The degradation of protein in the rumen of the sheep and redistribution of the protein nitrogen after feeding

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McDonald (1954) and McDonald & Hall (1957) showed that both zein and casein were digested in the rumen and that part of the nitrogen of these materials was apparently converted into microbial protein. McDonald (1948) and later Chalmers, Cuthbertson & Synge (1954) determined the course of ammonia formation in the rumen and showed how it can be of importance in the overall nitrogen retention of an animal on a particular diet. In the first parts of our work (Blackburn & Hobson, 1960*a–c*) an investigation of the proteolytic activities of different fractions of rumen contents and cultural investigations of proteolytic bacteria in the sheep rumen were described. It was shown that rumen contents could be adequately fractionated by centrifugation and that all fractions of the rumen microbial population, small bacteria, large bacteria and protozoa, had proteolytic activity, and that there was little activity in the cell-free rumen liquid. However, the numbers of proteolytic bacteria that could be cultured from the rumen were low compared with the total microbial population. The cultural counts of bacteria were made on samples taken at about the same time after feeding and it was thought that it would be of interest to extend these counts to different times during a digestion cycle of 7 h, and to link them to determinations of the proteolytic activity of the rumen contents. Since, when these experiments began, there were few data on the rate of breakdown of dietary protein and the redistribution of the protein nitrogen, the experiments were designed to obtain information on these subjects and also to link any variations in proteolytic activity with the disappearance of the foodstuff protein. To provide nitrogen mainly in one form, that of a known protein, a partly synthetic diet was used in which casein provided the main nitrogen source. Although the earlier experiments (Blackburn & Hobson, 1960*a*) suggested that differences in rumen proteolytic activity were not connected with the protein content of the diet, Chalmers *et al.* (1954) showed that the rate of ammonia formation in the rumen was related to the solubility of the casein in the diet, so casein in three different physical forms was provided. Although casein is not a protein found in 'natural' ruminant diets, it has been extensively used in feeding experiments, and the microbial populations sustained by the present experimental diets were similar to those of sheep on normal diets containing concentrates. Previous work (Blackburn & Hobson, 1960*a*, *b*) showed that micro-organisms from sheep on a variety of diets could digest casein, so no alteration in the enzymic properties of the rumen contents would be expected with these diets, and the results described here may be taken as representative of the happenings on a normal diet. The results of the counts of proteolytic

bacteria made during administration of these diets have been given (Blackburn & Hobson, 1960*c*), together with the results of a preliminary fractionation of the rumen contents of one sheep (sheep no. 47 on diet 1). In this paper a detailed description of the results for redistribution of nitrogen, pH values and volatile fatty-acid determinations are given, together with some determinations of proteolytic activity. In order to relate the loss of nitrogen from the rumen to the rate of passage of food from that organ, and to show any absorption of nitrogen, polyethylene glycol was used as a marker.

At about the time when these experiments began, Weller, Gray & Pilgrim (1958) described investigations of the partition of nitrogen in the rumen between feed and bacteria using $\alpha\epsilon$-diaminopimelic acid as a marker, and Moore & King (1958) using a technique similar to that described here investigated the decrease in soluble protein and non-protein nitrogen after feeding steers on hay with concentrates and with a protein hydrolysate.

A preliminary mention of some of the results described here was made by Hobson (1959).

EXPERIMENTAL

Sheep. Two 2-year-old Cheviot sheep, nos. 47 and 6, fitted with permanent rumen cannulas were used. While diets 1 and 2 were being given and nitrogen balances were being recorded the sheep were maintained in metabolism crates; otherwise they were kept in separate open pens with sawdust bedding.

Diet. The basal diet per feed consisted of: wheat straw 300 g, potato starch 75 g, sucrose 25 g, minerals (McDonald & Hall, 1957) 15 g, Adisco vitamin supplement (I. Spenser and Co., Aberdeen) 88 mg. To it 60 g casein were added: to diet 1, Glaxo C casein (Glaxo Laboratories Ltd, Greenford, Middlesex); to diet 2 hardened Genatosan casein (Chalmers *et al.* 1954) to diet 3 dissolved Glaxo C casein. Diets 1 and 2 were supplemented with 10 g and diet 3 with 15 g molasses to increase the palatability. For diets 1 and 2 all the constituents except the straw were suspended in 600 ml water and the straw was then mixed with the suspension to give a homogeneous damp feed. In diet 3 the casein was already in solution in a volume of 600 ml. This solution was achieved by suspending 700 g of casein in 3·5 l. water, adding 350 ml 0·1 N-NaOH and stirring. The temperature was then brought to 80°, by which time the casein had dissolved and the pH was about 6·7. The volume was made up to 7 l. with water and the solution stored at 4° until needed. The casein in diet 2 was ground in a hammer mill fitted with a 1 mm grid. The wheat straw was chopped and put through a coarse grid hammer mill which reduced it to $\frac{1}{2}$ in. lengths.

The sheep were fed at 9 a.m. and 4 p.m. each day and no water was allowed between these hours. The animals were allowed to accustom themselves to the diets, and then 1 week of regular eating on each diet was allowed before experiments proceeded.

Nitrogen balance. Each nitrogen-balance experiment was run concurrently with the other determinations on diets 1 and 2. The balance experiments were divided into four periods, of 3 or 4 days each, urine and faeces being pooled for each sheep in each period. Macro-Kjeldahl digestions followed by micro-distillation of a sample were

made, and for the faeces nitrogen less than ±4% deviation from the mean of three determinations was accepted. The nitrogen contents of random samples of the constituents of the diet were found by similar methods.

Rumen samples. Samples were removed through the rumen cannula with a 20 × 1 cm glass tube fitted with a large rubber suction bulb. The tube was moved so as to take portions of the sample from a wide area of the rumen.

Fractionation of rumen fluid. A sample of rumen fluid (100 ml) was strained through two thicknesses of surgical gauze to give liquid F_1. The coarse debris thus removed was resuspended in 0·85% (w/v) NaCl solution saturated with chloroform, diluted to 100 ml and strained again (liquid F_2). (The debris was squeezed each time to remove as much liquid as possible.) Liquid F_3 consisted of 10 ml portions of F_1 and F_2. A 10 ml portion of F_3 was centrifuged at 114 ***g*** for 5 min and the deposit (*a*) washed in saline. The supernatant liquid and washings were then recentrifuged at 19 000 ***g*** for 20 min and the deposit (*b*) washed once in saline. Liquid F_3 was called the whole fraction, deposit (*a*) the protozoal fraction, (*b*) the bacterial fraction, and the remaining supernatant liquid and washings the clear fluid fraction. F_1 clarified by centrifuging at 19000 ***g*** was used to determine levels of protein and non-protein nitrogen (NPN) in the rumen fluid. Compared with F_1 the clear fluid fraction had an identical ratio of protein N to NPN and there was shown to be no loss of ammonia or changes in constituents due to washing. Protein nitrogen was precipitated by trichloroacetic acid (TCA) in a final concentration of 0·36N and the precipitate washed once. Non-ammonia, non-protein nitrogen (NANPN) was obtained by difference between NPN and ammonia nitrogen.

Proteolytic activity. The proteolytic activity of liquid F_1 was determined by its capacity to hydrolyse casein, the decrease in protein precipitated by TCA and increase in 'tyrosine' not precipitated by TCA being measured in standard digests (Blackburn & Hobson, 1960*a*, *c*). The standard digests contained 5 ml of 2% (w/v) casein solution (Eastman casein, Kodak Ltd, Hemel Hempstead, Herts), 3 ml of 0·1M-phosphate buffer, pH 6·6, 1 ml of 0·6% (w/v) L-cysteine hydrochloride solution and 1 ml of liquid F_1, and were incubated under toluene for 24 h at 37°. Control digests without casein, and without rumen fluid but with casein, were also used. Portions consisting of 1 ml of the digests before and after incubation were added to 9 ml 0·72N-TCA for determination of protein with biuret reagent (Blackburn & Hobson, 1960*a*), and 'tyrosine'. To reduce the high blank values initially found in the 'tyrosine' determinations the following procedure, based on that of Tracey (1948) to oxidize reducing substances in plant extracts, was used. TCA was added to a measured portion of the digest and the mixture filtered through Whatman no. 42 paper and a 2 ml portion of the filtrate transferred to a graduated test tube. To it was added 0·1 ml starch solution (Pope & Stevens, 1939) and sufficient of a solution of 0·25% (w/v) iodine in 0·5% (w/v) aqueous potassium iodide to give a dark coloration. The tube was left at room temperature for 30 min, and the excess iodine then removed by titration with 0·005M-sodium thiosulphate. This procedure oxidized cysteine and other reducing substances which reacted with the Folin reagent. Some 0·55M-sodium carbonate (5 ml) was then added, the volume made up to 10 ml, and 1 ml of one-third strength Folin reagent

(British Drug Houses Ltd, Poole, Dorset) added. The contents of the tube were rapidly mixed, incubated at 30° for 30 min and the absorption at 660 mμ was measured on a Unicam colorimeter (Hagihara, Matsubaro, Nakai & Okunuki, 1958). Under these conditions 0–160 μg pure tyrosine gave a linear relationship with 0–0·83 colorimeter units.

Nitrogen. Total nitrogen was determined by a micro-Kjeldahl procedure (Chibnall, Rees & Williams, 1943) on duplicate samples of appropriately diluted fractions of the rumen fluid and ammonia nitrogen by a microdiffusion method (Conway, 1957) on liquid F_1.

Volatile fatty acids. The acids were determined on portions of the clear centrifuged fluid F_1 by acidification and distillation in the presence of magnesium sulphate and sodium tungstate (Friedemann, 1938). The steam distillate was then analysed qualitatively by paper chromatography (Elsden & Lewis, 1953) and quantitatively by chromatography on 'Celite' columns (Bueding & Yale, 1951).

Polyethylene glycol. Polyethylene glycol 4000 (PEG) (Oxirane Ltd, Manchester) (4 g) was dissolved in a small amount of water and mixed with the morning feed. To determine its distribution in the rumen 5 ml strained rumen fluid (F_1) were added to 5 ml 0·1 N-HCl, the mixture was diluted to 50 ml and filtered through Whatman no. 1 paper. Samples of the filtrate (5 ml) were deproteinized and the polyethylene glycol was determined by the method of Corbett, Greenhalgh, Gwynn & Walker (1958).

Sampling and general experimental procedure. Samples were taken from each sheep simultaneously at a different time after the 9 a.m. feed each day. Except for the runs on diet 2, when the samples were not taken in chronological order, samples were always taken 1 h later each day. Usually a run lasted 2 weeks, no samples being taken over the weekends. Each sample was fractionated as described above and the nitrogen content of the fractions, the proteolytic activity, ammonia concentration and viable proteolytic bacteria (Blackburn & Hobson, 1960*c*) were determined.

Before and after each run the ammonia concentration, PEG concentration and pH values were determined on 20 ml samples removed at increasing time intervals after feeding on 1 day. These were the only occasions on which PEG was included in the diet.

RESULTS

Feeding of sheep and nitrogen-balance experiments. It took some time for the sheep to adjust themselves to diet 1, but once accustomed to it they ate it and the other diets generally within 10 min. Sheep no. 47 drank an average of 700 ml water overnight and sheep no. 6 of 1700 ml.

Fig. 1 illustrates the nitrogen balances for the second runs on diet 2. The histograms of the balances for diet 1 were not substantially different. The amount of nitrogen retained is shown in Table 1. There was some indication that on diet 1 a decreased retention of nitrogen was associated with a lower than normal level of rumen micro-organisms. There was also a lower micro-organism population on diet 2 than on diet 1, which might account for the fact that a poorer nitrogen retention was found with the hardened casein.

Distribution of rumen nitrogen. Table 2 shows the amount of nitrogen present in each constituent of the diet. Wheat straw was chosen as roughage because of its low nitrogen content and the low availability of the nitrogen (Woodman, 1957). It was hoped that with diet 1 the casein, the main nitrogen source, which in diets 1 and 2 was in a finely divided form, would be separated from the straw, dispersed throughout the rumen, and dissolved fairly rapidly. On the assumption that the rumen volume was

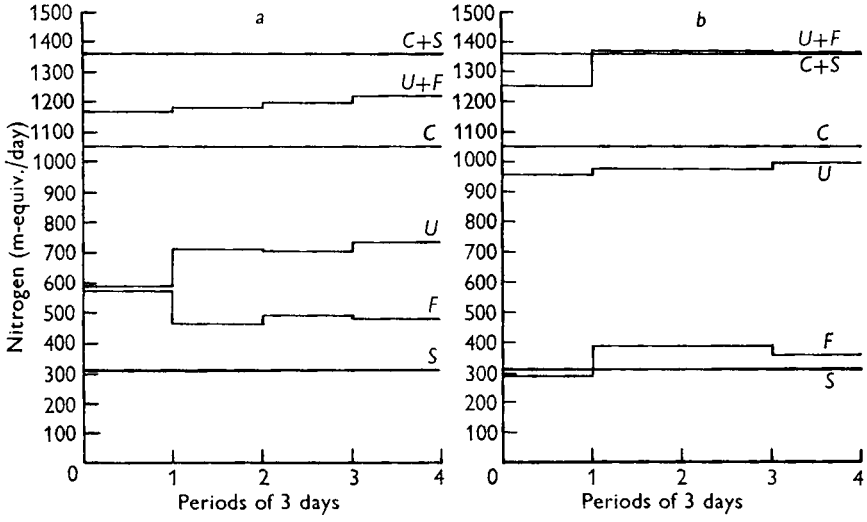

Fig. 1. Nitrogen eaten and excreted on diet 2 ('insoluble casein') by (*a*) sheep no. 47 and (*b*) sheep no. 6. *S*, nitrogen in straw; *C*, nitrogen in casein and other constituents of the diet; *F*, nitrogen excreted in faeces; *U*, nitrogen excreted in urine.

Table 1. *Nitrogen-balance experiments with sheep on diets* 1 (*'soluble casein'*) *and* 2 (*'insoluble casein'*)

	Food nitrogen retained over a 12-day period (%)	
Diet	Sheep no. 47	Sheep no. 6
1	16·9	10·2
2	13·1	2·5

Table 2. *Nitrogen contents of sheep diets* 1 (*'soluble casein'*), 2 (*'insoluble casein'*) *and* 3 (*'dissolved casein'*)

	Amount of N provided per day (m-equiv.)		
Dietary constituent	Diet 1	Diet 2	Diet 3
Casein	1105	1077	1190
Straw	313	313	486
Minerals, Starch, Sucrose	5	5	5
Molasses	45	45	67

8 l., which was about the value determined from PEG concentrations, the casein in the diet was expected to give an increment of approximately 70 μ-equiv. nitrogen/ml rumen fluid. This increase was attained with diets 1 and 3 suggesting that the casein was rapidly dispersed. With the insoluble casein of diet 2 the increment was only a small fraction of the theoretical amount, suggesting that the hardened casein granules were not well distributed in the rumen. Tests on the debris strained from the rumen

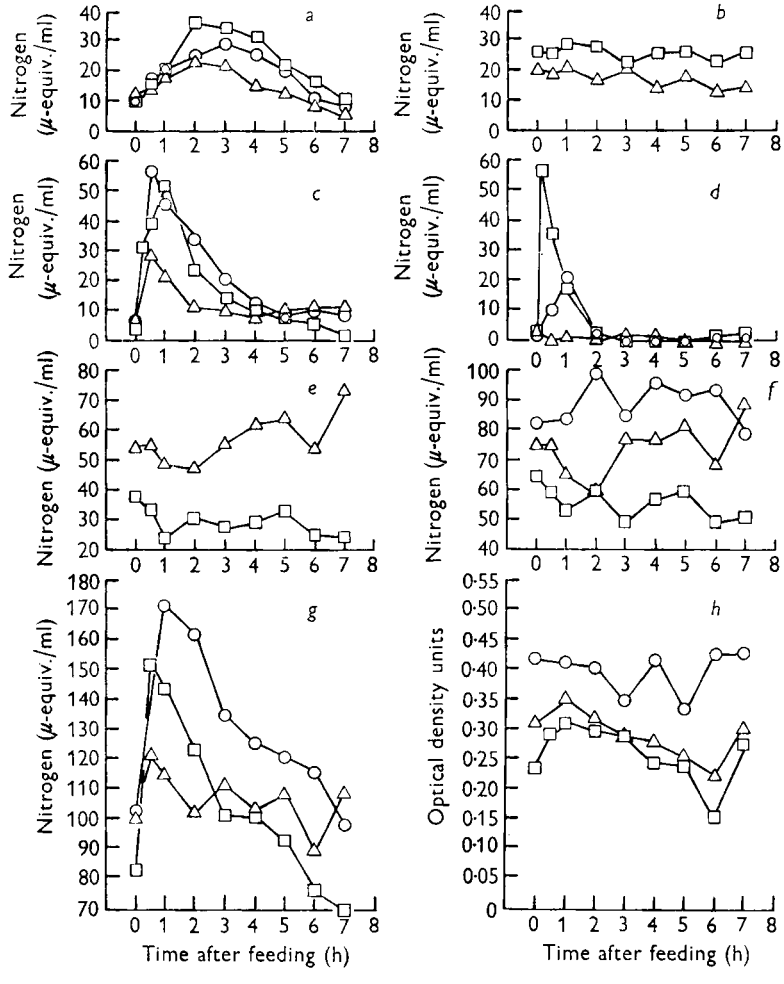

Fig. 2. Distribution of nitrogen and proteolytic activity in rumen contents. *a*, ammonia-N; *b*, bacterial-N; *c*, non-ammonia, non-protein-N; *d*, protein-N; *e*, protozoal-N; *f*, protozoal-+ bacterial-N; *g*, total N; *h*, proteolytic activity. o—o, diet 1, mean of three readings, two on sheep no. 47, one on sheep no. 6; $\triangle$—$\triangle$, diet 2, mean of two readings, one on sheep no. 47, one on sheep no. 6; □—□, diet 3, mean of four readings, two on sheep no. 47, two on sheep no. 6.

fluid showed that part of the casein was contained in the debris. However, all the casein could not be accounted for in this way, and samples taken from the reticulum suggested that part of the casein granules rapidly found their way into this organ and were not sampled by the usual procedure.

The results of the nitrogen determinations on the fractionated rumen contents are shown in Fig. 2*a–g*. Two runs were made with sheep no. 47 and one run with sheep no. 6 on diet 1, one run with each sheep on diet 2, and two runs with each on diet 3. With diet 1 the nitrogen contents of the bacteria and the protozoa were not separately determined. In general there was more similarity between the results for the two sheep on the same date on a given diet than between the results from a single sheep on the same diet on different dates. Approximate estimates of standard deviations among repeat values obtained on different runs for sheep on the same diet were made. These were: ammonia nitrogen ± 3·1, protein nitrogen ± 2·5, NANPN ± 4·4, protozoal plus bacterial nitrogen ± 13, protein plus non-protein nitrogen ± 5·5 μ-equiv.; proteolytic activity, Folin reagent ± 0·06 colorimeter units.

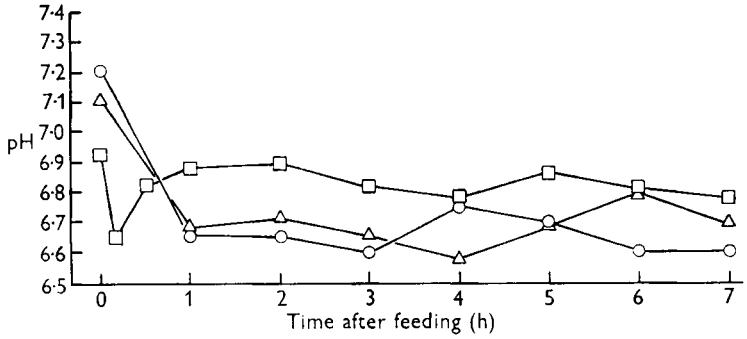

Fig. 3. pH values in sheep rumen fluid. ○—○, on diet 1 (mean of one reading on each of sheep nos. 47 and 6); △—△, on diet 2 (mean of two readings on each of sheep nos. 47 and 6); □—□, on diet 3 (mean of two readings on each of sheep nos. 47 and 6).

Proteolytic activity. The proteolytic activity as measured by the Folin reagent is shown in Fig. 2*h*. The values determined by the biuret reagent were similar (Blackburn & Hobson, 1960*c*).

Volatile fatty acids and pH values. The mean pH values of the rumen fluid from the two sheep are shown in Fig. 3. The pH fell somewhat in the 1st h after feeding and then remained relatively constant, but the values with diet 3 were generally higher than the others, which might have been correlated with the higher ammonia concentration in the rumen with that diet. The values for total volatile fatty acids (VFA) in rumen fluid are shown in Fig. 4. These values were determined on one sheep only. The concentrations of VFA rose to a maximum about 2–3 h after feeding and then slowly decreased. An analysis of the acids produced on diet 1 before feeding showed that the totals were made up of butyric and higher acids 11·3, propionic acid 14·3, acetic acid 39·6 μmoles/ml. Three hours after feeding the total acid consisted of butyric and higher acids, 21·0, 16·0 and 21·9 μmoles/ml; propionic acid, 23·9, 26·1 and 37·8 μmoles/ml; and acetic acid, 49·1, 47·6 and 49·8 μmoles/ml with diets 1, 2 and 3 respectively. Most of the increased VFA with diet 3 was thus made up of propionic acid at that time, but the proportions may have changed during the day. Qualitative analysis showed the higher fatty-acid fraction to consist mainly of valeric

acid and it appeared to be in the highest concentration with diet 3. On diets 1 and 2, which were tested extensively, the concentration of valeric acid seemed to decrease towards 7 h after feeding.

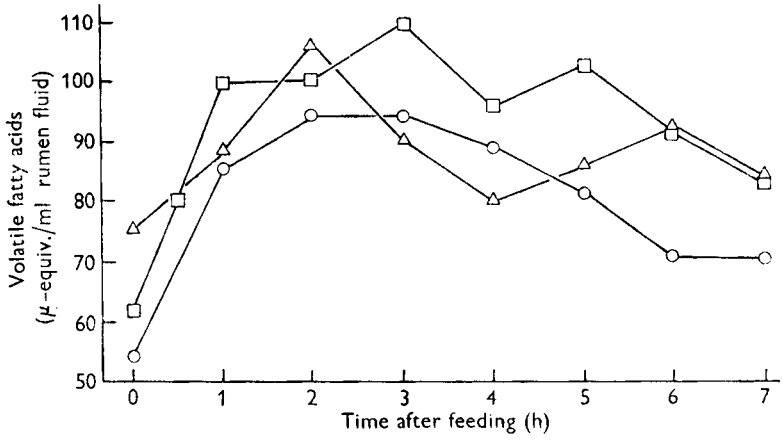

Fig. 4. Volatile fatty acids in the rumen fluid of sheep no. 47 on diet 1 (○—○), diet 2 (△—△) and diet 3 (□—□).

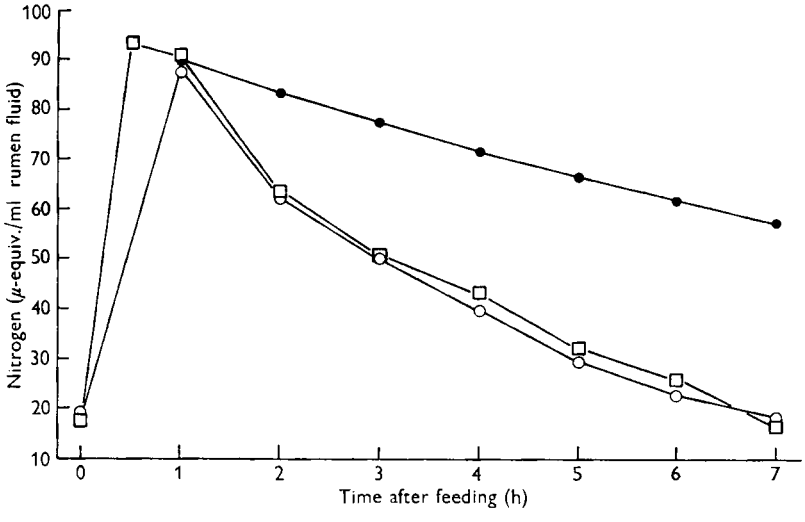

Fig. 5. Soluble nitrogen (protein + non-protein) in sheep rumen fluid. ○—○, on diet 1 (mean of two readings for sheep no. 47 and one on sheep no. 6); □—□, on diet 3 (mean of two readings on each of sheep nos. 47 and 6); ●—●, levels that would theoretically be expected from an initial value of 90 μ-equiv./ml if soluble N loss from the rumen were only by passage to the omasum and abomasum at the same rate as the polyethylene glycol used as marker, i.e. a fall in concentration in any hour of 7·3 % of the value at the beginning of that hour.

Polyethylene glycol. In all, six determinations of the rate of elimination of PEG from the rumen were made on each sheep at intervals over the experimental period. Typical results are shown in Fig. 5.

Each of the twelve runs could be described by linear regression equations of concentration or the logarithm of concentration on time. The former is appropriate for a constant rate of fall in concentration and the latter for a rate of fall proportional to concentration, but since the ratio of maximum to minimum concentration in each run was only of the order of 3:2, the values could not be expected to show clearly which hypothesis was the more correct. The regression coefficients were less homogeneous for the concentration equations than for the log concentration equations, and tended to be numerically greater for those runs with higher mean concentrations. This is what would be expected on the second hypothesis, and the log concentration equations are therefore preferred. Although the slopes did not differ significantly among the twelve runs, there were considerable differences between runs in the values of log concentration at any given time after feeding. This finding suggests that the effective volume of rumen contents may have differed between runs, even for the same sheep.

DISCUSSION

Some nitrogen in the form of urea and mucoprotein is added to the rumen by the saliva. No good estimate of it can be obtained, but it may be of the order of 1·5 g/24 h and may be neglected in discussing the results found here. Fig. 2*d* shows that soluble protein was rapidly broken down. The fact that no initial rise in the values was found on diet 2 must have been due to the low solubility of the casein, any small amount getting into solution being rapidly hydrolysed, whereas with the other two diets more casein was dissolved than could be immediately degraded. This rapid breakdown of soluble protein might be expected from the determinations of proteolytic activity. The activity was relatively high even before feeding. In conjunction with it the numbers of proteolytic bacteria that could be cultured were relatively constant over the same period (Blackburn & Hobson, 1960*c*). Fig. 2*f*, *h* shows that when the proteolytic activities and the concentrations of micro-organisms observed with the three different diets were plotted against time after feeding, the graphs obtained lay in much the same relative positions.

The amount of NANPN rose rapidly after feeding, most of the increase being due to hydrolysis of protein, since the amount of NPN extracted from the feeds by grinding and macerating them in a buffer resembling sheep saliva gave only about 9 μ-equiv./ml rumen contents, made up by 6 or 7 μ-equiv. from the straw and about 2 from the rest of the diet, most likely from the molasses. With a diet similar to those described here but without casein, no significant rise in non-protein nitrogen occurred, indicating that little came from the straw or other constituents of the diet or from saliva or other secretions. An appreciable amount of NANPN was formed on diet 2, showing that breakdown of this hardened casein was taking place. Since the protein was broken down and disappeared at a greater rate than NANPN it would seem that the degradation of protein is not generally the step limiting availability of food nitrogen for the rumen micro-organisms. Increase in ammonia nitrogen followed the increase in NANPN (Fig. 2*a*, *c*). The levels of bacterial and protozoal nitrogen remained fairly constant, which must have been due to the rate of microbial growth balancing the loss

of cells to the abomasum. The bacteria might be expected to be fairly evenly distributed throughout the rumen, and Boyne, Eadie & Raitt (1957) found that the concentrations of protozoa in the top and bottom portions of the rumen contents were similar. The micro-organisms, then, should pass from the rumen at the same rate as the dissolved PEG (Fig. 5). The rate of disappearance of the total nitrogen (Fig. 2*g*), or soluble protein plus NPN (Fig. 5), on diets 1 and 3 was greater than this rate, indicating that absorption of nitrogen from the rumen was occurring. Since the fall in NANPN can probably be ascribed mainly to deamination and utilization by micro-organisms, the absorbed nitrogen was probably mainly in the form of ammonia. Although some rumen bacteria will utilize ammonia alone for growth (Phillipson, Dobson & Blackburn, 1959; Gilroy, 1957) others appear to need some amino acids (Gilroy, 1957) and peptides (Bryant, Small, Bouma & Chu, 1958). Annison (1956) found no evidence for absorption of amino acids through the rumen epithelium. Calculations from the rate of disappearance of PEG compared with that of soluble nitrogen and the nitrogen uptake into micro-organisms suggest that the most rapid absorption took place 1–2 h after feeding. It may be that the most rapid absorption of VFA also took place around that time of maximum production. Most of the production of VFA can probably be ascribed to breakdown of the carbohydrates of the diet by the micro-organisms. Cline, Hershberger & Bentley (1958) have shown that valeric acid is produced from starch in the rumen, but some valeric, as well as lower VFA, could be produced by deamination of the NANPN which was disappearing rapidly about the time of maximum production of VFA (Dehority, Johnson, Bentley & Moxon, 1958; Sirotnak, Doetsch, Brown & Shaw, 1953). Although no strict equivalence between the values might be expected, the values for ammonia and VFA concentrations at 3 h after feeding supported the suggestion of deamination. The values for both increased in the order of rations 2, 1, 3, and if the values for ration 2 are taken as zero, the values for VFA concentration on diet 1 were 4·3 and on diet 3 19·8 μ-equiv./ml higher, whereas the NH_3 values for diets 1 and 3 were 7·2 and 13·0 μ-equiv./ml higher.

The increase and decrease in total nitrogen on diet 2 was not as great as on the other diets, indicating that the hardened casein was not being properly sampled and was either remaining in the rumen debris or in the reticulum, as already mentioned. Since the level of NANPN tended to be more constant and to remain rather higher between 5 h and 7 h after this diet than after the others (Fig. 2*c*) it is probable that the hardened casein in the debris was being gradually degraded and becoming available for the rumen metabolic processes.

On all three diets, however, during the first 3 h the level of NANPN fell rapidly from the level found immediately after feeding, and Moore & King (1958) found a similar decrease in NANPN in the 3 h after feeding three steers on diets of hay and grain, or hay, grain and tryptone, although the experimental conditions were somewhat different from those described above. These authors also found that the decrease in NANPN was accompanied by an increase in ammonia concentration in the rumen. Weller *et al.* (1958) also found that there was little soluble nitrogen in the rumens of sheep fed on wheaten hay and slaughtered between 2 and 24 h after feeding; most of

the nitrogen was in the form of microbial or plant nitrogen. All these results show that there is a very rapid utilization and degradation of soluble protein and non-protein material in the rumen and the rate of utilization of nitrogenous material is governed by its rate of liberation from the insoluble foodstuff particles.

The ammonia determinations gave similar curves when each point was obtained on a different day and also when all the points were obtained on one day, and the curves support the conclusion of Chalmers *et al.* (1954) that the physical state of the casein affects the rumen ammonia level (Fig. 2*a*).

SUMMARY

1. Two sheep were fed on partly synthetic diets differing only in the type of casein they contained, i.e. dissolved, partly soluble, or insoluble. The proteolytic activity of the micro-organisms, numbers of proteolytic bacteria, redistribution of the protein nitrogen, volatile fatty acids and pH values in the rumen were measured at intervals after feeding. The rate of passage of rumen contents to the abomasum was measured by determining rumen concentrations of polyethylene glycol introduced in the feed.

2. The rate of decrease of total nitrogen in the rumen was greater than that of polyethylene glycol, indicating an absorption of nitrogen.

3. Soluble protein was rapidly degraded, and except for a short time after feeding there was no measurable amount in the rumen contents. Compounds of non-ammonia, non-protein nitrogen (NANPN) were likewise broken down, but at a slower rate. The insoluble casein provided a more constant supply of NANPN than the other caseins.

4. Levels of protozoal and bacterial nitrogen remained fairly constant, indicating that growth of micro-organisms was almost balanced by the loss of cells to the abomasum.

5. Proteolytic activity varied with the diet, but did not change appreciably immediately after feeding.

6. After an initial fall on feeding, the rumen pH values remained relatively constant for the next 7 h. Total volatile fatty-acid concentration reached maximum values 3 h after feeding and there was some evidence that part of the volatile acids were formed by deamination of amino acids.

7. Rumen-ammonia concentrations depended on the state of the casein in the diet.

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