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## QUANTITATIVE STUDIES OF DIGESTION IN THE RETICULO-RUMEN

### I. TOTAL REMOVAL AND RETURN OF DIGESTA FOR QUANTITATIVE SAMPLING IN STUDIES OF DIGESTION IN THE RETICULO-RUMEN OF CATTLE

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#### SUMMARY

Quantitative sampling of the contents of the reticulo-rumen of cattle by total removal through a fistula, weighing, mixing, sampling and return of the digesta is described. An experiment in which a fasting cow was given a single feed of cut, fresh red clover and sampled in that manner before and after feeding and 3, 6, 12 and 22 hours later, is described and the results given, including the wet weight of and the dry matter, Na, K and VFA contents of the digesta, as well as the estimated rates of net inflow of water to the reticulo-rumen, and of outflow to the omasum. The effects of removal and return of the digesta on the animals and on the breakdown and passage of food, are briefly discussed, together with problems of sampling.

#### INTRODUCTION

A COMMON PRACTICE in studies of digestion in the reticulo-rumen is to take a series of samples of digesta through a rumen fistula and to follow the changes in concentration of a particular metabolite or metabolites. Often, the concentration of the metabolite will be compared with that of a non-fermented substance, usually lignin. The change in this ratio is then interpreted as an index of digestion of the metabolite.

Concentration changes alone do not permit quantitative estimations to be made, since the samples on which the analyses are performed represent an unknown proportion of the pool from which they were taken. Further, the concentration of a given substance in the reticulo-rumen can fall for one or any combination of five reasons:

- (1) The rate of production (or ingestion) has decreased.
- (2) The volume of the system in which the substance is dispersed has increased — the animal may have had a drink.
- (3) Utilization or destruction of the substance in the reticulo-rumen has increased.
- (4) The rate of absorption from the reticulo-rumen has increased.
- (5) The rate of loss by passage out of the rumen has increased.

Thus the quantitative interpretation of a particular concentration change presents great difficulty: not surprisingly, as will be seen from examples given in this and the two succeeding papers (Bailey, 1965; Clarke, 1965), concentration changes can be quite misleading.

The use of markers adds another measurement parameter—comparison of the disappearance rates of metabolite and marker. However, the ratio so obtained will depend on the nature of the marker used. Water-soluble markers are diluted and washed out in the flow of water that passes continually through the reticulo-rumen. The disappearance of insoluble markers, however, depends on their specific gravity and particle size (King and Moore, 1957; Balch, 1961; Campling and Freer, 1962). At the outflow of the reticulo-rumen is a selecting mechanism which favours small sized particles of approximately 1.2 specific gravity. Larger sized particles, even if of appropriate specific gravity, do not pass; they must remain until either they are degraded to water-soluble substances (or to gases), or are reduced to fragments small enough to pass out.

The essential information that is needed for quantitative studies are the amounts of water and of dry matter (D.M.) in the digesta. They may be readily calculated given the D.M. ratio and either the total weight of ingesta, or the volume of water (*e.g.*, Ulyatt, 1964). The volume of water can be estimated from the dilution of known amounts of a water-soluble marker such as polyethylene glycol (PEG) when added to the digesta (see Hyden, 1961). However, this method is only satisfactory if the volume and the rates of flow remain constant through the experiment: if they are changing, then fresh doses of marker must be given at each sampling, and the problem of adequate mixing of the marker through the digesta is met. Even if the water volume can be measured, there remains the difficulty of obtaining representative samples of digesta for dry matter determinations. This arises from the uneven distribution of the dry matter in the digesta, there being a distinct tendency towards layering in the rumen, particularly in cattle (Bryant, 1964).

One solution to the sampling problem is to slaughter groups of animals at various intervals after feeding (Gray, 1947; Paloheimo *et al.*, 1955; Gray *et al.*, 1958). This has the advantage that the entire digestive tract is available for sampling. It has the disadvantage that large numbers of animals are required to obtain statistically significant

results, and that only one experiment can be performed with a particular animal.

An alternative solution is to use animals with large rumen fistulae and to remove the entire contents of the reticulo-rumen for sampling. The digesta are manually transferred to a large container, weighed, mixed, sampled, and the remainder returned to the animal. This method has been used primarily in cattle (Kick and Gerlaugh, 1935; Hale *et al.*, 1940, 1947), but recently it has also been adapted to use in the sheep by Watanabe and Umeza (1962, 1963). However, although occasionally employed as a method of direct measurement of the quantity of digesta in the reticulo-rumen (*e.g.*, Campling *et al.*, 1961), it has not found wide application in digestion studies. This, it seems, has been largely due to fear of the possible deleterious effects on the animal and on the rumen micro-organisms, particularly the effects of the exposure of the latter to the air. Nevertheless, against such potential disadvantages, the method offers the important advantages that serial sampling can be carried out, and experiments repeated in the same animal.

To the complications of sampling, there must be added one more that is consequent on the manner in which feed is digested in the reticulo-rumen. Whereas the dissimilation of water-soluble substances in the feed is usually rapid during and after feeding, the breakdown of insoluble material is relatively slow (Bailey, 1965). Under free-feeding, breakdown of insoluble material will be incomplete before the next meal is taken. As a result, there is present in the reticulo-rumen at any one time the residues of several meals in different stages of digestion. The problem arises as to how to distinguish between the contributions of these different meals. One solution is to give the animal a meal in which the metabolites of interest are labelled in some fashion. The alternative is to restrict feeding and to follow the changes associated with the ingestion of an isolated meal. While this is a satisfactory practical solution and is widely used, it must be remembered that restricted feeding is an abnormal circumstance for ruminants, and results in conditions in the reticulo-rumen that can differ markedly from those found under free feeding.

At Plant Chemistry Division, methods have been developed for the quantitative study of digestion in the reticulo-rumen. The standard combination employed at present comprises:

- (1) Restricted feeding: isolated meals given to animals accustomed to having one meal a day.

- (2) Sampling the contents of the reticulo-rumen by the total removal technique (called here "bailing").
- (3) Estimation of the flow of water through the reticulo-rumen by the use of the water-soluble marker PEG.
- (4) Recording of jaw movements by a balloon under the jaw and connected to a tambour writing on a kymograph.

An experiment of this kind will be described below. Brief reference will be made to the changes found in the total wet weight of, and the D.M., Na, K, and volatile fatty acids contents of the digesta, and the estimated rates of inflow and outflow of water.

## METHODS

### ANIMAL

The animal used was a 5-year-old Ayrshire cow, which had a large rumen fistula closed by a rubber cannula of the Balch and Johnson (1948) pattern. She was housed indoors in a stall alongside her twin mate. She had been fed cut, fresh red clover, the experimental feed, for 6 weeks prior to the experiment; for the last 2 weeks, she had been given one 2-hour feed a day.

### FEED

On the day of the experiment, the D.M. content of the red clover was 19.64%. During the two hours in which she was fed, the animal ate 24.88 kg wet weight = 4.85 kg D.M. Water was offered after the feed was removed: the animal drank 8.40 litres in the first 3 hours after feeding, none thereafter until the end of the experiment.

Salt lick had been available to the animal, but had been removed 24 hours before the start of the experiment.

### SAMPLING

Sampling from the reticulo-ruminal contents was carried out before feeding, at the end of feeding, and then 3, 6, 12 and 22 h later. Reckoned from the start of feeding, these sampling times were 0, 2, 5, 8, 14 and 24 h.

A team of 3 was required for sampling — two to bail out the animal, one to handle the samples after they had been taken from the bulk of digesta.

The main equipment used for bailing out was a large galvanized-iron dust bin, a plastic bucket, two plastic dip-

pers (600 ml and 250 ml), a plastic drape with a hole in it, a short gutter made of polythene sheet 3.5 mm thick, and a round-bottomed bowl. Before each bailing, this equipment was weighed to obtain the first tare weight.

Bailing proceeded as follows: The large container was set up alongside the animal at the level of the rumen fistula, standing on dry wooden blocks, and with a blanket of 2.5 cm thick foamed plastic wrapped around it for insulation. The container was warmed by rinsing with hot water, and, immediately prior to bailing, gassed out with carbon dioxide. The plastic drape was placed around the cannula and clipped to the lip of the container: its purpose was to reduce spillage. The cannula plug was then removed and bailing commenced. More solid masses of ingesta were pulled out by hand, while the liquid was bailed out with the dippers. An inspection light was used to check progress. When all that could be readily bailed out had been removed, the amount remaining behind — of the order of 0.5 to 1.0% of the total wet weight — was assessed by eye.

Removal finished, the cannula was closed, and the drape detached from the animal and used to cover the stomach contents in the container. The container, together with dippers, etc., was now weighed again (gross weight). From the net weight (gross weight — tare weight), by correction for the amount left behind, for losses such as spillage on the floor, and for additions such as PEG solution, was estimated the weight of contents of the reticulo-rumen at the time of bailing.

The digesta in the container were mixed by arm, taking care not to introduce air. Portions were then taken from the top, middle and bottom of the container, and pooled to make one large composite sample and weighed. Only the minimum amount needed for the purpose of the experiment — usually 1.0 to 1.5 kg — was taken. The third member of the team immediately mixed the sample in the round-bottomed bowl and proceeded to divide it into subsamples for D.M. determinations, and the various chemical analyses and physical measurements.

The sample having been taken, return of the rest of the digesta was started. The drape was placed around the cannula again, the cannula unplugged, and the gutter inserted in the opening. The digesta were then pushed or poured down the gutter and so returned to the stomach. Care was taken to distribute more solid material around the rumen; liquid was simply bucketed in. When all had been returned, the cannula was closed and the container,

dippers, bucket, drape and gutter weighed again (second tare weight). The difference between the first and second tare weights gave the amount of digesta left on the collecting equipment. The weight of digesta in the reticulo-rumen after returning was then calculated as the net weight *plus* the amount left behind at the end of removal, *less* the amount taken for a sample, the amount left on the equipment, and any losses that occurred during replacement.

The time taken for bailing varied according to the state of the contents, being longest immediately after feeding, and becoming progressively shorter thereafter. The actual times taken during the present experiment are shown in Table 1.

TABLE 1: TIME TAKEN FOR BAILING

<i>Bailing</i>	<i>Removal (min)</i>	<i>Sampling (min)</i>	<i>Return (min)</i>	<i>Total Time (min)</i>
0 h	15	3	4	22
2 h	21	4	10	35
5 h	18	4	6	28
8 h	17	3	6	26
14 h	16	3	5	24
24 h	14	3	4	21

A standard form was used to record weights, times, the state of the digesta, and any other pertinent information.

#### DRY MATTER DETERMINATION

Dry matter was determined on 3 subsamples of 300 g. Samples were placed on tinfoil in dishes and dried at 90°C in an oven with a circulating fan. After 24 h, the samples were turned over and the tinfoil stripped back. The standard drying time used in this experiment was 36 h, but weighings were also made at 24 and 48 h for checking purposes.

#### WATER-SOLUBLE MARKER

A solution of PEG (Light & Co.: average molecular weight 4000) in water, made up to 1 litre, was added to the large container at the start of the first bailing. At the same time a small sample of digesta was taken directly from the animal, weighed, and used for a blank. PEG was determined by the turbidometric method of Hyden (1956) as modified by Ulyatt (1964).

## RECORDING JAW MOVEMENTS

The balloon for recording jaw movements was incorporated in a leather head halter. The animal wore this halter for a week before the experiment. Actual recording was carried out during the last 3 days before the experiment as a control period for the record made during the experiment itself. From the records obtained, the time spent ruminating between the successive samplings was determined, as well as the total time.

## RESULTS

## THE WET AND DRY WEIGHTS OF THE DIGESTA

The wet and dry weights of the contents of the reticulorumen before and after feeding in this experiment are shown in Figs. 1 and 2. Each curve has two points plotted for all but the last sampling. The upper point represents the weight of contents calculated to be present at the time of their removal, the lower point the weight of contents present after replacement.

The wet weight of the contents was increased by a factor of about 1.8 times as a result of feeding. It was further, but only slightly, increased following drinking.

The dry weight of the contents was estimated from the D.M.% of the samples. The D.M.% in this experiment did not exceed 10, the actual figures being: 0 h — 6.72% ; 2 h — 9.91% ; 5 h — 9.24% ; 8 h — 8.67% ; 14 h — 7.60% ; 24 h — 5.71%. In Fig. 2, the dry weight is shown plotted on a logarithmic scale. This demonstrates the almost constant rate of loss of D.M. after the 5 h sampling in this experiment. If it is assumed that the loss is exponential, the slope of the line — the instant rate of D.M. loss — may be calculated from the formula:

$$k = (\log_e W_1/W_2)/T$$

where  $k$  = the slope of the line

= the instant rate of D.M. loss

$W_1$  = dry weight of contents at start of period

$W_2$  = dry weight of contents at end of period

$T$  = duration of period

In this experiment, the instant rates of loss were found to be: period 2–5 h — 1.40% ; 5–8 h — 6.14% ; 8–14 h — 4.95% ; 14–24 h — 5.02%. The average rate of loss for the period 5–24 h was 5.37%. The likely error in estimating D.M. will be discussed below.

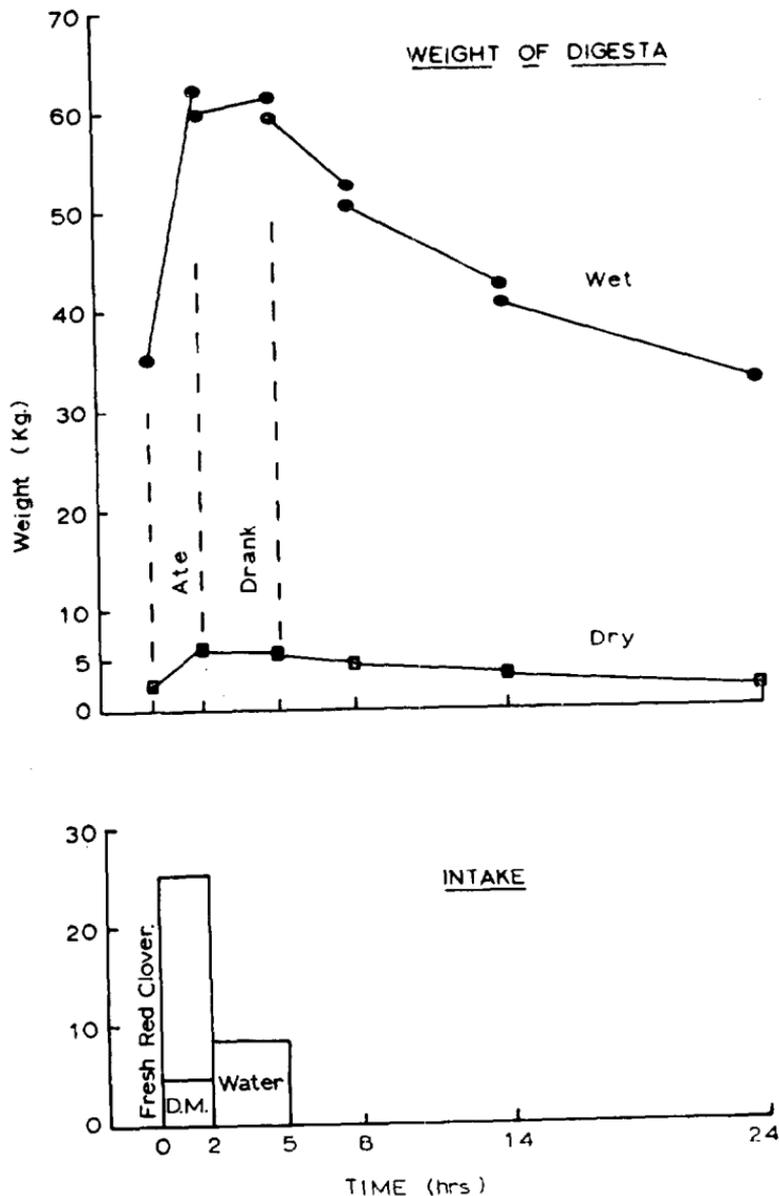


Fig. 1: Changes in the wet and dry weights of the contents of the reticulo-rumen of cow 30, following a 2 h feed on fresh cut red clover. The upper point at each sampling represents the weight of contents at removal, the lower point, the weight of contents at return. Expt. A8, 11/12.12.64.

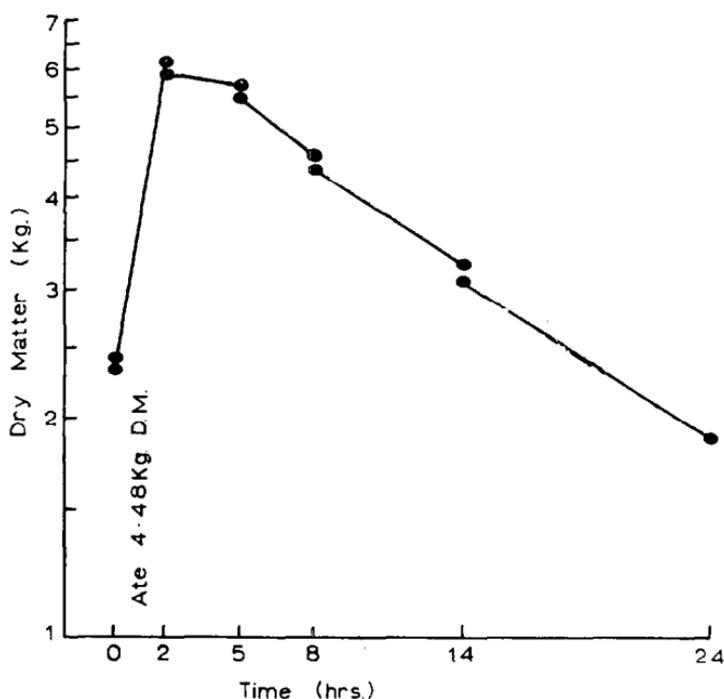


Fig. 2: Changes in the dry weight of the contents of the reticulo-rumen of cow 30 during the experiment shown in Fig. 1, plotted on a logarithmic scale.

#### THE AMOUNTS OF Na AND K IN THE FREE AQUEOUS PHASE

An example of how misleading concentration changes can be in relation to the total amount of a substance in the contents of the reticulo-rumen is given by the changes that occurred in the concentration and amounts of Na and K in the free aqueous phase. Following high-speed centrifugation, samples of liquor from the contents were analysed for Na and K by flame photometry. The concentrations found are shown in the upper section of Fig. 3, while the estimated total amounts are shown in the lower section. From the appearance of the concentration curves, it might be assumed that the amount of K present in the free aqueous phase had been increased by feeding, whereas the amount of Na had been decreased. However, when the total amounts are estimated, it is clear that, not only did the expected tide of K occur, but also there was a definite if small, increase in the amount of Na.

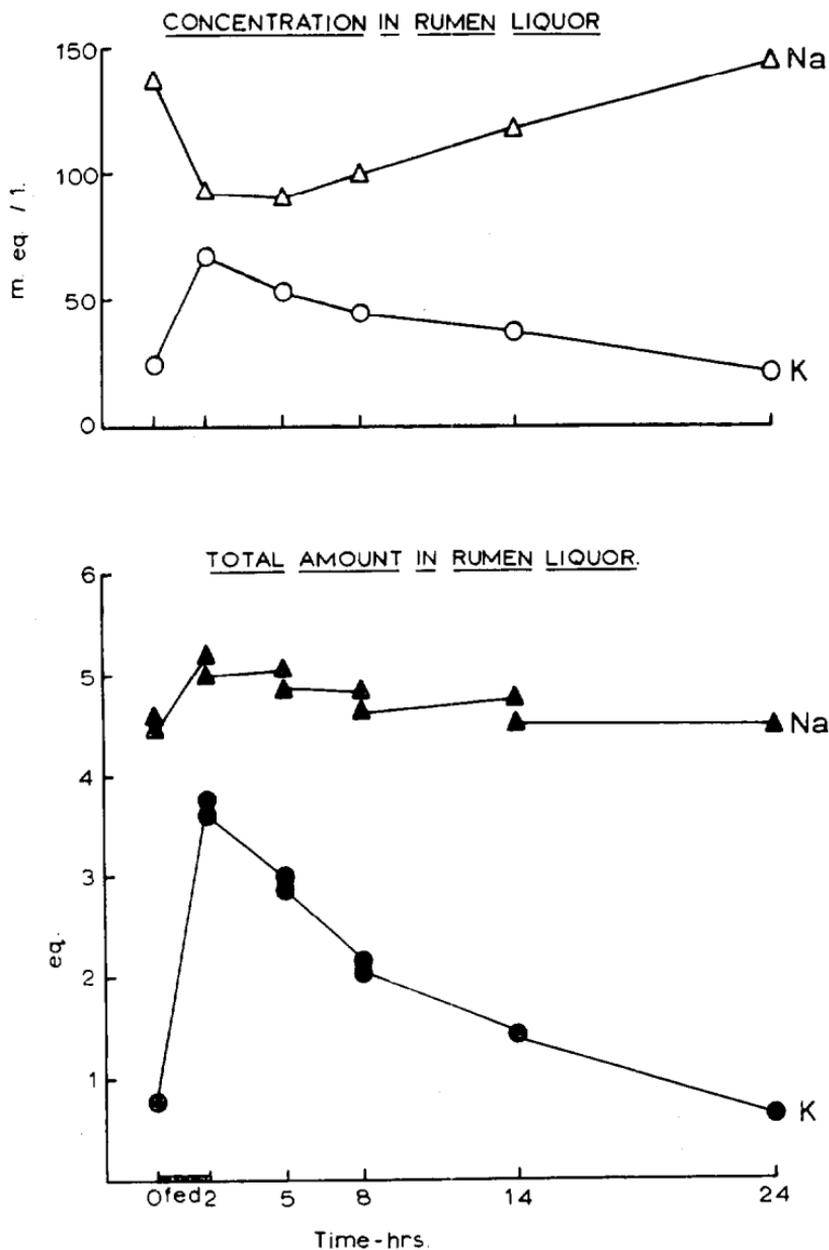


Fig. 3: Changes in the concentrations (above) and amounts (below) of sodium and potassium in centrifuged liquor from the contents of the reticulo-rumen of cow 30, during the experiment shown in Fig. 1.

## THE PASSAGE OF WATER THROUGH THE RETICULO-RUMEN

From the changes in the volume of water and the changes in the concentration of the water-soluble marker PEG in the contents, it is possible to calculate the net inflow to the reticulo-rumen and the outflow to the omasum. The formulae\* used here are:

$$\text{Net inflow (litres/hour)} = \frac{(V_1 - V_2)/T \times \log(c_1/c_2)}{\log(V_1/V_2)}$$

$$\begin{aligned} \text{Outflow to omasum (litres/hour)} \\ = \text{Net inflow} + (V_1 - V_2)/T \end{aligned}$$

where  $V_1$  = volume at start of the period (litres)

$V_2$  = volume at end of period (litres)

$c_1$  = concentration of PEG at start of the period (mg/ml)

$c_2$  = concentration of PEG at end of period (mg/ml)

$T$  = time (h).

The net flow through the reticulo-rumen is equivalent to the average change in volume during the period — *i.e.*,  $(V_1 - V_2)/T$ .

The estimated rates of flow in the present experiment are shown in Fig. 4.

The net inflow includes saliva, ingested water, and the net flux across the stomach wall. In Fig. 4, the endogenous contribution (solid) has been distinguished from the ingested water (barred). It will be seen that there was a high rate of inflow, derived from both endogenous sources and ingested clover during the 2 h of feeding. The rate of inflow was still moderately high during the next 3 h, but after that, it appears to have fallen to a steady level of about 2 litres/hour.

The changes in the rate of outflow were smaller than the changes in inflow. Outflow was highest during the first 5 h, but did not exceed the rate of inflow; thereafter the rate of outflow, although decreasing, was always greater than the rate of inflow. Not surprisingly, the net flow shows a high rate of gain during feeding and a change over to net loss after 5 h.

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\* The formulae for inflow and outflow were derived by A. C. Glen-day, Applied Mathematics Laboratory, D.S.I.R., Palmerston North.

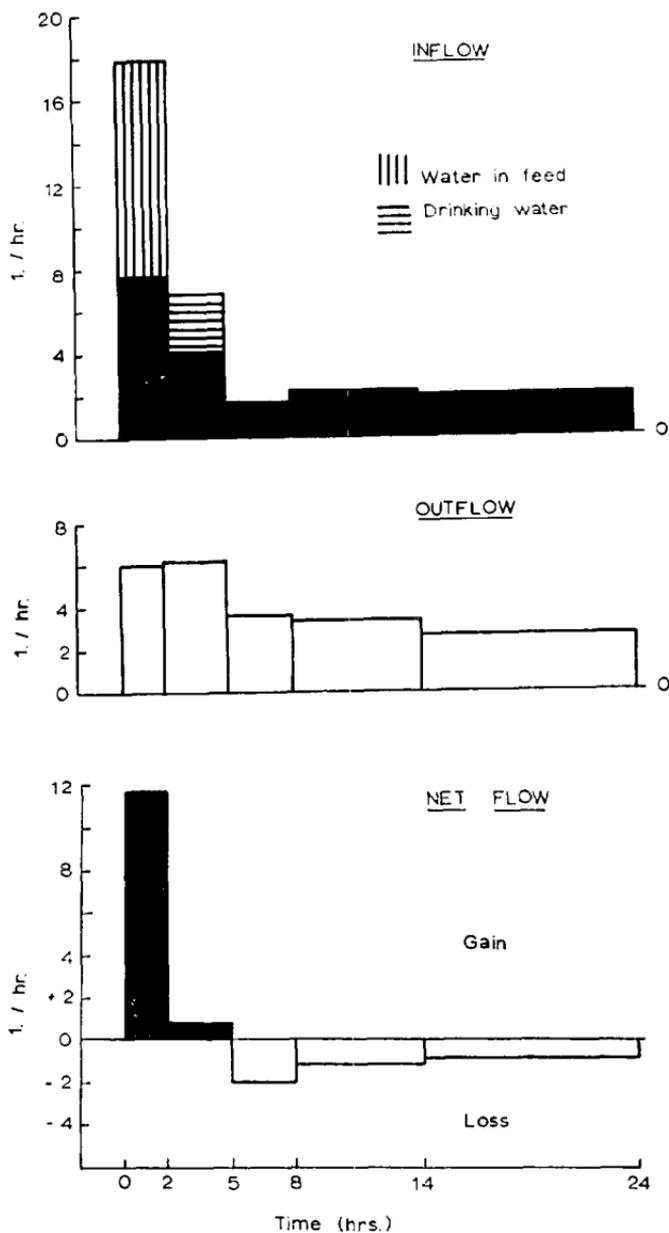


Fig. 4: Estimated average rates of net inflow to the reticulo-rumen (top), outflow through the reticulo-omasal orifice (middle), and of net flow to the reticulo-rumen (bottom), during the experiment shown in Fig. 1.

### THE MINIMAL RATE OF PRODUCTION OF VFA

As was pointed out in the introduction, water-soluble substances are continually lost from the reticulo-rumen in the flow of water through the reticulo-omasal orifice. Correction for this loss is possible by reference to the rate of loss of PEG. In the upper section of Fig. 5, the amounts of VFA and PEG present in the free aqueous phase of the contents has been plotted on a logarithmic scale. If it is assumed that the rate of loss of VFA by passage to the omasum is the same as the rate of loss of PEG, then the loss of VFA by this route can be estimated from the slopes of the two curves. This can be done mathematically by calculating the slopes in the same way that the rate of loss of D.M. was calculated above. A simple alternative method is to draw from the VFA point at the start of each interval, a line parallel to the PEG line: where the parallel line cuts the co-ordinate representing the end of the period indicates the amount of VFA that would have been present had there been no production and no loss other than passage out. The difference between this point and that representing the actual amount found gives the minimal production of VFA during the interval. The minimal rate of production of VFA is shown in the lower section of Fig. 5. This is the rate of production of VFA required to produce the VFA curve seen in the upper section of the figure, in the face of the loss in the outflow to the omasum. The histograms do not represent gross production. They do *not* indicate that production of VFA has ceased 3 h after the end of feeding, only that production then closely approximates to loss by all routes other than passage.

### THE RELATIVE RATES OF LOSS OF PEG AND LIGNIN

It is generally considered that lignin suffers little if any attack by the micro-organisms in the forestomachs (Gray, 1947). The loss of lignin from the reticulo-rumen is, therefore, related to the physical detrition of the plant residues of which it is a component. Some indication of the relative rates of passage out of the reticulo-rumen of water-soluble substances and of comminuted plant material will be given by the changes in the ratio of the amounts of PEG and lignin in the contents. This ratio, expressed as a percentage of the ratio found immediately after feeding, has been plotted in Fig. 6, using the lignin estimations made by Bailey (1965). A similar curve is shown by Weller *et al.* (1962). As would be expected, the ratio falls rapidly, reflect-

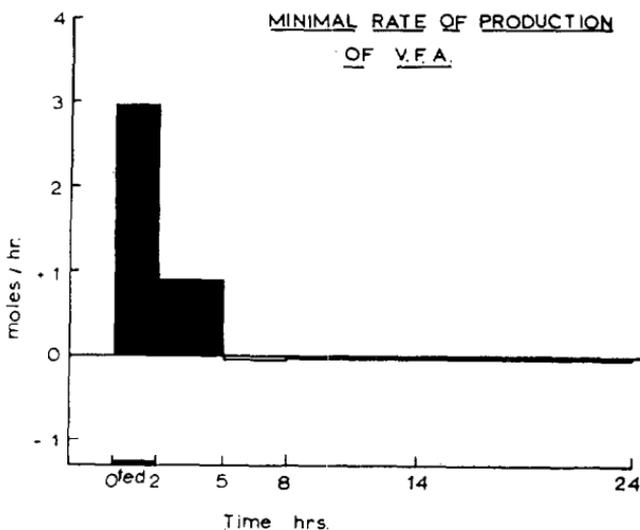
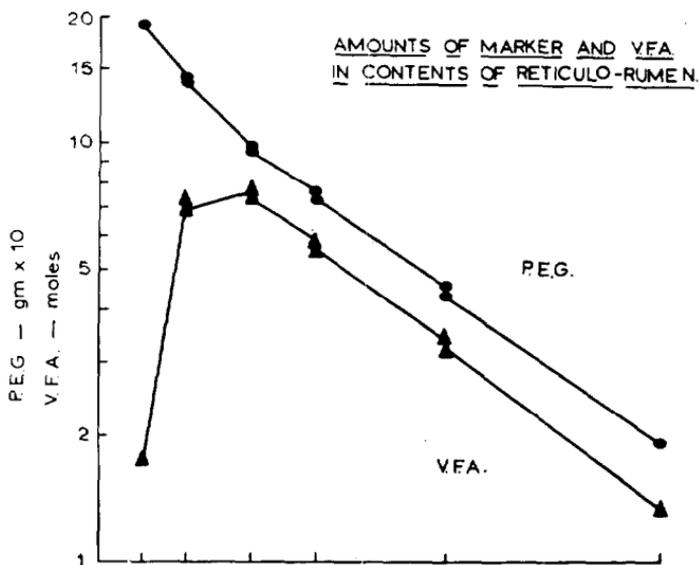


Fig. 5: Top—changes in the amounts of water-soluble marker (PEG) and of steam-volatile fatty acids (VFA) in the contents of the reticulo-rumen of cow 30 during the experiment shown in Fig. 1. Bottom—minimal rate of production of VFA, calculated from the PEG and VFA curves above (see text).

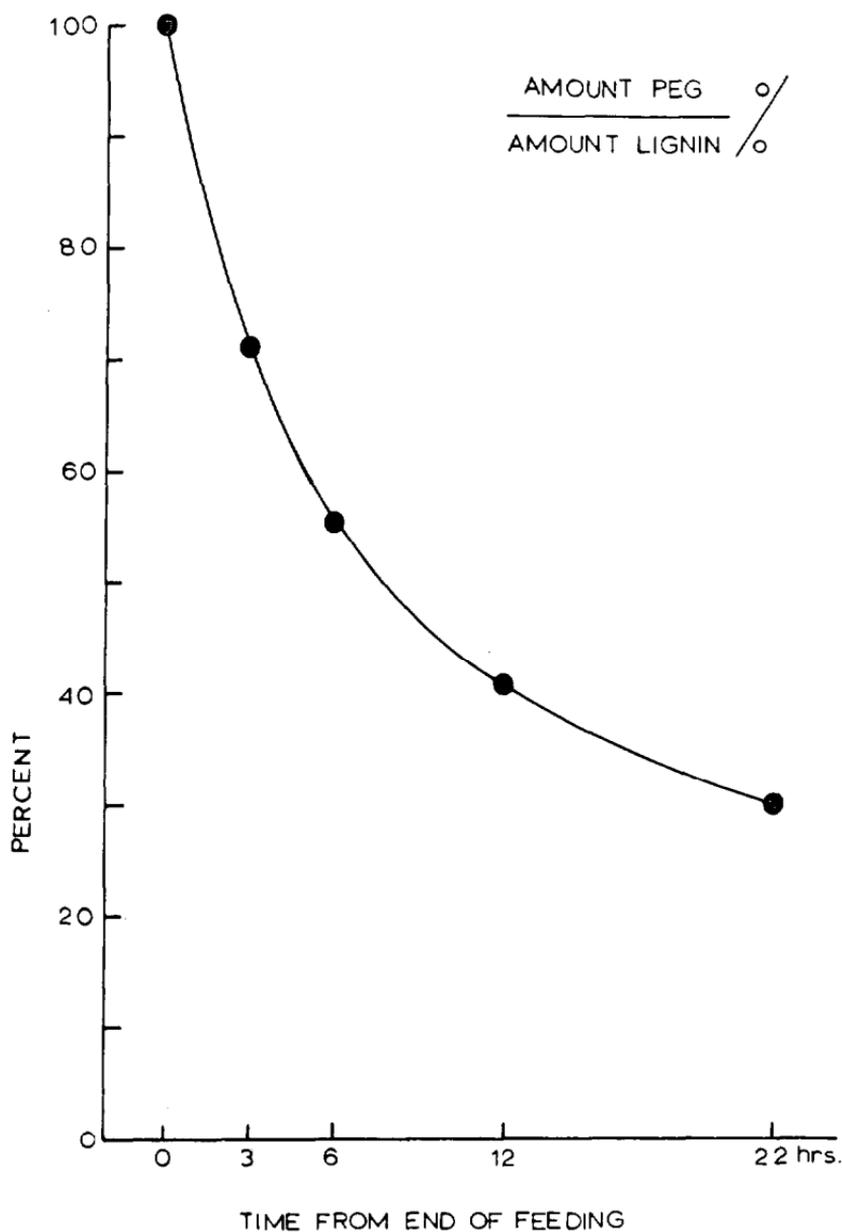


Fig. 6: Changes in the ratio of the amount of PEG: the amount of lignin, expressed as a percentage of the ratio at the end of feeding. Cow 30, same experiment as Fig. 1: note different time scale.

ing the faster rate of loss of the water-soluble marker. However, the rate of fall of the ratio decreases progressively as the time since the end of feeding lengthens and as the rate of loss of lignin, at first small, increases (Bailey, 1965).

## DISCUSSION

In assessing the value of the method of quantitative sampling described in this paper, three critical questions must be asked: "What effect has the removal and return of the contents of the reticulo-rumen on the well-being of the animal?", "What effect has it on the processes under investigation?", and "How representative is the sample obtained?" Only partial, if promising, answers can be given at present.

### THE EFFECTS OF BAILING

Removal and return of the contents of the reticulo-rumen has now been carried out some 200 times over the past two years. No deleterious effects on any of the 8 cows involved have been observed. Animals accustomed to bailing usually appear quite disinterested in the procedure. The only sign of discomfort has been occasional restlessness associated with distortion of the rumen fistula, as might happen when the most anterior regions of the rumen or the reticulum were being emptied. Removal of the contents of the reticulo-rumen would be expected to result in at least a temporary displacement of the internal organs — *e.g.*, the abomasum — with the attendant risk of torsion: no evidence of abnormality of this kind has been found. There have been no adverse effects on the appetite of the animals: on the day following an experiment, intake is usually increased slightly, thereafter returning to normal levels.

Interference with the processes of digestion in the reticulo-rumen consequent on removal and return of the contents, could arise in three ways:

(1) *Microbiologically*: Exposure of the digesta to the air will allow access of oxygen, loss of carbon dioxide, changes in pH and in solute concentration, as well as cooling, all of which effects may influence the activity of the micro-organisms. Anaerobes such as the cellulolytic bacteria are especially likely to be adversely affected by this treatment.

(2) *Mechanically*: The handling of the digesta entailed in removal, mixing and returning will itself result in some breakdown of the feed residues. Further, the normal pattern

of distribution of the components within the digesta mass — *e.g.*, layering of solid residues, gradients of solute concentration — will be disrupted.

(3) *Physiologically*: The deflation and redistension of the stomach, the localized stretching of the stomach wall, the movement of digesta, dippers and hands across the stomach lining are all forms of sensory stimulation capable of reflexly eliciting changes in a range of physiological functions. These include the composition and rate of secretion of saliva, and the form, strength and frequency of gastric contractions (Comline and Titchen, 1961; Reid, 1963; Titchen and Reid, 1965). The existence of such sensory stimulation would seem confirmed by the fact that animals commonly ruminate, or attempt to ruminate, when the reticulum is being bailed out. Other likely consequences of bailing are changes in the rate of uptake of solutes by the mucosa (see Dobson, 1961) and in the rate of outflow through the reticulo-omasal orifice.

Some of these effects — *e.g.*, an increase in the rate of gastric contractions — might be expected to increase the rate of breakdown and/or rate of passage of feed residues; others — *e.g.*, a depression of cellulolytic activity — might be expected to decrease it. However, knowledge of their magnitude, persistence and net result, is lacking.

That there was no gross depression of the rates of breakdown and passage of feed residues in the present case is indicated by the amount of D.M. found in the reticulo-rumen at the end of the experiment. Such a depression would result in reduction of the rate of loss of D.M., and, provided that the intake on the day of the experiment was similar to that of the day before, the amount of D.M. present at the end of an experiment (24 h) would be greater than the amount present at the start (0 h). In fact, as will be seen in Fig. 2, the amount of D.M. found at the end of this experiment was *less* than that at the start, although intakes on the two days were similar, the difference being in the order of the amount taken for samples during the experiment. This pattern was also shown by the carbohydrate analyses (Bailey, 1965); it has been repeatedly observed in other experiments.

#### SAMPLING ERRORS

The effectiveness of bailing as a method of quantitative sampling depends on two factors: the completeness with

which the digesta can be removed or accounted for, and the errors involved in the sampling from the mass of digesta once it has been removed from the stomach.

Emptying the reticulo-rumen is not difficult: it is easy to remove 95% of the contents, with practice more than 98%. Nor is it difficult to estimate to the nearest 100 g what is left behind. However, the amount of digesta present at any one time is the resultant of opposing activities. Some, such as the ingestion of food, drinking, and salivation, tend to increase the contents; others, such as absorption and passage to the omasum, tend to decrease them. Bailing out the reticulo-rumen is not to be likened to emptying a bucket, but to emptying a pool in a rather sluggish stream. If it is assumed that, throughout the time taken for removal of the digesta, the rates of inflow to and outflow from the reticulo-rumen remain the same as the average rates in the interval since the previous sampling, an estimate of their effects can be made. The results of such calculations for the experiment described in this paper are given in Table 2.

TABLE 2: THE EFFECTS OF INFLOW AND OUTFLOW ON THE QUANTITY OF DIGESTA FOUND IN THE RETICULO-RUMEN

	<i>Sampling Time</i>				
	<i>2 h</i>	<i>5 h</i>	<i>8 h</i>	<i>14 h</i>	<i>24 h</i>
Inflow .....	+4.3*	+2.0	+1.0	+1.5	+1.5
Outflow .....	-3.4	-3.0	-2.0	-2.2	-1.9
Net effect	+0.9	-1.0	-1.0	-0.7	-0.4

\* Calculated from the time taken to remove the digesta and the average rates of endogenous inflow and of outflow over the period since the previous sampling. Expressed as a percentage of the wet weight of digesta found.

Unfortunately, the assumption that the rates of inflow and outflow will be maintained at pre-existing levels or relative proportions is almost certainly not valid. As mentioned above, the manipulations of the stomach during bailing are potent sources of sensory stimulation, capable of reflex effects on several factors affecting the flows. Further, the changes in pressure relationships and the lowering of the level of the liquid contents will reduce the rate of outflow through the reticulo-omasal orifice, at least during the later stages of emptying. The net effect of this complex situation is likely to vary from bailing to bailing. So far, no satisfactory method of measuring it has been devised.

Mixing and sampling the removed digesta has also provided difficulties. Mixing by arm a fibrous mass weighing 60 kg or more is not physically easy. It is made the more difficult by the need to keep aeration and mechanical breakdown of the digesta to a minimum. Added to this, there is a tendency for the liquid to separate from the solid residues, which sometimes seems to be increased by any attempts made to correct it by further agitation. Perfect mixing of the digesta is thus hard to achieve. It follows that some form of multiple sampling is essential. However, practical limits are set, on the one hand, by the need to keep to a minimum the amount taken at any one sampling in experiments involving serial sampling, and on the other, by the difficulty of obtaining small representative samples from a fibrous mass of digesta. The procedure adopted so far has been described above — pooling of 3 handfuls (approximately 500 g each) taken from different regions in the container. Standard errors of mean D.M. ratios for various numbers of main (pooled) samples and subsamples, taken and prepared according to the methods described in this paper, have been calculated from the results of an experiment in which 3 main samples were taken at bailings before and after feeding. The same animal was used as in the present experiment, fed fresh red clover cut from the same stand. It will be seen (Table 3) that the major error was associated with taking the main samples. The probable source was heterogeneity of the mass of digesta; that is, mixing in the large container was imperfect.

TABLE 3: STANDARD ERROR OF MEAN D.M. RATIO EXPRESSED AS A PERCENTAGE OF THE MEAN D.M. RATIO

<i>No. of Samples</i>	<i>No. of sub-samples</i>		
	<i>1</i>	<i>2</i>	<i>6</i>
1	±4.0	±4.0	±3.9
2	±2.8	±2.8	±2.8
3	±2.3	±2.3	±2.3
4	±2.0	±2.0	±2.0
5	±1.8	±1.8	±1.7
6	±1.6	±1.6	±1.6

From the above considerations, it will be seen that no reliable figure for the overall errors involved in bailing as a quantitative sampling method can yet be given. Nevertheless, the method is practical and it allows useful results to be obtained. No equally satisfactory alternative seems

available. The value of the method is borne out by the results that have been reported in this paper as well as those reported in the papers of Bailey (1965) and Clarke (1965).

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