Rate of passage of digesta in sheep

6. The effect of level of food intake on mathematical predictions of the kinetics of digesta in the reticulorumen and intestines

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1. Five sheep were given 400, 625, 850, 1075 and 1300 g lucerne chaff/d according to a 5 × 5 Latin square design to perturbate a mathematical model describing the rate of passage of digesta in terms of rate constants for the reticulo-rumen (k_1) and caecum and proximal colon (k_2), and a transit time (TT) for the intestines. These values were obtained from the concentration curves of ^{51}Cr EDTA and ^{144}Ce-^{144}Pr (^{144}Pr) excretion in faeces for comparison with similar measurements obtained directly.

2. The retention times of markers in the reticulo-rumen, caecum and proximal colon and intestines all decreased by approximately 50 % as intakes were increased from 400 to 1300 g/d. For both markers, the direct and indirect measures of half-time in the reticulo-rumen were essentially identical. The predicted (indirect) values for half times in the caecum and proximal colon were smaller than the direct values ($P < 0{\cdot}005$) but the correlations between them were significant ($P < 0{\cdot}01$) for ^{51}Cr EDTA (r 0·66) and ^{144}Pr (r 0·78). The direct measures of transit time were smaller ($P < 0{\cdot}025$) by 5–10 % than the predicted values but correlations between them were significant ($P < 0{\cdot}01$) for ^{51}Cr EDTA (r 0·91) and for ^{144}Pr (r 0·93). Thus the model predicted the changes produced in the rate of passage of digesta and its usefulness and limitations are discussed.

3. With ^{144}Pr $T_{\frac{1}{2}}k_1$ for the reticulo-rumen and $T_{\frac{1}{2}}k_2$ for the caecum and proximal colon were both positively correlated ($P < 0{\cdot}025$) with the organic matter digestibilities. $T_{\frac{1}{2}}k_2$ decreased at half the rate of $T_{\frac{1}{2}}k_1$ with increasing intakes. This would have favoured compensatory digestion in the large intestine.

This experiment in pertubation analysis was designed to evaluate further a mathematical model of digesta passage through the alimentary tract of the sheep. Grovum & Williams (1973*c*) showed that the rate of passage of digesta in sheep given either 800 g lucerne chaff or diets consisting of wheat and lucerne chaff could be described with an equation having as independent variables: the transit time of marker through the intestinal tract (TT), the half-time of marker in the reticulo-rumen ($T_{\frac{1}{2}}k_1$) and the half-time of marker pertaining mainly to the caecum and proximal colon ($T_{\frac{1}{2}}k_2$).

In the experiment now reported, the rate of passage of digesta through the gut was increased by increasing the level of food intake and values of TT, $T_{\frac{1}{2}}k_1$, and $T_{\frac{1}{2}}k_2$ were compared with corresponding measurements made more directly. The biological relevance of these values for rate of passage was studied by relating them to the apparent digestibility of the food, the concentrations of volatile fatty acids (VFA) in the reticulo-rumen and the water content of the faeces.

EXPERIMENTAL

Sheep

Three Merino wethers (castrated males) (sheep nos. 1, 2 and 3) and two ewes (sheep nos. 4 and 5) with permanent rumen (Hecker, 1969) and abomasal (Jarrett, 1948) fistulas were

used. Their body-weights while being fed on 800 g lucerne chaff/d were 36, 48, 41, 34 and 48 kg respectively. Their faeces and food were free of gastrointestinal parasites before the experiment began.

Feeding and housing

Lucerne chaff for the entire experiment was bulked and mixed before experimental work began. Daily rations for each sheep were weighed into plastic bags at the beginning of each 21 d period. The dry-matter (DM) content of the food for each sheep was determined for each period.

Three automatic feeders (Minson & Cowper, 1966) were mounted above metabolism cages to give each sheep approximately one-twenty-fourth of its daily ration at hourly intervals. Food that was spilt on the floor and on the bottom of the metabolism cages was returned to the food bucket for the sheep concerned. Water was freely available. The experimental room was illuminated continuously.

Design of the experiment

All sheep were given 800 g lucerne chaff/d for 14 d before the experiment commenced. Period, sheep and food treatments were arranged in a 5×5 Latin square design. Each period consisted of 21 d, 10 d being the preliminary period and the remaining 11 d the collection period. The treatments were 400, 625, 850, 1075 and 1300 g air-dry lucerne chaff/d. The outputs of wet and dry faeces were determined daily throughout each period. The residual effects of treatments within sheep did not appear to be a problem as in each period the daily outputs of faeces fluctuated about the mean value for the 11 d collection period with no trend being evident over the time period of the collection. Sheep no. 4 did not consume 1300 g lucerne chaff/d and therefore there was one missing observation in period no. 4 for all measurements made.

The time-sequence of marker injection, and of digesta and faeces sampling, during each experimental period is shown in Table 1.

Water intake and urine and faeces outputs

Daily intakes of water and outputs of urine and faeces were measured at 16.00 hours throughout the experiment. Faeces outputs from days 10 to 21 in each period were adjusted for samples taken to analyse the rate of passage of markers and then used to calculate coefficients of organic matter digestibility (DOM).

DM and ash contents of food and faeces

The daily faeces DM output was obtained for each sheep from duplicate 100 g subsamples of fresh faeces dried at 100° for 4–8 d. No check was made to determine DM losses due to heating the samples. Representative samples of food given to each sheep during each period were dried at 100° for 4 d and were found to contain 845 ± 7 g DM/kg (mean $\pm$ SD; n 25).

Samples of dry food and faeces were ashed in duplicate at 800° for 24 h to calculate coefficients of DOM. The amounts of sodium and potassium volatilized would have been a negligible proportion of the DM.

Markers

The marker used for the water phase of digesta was ^{51}Cr EDTA (Downes & McDonald, 1964) and ^{144}Ce–^{144}Pr (^{144}Pr) was used as the marker for the particulate matter (Miller, Perry, Chandler & Cragle, 1967; Ellis & Huston, 1968; Huston & Ellis, 1968).

Table 1. *The schedule for each experimental period in an experiment in which five sheep were studied in a* 5×5 *Latin square design*

Experimental period (d of experiment)	Experimental procedure
1–9	Preliminary feeding period
9	Single injection of ^{51}Cr EDTA into the abomasum at 24.00 hours
10	Faeces were sampled every 0·5 h from 07.00 to 18.00 hours and every hour from 19.00 to 24.00 hours
11 and 12	Faeces were sampled hourly from 07.00 to 24.00 hours
12	Single injection of ^{51}Cr EDTA and ^{144}Ce–^{144}Pr into the reticulo-rumen at 24.00 hours
13	Reticulo-rumen contents were sampled at 01.00, 09.00, 14.00, 17.00 and 24.00 hours Faeces were sampled half-hourly from 07.00 to 24.00 hours
14	Reticulo-rumen contents were sampled at 09.00, 14.00, 17.00 and 24.00 hours Faeces were sampled half-hourly from 00.00 to 03.00 hours and from 08.00 to 24.00 hours
15	Reticulo-rumen contents were sampled at 18.00 hours Faeces were sampled hourly from 16.00 to 18.00 hours
16	Reticulo-rumen contents were sampled at 09.00, 14.00, 17.00 and 24.00 hours Faeces were sampled hourly from 07.00 to 22.00 hours
17	Faeces were sampled hourly from 07.00 to 22.00 hours
18	Faeces were sampled hourly from 07.00 to 22.00 hours Rumen contents were sampled once for volatile fatty acid (VFA) analysis
19	Faeces were sampled hourly from 07.00 to 22.00 hours
20	Faeces were sampled hourly from 07.00 to 22.00 hours Rumen contents were sampled once for VFA analysis
21	Faeces were sampled hourly from 07.00 to 22.00 hours
9–12	Faeces sampled for measurement of $T_{\frac{1}{2}CPC}$*
12–21	Faeces sampled for measurement of $T_{\frac{1}{2}}k_1$, $T_{\frac{1}{2}}k_2$ and TT*
13–18	Rumen contents sampled for measurement of total rumen water and dry matter, and half times of ^{51}Cr EDTA and ^{144}Ce–^{144}Pr
11–21	Water intake and urine output measured. The outputs of faeces were used to calculate digestibility coefficients

* For definition of variables, see p. 428.

Preparation of the reticulo-rumen samples

The reticulo-rumen samples were collected into bottles containing mercuric chloride crystals, shaken and then frozen. The samples were thawed and the digesta were centrifuged at 2500 *g* for 30 min. The supernatant fluid was used to determine the concentrations of ^{51}Cr EDTA and total VFA. The particulate matter from appropriate samples was dried in an oven at 100° for 6 d and then ground with a steel rod for ^{144}Pr determination.

VFA determination

The total concentration of VFA in reticulo-rumen supernatant fluid was determined in a Markham still (Briggs, Hogan & Reid, 1957).

^{51}Cr EDTA and ^{144}Pr determinations in reticulo-rumen contents and faeces DM

The isotopes were determined individually or together. The method given by Grovum & Williams (1973*b*) was used to adjust for the interference of one isotope in the determination of the other when they were determined together.

Samples of reticulo-rumen fluid (3 ml) were analysed for radioactivity contents of ^{51}Cr and ^{144}Pr. Adjustments were made to the ^{51}Cr value for the small amount of interference from ^{144}Ce and ^{144}Pr. Ground samples of DM from the reticulo-rumen were added to plastic scintillation-tubes to constant depth and analysed for ^{51}Cr and ^{144}Pr. The ^{144}Pr values were adjusted for ^{51}Cr interference.

Values for half-times of ^{51}Cr and ^{144}Pr in the reticulo-rumen were determined by regression analyses on the natural logarithms of their concentrations in fluid and DM respectively after the effects of background and interference were subtracted.

For faeces DM the methods used were described by Grovum & Williams (1973*b*).

Measurement of total rumen contents

Water. The equation given by Warner & Stacy (1968) was used. The ^{51}Cr EDTA concentration changes in the reticulo-rumen were determined from samples collected over a 4 d period as shown in Table 1. Extrapolation errors were not checked because the sheep were fed every hour.

DM. Equation no. 1 was used to estimate the quantities of DM in the reticulo-rumen. This method does not account for the additions to the food in the rumen of electrolytes from saliva or for the absorption of a portion of the end-products of fermentation.

$$\text{DM (g)} = \frac{\text{food DM intake (g/min)} \times T_{\frac{1}{2}} \text{ for } ^{144}Pr \text{ in the rumen (min)}}{0{\cdot}693}. \quad (1)$$

Flow-rate of water from the reticulo-rumen

This was determined from the following equation:

$$\text{flow-rate (ml/min)} = \text{total volume (ml)} \times \frac{0{\cdot}693}{T_{\frac{1}{2}} \text{ for } ^{51}Cr \text{ EDTA (min)}}. \quad (2)$$

Rate of passage of digesta estimated from changes in marker concentration in faeces

Caecum-proximal colon. A single injection of ^{51}Cr EDTA in 40 ml distilled water was given into the abomasum and duplicate three-pellet samples of voided faeces were collected, dried and counted. A value of half-time of digesta in the caecum and proximal colon ($T_{\frac{1}{2}CPC}$) was obtained from the terminal part of the concentration curve of marker in faeces DM (Grovum & Williams, 1973*b*). The values in the 'increasing' and 'peak' portions of the curve were ignored to eliminate any influence of marker passage through the abomasum and small intestine.

Reticulo-rumen ($T_{\frac{1}{2}}k_1$) *and caecum and proximal colon* ($T_{\frac{1}{2}}k_2$)

A single injection of ^{144}Pr and ^{51}Cr EDTA in 200 ml distilled water was given into the reticulo-rumen and duplicate three-pellet samples of faeces were collected, dried and counted. The concentration changes of markers in faecal DM were analysed (Grovum & Williams, 1973*c*) and described in their entirety by equation no. 3:

$$y = Ae^{-k_1(t-TT)} - Ae^{-k_2(t-TT)}, \quad (3)$$

where y and A are concentrations of marker in faeces DM, k_1 is the rate constant describing marker kinetics in the reticulo-rumen, k_2 is the rate constant pertaining mainly to digesta kinetics in the caecum and proximal colon (in this paper $T_{\frac{1}{2}}k_1$ and $T_{\frac{1}{2}}k_2$ are used instead of k_1 and k_2 respectively ($T_{\frac{1}{2}} = 0{\cdot}693 \div k$), t is the period after injection of marker, calculated as the mid-point between successive periods of sampling faeces, and TT is the transit time of marker through the omasum and small and large intestines.

Correlations between measured variables

To avoid high spurious correlations between measured variables, such as VFA concentration in the rumen and $T_{\frac{1}{2}}k_1$, it was necessary to do the regression analyses on 'refined' observations devoid of the effects of sheep, periods and treatments. A 'refined' observation was thus equal to the observation plus three times the general mean from the analysis of variance minus the period mean including the observation, minus the treatment mean

minus the sheep mean. These adjustments to the observations were not made, however, in correlating DOM *v.* $T_{\frac{1}{2}}k_1$ and $T_{\frac{1}{2}}k_2$ for ^{144}Pr since it is argued that digestibility decreased as intake was increased because the period available for digestion and absorption decreased.

RESULTS

There were simple positive correlations (Table 2) between the amount of food consumed and the weight of water ($P < 0{\cdot}05$) and dry digesta ($P < 0{\cdot}005$) in the reticulo-rumen, the flow rates of water from the reticulo-rumen ($P < 0{\cdot}005$) and the total concentrations of VFA in rumen fluid ($P < 0{\cdot}005$). Simple negative correlations (Table 2) existed between food intake and the apparent DOM, all measures of the transit time of markers through the intestines $T_{\frac{1}{2}CPC}$, $T_{\frac{1}{2}}k_1$ and $T_{\frac{1}{2}}k_2$ for both markers ($P < 0{\cdot}005$). The rate of decrease of $T_{\frac{1}{2}}k_2$ with increases in food intake was about half that for $T_{\frac{1}{2}}k_1$ (Table 2). The level of food intake was not significantly correlated in a rectilinear manner with the proportion of DM in faeces (Table 2). The mean values for the various intakes of food are given in Table 2.

Rate of passage measurements

Comparison of direct and indirect values. The direct and indirect measures of retention times of either ^{144}Pr or ^{51}Cr EDTA in the reticulo-rumen were not significantly different in magnitude according to Student's paired *t* test (Table 3). The values of $T_{\frac{1}{2}}k_2$ for either ^{144}Pr or ^{51}Cr EDTA were significantly less than corresponding values for $T_{\frac{1}{2}CPC}$ ($P < 0{\cdot}005$) but for each comparison values of half-times were significantly correlated ($P < 0{\cdot}01$; Table 3). The regression equations were as follows:

$$T_{\frac{1}{2}CPC} = 25 + 1{\cdot}20 \pm 18\,\% \; T_{\frac{1}{2}}k_2 \text{ for } ^{144}\text{Pr (residual standard deviation (RSD) 103)}, \quad (4)$$

$$T_{\frac{1}{2}CPC} = 143 + 1{\cdot}07 \pm 24\,\% \; T_{\frac{1}{2}}k_2 \text{ for } ^{51}\text{Cr EDTA (RSD 122)}. \quad (5)$$

The range in values of $T_{\frac{1}{2}}k_2$ for ^{144}Pr were 165–768 min and corresponding values for ^{51}Cr EDTA were 57–760 min. Since $T_{\frac{1}{2}CPC}$ was the same for both ^{144}Pr and ^{51}Cr EDTA (Grovum & Williams, 1973*b*) it was meaningful to correlate $T_{\frac{1}{2}}k_2$ values for ^{144}Pr with $T_{\frac{1}{2}CPC}$ of ^{51}Cr EDTA.

The intervals after which markers first appeared in faeces after single injections into the rumen were significantly less than the values of *TT* for either ^{144}Pr or ^{51}Cr EDTA ($P < 0{\cdot}025$) but the values were highly correlated ($P < 0{\cdot}01$; Table 3).

Comparison of markers. The values of $T_{\frac{1}{2}}k_1$, $T_{\frac{1}{2}}k_2$ and *TT* with ^{144}Pr were significantly greater than corresponding measurements with ^{51}Cr EDTA (Table 3) according to Student's paired *t* test ($P < 0{\cdot}001$, $P < 0{\cdot}005$, $P < 0{\cdot}025$ respectively). However, significant correlations existed ($P < 0{\cdot}01$) between values for the two markers for $T_{\frac{1}{2}}k_1$ (r 0·94), $T_{\frac{1}{2}}k_2$ (r 0·73) and *TT* (r 0·97). The amount of food consumed did not affect the magnitude of the differences between $T_{\frac{1}{2}}k_1$ of ^{144}Pr and ^{51}Cr EDTA. However, the mean differences were approximately 6 and 14 % of $T_{\frac{1}{2}}k_1$ values for ^{144}Pr at 400 and 1300 g/d respectively (Table 2).

DOM in relation to $T_{\frac{1}{2}}k_1$ *and* $T_{\frac{1}{2}}k_2$

There was a positive correlation between apparent DOM and $T_{\frac{1}{2}}k_1$ for ^{144}Pr which was significant (equation no. 6; $P < 0{\cdot}005$). The relationship between DOM and $T_{\frac{1}{2}}k_2$ was more variable but was significant (equation no. 7; $P < 0{\cdot}025$). The equations were as follows:

$$\text{DOM} = 60{\cdot}30 + 0{\cdot}0049 \pm 20\,\% \; T_{\frac{1}{2}}k_1 \text{ for } ^{144}\text{Pr (RSD 1·09, } r + 0{\cdot}73), \quad (6)$$

$$\text{DOM} = 62{\cdot}62 + 0{\cdot}0055 \pm 38\,\% \; T_{\frac{1}{2}}k_2 \text{ for}_2 \; ^{144}\text{Pr (RSD 1·39, } r + 0{\cdot}49). \quad (7)$$

Table 2. *Summary of analyses of variance of a 5 × 5 Latin square experiment to study the effect of food intake on digesta kinetics in sheep, with complementary regression relationships of measured variables on dry-matter* (DM) *intake*

Measurement (y)	n	Treatment means†					Treatment effect	SE of treatment means	y intercept	Regression of y v. DM intake† Slope	SE of slope (%)	r	Statistical significance of r
Food (g/d)	—	400	625	850	1075	1300	—	—	—	—	—	—	—
DM intake (g/d)	1	339	529	716	907	1098	****	4	—	—	—	—	—
OM digestibility	1	0·657	0·651	0·645	0·634	0·631	****	0·0088	0·6701	−0·000037	25	−0·64	****
Reticulo-rumen VFA (mmol/l)	2	72·1	86·1	95·0	99·7	116·0	****	8·08	57·21	0·050	21	+0·72	****
Rumen volume (g water)	1	5041	5690	6660	7478	7214	*	1156	4139	3·17	43	+0·44	*
Flow-rate of water (ml/min)	1	3·14	4·47	6·35	7·79	9·21	****	0·94	0·29	0·0083	9	+0·92	****
Dry rumen contents (g)	1	387	484	613	623	702	****	72	281	0·39	20	+0·73	****
Water intake (g/d)	11	842	1215	1702	2095	2596	****	96	58·9	2·26	7	+0·96	****
Urine output (ml/d)	11	344	549	745	798	951	****	83	125	0·77	10	+0·91	****
Interval before first appearance (min)‡	2	953	843	700	584	490	****	115	1144	−0·59	18	−0·77	****
$T_{\frac{1}{2}CPC}$ (min)	1	637	484	361	291	252	***	75	768	−0·43	11	−0·82	****
TT§: ^{144}Pr (min)	1	1076	895	799	684	534	****	125	1267	−0·64	18	−0·76	****
^{51}Cr EDTA (min)	1	1003	861	746	661	509	****	82	1185	−0·59	16	−0·80	****
$T_{\frac{1}{2}}k_1$§: ^{144}Pr (min)	1	1169	931	790	657	602	****	106	1363	−0·74	13	−0·86	****
^{51}Cr EDTA (min)	1	1103	876	699	603	519	****	114	1308	−0·77	14	−0·83	****
$T_{\frac{1}{2}}k_2$§: ^{144}Pr (min)	1	504	355	272	264	211	**	106	575	−0·35	23	−0·69	****
^{51}Cr EDTA (min)	1	429	296	209	178	129	****	84	508	−0·36	25	−0·65	****
Faeces (g DM/kg)	11	355	401	407	394	376	**	22					NS
Faeces ash (g/kg DM)	2	121	113	112	112	108	****	3	124·2	−0·016	26	−0·63	****

OM, organic matter; VFA, volatile fatty acids; ^{144}Pr, ^{144}Ce–^{144}Pr; n, no. of replications contributing to each observation; NS, not significant.
* $P < 0·05$, ** $P < 0·025$, *** $P < 0·01$, **** $P < 0·005$.
† The missing observations calculated for the analyses of variance were included in the treatment means but were not used in the regression analyses.
‡ Time taken for markers to pass through the omasum and intestines and first appear in faeces.
§ For definition of variables, see p. 428.

Table 3. *Comparisons of various retention times of digesta obtained directly and indirectly with sheep given lucerne chaff intakes ranging from* 400 *to* 1300 *g/d*

	Retention time (min)								
	Reticulo-rumen			Caecum and proximal colon			Transit time (min)		
Variable† . . . Marker	*n*	Direct $T_{\frac{1}{2}}$	Indirect $T_{\frac{1}{2}}k_1$	*n*	Direct $T_{\frac{1}{2}CPC}$	Indirect $T_{\frac{1}{2}}k_2$	*n*	Direct First appearance‡	Indirect *TT*
^{144}Ce–^{144}Pr	22	857	836	23	396	308	24	730	815
Statistical significance of difference (paired *t* test)		NS			***			*	
r		0·86(**)			+0·78(**)			+0·93(**)	
^{51}Cr EDTA	23	775	760	23	396	237	24	730	772
Statistical significance of difference (paired *t* test)		NS			***			*	
r		0·91(**)			+0·66(**)			+0·91(**)	

* $P < 0{\cdot}025$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}005$.
NS, not significant.
† For definition of variables, see p. 428.
‡ Time taken for the marker which was injected into the reticulo-rumen to first appear in faeces.

Table 4. *Summary of transit times of* ^{51}Cr *EDTA through the intestinal tract and of retention times of* ^{51}Cr *EDTA and digesta in the small and distal large intestines respectively of sheep given lucerne chaff intakes ranging from* 400 *to* 1300 *g/d*

(The no. of observations are indicated in parentheses)

			Retention time (min)		
Lucerne chaff intake (g/d)	Transit time of ^{51}Cr EDTA (min)	Lucerne chaff intake (g/d)	Small intestine* (*a*)	Large intestine† (*b*)	Total *a*+*b*
400	1003 (5)	400	136 (5)	639 (7)	775
850	746 (5)	800	—	488 (8)	—
1300	545 (4)	1200	91 (5)	285 (6)	376

* From Grovum & Williams (1973*a*).
† Values for the distal 80 % of the large intestine from Grovum & Hecker (1973).

Relationships between other measured variables

After removing the effects of food intake, sheep and periods from the observations, significant correlations were found between DOM and VFA concentration in the reticulo-rumen (r +0·44, $P < 0{\cdot}05$), and between *TT* for ^{51}Cr EDTA and the ash content of faeces (r −0·45, $P < 0{\cdot}05$) and the DM content of faeces (r +0·48, $P < 0{\cdot}05$). However, the correlation between VFA concentration in the reticulo-rumen and $T_{\frac{1}{2}}k_1$ for ^{51}Cr EDTA was not significant.

DISCUSSION

Weights of rumen contents and flow-rates of water

The amounts of dry digesta calculated to be in the reticulo-rumen averaged 18 % greater than those found in 'slaughter' experiments with sheep given 400, 800 and 1200 g lucerne

chaff, the values being (g; mean ± SE) 339 ± 21 (*n* 7), 456 ± 42 (*n* 6), 598 ± 51 (*n* 5) respectively (Grovum & Williams, unpublished results). The regression of amount of dry digesta *v.* food intake in the 'slaughter' experiment was significant ($P < 0{\cdot}005$; $r = 0{\cdot}78$) and the value for the regression coefficient, 0·32, agreed reasonably well with that of 0·39 reported in Table 2. The volumes of water in the reticulo-rumen (Table 2) were larger by 66 % than those found in the 'slaughter' experiment in which they were (ml; mean ± SE) 3684 ± 255 (*n* 7), 3315 ± 385 (*n* 6) and 4402 ± 327 (*n* 5) respectively. The regression of volume of water *v.* intake in the 'slaughter' experiment was not significant. The reason for these discrepant results is not known. However, the rates of flow of water from the reticulo-rumen (Table 2) are in good agreement with those reported by Grovum & Williams (1973*a*) for sheep given 400 and 1200 g lucerne chaff/d.

Marker kinetics in the digestive tract

General. The fact that the direct and indirect measures of the rates of passage of digesta in the reticulo-rumen, the caecum and proximal colon and the remainder of the intestines were significantly correlated, demonstrated further the biological relevance of k_1, k_2 and *TT*. The increased rate of passage of marker through the gut with increased intakes of food in the experiment now reported agrees with other results for sheep (Blaxter, Graham & Wainman, 1956; Coombe & Tribe, 1963; Leaver, Campling & Holmes, 1969; Bawden, 1970), cattle (Campling, Freer & Balch, 1961; Shellenberger & Kesler, 1961; Balch & Campling, 1965) and pigs (Castle & Castle, 1957). Increased rates of passage of digesta with increased intakes have also been demonstrated in the reticulo-rumen of cows (Mäkelä, 1956; Paloheimo & Mäkelä, 1959; Minson, 1966) and in the intestines of sheep (Coombe & Kay, 1965; Grovum & Hecker, 1973; Grovum & Williams, 1973*a*) and cattle (Campling, Freer & Balch, 1962). Since the increased rates of passage occurring with increasing intakes were detected as well with the indirect measures of rate of passage (k_1, k_2, *TT*) as with the direct measurements, it follows that either may be used at least for qualitative studies where only the measurement of a change is important.

Blaxter *et al.* (1956) described the cumulative percentage recovery curve of stained food particles in faeces using a mathematical equation containing two rate constants. They also discussed equation no. 3 used in this experiment to describe the concentration curves of ^{51}Cr EDTA and ^{144}Pr in faecal dry matter. A comparison of the effect of level of food intake on rate constants described by them and in this paper is complicated because different markers were used. The elimination of stained particles from the reticulo-rumen as illustrated by Balch (1950) could not be described by first-order kinetics which applied to the markers used in this study (Grovum & Williams, 1973*b*) and hence this would preclude a simple explanation for one, if not both, of their rate constants. However, increasing the level of food intake in their study increased their values of k_1, and k_2 and reduced their values of τ equivalent to *TT* in this paper. Blaxter *et al.* (1956) did not produce evidence to account for the biological significance of their values of k_1, k_2 and τ.

k_1. There is no doubt that k_1 is quantitatively an accurate measure of the rate of turnover of marker in the reticulo-rumen. The ability to predict the retention time of marker and food in the reticulo-rumen by administering marker in a drench and collecting faeces will eliminate the need of surgical intervention in some experimental animals and it will facilitate studies of rates of passage in expensive breeding stock or in other valuable animals. As yet the heritability of turnover rate of reticulo-rumen contents has not been examined. Thornton & Minson (1972) found significant negative relationships between the retention time of dry matter in the reticulo-rumen and intake of dried-grass diets by sheep. Although in this instance the effect of retention time on intake may have been

Table 5. *Effect of level of lucerne chaff intake by sheep on the retention time (RT) of digest in the caecum-proximal colon and on half-times** $T_{\frac{1}{2}}k_2$ *and* $T_{\frac{1}{2}CPC}$ *(min) of* ^{51}Cr *EDTA*

(Values for no. of observations indicated in parentheses)

Food intake (g/d)	^{51}Cr EDTA $T_{\frac{1}{2}}k_2$ (min)	^{51}Cr EDTA $T_{\frac{1}{2}CPC}$ (min)	Food intake (g/d)	RT (min)†	Adjusted RT (min) (RT × 0·693‡)
400	429 (5)	681 (5)	400	1098	761
850	209 (5)	361 (5)	800	568	394
1300	162 (4)	243 (4)	1200	407	282

* For definition of variables, see p. 428.
† From Grovum & Hecker (1973); the values are for the first 20 % of the large intestine.
‡ Approximates to a half-time.

indirect through the lignin contents of the diets, it may be possible to select animals with a fast turnover rate of reticulo-rumen contents to increase intakes of roughages.

TT. The values of *TT* for ^{51}Cr EDTA and ^{144}Pr were significantly greater than the interval before first appearance (1·06 and 1·12 times greater respectively) but any one of these values is a satisfactory measure of the time taken for digesta passage through the omasum and the intestines since the differences between the measurements were small and consistent. The values of *TT* may have been greater than the values for the interval before first appearance because equation no. 3 did not describe the small 'tail' generally found at the beginning of the distribution curve of marker concentration in faeces.

A comparison of the effect of level of food intake on the retention times of digesta in the small and large intestines (Table 4) illustrates clearly that the decrease in *TT* with increasing intakes is accounted for mainly by a faster passage of digesta through the distal 80 % of the large intestine. The discrepancy between *TT* and the total of retention times in the small and large intestines may be due to a small omasal component in *TT* and to an underestimate in the retention time in the large intestine because the distal edge of the mixing pool in the caecum and proximal colon has not been defined. In this argument it is assumed that a mixing pool has a *TT* value of 0 min because marker should begin leaving the pool shortly after entry.

k_2. The quantitative significance of k_2 is somewhat uncertain. There is strong evidence that k_2 is a good qualitative measure of the rate of passage of digesta through the caecum and proximal colon as first-order kinetics apply to the elimination of marker from the organ (Grovum & Williams, 1973*b*) and in a physical model of the digestive tract k_2 accurately predicted the rate of turnover of the model caecum and proximal colon (Grovum & Phillips, 1973). Besides, what organ in the digestive tract of the sheep other than the caecum and proximal colon is capable of retaining digesta for an average of $\frac{1}{k_2}$ min? From Table 5 it is evident that the reduced retention times of contents in the caecum and proximal colon of sheep resulting from increasing the level of food intake were detected equally well by $T_{\frac{1}{2}}k_2$, $T_{\frac{1}{2}CPC}$ and by retention times obtained from 'slaughter' experiments. The retention times apply to the first 20 % of the large intestine which has a relatively large lumen diameter (Grovum & Hecker, 1973). The values were multiplied by 0·693 to equate them to values of $T_{\frac{1}{2}}$ because dividing the volume of a pool by the flow rate approximates retention time $\left(\frac{1}{k}\right)$ whereas $T_{\frac{1}{2}} = \frac{0{\cdot}693}{k}$. The mean values for $T_{\frac{1}{2}CPC}$ were slightly smaller than the adjusted retention times. The values of $T_{\frac{1}{2}CPC}$ and the adjusted retention times should

theoretically have been identical if they were obtained from the same mixing pool. The difference between the two values may be accounted for if the distal end of the mixing pool of digesta in the caecum and proximal colon ended proximal to the 20 % point mentioned previously. However, information on this is not available so precise quantitative comparisons between these two methods cannot presently be made.

Equation no. 3 described equally well the distribution curves of marker concentration resulting from the passage of marker through the sheep (Grovum & Williams, 1973*c*) and the physical model of its digestive tract (Grovum & Phillips, 1973). However, $T_{\frac{1}{2}}k_2$ with ^{144}Pr and ^{51}Cr EDTA in the sheep averaged 0·78 and 0·60 respectively of $T_{\frac{1}{2}CPC}$, whereas in the model, $T_{\frac{1}{2}}k_2$ and an estimate of $T_{\frac{1}{2}CPC}$ were equal in magnitude. It seemed reasonable, based on results from the model, that if the concentration curve of marker in faeces was described accurately then the rate constants k_1 and k_2 would also accurately predict the retention times of marker in the rumen and caecum and proximal colon. If anything it was expected that $T_{\frac{1}{2}}k_2$ in the sheep would have been slightly greater than $T_{\frac{1}{2}CPC}$ because of the possible influence of the abomasum and the fact that a single rapid injection of marker into the duodenum was dispersed in passing through the small intestine so that it was collected in a period of approximately 2 h from the ileum (Coombe & Kay, 1965). These influences would tend to broaden the peak of the concentration curve of marker excretion in faeces and hence would increase the value of $T_{\frac{1}{2}}k_2$ needed to make the simulated curve fit the results as described by Grovum & Williams (1973*c*). Clearly this was not the situation. The discrepancy between $T_{\frac{1}{2}}k_2$ and $T_{\frac{1}{2}CPC}$ in the sheep may be due to a streamlined flow of digesta through the caecum and proximal colon resulting from imperfect mixing. An analogous situation may be the laminar flow of blood in vessels. This concept implies that some digesta on entering the caecum and proximal colon does not mix with all the contents of this organ but are moved on to the spiral colon. Marker injected into the reticulo-rumen and passed distally to the caecum and proximal colon for the duration of the experiment may take this route continuously and in effect be mixed with less than the total amount of digesta in the caecum and proximal colon. In contrast when marker was injected into the abomasum to determine $T_{\frac{1}{2}CPC}$, all of it would be delivered to the caecum and proximal colon within approximately 10 h, allowing time for it to be eliminated from the abomasum and small intestine (Grovum & Williams, 1973*a*, *b*). A portion of this marker would take the proposed route of streamlined flow through the core of the caecum and proximal colon but the rest would be distributed throughout the remainder of the pool which presumably may be turning over at a slower rate. This rate may be detected as $T_{\frac{1}{2}CPC}$ when the terminal portion of the excretion curve in faeces is analysed.

Differences between markers. The smaller values of $T_{\frac{1}{2}}k_1$ for ^{51}Cr EDTA than for ^{144}Pr are consistent with the more direct observation that ^{51}Cr EDTA was eliminated from the reticulo-rumen at a faster trate than ^{144}Pr (Grovum & Williams, 1973*b*). The smaller values of $T_{\frac{1}{2}}k_2$ for ^{51}Cr EDTA than for ^{144}Pr probably reflects either a difference in the 'mixing times' of these two isotopes in the reticulo-rumen or the differential rates of elimination of ^{144}Pr and ^{51}Cr EDTA from the abomasum (Grovum & Williams, 1973*b*). Values of *TT* for ^{144}Pr may have been slightly greater than those for ^{51}Cr EDTA because of a difference in transit times through the omasum.

DOM

The positive correlations between DOM and $T_{\frac{1}{2}}k_1$ and $T_{\frac{1}{2}}k_2$ is new information although it was known previously that DOM and the retention of stained particles in the entire digestive tract were related in a curvilinear manner (Blaxter *et al.* 1956). Due perhaps to technical problems in estimating retention time, Phillips (1961) was unable to account for the greater digestibility of hay in Zebu than in Hereford steers in terms of the period available for

digestion to occur. Since $T_{\frac{1}{2}}k_2$ decreased at roughly half the rate of $T_{\frac{1}{2}}k_1$ with increasing levels of intake (Table 2) it might be expected the caecum and proximal colon are relatively more important in digestion when intake is high than when it is low. Examples of compensatory digestion in the intestines have been reported (Yadava & Bartley, 1964; Porter & Singleton, 1966; Ulyatt, Blaxter & McDonald, 1967) and this is not surprising since the values for $T_{\frac{1}{2}}k_2$:$T_{\frac{1}{2}}k_1$ averaged 0·39 for ^{144}Pr and 0·33 for ^{51}Cr EDTA. The potential for digestion in the caecum and proximal colon is substantial considering these values. This may also account for the fact that, of the cellulose normally digested in the ruminant, from 7–30 % occurred in the intestines (Hale, Duncan & Huffman, 1940; Gray, 1947; Ridges & Singleton, 1962; MacRae & Armstrong, 1969) even though the digesta had already been degraded by fermentation in the reticulo-rumen.

In this study it was remarkable that values of $T_{\frac{1}{2}}k_1$, $T_{\frac{1}{2}}k_2$ and TT all decreased by approximately 50 % as the intake was increased from 400 to 1300 g/d but yet the apparent DOM only decreased from 0·657 to 0·631. The perturbation analysis demonstrated further that k_1, k_2 and TT were responsive to changes produced in the rate of passage of digesta. Their use is attractive in that they effectively partition the average retention time of food in the digestive tract into three important components which are biologically meaningful. In addition they can be obtained from normal sheep without the need for surgical interference with the gut.

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REFERENCES

Balch, C. C. (1950). *Br. J. Nutr.* **4**, 361.
Balch, C. C. & Campling, R. C. (1965). In *Physiology of Digestion in the Ruminant*, p. 108 [R. W. Dougherty, editor]. Washington: Butterworths.
Bawden, R. J. (1970). *Br. J. Nutr.* **24**, 291.
Blaxter, K. L., Graham, N. McC. & Wainman, F. W. (1956). *Br. J. Nutr.* **10**, 69.
Briggs, P. K., Hogan, J. P. & Reid, R. L. (1957). *Aust. J. agric. Res.* **8**, 674.
Campling, R. C., Freer, M. & Balch, C. C. (1961). *Br. J. Nutr.* **15**, 531.
Campling, R. C., Freer, M. & Balch, C. C. (1962). *Br. J. Nutr.* **16**, 115.
Castle, E. J. & Castle, M. E. (1957). *J. agric. Sci., Camb.* **49**, 106.
Coombe, J. B. & Kay, R. N. B. (1965). *Br. J. Nutr.* **19**, 325.
Coombe, J. B. & Tribe, D. E. (1963). *Aust. J. agric. Res.* **14**, 70.
Downes, A. M. & McDonald, I. W. (1964). *Br. J. Nutr.* **18**, 153.
Ellis, W. C. & Huston, J. E. (1968). *J. Nutr.* **95**, 67.
Gray, F. V. (1947). *J. exp. Biol.* **24**, 15.
Grovum, W. L. & Hecker, J. F. (1973). *Br. J. Nutr.* **30**, 221.
Grovum, W. L. & Phillips, G. D. (1973). *Br. J. Nutr.* **30**, 377.
Grovum W. L. & Williams, V. J. (1973*a*). *Br. J. Nutr.* **29**, 13.
Grovum, W. L. & Williams, V. J. (1973*b*). *Br. J. Nutr.* **30**, 231.
Grovum, W. L. & Williams, V. J. (1973*c*). *Br. J. Nutr.* **30**, 313.
Hale, E. B., Duncan, C. W. & Huffman, C. F. (1940). *J. Dairy Sci.* **23**, 953.
Hecker, J. F. (1969). *Aust. vet. J.* **45**, 293.
Huston, J. E. & Ellis, W. C. (1968). *J. agric. Fd Chem.* **16**, 225.
Jarrett, I. G. (1948). *J. Coun. scient. ind. Res. Aust.* **21**, 311.
Leaver, J. D., Campling, R. C. & Holmes, W. (1969). *Anim. Prod.* **11**, 11.
MacRae, J. C. & Armstrong, D. G. (1969). *Br. J. Nutr.* **23**, 377.
Mäkelä, A. (1956). *Suom. maatal. Seur. Julk.* **85**, 1.
Miller, J. K., Perry, S. C., Chandler, P. T. & Cragle, R. G. (1967). *J. Dairy Sci.* **50**, 355.
Minson, D. J. (1966). *Br. J. Nutr.* **20**, 765.
Minson, D. J. & Cowper, J. L. (1966). *Br. J. Nutr.* **20**, 757.
Paloheimo, L. & Mäkelä, A. (1959). *Suom. maatal. Seur. Julk.* **94**, 1.
Phillips, G. D. (1961). *Res. vet. Sci.* **2**, 202.

Porter, R. & Singleton, A. G. (1966). *J. Physiol., Lond.* **186**, 145*P*.
Ridges, A. P. & Singleton, A. G. (1962). *J. Physiol., Lond.* **161**, 1.
Shellenberger, P. R. & Kesler, E. M. (1961). *J. anim. Sci.* **20**, 416.
Thornton, R. F. & Minson, D. J. (1972) *Aust. J. agric. Res.* **23**, 871.
Ulyatt, M. J., Blaxter, K. L. & McDonald, I. (1967). *Anim. Prod.* **9**, 463.
Warner, A. C. I. & Stacy, B. D. (1968). *Br. J. Nutr.* **22**, 369.
Yadava, I. S. & Bartley, E. E. (1964). *J. Dairy Sci.* **47**, 1352.

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