Rate of passage of digesta in sheep

3.* Differential rates of passage of water and dry matter from the reticulo-rumen, abomasum and caecum and proximal colon

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1. Sheep were given 800 g lucerne chaff/d and the mean half-times of cerium-144 – praseodymium-144 and the complex of chromium-51 with EDTA were 811 and 604 min in the reticulo-rumen, 37 and 17 min in the abomasum and 413 and 406 min in the caecum and proximal colon respectively. The average time available for digestion and absorption in the abomasum was therefore very short relative to that in the other two organs.

2. The half-times of the markers also indicated that particulate matter and water had different rates of turn over in the reticulo-rumen and abomasum but not in the caecum and proximal colon.

3. Evidence is presented which suggests imperfect mixing of the digesta in the caecum and proximal colon.

First-order kinetics apply to the elimination of a single injection of non-absorbed marker from the reticulo-rumen (Bullen, Scarisbrick & Maddock, 1953; Downes & McDonald, 1964) and the caecum and proximal colon (Hecker, 1971) but it is not known whether this also applies to the abomasum.

Water and dry matter were eliminated from the reticulo-rumen at different rates in the experiment conducted by Weller, Pilgrim & Gray (1962). However, the rates of passage of water and dry matter through the large intestine are similar (Coombe & Kay, 1965). No comparable information could be found for the abomasum, but Mylrea (1966) showed that most of the whey from milk ingested by calves left the abomasum before the curd. Fat and water in a mixed meal separated in the stomach of humans and passed at different rates through the pylorus (Wiggins & Dawson, 1961; Chang, McKenna & Beck, 1968). James (1957) cited references concerning the differential passage of fluid and particulate food from the stomachs of humans and cats.

The experiments now reported give information on the kinetics of water and particulate matter in the reticulo-rumen, abomasum and caecum and proximal colon of the sheep.

EXPERIMENTAL

Sheep

Mature Merino wethers (castrated males) were used in Expt 1 (sheep 1, 2, 3). Mature Merino–Romney Marsh wethers were used in Expt 2 (7, 8, 9).

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Surgery

Each sheep was fitted with a permanent abomasal cannula (Jarrett, 1948) with an internal diameter of 5 mm. Plastic cannulas were also inserted into the large intestine of sheep 7, 8, and 9. Sheep 7 had the fistula 5 cm from the ileo-caecal junction within the caecum. In sheep 8 it was opposite the ileo-caecal junction. The position of the fistula was not determined in sheep 9.

Housing and feedings

The sheep were kept in metabolism cages in an animal house. Faeces and urine were separated. The experimental room was illuminated continuously. The sheep were given 800 g lucerne chaff/d in approximately equal amounts at hourly intervals from an automatic feeder (Minson & Cowper, 1966). Water was available *ad lib.*

Radioactivity counting

Markers. An aqueous solution of the complex of chromium-51 with EDTA (^{51}Cr EDTA) was used to mark the water phase of digesta (Downes & McDonald, 1964). Carrier Cr EDTA was not added to this solution. An aqueous solution of ^{144}Ce–^{144}Pr was used to mark the particulate matter (Miller, Perry, Chandler & Cragle, 1967; Ellis & Huston, 1968; Huston & Ellis, 1968) as these isotopes are readily and strongly adsorbed on to food particles. It was assumed that only 3–5 % of the ^{51}Cr EDTA injected into the digestive tract was absorbed and excreted in the urine (Downes & McDonald, 1964). The isotopes ^{144}Ce and ^{144}Pr were inseparable, but only the radiation from ^{144}Pr was counted to determine their rates of passage in different parts of the gut.

Equipment. The isotopes were counted individually or together in a Packard model 3002 Tricarb Scintillation Spectrometer (Packard Instrument Company Inc., Illinois 60515, USA).

Measurements of interference. ^{51}Cr and ^{144}Pr standards were prepared in 3 ml distilled water. ^{51}Cr was counted in the green channel with a 40 % gain, and discriminator settings from 292 to 312 keV. ^{144}Pr was counted in the red channel with a 20 % gain and discriminator settings from 350 to 850 (700–1700 keV). The percentage interference of ^{51}Cr on the ^{144}Pr counts was calculated from equation (1) by counting the ^{51}Cr standards:

$$e = \frac{(^{51}\text{Cr interfering counts in red channel} - \text{background}) \times 100}{^{51}\text{Cr standard counts in the green channel} - \text{background}}, \qquad (1)$$

e was usually about 0·75 %.

The percentage interference of ^{144}Pr and ^{144}Ce on ^{51}Cr counts was determined from equation (2) by counting the ^{144}Pr standards:

$$f = \frac{(^{144}\text{Ce and } ^{144}\text{Pr interfering counts in the green channel} - \text{background}) \times 100}{^{144}\text{Pr standard counts in the red channel} - \text{background}}. \qquad (2)$$

f was usually about 5 % but did increase slightly for ^{144}Pr counting rates above 300/sec. However, no corrections were made to *f* for the counting rate of samples.

The ^{144}Pr counting rate was positively related to that of ^{144}Ce.

Adjustment for height of sample in the counting tube. This step was necessary because the efficiency with which a radioactive sample could be counted varied with its height in the scintillation tube. Mathematical relationships were obtained for the efficiency of counting a constant amount of ^{51}Cr EDTA in different depths of water in a scintillation tube. The heights of samples consisting of three faecal pellets were measured and the counts of ^{51}Cr and ^{144}Pr were adjusted to the efficiency of counting at a height of 28 mm (equivalent to 3 ml).

Preparation of samples

Abomasal digesta. The abomasal digesta samples collected in Expt 1, parts 1 and 2, were centrifuged at 2500 *g* for 30 min, and ^{51}Cr was counted in 3 ml portions of the supernatant fraction. The interference of ^{144}Pr and ^{144}Ce in these counts was negligible.

The sediment from each of the centrifuged abomasal samples was washed with distilled water until the washings were nearly free from colour. The residues were dried in tared plastic scintillation tubes in a vacuum oven at 73° and then counted for ^{144}Pr.

Caecal digesta samples. These were collected into tared plastic counting tubes, counted while wet for ^{144}Pr and ^{51}Cr and then dried in a vacuum oven at 73° to determine the weight of sample dry matter. The drying process sucked the digesta up the tubes and the samples from sheep 9 were lost.

Faecal samples. These were dried in plastic counting tubes in a vacuum oven, transferred to a desiccator and weighed before counting.

Statistical methods

Regression analyses were done on the natural logarithms of the adjusted counts to obtain half-times ($T_{\frac{1}{2}}$) that were descriptive of the changes in concentration of the marker.

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.

The value of $T_{\frac{1}{2}}$ in min was calculated from equation (3):

$$T_{\frac{1}{2}} = \frac{-0{\cdot}693}{\text{regression coefficient}}. \qquad (3)$$

$T_{\frac{1}{2}}$ is 0·693 of the cycle time or average time available for digestion or absorption in a pool (Hungate, 1966).

Expt 1

Studies were made of the differential rates of passage of water and particulate matter from the reticulo-rumen (part 1), abomasum (part 2) and caecum and proximal colon (part 3) in sheep 1, 2 and 3 using a single-injection technique.

Part 1. ^{51}Cr EDTA and ^{144}Pr in 200 ml distilled water were administered to the

reticulo-rumen by stomach tube at 24.00 hours. Three samples of abomasal digesta were then collected from each sheep at about 11.00, 17.00 and 24.00 hours on each of the next 4 d.

Part 2. Both isotopes were administered to the abomasum via the cannula, in 40 ml distilled water. The right side of each sheep was then gently palpated to facilitate mixing of the markers in the abomasal contents. Seven 20 ml samples of abomasal digesta were removed from each sheep at 30 min intervals.

Part 3. Both isotopes were injected into the abomasum as in Part 2 and duplicate three-pellet samples of voided faeces were obtained from each sheep at short intervals over the next 4 d.

Expt 2

The purpose was to study the mixing of digesta in the caecum and proximal colon of the sheep.

Part 1. The patterns of elimination of ^{51}Cr EDTA and ^{144}Pr from the caecum and proximal colon were compared with the subsequent changes in concentration of the markers in faeces.

^{51}Cr EDTA was administered as a single injection into the abomasum of sheep 7, 8, and 9 as in Expt 1, part 2. About 3 h later a 6 ml solution of ^{144}Pr in distilled water was injected via the cannula into the caecum and proximal colon of each of these animals. Single digesta samples were obtained from the caecum and proximal colon region of the large intestine, and duplicate three-pellet samples of faeces were collected at short intervals over the next 4 d.

Part 2. ^{144}Pr was continuously infused, first into the apex of the caecum and secondly opposite the ileo-caecal junction and in each instance between-pellet variability in concentration of marker in the faecal dry matter was determined for 2 d after plateau conditions had been achieved. Ten single pellets were counted in each of thirteen and eighteen faecal samples respectively. One sheep was used for both infusions. All counts were corrected for background and adjusted to the average dry weight of the single pellets counted in the experiments.

The variances of ^{144}Pr concentrations within samples were pooled to calculate a coefficient of variation for each infusion site.

RESULTS

Expt 1. *Differential passage of markers from the reticulo-rumen, abomasum and caecum and proximal colon*

Part 1. *reticulo-rumen.* The $T_{\frac{1}{2}}$ for ^{51}Cr EDTA was less than that for ^{144}Pr in each sheep (Fig. 1). The slopes (b) of the respective regression lines were significantly different ($P < 0{\cdot}005$). The standard deviation of regression coefficient (S_b) ranged in magnitude from 2 to 6% of b.

Part 2. *abomasum.* The $T_{\frac{1}{2}}$ of ^{51}Cr EDTA in the abomasum was markedly less than that for ^{144}Pr in sheep 2 and 3 ($P < 0{\cdot}005$) but not in sheep 1 (not significant) (Fig. 1). The standard deviation S_b ranged from 2 to 8% of b. Sheep 1 had a poor appetite

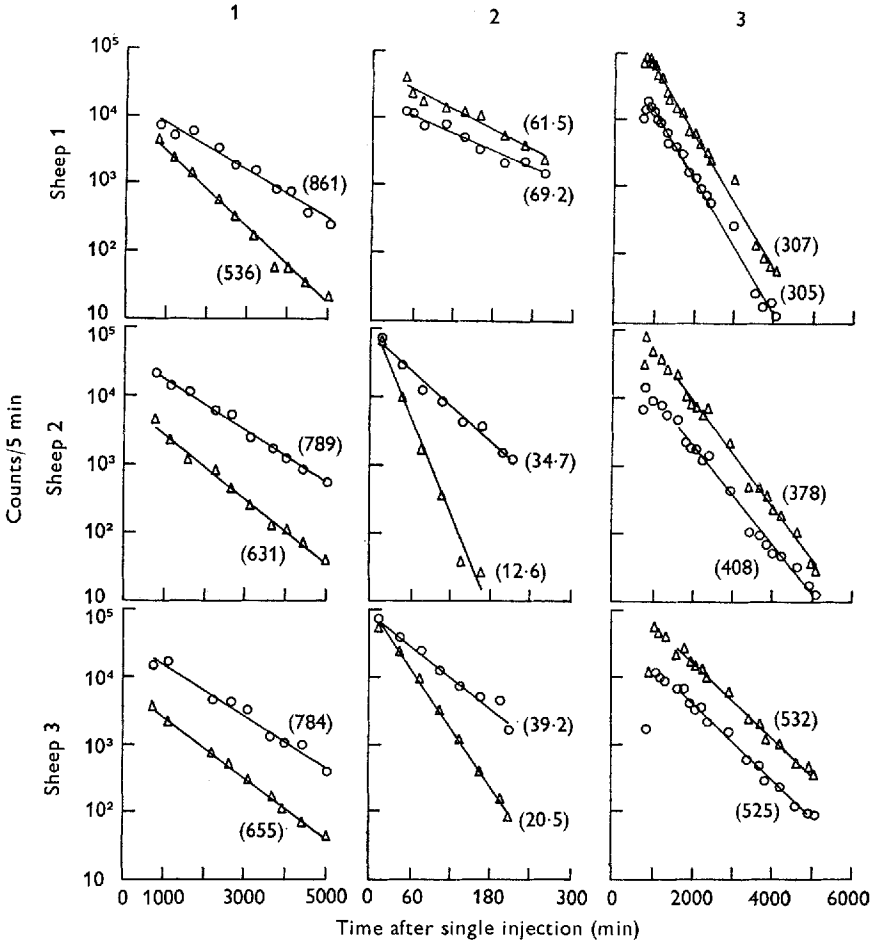

Fig. 1. Elimination rates of ^{51}Cr EDTA and ^{144}Pr from the reticulo-rumen (1), abomasum (2), and caecum and proximal colon (3) in sheep. Numbers in parentheses are half-times in min.

Counts ^{144}Pr (○): (1) injected into reticulo-rumen – counts/0·50 g abomasal dry matter (DM); (2) injected into the abomasum – counts/0·50 g abomasal DM; (3) injected into the abomasum – counts/0·50 g faecal DM. The values were divided by 10 for plotting.

Counts ^{51}Cr EDTA (△): (1) injected into the reticulo-rumen – counts/3 ml abomasal fluid; (2) injected into the abomasum – counts/3 ml abomasal fluid; (3) injected into the abomasum – counts/0·50 g faecal DM.

while the abomasal samples were collected. Also the half-times for both isotopes were relatively large in sheep 1.

Part 3. caecum and proximal colon. There was no consistent difference between the half-time for ^{51}Cr EDTA and ^{144}Pr obtained from the terminal portions of the marker concentration curves in faeces (Fig. 1). The standard deviation S_b ranged from 2·2 to 2·9% of b.

Relative marker half-times in different organs of the alimentary tract. The average half-times for ^{51}Cr EDTA in the reticulo-rumen, abomasum and caecum and proximal colon were 607, 17 and 406 min respectively. For ^{144}Pr they were 811, 37 and 413 min respectively.

The abomasal values from sheep 1 were excluded from these calculations.

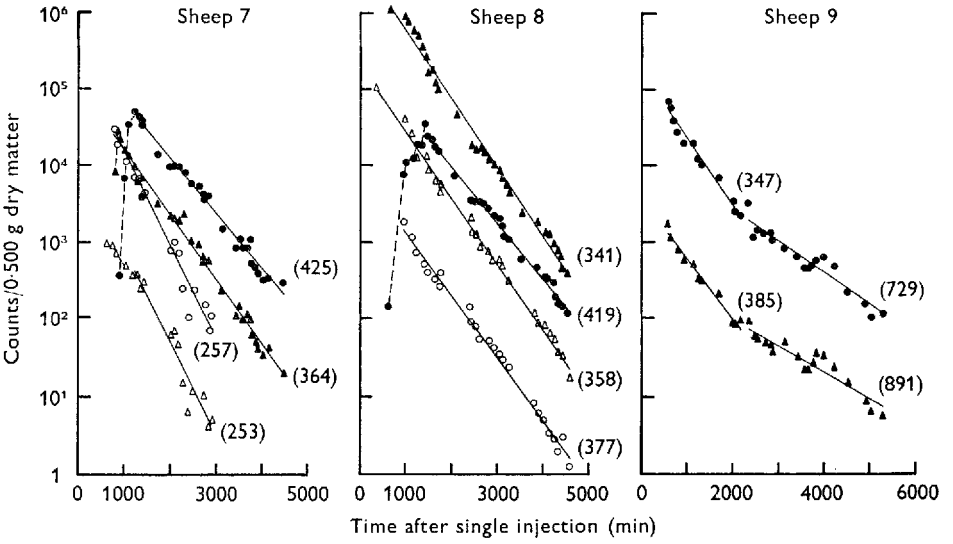

Fig. 2. Relationship between marker kinetics in the caecum and proximal colon of sheep and that observed in faeces. Numbers in parentheses are half-times in min.

^{51}Cr EDTA was injected into the abomasum and sampled in digesta from the caecum and proximal colon (△) and in faeces (▲). The true concentrations in the faecal dry matter of sheep 8 were multiplied by 10 and those for sheep 9 were divided by 10 for ease of plotting.

^{144}Pr was injected into the caecum and proximal colon and sampled in digesta from the caecum and proximal colon (○) and in faeces (●). The ^{144}Pr concentrations in digesta dry matter of sheep 8 were divided by 10.

Expt 2. Mixing of digesta in the caecum and proximal colon

Part 1. Single first-order equations described the terminal concentration changes of ^{144}Pr and ^{51}Cr EDTA in the dry matter from the caecum and proximal colon and in the faeces of sheep 7 and 8 (Fig. 2).

The half-times of markers in digesta in sheep 7 were less than those in the faeces. This was not observed in sheep 8.

The faecal concentration changes of ^{144}Pr and ^{51}Cr EDTA in sheep 9 were curvilinear on semilogarithmic graph-paper. The curve was divided into two exponential processes by eye and separate regression analyses were done to determine the half-times given in Fig. 2.

Part 2. The coefficient of variation for ^{144}Pr concentrations in the dry matter of single faecal pellets was larger (27·4 %) when marker was infused into the apex of the caecum than when it was infused opposite the ileo-caecal junction (16·4 %). However, the range of concentrations was 2400–7500 counts/2 min per average pellet weight for both infusion sites.

A coefficient of variation of 1·6 % was obtained for ten counts on a pellet with a counting rate near the average for this experiment.

DISCUSSION

^{51}Cr EDTA and ^{144}Pr as digesta markers

Warner (1969) showed that ^{51}Cr EDTA was partly adsorbed onto dry matter in the reticulo-rumen of some sheep. The results of Downes & McDonald (1964) show that the $T_{\frac{1}{2}}$ of polyethylene glycol (PEG) in the reticulo-rumen was slightly smaller than that of ^{51}Cr EDTA in one sheep, but the significance of this was not discussed. Some ^{51}Cr EDTA was also bound by casein in the abomasum of milk-fed calves (Smith & Hill, 1967). These findings indicate that ^{51}Cr EDTA is not a perfect marker of fluid digesta. Therefore, the differences in half-times between ^{144}Pr and ^{51}Cr EDTA for the reticulo-rumen in Expt 1 may be qualitative rather than quantitative.

Expt 1, part 1. Differential rates of passage of particulate matter and water from the reticulo-rumen

^{144}Pr and ^{51}Cr EDTA were injected into the reticulo-rumen, and the elimination rates of the markers from the organ were obtained by sampling abomasal contents. This procedure was justified from preliminary studies (unpublished) in which the half-times of ^{51}Cr EDTA in the reticulo-rumen supernatant faction of four sheep (933, 749, 795, 744 min) given 800 g lucerne chaff were not significantly different from that determined by counting whole abomasal contents (936, 773, 794, 791 min respectively). The digesta samples were collected over 4 d.

Water in the reticulo-rumen of cows can move to some extent independently of the particulate matter (Schalk & Amadon, 1928) and some drinking-water flows direct into the abomasum of sheep (Phillipson & Ash, 1965). The cumulative excretion curves for faecal recoveries of PEG and ^{144}Ce–^{144}Pr obtained by Ellis & Huston (1968) indicated that PEG was eliminated faster from some part or parts of the sheep digestive tract than was ^{144}Ce–^{144}Pr. The evidence obtained in this experiment that water left the reticulo-rumen in sheep at a faster rate than particulate matter is also supported by the work of Weller *et al.* (1962) with PEG and lignin markers.

The effect of water intake, quantity and maturity of roughage consumed, and type or form of ration on differential passage of water and dry matter from the reticulo-rumen is unknown.

Expt 2, part 2. Differential rates of passage of water and particulate matter from the abomasum

Hydén (1961) calculated a 'transit time' of 0·5–1 h for PEG in the abomasum of sheep. These values were apparently obtained by dividing the volume of water in the abomasum (V) by the flow rate (F) and would therefore be equivalent to the reciprocal of the rate-constant ($1/k = V/F$) or about 1·44 times the $T_{\frac{1}{2}}$ used in this experiment. The half-times in this work are therefore in good agreement with the 'transit times' of Hydén (1961). However, it was not known previously that first-order kinetics applied. The term 'transit time' is therefore no longer descriptive of digesta movement

through the abomasum, but it is useful in describing passage of digesta through tubular organs such as the small intestine.

The rate of elimination of ^{144}Pr from the abomasum was slower than that for ^{51}Cr EDTA. This may have been caused by sedimentation of particulate matter in the body of the organ, by gastric secretion or by both. The separation of particulate matter from the fluid of abomasal digesta removed through a cannula is surprisingly rapid.

Expt 1, part 3. The kinetics of water and particulate matter flowing through the caecum and proximal colon

Most of the ^{51}Cr EDTA and ^{144}Pr added to the abomasum should have been delivered to the caecum and proximal colon within 10 h because the half-times of the markers in the abomasum were short and movement of digesta through the small intestine requires 2·25–4·50 h (Coombe & Kay, 1965). The possible influence of the abomasum and small intestine on the half-times of these markers in faeces was avoided by ignoring values before and around the narrow peak of marker concentration in faecal dry matter (Fig. 1). The caecum and proximal colon was the most likely site of generation of the half-times observed in faeces because its lumen diameter is relatively large and the digesta in it have the consistency of a thin paste. First-order equations described the elimination of ^{144}Pr and ^{51}Cr EDTA from the caecum and proximal colon of two of the three sheep used in Expt 2, part 1. This proves that the digesta in the caecum and proximal colon behave to some extent as a mixing pool.

The amount of binding of ^{51}Cr EDTA onto particulate matter in large-intestinal digesta is unknown. Therefore from the results presented in Fig. 1 we can only conclude that ^{51}Cr EDTA and ^{144}Pr behave similarly in the proximal large intestine. However, Coombe & Kay (1965) found similar rates of faecal excretion of PEG, stained straw and plastic particles added to the ileum of sheep, which suggests that digesta in the hind-gut act as a single phase. Also, differential passage of water and dry matter in the caecum and proximal colon of normal sheep is unlikely because of the pasty nature of the digesta.

Expt 2. Mixing of digesta in the caecum and proximal colon

Single first-order equations described terminal changes in marker concentration in digesta from the caecum and proximal colon and in the faeces of two out of three sheep used in part 1. The curvilinear results obtained for sheep 9 were attributed to a change in motility of the gut or in flow–volume relationships rather than the recycling of the isotopes. This sheep may have been upset by the sampling of digesta from the caecum and proximal colon. However, the animal ate all its food during the experiment.

The differences between the half-times of markers in the caecum and faeces of sheep 7 are difficult to explain. Digesta were sampled from within the caecum of sheep 7 but in sheep 8 from opposite the ileo-caecal junction. The effect of this is not known. Cannulation of the caecum and proximal colon might disturb the normal flow-pattern of digesta entering the large intestine. The half-time obtained by injecting markers into the abomasum and collecting faeces may therefore be a more

reliable index of digesta kinetics in the caecum and proximal colon than direct measurements that involve surgical intervention with the organ.

The ^{144}Pr injected into the caecum and proximal colon in Expt 2 part 1 must have been slowly mixed throughout the digesta because the concentrations of ^{144}Pr in faecal dry matter rose to a peak about 300 and 600 min after the time of first appearance in sheep 7 and 8 respectively (Fig. 2). The variation in marker concentration between single faecal pellets in Expt 2, part 2, was much greater than could be explained by counting error, and therefore must have arisen from inadequate mixing of ileal digesta flowing into the large intestine with that present in the caecum and proximal colon. Coombe & Kay (1965) injected markers into the intestines of sheep and found small peaks in marker concentration on the tail-end of the excretion curve of the reference materials in faeces. They attributed these to the storing, mixing and intermittent release of the digesta in the proximal large intestine. The mixing of digesta in the caecum and proximal colon must be imperfect because a gradient in water content has been shown from the apex of the caecum to the start of the spiral colon (Grovum & Hecker, 1973).

Neimeier (1941) (cited by Dukes, 1955) found that in pigs the colon usually received its contents from the caecum but sometimes the colon received the ileal digesta before the caecum was filled. Berehoiu (1966) and Ruckebusch (1970) have noted that the caecum in sheep sometimes undergoes total contraction which completely empties its content into the colon. Thus all the evidence available points to some irregularity in movement of digesta through the caecum and proximal colon.

Kinetics of digesta in the reticulo-rumen, abomasum and caecum and proximal colon

The half-times of ^{51}Cr EDTA and ^{144}Pr were large in the reticulo-rumen and caecum and proximal colon and relatively small in the abomasum. Single injections of markers administered to the abomasum or duodenum are excreted in faeces faster than similar markers placed into the reticulo-rumen of lambs (Lenkeit, 1932, as cited by McAnally & Phillipson, 1944), adult cattle (Balch, 1950; King & Moore, 1957; Campling, Freer & Balch, 1961; Campling & Freer, 1962), sheep (Weston, 1968), calves (Miller, Moss, Hall & Gorman, 1969) and goats (Castle, 1956). The 'transit times' of marker in the abomasum and caecum of sheep (Hydén, 1961) are in about the same proportion as the half-times reported in this paper. Also, McAnally & Phillipson (1944) cited radiographic experiments which indicate that digesta are retained for a shorter period of time in the abomasum than in the large intestine of goats.

The use of first-order kinetics to describe the elimination of marker from the reticulo-rumen, abomasum and caecum and proximal colon of sheep is of fundamental importance in developing a mathematical model of passage of marker through the alimentary tract. The rate-constants of elimination of marker from these digesta pools can also be used to calculate the average times available for digestion ($1/k$; Hungate, 1966) and hence to measure the amount of digestion per unit of retention time. The latter may be a useful index when comparing rates of fermentation or digestion either within or between species.

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