Diurnal Changes in the Concentrations of Micro-organisms in the Rumens of Sheep Fed Limited Diets Once Daily

WITH AN APPENDIX ON THE KINETICS OF RUMEN MICROBES AND FLOW

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SUMMARY

The pattern of change of concentration of different groups of micro-organisms in the rumen was found to be characteristic of the group and little affected by the time of day, the nature of the diet, or the host animal. The dilution rate of rumen liquor and the rate of change of concentration of several groups of micro-organisms were measured at intervals following feeding; this allowed calculation of the apparent specific growth rate. The changes in concentration of the ophryoscolecoid ciliate protozoa, the selenomonads and the oval organism described by Eadie could be explained as the resultant of the changes in dilution rate due to the act of eating and in growth rate in response to incoming nutrients. Rates of change in concentration greater than could be accounted for on the basis of growth and dilution alone were found with the peptostreptococci, the polymastigote flagellate protozoa and the holotrich ciliate protozoa. It is suggested that the peptostreptococci underwent lysis or engulfment by other organisms and that the polymastigates became sequestered, probably close to the rumen wall. The concentration changes of the holotrichs were more difficult to understand, but it would appear that little division took place for some 18 hr after feeding, followed by several divisions in quick succession. The oscillospirae showed two peaks in concentration; no explanation can be offered for this.

INTRODUCTION

Warner (1962*b*) found that the concentrations of different groups of micro-organisms in the rumen of a single sheep fed once daily changed with time in different ways, with different amplitudes and reaching maxima and minima at different times. The present studies extend this work to other sheep, diets and times of feeding. The rates of change of concentration are compared with the dilution rate of the rumen liquor, allowing calculation of the growth rate of the micro-organisms. Some tentative explanations of the various patterns of concentration change are offered. Subsequent papers describe studies made of sheep offered limited quantities of feed at frequent intervals throughout the day (Warner, 1966*a*) and of sheep with unlimited quantities of feed available, either in pens or at pasture (Warner, 1966*b*).

METHODS

Sheep. In the main experiments English Leicester–Merino crossbred ewes or wethers were used; in the minor experiments other Merino ewes were used. The sheep were trained to eat their ration within 1 hr.

Enumeration of micro-organisms. The standard counting techniques of Warner (1962*a*) were used.

Identification of organisms. Genera of ophryoscolecoid protozoa were identified and named following Lubinsky (1957). It should be noted that the organisms described as *Metadinium* sp. by Warner (1962*b*) were misnamed; following Eadie (1962*a*), they should have been described as *Polyplastron* sp.

Polymastigates and selenomonads were identified as previously (Warner, 1962*b*).

Peptostreptococci were the organisms given the number 27 by Moir & Masson (1952) and described simply as streptococci by Warner (1962*b*). They are here renamed to avoid confusion with *Streptococcus bovis*, believed to be present in some of these specimens, but not positively identified or counted.

Table 1. *Major experiments*

Expt. no.	Sheep no.	Protozoal type*	Diet†	Time of feeding (hr)
1	SO74	A	700 g. R9	09.00
2	SO74	A	700 g. R9	15.30
3	SO74	A	700 g. F19	09.00
4	SO74	A	700 g. F19	15.30
5	1319	B	700 g. R9	09.00
6	B586	A	700 g. M2	09.00

* Ophryoscolecoid protozoal population type (Eadie, 1962*a, b*); polyplastrons and diploplastrons were seen in type A populations, epidinia, diplodinia and eudiplodinia in type B.

† Diet R9 consisted of 50 % wheaten chaff, 50 % lucerne chaff. Diet F19 consisted of 50 % lucerne chaff, 20 % wheat, 10 % linseed meal, 10 % coconut meal, 10 % oats. Diet M2 consisted of 50 % lucerne chaff, 50 % oats.

The organisms described here as Eadie's ovals were described by Eadie (1962*a*) in Scotland and independently as 'flagellates' by Warner (1962*b*) in Australia; they have since been described in Egypt by Abou Akkada & El-Shazly (1964).

Experimental design. Six major experiments were carried out in which samples of rumen contents were collected at intervals of about 2 hr for 24 hr or more, and examined thoroughly for all important groups of organisms. These experiments are described in Table 1.

Other sheep were fed 800 g. of diet R9 with or without 1.25 % added NaCl at either 09.00 hr or 14.00 hr, and were used in minor experiments as described later, only one group of micro-organisms being examined at a limited number of times.

In another experiment, the dilution rate of the rumen liquor was determined. The ⁵¹Cr complex of ethylenediaminetetra-acetic acid (⁵¹CrEDTA) was added to the rumen of sheep SO74 and the concentration of ⁵¹Cr determined from time to time as described by Downes & McDonald (1964). The dietary regimen was 700 g. diet R9 fed at 14.00 hr.

In all these experiments, access to water was allowed at all times. Animals were fed according to the experimental schedule for at least 3 weeks before sampling, and were thoroughly accustomed to the routine.

EXPRESSION OF RESULTS

It is shown in the appendix to this paper that plots of the logarithm of the concentration of a reference solute such as $^{51}\text{CrEDTA}$ and of the micro-organisms against time can be used to calculate the dilution rate of the rumen liquor and the growth rate of the micro-organisms. Monod (1949) pointed out the convenience of using logarithms to the base 2 for graphical presentation of work with bacterial cultures; similar considerations apply to flowing systems and hence to work with organisms in the rumen.

It was found previously (Warner 1962*b*) that under apparently constant conditions of nutrition the concentration of any one species of micro-organism in the rumen at a specified time after feeding can stabilize at any of a wide range of concentration values. However, it was expected that the pattern of growth behaviour of a given organism over the 24 hr feeding cycle would be largely independent of its initial concentration, so to compare concentration curves at different concentration levels the following procedure was adopted to plot the results of any one experiment.

(i) The concentration of each group of organisms was estimated for each sample of rumen liquor, and the $\log_2$ concentration was computed, using the loglog scales of a slide rule and units chosen to give a minimum concentration between 1 and 10.

(ii) The mean of these $\log_2$ concentrations for each group of organisms was calculated; the concentration corresponding to this mean was called the basal concentration, and is recorded in the legends to the figures.

(iii) The deviations of the $\log_2$ concentrations from the mean were computed and plotted as the relative $\log_2$ concentrations about the mean as arbitrary zero.

Examples of sets of individual curves plotted in this way are shown in Figs. 1, 11 and 13. A mean curve for each organism was then constructed from the corresponding set of individual curves. It had been inconvenient to collect specimens in the various experiments at identical times after feeding. Consequently, these mean curves were plotted by computing values for 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 23 hr after feeding, from each individual curve, and then taking the mean for each time. Thus Fig. 2 contains the mean curve for the individual curves plotted in Fig. 1. Other mean curves were plotted similarly. It should be noted that the zero line of this mean curve has not a simple meaning.

These curves were then used to calculate the relative rates of increase of concentration, r , as described in the appendix. The sum of this rate and the dilution rate D equals the net rate of formation of microbial cells ρ ; for a randomly dividing population when the death rate λ is negligible, this is the same as the specific growth rate ν .

An alternative calculation of ν can also be made from the division index, the proportion of dividing organisms. Warner (1962*b*) used an equation of Crick (1948) as cited by Hughes (1952) to determine the doubling time, g , of entodinia in the rumen. Smith & Dendy (1962) showed that the equation should have been $g = T \ln 2 / \ln (1 + I)$ instead of that misquoted by Hughes (1952),

$$g = T \ln 2 / \ln [(1 + 2I)/(1 + I)],$$

where T is the time needed to complete division and I is the proportion of dividing cells; for small values of I the two formulae give the same result. Hungate, Bryant & Mah (1964) correctly criticized the use of this formula to calculate genera-

tion times of entodinia at various times of the day, since some degree of synchronicity was present; however, the equation can be used to give a simple measure of the rate of increase of numbers at any instant, without any implications about the time between successive divisions. From the equation of Smith & Dendy (1962) above, it can be seen that $\nu = \ln 2/g = \ln (1+I)/T = I/T$ approximately for small values of I . In the work reported here, the observer who estimated the proportion of dividing cells used different criteria from those used in the studies of Warner (1962*b*) and it is believed that $I' = 0.75I$, where I' is the proportion of cells recognizable as dividing in the living preparations used to determine the time to complete division T , and I is the proportion of cells recognizable as dividing in fixed preparations, as recorded in this paper. For entodinia, therefore, $\nu = I'/T = 3I \text{ hr}^{-1}$, since $T = 0.25 \text{ hr}$ (Warner, 1962*b*).

The time of day is expressed on the 24 hr clock as 01.00 hr, etc.; the time after presentation of feed, briefly the time after feeding, is expressed as 1 hr, etc.

RESULTS

The concentrations of micro-organisms were found to change with time in patterns characteristic of the species, as shown by Figs. 1–14. Closely similar curves were obtained despite variations in basal concentrations, as shown by the curves for entodinia in Expts. 3 and 4 (Fig. 1), dasytrichs in Expts. 1 and 5 (Fig. 11) and oscilospirae in Expts. 2 and 4 (Fig. 13).

Altering the time of feeding shifted the phase of these patterns by an equivalent amount, as is seen by comparing pairs of curves for Expts. 1 and 2 (Figs. 1*a*, 11*a*, and 13*a*) and for Expts. 3 and 4 (Figs. 1*b*, 11*b* and 13*b*). No evidence was seen of the influence of any diurnal rhythm other than that of feeding.

No effect on the patterns of concentration change due to the host animal could be detected in comparing the results of Expts. 1 and 5; examples may be seen in Figs. 1 and 11. The patterns obtained with the three different diets, R9 (Expts. 1, 2 and 5; Figs. 1, 11 and 13), F19 (Expts. 3 and 4; Figs. 1, 11 and 13) and M2 (Expt. 6, Figs. 1 and 11) were very similar, though small differences in magnitude (Figs. 1*b*, 1*c*, 11) or in timing (Figs. 1*c* and 13) can be seen.

Similar findings to the above were made with the other micro-organisms, suggesting that the pattern of concentration change was primarily determined by the species of micro-organism under consideration, and little affected by its initial concentration, the time of day, the nature of the diet, or the host animal. Consequently, it was felt to be justifiable to construct the mean curve as described above. It should be noted, however, that the minor differences in timing previously mentioned tend to make the mean curve flatter than the individual curves, with lower maxima and higher minima (compare, particularly, individual curves in Figs. 11 and 13 with the mean curves in Figs. 12 and 14). Consequently, in reporting the results of many experiments, data from the unpublished individual curves as well as the published mean curves are given.

Entodinia. Immediately following feeding, the concentration of entodinia decreased at a rate $r = -0.16 \text{ hr}^{-1}$ (Fig. 2) and continued to decrease for the first 2–4 hr. However, the proportion of dividing organisms and hence the growth rate started to increase immediately and increased to a maximum of some four times the

initial rate at about 8 hr after feeding, after which it decreased gradually over the remaining 16 hr. The concentration increased from its minimum at 2–4 hr to a maximum at 16–20 hr at a rate of about $r = 0.05 \text{ hr}^{-1}$ (range, from Fig. 1, about $0.03\text{--}0.12 \text{ hr}^{-1}$). During the last 2–6 hr before feeding, when the growth rates were minimal, the concentration decreased at a mean rate of $r = -0.07 \text{ hr}^{-1}$.

Other ophryoscolecoid protozoa. The diploplastrons (Fig. 3), polyplastrons (Fig. 4) and epidinia (Fig. 5) all showed patterns of concentration change similar to the entodinia, a rapid decrease in concentration after feeding, then a slower increase to

Fig. 1. Changes in concentration of entodinia in individual experiments (basal concentrations of entodinia in numbers/ml. are given in brackets):

- (a) ○ Expt. 1 sheep SO74 fed diet R9 at 09.00 hr (1.3×10^5).
● Expt. 2, sheep SO74 fed diet R9 at 15.30 hr (2.0×10^5).
- (b) △ Expt. 3, sheep SO74 fed diet F19 at 09.00 hr (7.3×10^5).
▲ Expt. 4, sheep SO74 fed diet F19 at 15.30 hr (4.4×10^5).
- (c) □ Expt. 5, sheep 1819 fed diet R9 at 09.00 hr (3.9×10^5).
■ Expt. 6, sheep B586 fed diet M2 at 09.00 hr (2.6×10^5).

a maximum a few hours before the next feed, followed by a slow decline. The diplo-dinia (Fig. 5) were examined in only one experiment; this seemed to indicate an absence of the rapid initial decrease in concentration. The organisms were, however, present in very low concentration and this seems to lead to increased counting error, even though the total number of organisms counted was similar to that counted in

the other experiments. It seems possible that, in fact, the diplodinia suffer similar concentration changes to the other ophryoscolecids. The polyplastrons, diplodinia and epidinia (Figs. 4, 5) changed in concentration over a wider range than the entodinia or diploplastrons (Figs. 2, 3).

In Expt. 5 (Fig. 5), the epidinia and diplodinia were present in low concentration, making the estimation of the proportion of dividing organisms difficult and inaccurate, but for both organisms peak proportions exceeding 0.10 were noted.

Fig. 2. Mean curves for concentration (O), and proportion of dividing organisms and specific growth rate v (●) for entodinia; data from the experiments described in Fig. 1 were used.

Fig. 3. Mean curves for concentration (O) and proportion of dividing organisms (●) for diploplastrons; data used were from the following experiments (basal concentrations of diploplastrons in numbers/ml. are given in brackets): Expt. 2, sheep SO74 fed diet R9 at 15.30 hr (5.1×10^3); Expt. 3, sheep SO74 fed diet F19 at 09.00 hr (3.6×10^4); Expt. 4, sheep SO74 fed diet F19 at 15.30 hr (8.7×10^3); Expt. 6, sheep B586 fed diet M2 at 09.00 hr (5.6×10^4).

The mean proportion of dividing organisms found for all the higher ophryoscolecids was greater than that noted for the entodinia (Fig. 2).

Selenomonads. The concentration of selenomonads decreased after feeding (Fig. 6) at a mean rate of about $r = -0.22 \text{ hr}^{-1}$ (range -0.1 to -0.5 hr^{-1}) to a minimum

usually within the first 2 hr. Concentrations then remained fairly steady for 8–12 hr and then increased more rapidly ($r = 0.03\text{--}0.11$, mean 0.05 hr^{-1}) to a peak 16–24 hr after feeding, then perhaps slowly declining.

Fig. 4

Fig. 5

Fig. 4. Mean curves for concentration (○) and proportion of dividing organisms (●) for polyplastrons; data used were from the following experiments (basal concentrations of polyplastrons in numbers/ml. are given in brackets): Expt. 3, sheep SO74 fed diet F19 at 09.00 hr (3.5×10^3); Expt. 4, sheep SO74 fed diet F19 at 15.30 hr (4.0×10^3).

Fig. 5. Changes in concentration of micro-organisms in Expt. 5, sheep 1319 fed diet R9 at 09.00 hr (basal concentrations of micro-organisms in numbers/ml. are given in brackets): (○) epidinia (4.2×10^3); (●) diplodinia (2.9×10^3).

Fig. 6

Fig. 7

Fig. 6. Mean curve for concentration of selenomonads; data used were from the following experiments (basal concentrations of selenomonads in numbers/ml. are given in brackets): Expt. 1, sheep SO74 fed diet R9 at 09.00 hr (2.8×10^7); Expt. 2, sheep SO74 fed diet R9 at 15.30 hr (5.5×10^7); Expt. 3, sheep SO74 fed diet F19 at 09.00 hr (3.4×10^8); Expt. 4, sheep SO74 fed diet F19 at 15.30 hr (5.8×10^7); Expt. 5, sheep 1319 fed diet R9 at 09.00 hr (7.6×10^8).

Fig. 7. Changes in concentration (○) and proportion of dividing organisms (●) for Eadie's ovals in Expt. 1, sheep SO74 fed diet R9 at 09.00 hr (basal concentration 4.1×10^6 /ml.)

Eadie's ovals. These organisms were observed in only a few experiments. While the concentration curves were generally similar, the maxima and minima occurred at somewhat different times and this made the mean curve misleading; consequently, figures for one experiment only are given in Fig. 7. The concentration decreased

after feeding at a rate of about $r = -0.2 \text{ hr}^{-1}$ to a minimum at 6–12 hr, then rising at a rate of about 0.17 hr^{-1} to a plateau at 12–20 hr, remaining fairly constant thereafter, at a level about four times the minimum. The proportion of dividing organisms was very low in the fasting animal, rising very rapidly after feeding to a maximum at about the time of minimum concentration, declining again rather more slowly.

Total bacterial counts. There was a decrease in concentration (Fig. 8) after feeding at a mean rate $r = -0.14 \text{ hr}^{-1}$ (range 0.0 to -0.19) to a minimum at 1–4 hr, then a slow increase at a mean rate $r = 0.03 \text{ hr}^{-1}$ to a maximum at 12–20 hr; from about 20 hr after feeding, the concentration usually decreased at a rate about -0.03 hr^{-1} . For Expts. 1 and 6, the curves were very flat, the maxima being only some 15 % greater than the minima; in Expt. 5, however, the range was twofold.

Fig. 8

Fig. 9

Fig. 8. Mean curve for concentration of total bacteria; data used were from the following experiments (basal concentrations of bacteria in numbers/ml. are given in brackets): Expt. 1, sheep SO74 fed diet R9 at 09.00 hr (2.0×10^{10}); Expt. 2, sheep SO74 fed diet R9 at 15.30 hr (1.8×10^{10}); Expt. 3, sheep SO74 fed diet F19 at 09.00 hr (2.7×10^{10}); Expt. 4, sheep SO74 fed diet F19 at 15.30 hr (2.3×10^{10}); Expt. 5, sheep 1319 fed diet R9 at 09.00 hr (2.6×10^{10}); Expt. 6, sheep B586 fed diet M2 at 09.00 hr. (2.6×10^{10}).

Fig. 9. Mean curve for concentration of peptostreptococci; data used were from the following experiments (basal concentrations of peptostreptococci in numbers/ml. are given in brackets): Expt. 1, sheep SO74 fed diet R9 at 09.00 hr (8×10^4); sheep 1184 fed diet R9 at 14.00 hr (4×10^4); sheep 2714 fed diet R9 at 14.00 hr (1×10^4); sheep MA94 fed diet R9 at 09.00 hr (2×10^4).

Peptostreptococci. This organism is multicellular, and was counted as the number of chains. There was a great decrease in concentration after feeding (Fig. 9) at a mean rate $r = -1.7 \text{ hr}^{-1}$ (range -0.7 to -2.8 hr^{-1}) to a minimum 1–4 hr after feeding, then a steady increase at a mean rate of about 0.4 hr^{-1} to a maximum at about 12–18 hr, and finally a decrease at a slowly increasing rate up to about $r = -0.8 \text{ hr}^{-1}$. The number of cells per chain appeared to be relatively constant. In Expt. 1, 40 chains were examined in each specimen. In all specimens the mean number of cells per chain was between 5 and 6, with standard deviations about 2. More than half the chains had four cells, more than a quarter eight; other numbers found in this series were, in decreasing order of frequency, 6, 2, 3, 5 and 12. Accurate counts have not been made, but it appears that, in other experiments, the mean chain length may have been somewhat different; in some experiments, 16 or more

cells per chain were not rare. Chain length may be a characteristic of a particular strain of peptostreptococcus, or perhaps of particular environmental conditions.

Polymastigates. These flagellates showed a large increase in concentration 1–2 hr after feeding (Fig. 10) at a mean rate $r = 1.4 \text{ hr}^{-1}$; in one experiment, $r = 3.8 \text{ hr}^{-1}$, and the concentration increased 50-fold in 3 hr. This increase was followed by an almost equally rapid decrease in concentration to a minimum at 6–12 hr after feeding, followed by a fluctuating concentration value. In most experiments, the ratio of maximum to minimum concentration exceeded 25; the averaging process used to draw the mean line masked this, showing only an eightfold range, due mainly to differences in timing of the minima and the subsequent four- to eightfold fluctuations.

Fig. 10. Mean curve for concentration of polymastigates; data used were from the following experiments (basal concentrations of polymastigates in numbers/ml. are given in brackets): Expt. 1, sheep SO74 fed diet R9 at 09.00 hr (7×10^3); Expt. 2, sheep SO74 fed diet R9 at 15.30 hr (3×10^3); Expt. 3, sheep SO74 fed diet F19 at 09.00 hr (1×10^4); Expt. 4, sheep SO74 fed diet F19 at 15.30 hr (5×10^3); Expt. 5, sheep 1319 fed diet R9 at 09.00 hr (1×10^3); Expt. 6, sheep B586 fed diet M2 at 09.00 hr (2×10^4).

This great increase in concentration after feeding suggested that organisms might perhaps be entering the rumen with the feed or the drinking water. However, no polymastigates were seen in any of the following preparations: the deposit from centrifuged water from the drinking troughs; unfiltered aqueous extracts of the feed; cultures of feed incubated with water or centrifuged rumen liquor for 24 hr at 39° in completely filled glass-stoppered bottles, presumed to be anaerobic; cultures of water from the drinking troughs incubated as above either alone or mixed with an equal volume of centrifuged rumen liquor. No organisms suggesting cysts of these polymastigates were noticed, confirming Braune (1913).

In an attempt to identify the stimulus for the increase in concentration, four extracts of the feed were prepared as follows:

(1) 500 g. coarsely ground diet R9 mixture was extracted for 45 min. with water in a shaking machine, and the extract was filtered.

(2) A similar extract was dialysed against running tap water overnight, then against several changes of distilled water in the refrigerator.

(3) Following the procedure of Smith & Benitez (1955), 500 g. finely ground diet R9 mixture was extracted with 1500 ml. 80% (v/v) acetone in water, filtered on a Buchner funnel and washed with 3×150 ml. 80% (v/v) acetone in water. To the filtrate (about 1000 ml.), 320 g. talc and 1000 ml. water were added and the mixture

filtered through a layer of talc on a Buchner funnel. The filtrate was concentrated in a rotary evaporator under reduced pressure, using a silicone oil anti-foam. The concentrate was divided into two portions and each was evaporated to dryness to remove acetone, taken up in 100 ml. water and centrifuged. The supernatant fluid in each portion contained about 8.0 g. dry matter (about 150 mg. N). One portion was used without further treatment.

(4) The second portion was mixed with 250 ml. 6N-HCl and hydrolysed overnight. The HCl was removed by repeated evaporation under reduced pressure, the mixture brought to 100 ml. with water and filtered. It was found to contain about 3.8 g. dry matter (about 135 mg. N).

Table 2. *Effect of intra-ruminal administration of feed extracts on the concentration of polymastigates in the rumen*

The normal feed was given immediately after the 2 hr sample

Sample	Concentration of polymastigates (thousands/ml.)			
	Water extract	Dialysed water extract	Acetone extract	Hydrolysed acetone extract
Pre-dosing	6	4	6	10
1 hr post-dosing	43	11	120	13
2 hr post-dosing	32	7	90	23
1 hr post-feeding	12	29	15	35

These four extracts were injected into the rumens of sheep held 24 hr without feed; 2 hr later, feed was given. Samples of rumen liquor were taken before, and 1, 2 and 3 hr after, the extracts were injected, and examined for polymastigates. The results are given in Table 2. Both aqueous and acetone extracts of feed elicited the response of rapid increase in concentration of the polymastigates followed by a slower decline; subsequent feeding did not reverse the decline. The dialysed (2) and hydrolysed (4) extracts elicited little if any response and subsequent feeding produced a small increase in concentration. No response was elicited by the injection of solutions of glucose or of bacteriological peptone.

Holotrich protozoa. The dasytrichs (Figs. 11, 12) reached a peak concentration at or shortly after the time of feeding, and then fell steadily in concentration (mean rate of fall $r = -0.11 \text{ hr}^{-1}$) to a minimum around 12–20 hr. This was followed by a rapid increase in concentration at a mean rate of 0.30 hr^{-1} , range $0.14\text{--}0.70 \text{ hr}^{-1}$. The isotrichs (Fig. 12) behaved similarly, though moving through a somewhat smaller range.

In several supplementary experiments in which one day's feed was withheld, it was found that concentrations of holotrichs increased rapidly from the minimum at about 20 hr to maxima at 22–28 hr after feeding. The subsequent rate of decrease appeared to be slightly less than in the main experiments, but sampling was terminated before this was determined with certainty.

Throughout the first 16 hr after feeding, the proportion of dividing holotrichs was very low, usually 0.001 or less. This proportion then rose to a maximum at or just after the time of minimum concentration, falling rapidly again. The maximum proportion of dividing holotrichs was usually about 0.10, but in a few instances even higher proportions, up to 0.28, of dividing dasytrichs have been seen.

Sporadically in this work, the dasytrichs have been seen in conjugation. The proportion of conjugating organisms has usually been 0.01 or less, but on a few occasions it has been over 0.10. So far, no pattern has been noted in this phenomenon, and it appears to be unrelated to normal division.

Oscillospirae. These multicellular organisms were counted as the number of trichomes, not the number of cells. Two peaks in concentration were found (see Figs. 13, 14), the first at about the time of feeding. Following this, the concentration declined at a mean rate $r = -0.09 \text{ hr}^{-1}$ to a minimum 6–8 hr after feeding, increased again at a mean rate $r = 0.04 \text{ hr}^{-1}$ to a second peak 6–14 hr after the first, and then

Fig. 11

Fig. 11. Changes in concentration of dasytrichs in individual experiments (basal concentrations of dasytrichs in numbers/ml. are given in brackets):

- (a) ○ Expt. 1, sheep SO74 fed diet R9 at 09.00 hr (2.2×10^4);
● Expt. 2, sheep SO74 fed diet R9 at 15.30 hr (1.2×10^4);
- (b) △ Expt. 3, sheep SO74 fed diet F19 at 09.00 hr (3.2×10^3);
▲ Expt. 4, sheep SO74 fed diet F19 at 15.30 hr (3.0×10^3);
- (c) □ Expt. 5, sheep 1319 fed diet R9 at 09.00 hr (6.3×10^3);
■ Expt. 6, sheep B585 fed diet M2 at 09.00 hr (1.4×10^4).

Fig. 12

Fig. 12. Mean curves for concentration of (○) dasytrichs (data from Fig. 11) and of (●) isotrichs, where the data used were from the following experiments (basal concentrations of isotrichs in numbers/ml. are given in brackets): Expt. 2, sheep SO74 fed diet R9 at 15.30 hr (2.2×10^3); Expt. 3, sheep SO74 fed diet F19 at 09.00 hr (1.2×10^4); Expt. 4, sheep SO74 fed diet F19 at 15.30 hr (6.8×10^3); Expt. 5, sheep 1319 fed diet R9 at 09.00 hr (1.2×10^3); Expt. 6, sheep B586 fed diet M2 at 09.00 hr (1.3×10^4).

declined once more at a mean rate $r = -0.03 \text{ hr}^{-1}$ to a second minimum about 18 hr after feeding, beginning to increase again at a mean rate about 0.07 hr^{-1} before the next feeding period.

It was not found practicable to count the number of cells per trichome, but the length of the trichomes was measured. This length was highly variable, with a coefficient of variation of 20–40 %, so 100 cells were measured in each sample; an ocular micrometer was used. The minimum length seen was about 6μ , the maximum over 100μ . After feeding, there was a rapid increase of about 20 % in the mean length, the cells became highly refractile and strongly iodophilic. The length then decreased, at first rapidly, then more slowly, while the trichomes gradually lost their

Fig. 13

Fig. 14

Fig. 13. Changes in concentration of oscillospirae in individual experiments (basal concentrations of oscillospirae in numbers/ml. are given in brackets):

- (a) ○ Expt. 1, sheep SO74 fed diet R9 at 09.00 hr (8.4×10^6);
- Expt. 2, sheep SO74 fed diet R9 at 15.30 hr (3.1×10^6);
- (b) △ Expt. 3, sheep SO74 fed diet F19 at 09.00 hr (1.1×10^6);
- ▲ Expt. 4, sheep SO74 fed diet F19 at 15.30 hr (6.9×10^4).

Fig. 14. Mean curves for concentration (○) and mean length of trichome (●) for oscillospirae; data from the experiments described in Fig. 13 were used.

refractility and internal polysaccharide. In Expt. 4 only, a statistically significant second peak in length was seen at the time of the second peak in concentration; no such second peak was seen in any other experiment and it may have been a chance effect.

Marker dilution. The results of the experiment using $^{51}\text{CrEDTA}$ as a marker of rumen dilution are shown in Fig. 15. Before feeding, the dilution rate was 0.063 hr^{-1} , and during the hour that the animal spent eating, the rate increased considerably to 0.234 hr^{-1} . Nearly all the 1800 ml. water drunk during the experiment was consumed within the times 14.30–15.30 hr, i.e. in the last portion of the feeding period and shortly thereafter. During the period 1–3 hr after feeding, a decreased dilution rate, 0.039 hr^{-1} , was observed; the rate then became fairly steady at 0.052 hr^{-1} for the remainder of the experiment. The overall average dilution rate was 0.059 hr^{-1} . The prefeeding volume of rumen liquor was calculated as about 7.5 l.; the sheep weighed about 42 kg.

Fig. 15. Dilution curve for the rumen marker ^{51}Cr EDTA in sheep SO74 fed diet R9 at 14.00 hr.

DISCUSSION

The nutritional economy of the ruminant animal depends on the absolute numbers of micro-organisms present in the rumen and flowing to the omasum. But the volume of rumen contents and the rate of flow are not constant (see Warner, 1964), so that the significance to the host of the concentration figures as presented here is uncertain (Clarke, 1965). However, the biology and ecology of the micro-organisms of the rumen are comparatively unaffected by changes in rumen volume and their discussion requires knowledge only of relative flow rates, i.e. dilution rates, and of concentrations.

The volume of rumen contents found for sheep SO74 was greater and the resting dilution rate less than in the sheep of similar breed and weight described by Stacy & Warner (1966). However, all the latter animals were relatively slow eaters and there is some (unpublished) evidence from these laboratories and elsewhere that more rapid eaters tend to have large rumen volumes. There is no evidence that the rate of secretion of saliva in the resting state, 4–7 ml./min. (Stacy & Warner, 1966) depends on this factor, so that resting dilution rates would be less for the more rapid eaters; Stacy & Warner (1966) found rates of 0.07–0.11 hr^{-1} , as compared with 0.05–0.06 hr^{-1} for sheep SO74 in this work. During feeding, the amount of saliva secreted per g. feed consumed seems to be independent of the animal, and about 2.5–3.0 ml./g. for hay-type diets (Bailey, 1961; Meyer, Bartley, Morrill & Stewart, 1964; Stacy & Warner, 1966), so that the rapid eaters would secrete more saliva per unit time while eating than the slow eaters, and hence the dilution rates would be similar; Stacy & Warner (1966) found rates of 0.19–0.28 hr^{-1} , as compared with 0.23 hr^{-1} found in the present work. For a short while after feeding, the flow of saliva and the dilution rate were diminished below the normal resting rate (Bailey & Balch, 1961; Stacy & Warner, 1966). Consequently it is believed that the results of the experiment described in Fig. 15 can be taken as typical for sheep SO74 and, on the basis of feeding rates, as probably typical for sheep 1319 and B586 in Expts. 1–6.

Warner (1962a) defined the standard count as the concentration of micro-

organisms found in strained rumen liquor, and the total count as the concentration found in whole rumen contents after the feed particles had been washed and disintegrated. The concentration of organisms as measured by the standard count can be affected by dilution of the rumen liquor, by division or death and lysis of organisms, by engulfment by other organisms, and by sequestration of organisms in and return from places not sampled at examination, such as close to the rumen wall, or in close contact with the solid mass of feed found with some diets.

A measure of sequestration is given by comparison of the standard count with the total count of micro-organisms. Results of such studies by Warner (1962*a, b*), with other unpublished data collected at the same time, showed conclusively that sequestration among the feed particles was of major importance only for the small bacteria included in the total bacterial counts. Individual bacterial groups could be represented in the standard count at only 12 % or less of their true concentration (Warner, 1962*a*); this proportion varied considerably with different groups. Sequestration among feed particles was of only minor significance for the larger organisms. A small increase in sequestration was noted shortly after feeding and again some 12–16 hr later (Warner, 1962*b*) for several groups of organisms, including the entodinia, oscillospirae and dasytrichs, while the peptostreptococci showed increased sequestration for some 8 hr after feeding; however, at most times the ratio of total to standard count was fairly constant and close to 1, that is, the standard counts approximated fairly well to the true counts for all the organisms discussed in this work, except for the small bacteria. Clarke (1965), who used a sampling technique expected to give counts corresponding to the total counts of this laboratory, and Johnson, Hamilton, Robinson & Garey (1944), Purser & Moir (1959) and Purser (1961), who used techniques similar to the standard count of this laboratory, all found patterns of concentration change for the ciliate protozoa similar to those found here. However, it should be noted that organisms sequestered among the papillae of the rumen wall would probably escape counting by any of these techniques.

Inspection of Figs. 1–14 shows a number of qualitatively different patterns of concentration change, and evidence is presented that this pattern depended primarily on the nature of the organism and the feeding regimen and was little affected by differences in the host animal or, within the range studied, the diet. This is confirmed by the finding of similar patterns for a different sheep fed on diet R9 (Warner, 1962*b*) and for grazing sheep where the grazing behaviour resembled the feeding behaviour of these pen-fed sheep (Warner, 1966*b*). Also, Johnson *et al.* (1944), Purser & Moir (1959) and Clarke (1965) found similar patterns for the ophryoscolecid protozoa, and Purser (1961) and Clarke (1965) found similar patterns for the holotrichs, with animals fed rather different diets. Total bacterial counts, where many very different species may be confounded, may be expected to be more affected by the nature of the diet, since the different diets would allow different species to dominate and these species might well have different growth behaviours. Such differences due to diet can be seen in the work of Nottle (1956), El-Tabey Shehata (1958) and Bryant & Robinson (1961).

Probably the simplest concentration pattern to interpret is where feeding is accompanied by a rapid decrease in concentration of micro-organisms, followed by a slow increase for some time and perhaps by a slow decrease just before the next feeding period. Such curves are seen with the ophryoscolecid protozoa, the entodinia

(Fig. 2), diploplastrons (Fig. 3), polyplastrons (Fig. 4), epidinia (Fig. 5) and possibly diplodinia (Fig. 5); the selenomonads (Fig. 6); Eadie's ovals (Fig. 7) and the total bacterial count (Fig. 8). The data are summarized in Table 3, and for the entodinia in Table 4.

The data for the entodinia (Table 4) are more detailed and probably more accurate than for the other groups of organisms. The rate of increase of concentration, r , as actually measured closely approximated to the value calculated from the difference between the growth rate ν and the dilution rate D . The calculation of ν assumes that T , the time needed to complete division, is independent of the species of entodinia

Table 3. *Mean rates of change of concentration of rumen micro-organisms*

Organism	Post-feeding rate (hr ⁻¹)	Time at minimum*	Rate during phase of increase (hr ⁻¹)	Time at maximum*	Pre-feeding rate (hr ⁻¹)
Entodinia	-0.16	2-4	0.05	14-20	-0.05
Diploplastrons	-0.16	2-10	0.06	16-22	-0.04
Polyplastrons	-0.18	4	0.07	18-20	-0.09
Epidinia	-0.35	4	0.17	14	-0.05
Diplodinia	0	0	0.15	14	-0.10
Selenomonads	-0.22	2-8	0.05	18-22	-0.01
Eadie's ovals	-0.21	6	0.17	14-24	0
Total bacteria	-0.14	2	0.03	12-20	-0.03
Peptostreptococci	-1.7	1	0.4	12-18	-0.3

* A single figure indicates a sharp minimum or maximum at the stated time after feeding; a range indicates the period during which the concentration was approximately constant.

Table 4. *Rates of increase of concentration of entodinia*

Values of the rate of increase r and the dilution rate D were calculated for the periods shown from Figs. 2 and 15 respectively. The values for the growth rate ν were averages calculated from the data of Fig. 2.

Time after feeding (hr)	r (hr ⁻¹)	ν (hr ⁻¹)	D (hr ⁻¹)	$\nu - D$ (hr ⁻¹)
0-24	0.000	0.053	0.059	-0.006
0-2	-0.10	0.04	0.15	-0.11
2-4	0.00	0.04	0.04	0.00
4-14	0.04	0.07	0.05	0.02
14-20	0.01	0.04	0.05	-0.01
20-24	-0.05	0.02	0.06	-0.04

and unvarying with time (i.e. changes in environment), which may not be completely true (James, 1960), though Warner (1962*b*) was unable to detect any differences in a limited series of experiments. The technique used to estimate T probably yields an overestimate because of unphysiological conditions on the microscope slide (cf. Browning, Varnedoe & Swinford, 1952); this would give an underestimate of ν and would perhaps account for the differences between r as measured and the calculated $\nu - D$. The closeness of these estimates of r nevertheless suggests that there was little sequestration or death of these organisms, so that $\lambda = 0$ and $\nu = \rho$. This confirms the statements made above based on the findings of Warner (1962*b*); dead entodinia were seen only very rarely in this work.

The simplest interpretation of the data appears to be that the initial decrease in concentration was caused by dilution due to the rapid influx of saliva into the rumen that accompanies feeding, and to the concurrent consumption of water. Clarke (1965) showed that this initial drop in concentration did not indicate destruction of organisms, since the total number of ophryoscolecoid protozoa in the rumen was practically unchanged over the feeding period. The growth rate then increased in response to the incoming nutrients and soon exceeded the dilution rate, thus allowing an increase in concentration. With the exhaustion of nutrients, the growth rate declined until it was less than the dilution rate, so that shortly before the next feeding period there was a decrease in concentration.

Both the rate of change of concentration and the ratio of maximum to minimum concentration were similar for the entodinia and the diploplastrons (Table 3, Figs. 2, 3), but the proportion of dividing organisms was at all times greater for the latter than for the former. This suggests that T , the time needed to complete division, was greater for the diploplastrons than for the entodinia. The ratio of maximum to minimum concentration for the polyplastrons (Fig. 4) was slightly greater and for the diplopinia and epidinia (Fig. 5) considerably greater than for the entodinia (Fig. 2) or diploplastrons (Fig. 3). This is reflected in the rates of change of concentration (Table 3) and indicates that the growth rates varied over a wider range; this is confirmed by the wider range for the proportion of dividing cells.

Purser & Moir (1959) claimed that the diurnal changes in concentration of the ophryoscolecoid protozoa could be largely explained by dilution following feeding and an inhibition of division due to the decrease in pH value. If they had calculated the proportion, rather than the absolute concentration, of dividing organisms as a measure of growth activity, they would have found that minimal growth occurred in the period 16–24 hr after feeding, when the pH value was increasing to its maximum, and maximal growth occurred at 8 hr after feeding when the pH value was still moderately low. However, they would also have found that the proportion of dividing organisms increased immediately after feeding and then decreased somewhat before rising to its maximum; this decrease was associated with the period of minimal pH value and may have been caused by it. No such phenomenon was noted in the present work, but it is likely that the pH value did not decrease much below pH 5.9 in these experiments, as compared with decreases to pH 5.4–5.7 in the work of Purser & Moir (1959). A puzzling feature of this latter work is revealed when the dilution rate is calculated from the equation $D = \rho - r$, making any reasonable assumption about the relation between $\rho = \nu$ and the proportion of dividing organisms as measured by these authors. High values for the dilution rate, exceeding 0.4 hr^{-1} , are found for the first hour after feeding, followed by low figures for a few hours, increasing again to a maximum exceeding 0.2 hr^{-1} at 6–8 hr after feeding, and finally decreasing to a very low value in the last period of the 24 hr. No such phenomenon was noted in the present work.

A superficially similar curve was shown by the peptostreptococci (Fig. 9), but the amplitude of the fluctuations (Table 3) was much greater. The considerable rate of decrease before feeding, and the very rapid decline following feeding, where in both cases the sum of r and D , i.e. the net growth rate ρ , was negative, indicate some means of removing the organisms in addition to dilution. The figures of Warner (1962*b*) indicated some sequestration on feed particles during most of the period

of low concentration, but this could not have accounted for all the decline in concentration; dissolution or engulfment must have occurred. The rate of increase found a short time after feeding, $r = 0.4 \text{ hr}^{-1}$, is readily compatible with the growth capabilities of the organism, as known from cultures, for doubling times of about 2 hr are all that would be required.

The polymastigote flagellate protozoa appeared to undergo sequestration, probably on the rumen wall, and return to the general rumen liquor, since otherwise 2–5 or more divisions would have needed to occur within the hour after feeding, followed by an almost equally rapid death rate, which seems unlikely. Dividing or dead forms have been positively identified only rarely. The stimulus for return was definitely associated with the feed, and appeared to be some substance of low molecular weight, soluble in acetone as well as water, and destroyed by acid treatment. Perhaps, during the fasting period, some similar substance diffused across the rumen wall from the blood in sufficient concentration to attract the organisms there. The normal mixing of the rumen and the rapid rate of locomotion of the organism would seem sufficient to account for the observed fluctuations in concentration.

The holotrich ciliate protozoa showed more puzzling behaviour (Figs. 11, 12). After reaching a maximum near feeding time, the concentration of dasytrichs decreased until 12–20 hr after feeding, at a mean rate of about $r = -0.11 \text{ hr}^{-1}$ (Fig. 12); in some experiments, rates of -0.3 hr^{-1} or lower were maintained for some hours. This was much greater than can be accounted for by dilution, even though the growth rate was effectively zero. Clarke (1965) suggested that this decline was due to bursting of the ciliates when overfull of synthesized polysaccharide (Sugden & Oxford, 1952). Use can be made of an expression analogous to that used in the calculation of growth rate, i.e. $\lambda = -\rho = -(r + D) = I'/T'$, where I' is the proportion of organisms showing signs of dissolution, and T' the time needed to complete this process, i.e. the time required for an apparently normal organism to become unrecognizable as a holotrich. In this work, very few bursting organisms were seen, and I' was of the order of 0.001 or less. This suggests that T' may have been considerably less than 1 min; there is no experimental evidence about this, but it would seem to be a very short time.

The alternative hypothesis of sequestration (Purser, 1961) does not seem to be any more likely. Previous work in this laboratory showed no evidence of sequestration amongst feed particles, and if it occurred amongst the papillae of the rumen wall, accumulations would be found in such concentrations as should be obvious to the naked eye; in other work, the rumen wall of sheep inspected at various times after feeding showed no such appearance.

During the period 20–28 hr after feeding, a sustained rapid rate of increase of concentration of dasytrichs was noticed, at a mean rate of about 0.3 hr^{-1} , while the concentration increased by some 4 to 20-fold or more within a few hours. This would imply a doubling time of 1.5–2.0 hr sustained over 2–4, or more, divisions. Similar large rapid increases were recorded by Purser (1961) and by Clarke (1965), though the latter author found the increase to occur slightly later. Now, the number of divisions undergone by a micro-organism per day is $24/g$, i.e. $24\nu/\ln 2$; since, considered over the 24 hr feeding cycle, the rumen approximates a steady-state system, this equals $24 D/\ln 2$ (see appendix), i.e. about $35 D$ divisions occur per day provided the death rate λ is negligible and no sequestration occurs. Hence, to main-

tain numbers against an overall mean dilution rate of $0.06-0.11 \text{ hr}^{-1}$, each organism must divide on average 2-4 times per day; perhaps an extra 1-2 divisions would be needed to make up numbers if the earlier decline were due to dissolution of organisms. It appears that nearly all the division in the holotrichs occurs within a few hours, since very few dividing organisms were seen at other times. Campbell (1930) noted that in cattle normally very few dividing isotrichs were seen but that on one occasion 0.6 of the population were dividing. Hence it would appear that the dasytrichs undergo 2-7 divisions within 4-8 hr, and then divide no more for about 18 hr; and the isotrichs behave similarly. It can be calculated that the time needed to complete division is of the same magnitude as for the entodinia.

It was noticed that dividing holotrichs never contained obvious feed particles (starch grains, etc.), but were never void of internal polysaccharide. It may therefore be postulated that nutrients must be metabolized to some particular state, a process requiring perhaps 18 hr, before division can take place, and that several divisions then occur in rapid succession. No analogous phenomenon is known to the author.

No evidence on the nature of the stimulus for conjugation among the holotrichs was found in the present work. No detailed cytological studies were made, but the process appeared to be identical with that described by Dogiel & Federowa (1925).

The oscillospirae (Figs. 13, 14) showed a pattern of concentration change difficult to interpret, there being two maxima in the 24 hr feeding cycle. The first peak, shortly after feeding, was associated with the maximum length of the trichomes. The second peak, 8-16 hr later, occurred with slowly decreasing trichome length. An interesting feature was that the concentration was rising before feeding, though the length was slowly decreasing. It was hoped that the trichome length would have given an indication of the number of cells per trichome, so that the concentration of trichomes would have increased when the length decreased and vice versa. However, this was not the case, and it would appear that the length of the trichomes reflected their nutritional rather than their reproductive status. Previous work (Warner, 1962*b*) indicated that much, but not all, of the variation in concentration of oscillospirae, as measured by the standard count, disappeared when the total count was used, suggesting that sequestration and return accounted for some part of the changes in concentration. This might account for the fact that the initial rate of decline in concentration exceeded the dilution rate. However, the double peak was still obvious in the total count and remains inexplicable.

It became apparent during this work that at least two strains or species of organisms were included in the group 'oscillospirae'. Only one strain was present in most animals, but in some a mixture occurred. The strains differ in diameter; one has a diameter near 7μ , one near 4μ , and there may be a third still thinner. Spores, when formed, appeared more uniform in size, being $6-7 \mu$ in diameter in all strains. The tendency to form spores also differed considerably; some strains formed spores very rarely, others more frequently. The number of spores per trichome also varied, some commonly forming one and only rarely two spores, others, particularly the thinner strains, forming up to six spores. This character is not mentioned for *Oscillospira guillermondii*, the only species described in *Bergey's Manual*. The behaviour of all these oscillospira strains, both in concentration and in trichome length, appeared to be similar, and they have not been differentiated in the present work.

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APPENDIX

Kinetic study of particles, solutes and micro-organisms in the rumen

Previous studies of the kinetics of feed particles in the rumen (Blaxter, Graham & Wainman, 1956; Brandt & Thacker, 1958; Garner, 1964) have made the basic assumption of steady-state flow, i.e. it has been assumed that movements of water into and out of the rumen were constant and equal, so that the volume remained constant. This assumption is at best a crude approximation under most circumstances (see Warner, 1964). Hydén (1961*a*) made use of an inert reference solute, polyethylene glycol, as a marker for water movements in the rumen. He also assumed steady-state flow for his main mathematical treatment, but showed how to calculate outflow of water from the rumen when the volume varied. Hydén (1961*b*) applied his treatment to estimate the net flux of water across the rumen wall, while Engelhardt (1963), who used a similar approach, endeavoured to measure the total flux by using tritiated water. No kinetic study of the micro-organisms in the rumen is known, but a considerable literature exists for continuous-flow cultures (see James, 1961), which are in many ways analogous to the rumen but again many parameters are constant. The present treatment has been developed without making any assumptions about the constancy of parameters pertaining to events in the rumen. The symbols agreed by Andreyev *et al.* (1964) have been used where applicable.

Fundamental kinetic equation

Consider particles, solute molecules, or micro-organisms that cannot cross the rumen wall, that move at the same rate as water into the omasum, and that may be formed or destroyed in the rumen but only at rates directly proportional to the numbers present. Instantaneous and complete mixing is assumed.

At the instant t , let J be the inflow rate of water down the oesophagus, H the concentration of the substance in this inflowing water, W the net rate of entry of water across the rumen wall (the concentration of the substance in this water is nil), F the net inflow rate of water into the rumen ($F = J + W$), V the volume of water in the rumen, C the concentration of the substance in the rumen, E the outflow rate of water to the omasum (the concentration of the substance in this water is C , due to the assumed perfect mixing), and k the net rate constant for formation and destruction of the substance in the rumen. During the interval from time t to time $t + \delta t$, a simple balance equation holds:

$$\begin{aligned} \text{Input} + \text{Formation} - \text{Output} &= \text{Accumulation} \\ J\delta t H + k\delta t VC - E\delta t C &= (V + \delta V)(C + \delta C) - VC \\ &= \delta VC + V\delta C + \delta V\delta C = (F - E)\delta t C + V\delta C + \delta V\delta C \end{aligned}$$

Divide by δt and take to the limit:

$$JH + kVC = FC + V \frac{dC}{dt}. \quad (1)$$

Special cases

Single-shot marker experiment. An inert reference substance such as polyethylene glycol is introduced into the rumen at time $t = 0$, and the concentration measured at intervals thereafter. In this case, $H = 0$, $k = 0$; let $C = c$. Then equation (1) becomes $0 = Fc + Vdc/dt$, or

$$\frac{1}{c} \frac{dc}{dt} = -\frac{F}{V} = -D, \quad (2)$$

where $D = F/V$ is the relative net inflow rate of water or dilution rate.

The curve of $\ln c$ plotted against t has an instantaneous gradient of

$$\frac{d \ln c}{dt} = \frac{1}{c} \frac{dc}{dt}$$

and hence, from equation (2), $-D$. This curve is usually approximately linear, i.e. D is approximately constant, for substantial periods of time, and hence

$$\ln c = \ln c_0 - Dt \quad (3)$$

equivalent to

$$c = c_0 e^{-Dt}, \quad (4)$$

where the subscript 0 refers to the instant $t = 0$. It is usually convenient to plot c against t on semi-logarithmic paper, or alternatively to plot against t the logarithm of c taken to some convenient base such as 2, and to fit a straight line by eye. An estimate is then made of the half time, t_1 , which is the time needed for the concentration to fall by one half or for the $\log_2$ concentration to fall by 1. Then

$$D = (\ln 2)/t_1. \quad (5)$$

Since the problem appears to involve the functional relationship between the variables rather than the estimation of the mean of one as a function of the other, it is doubtful whether the fitting of a regression line would lead to a more accurate estimate of D .

Ulyatt (1964), who worked with grazing sheep, fitted a quadratic rather than a linear expression to the plot of $\ln c$ against t , but he made no attempt to correlate departures from linearity with patterns of eating, drinking or ruminating.

Continuous infusion of marker. The reference substance is infused continuously into the rumen. Here, $k = 0$; let $H = h$, $C = c$. Then equation (1) becomes

$$Jh = Fc + Vdc/dt. \quad (6)$$

This expression is difficult to handle without further information.

Micro-organisms. Micro-organisms that are 'fixed' to the feed particles or sequestered close to the rumen wall would not in general move with water to the omasum and are therefore beyond the scope of this treatment. For the remaining organisms, $H = 0$; let $C = n$, the concentration in numbers of live cells per unit volume, and $k = \rho = \nu - \lambda$, where ν and λ are specific growth and death rates and ρ the net specific growth rate. Then equation (1) becomes $\rho Vn = Fn + Vdn/dt$, that is

$$\frac{1}{n} \frac{dn}{dt} = \rho - D = r, \quad (7)$$

where $r = \rho - D$, the relative rate of change of concentration. When r is constant, it can be determined as for D above, from the equation

$$\ln n = \ln n_0 + rt. \quad (8)$$

Experimental determination of r and D allows the calculation of ρ which, if the death rate λ is negligible, equals the specific growth rate ν .

Outflow. There is no outflow term in the general equation (1), but the outflow of water and of the substances that move with the water is of considerable importance to the economy of the animal. The amount of a substance of the type considered in this study that leaves the rumen is equal to the difference between the sum of the initial amount and what arrives there by inflow or formation, and the final amount in the rumen, that is

$$\int_0^t EC \, dt = C_0 V_0 + \int_0^t JH \, dt + \int_0^t kCV \, dt - CV. \quad (9)$$

In a single-shot marker experiment as described above, if E can be considered constant, equation (9) reduces to

$$E = \frac{c_0 V_0 - cV}{\int_0^t c \, dt}. \quad (10)$$

The integral $\int_0^t c \, dt$ could be estimated graphically, or, if D was also constant, from

$$\begin{aligned} \int_0^t c \, dt &= \int_0^t c_0 e^{-Dt} \, dt \quad (\text{from equation (4)}) \\ &= c_0 (1 - e^{-Dt})/D = (c_0 - c)/D \quad (\text{from equation (4)}) \\ &= (c_0 - c)t/(\ln c_0 - \ln c) \quad (\text{from equation (3)}). \end{aligned}$$

Hence

$$E = \frac{(c_0 V_0 - cV)(\ln c_0 - \ln c)}{(c_0 - c)t}. \quad (11)$$

The relative outflow rate or production rate M may be defined as E/V , analogous to D .

Steady state. Flow through the rumen approximates to a steady state if considered over a complete feeding cycle or for short periods of time that are well separated from times of eating, drinking or ruminating. Then F and E are equal and constant, V is constant; hence $D = M$.

If marker is infused at a constant rate, equation (6) reduces to $Jh = Fc$ = rate of infusion of marker. Experimentally this technique often gives a better estimate of F than can be obtained from calculations of V and D in a single-shot marker experiment.

For micro-organisms in a steady-state system, $r = 0$ and $\rho = D = M$.

Mean residence time. The average time spent by a particle in the rumen, the mean residence time θ , may be calculated as the time needed for the number of particles leaving the rumen to equal the average number in the rumen during the period of observation, provided there is no destruction of particles within the rumen. It can be readily calculated only for steady-state conditions, when $E\theta = V$, or

$$\theta = V/E = 1/M = 1/D. \quad (12)$$

This term is formally analogous to turnover time, as used in metabolic studies.

Dimensions and units

Note that k , D , M , ρ , ν , λ , and r are all rate constants, with physical dimensions of $[T^{-1}]$ and their units should be in conformity and the same for all, such as hr^{-1} or day^{-1} . The commonly applied unit for D , per cent per hour (Hydén, 1961*a*) can give a misleading impression if applied to, say, ρ , and the value cannot be used unchanged in expressions of the form of equation (4).

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