. 3) it is clear that TT, $T_{\frac{1}{2}k_2}$ and $T_{\frac{1}{2}k_1}$ responded to the effects of changes in diet and that equation (1) applied over large changes in the patterns of marker excretion.

Experiment 5. Differential rates of appearance of ^{51}Cr EDTA and ^{144}Pr in faeces

The smaller values of $T_{\frac{1}{2}k_1}$ for ^{51}Cr EDTA than for ^{144}Pr are a reflection of the smaller half-times of ^{51}Cr EDTA in the reticulo-rumen (Grovum & Williams, 1973). Ellis & Huston (1968) showed that the water-soluble marker polyethylene glycol passed through the digestive tracts of sheep faster than ^{144}Ce–^{144}Pr.

The mathematical analysis used in this paper to describe marker excretion from sheep partitions total mean retention time into TT, $T_{\frac{1}{2}k_1}$ and $T_{\frac{1}{2}k_2}$. Measurements such as the time for 50% excretion of marker have limited biological usefulness because all parts of the gut that affect marker excretion have different and probably changing roles in digestion, depending on diet, form of ration, level of food intake and so on. Thus, to correctly assess the potential for cellulose digestion in the reticulo-rumen and caecum and proximal colon it would probably be necessary to weight $T_{\frac{1}{2}k_1}$ and $T_{\frac{1}{2}k_2}$ respectively with estimates of the cellulolytic activity in these organs. Little information on this subject is available in the literature. Hecker (1971) reported similar rates of breakdown of cotton thread in the reticulo-rumen and caecum of sheep fed *ad lib.* on meadow hay, but Hungate, Phillips, McGregor, Hungate & Buechner (1959) found relatively little microbial fermentation in the contents of the large

intestines of several African herbivores. The cellulolytic activity is low in the reticulo-rumen of sheep (Moir & Harris, 1962; Coombe & Tribe, 1963; Hemsley & Moir, 1963) and cattle (Campling *et al.* 1961, 1962) given diets with a low nitrogen content. This activity is also low in cattle given either ground and pelleted roughage *ad lib.* (Campling, Freer & Balch, 1963) or concentrate diets (Freer & Campling, 1963). Thus large average retention times $(1/k)$ of marker in the rumen need not infer extensive digestion of cellulose. The amounts of digestion in different parts of the gut should be related to respective average retention times of marker to obtain the actual rates of digestion. Coombe & Tribe (1963) have shown a significant positive correlation between the retention time of stained food particles in the rumen of sheep and the time for 50% digestion of cotton thread in the rumen. Phillips, Hungate, McGregor & Hungate (1960) reported a significant negative correlation between rumen retention time and fermentation rate in cattle, but neither of these values was significantly related to digestibility. More research of this nature would contribute markedly to the understanding of the physiology of digestion.

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