Digestion of foodstuffs in the rumen of the sheep and the passage of digesta through its compartments

3.* The progress of nitrogen digestion

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In earlier papers we have reported on the extent of conversion of plant nitrogen (plant-N) into microbial nitrogen (microbial-N) in the rumen (Weller, Gray & Pilgrim, 1958) and on the passage of the residual and resulting nitrogenous compounds through the omasum and abomasum, to the small intestine (Gray, Pilgrim & Weller, 1958*b*). The experiments now described provide details of the progress of N digestion during the feeding cycle in sheep receiving two roughage diets containing widely different amounts of plant-N. Some analyses of omasal contents have been included in this report, and also a comparison of the passage of two indigestible markers—lignin and polyethylene glycol—from the rumen. Findings from these experiments have a bearing on previously stated views on the passage of digesta from the rumen to the omasum.

EXPERIMENTAL

Animals and diets. Ten merino sheep were divided into two groups of five animals: one group received a daily ration consisting of 650 g wheaten hay and 150 g lucerne hay (1·4% N) and the other group received a ration of 800 g lucerne hay (2·9% N). After a period of 2 weeks on these rations the sheep were slaughtered. For convenience the diets will be referred to as the wheaten hay diet and the lucerne hay diet, respectively.

A third group of twelve sheep, subdivided into four subgroups of three animals each, was fed on the wheaten hay diet. On the day of slaughtering, each of these animals was given an injection of 10 g polyethylene glycol (*M* about 4000) into the rumen, 4 h after the beginning of feeding.

All of the sheep used in these experiments were fed once a day, in the morning. They had been trained to eat quickly, the maximum time allowed being 3 h. The precise time occupied in eating was not measured, but usually the greater part of the ration was consumed in 1 h.

Sampling of stomach contents. Animals in each of the groups of five sheep were slaughtered at various times during the feeding cycle—3, 6, 10, 16 and 24 h after the beginning of feeding. The contents of the rumen, among which were included the

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contents of the reticulum, and of the omasum were removed and sampled and the samples prepared for analysis by procedures previously described (Gray *et al.* 1958*a*; Weller *et al.* 1958).

Animals in the remaining group were slaughtered 4, 8, 14 and 24 h after the beginning of feeding and the rumen contents removed and sampled. Rumen fluid was obtained by filtering some of the contents through muslin.

Methods of analysis. The methods used for determination of lignin and N, and the distribution of N between plant-N, bacterial-N, protozoal-N and soluble-N have been described earlier (Gray *et al.* 1958*a*, *b*; Weller *et al.* 1958). The presence of diaminopimelic acid in rumen bacteria and its absence from plants and rumen protozoa form the basis for determining the amount of bacterial-N in the rumen contents (Weller *et al.* 1958). The concentration of diaminopimelic acid-N in the bacterial-N was measured in each of the samples of rumen contents. The close similarity of these concentrations in all the sheep fed on the wheaten hay diet warranted the use of the same values in analyses of the omasal contents. But the concentrations found in sheep fed on lucerne hay were more variable at different times of the day and were not applied to the omasal contents of those animals.

Polyethylene glycol was estimated gravimetrically in the rumen fluid by the method of Shaffer & Critchfield (1947) as modified by Sperber, Hydén & Ekman (1953).

RESULTS

Progress of changes in N distribution in the rumen

The amounts of N in the rumen and the distribution between plant-N, microbial-N and soluble-N at the various times during the feeding cycle are shown in Fig. 1. The concentrations of diaminopimelic acid-N on which these distributions are based are given in Table 1. Although curves are presented in Fig. 1, it must be remembered that the values for each of the sampling times were derived from different animals. However, it seems clear from the figure that the microbial attack on plant nitrogenous compounds was extremely rapid. In sheep fed on the wheaten hay diet the rumen probably contained less than 1 g plant-N before feeding, and within 3 h of the ingestion of 11·2 g of new plant-N only 6 g were left—and at 6 h, only 3 g. With sheep fed on lucerne hay the speed of the attack was even more striking. Before feeding, plant-N in the rumen amounted to about 2 g, and 23 g of new plant-N were eaten: 3 h after

Table 1. *Diaminopimelic acid nitrogen (DAP-N) in bacterial nitrogen in the rumen of sheep*

Time after beginning of feeding (h)	DAP-N in rumen bacterial-N (%)	
	Wheaten hay ration	Lucerne hay ration
3	0·69	0·44
6	0·69	0·60
10	0·69	0·62
16	0·63	0·54
24	0·67	0·69

the beginning of feeding only 9–10 g of plant-N remained. In considering the rate of attack, account must also be taken of the time needed to consume the ration—usually between 1 and 2 h.

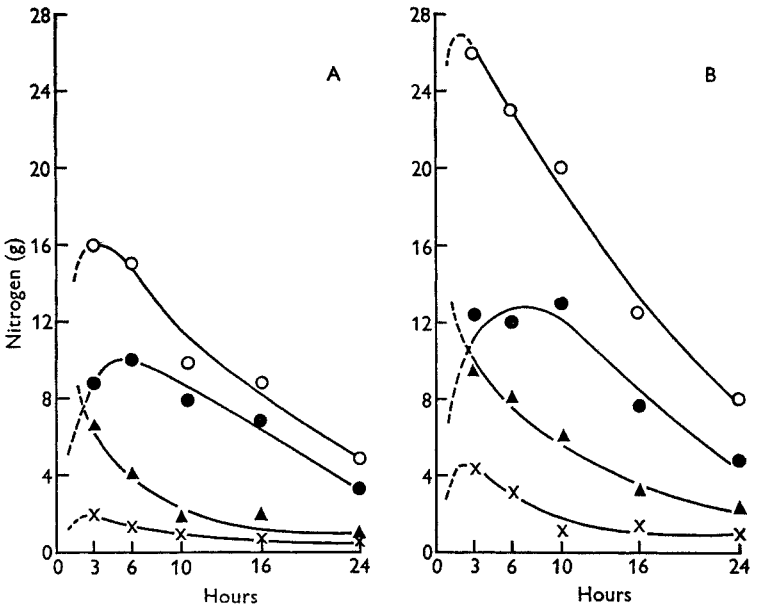

Fig. 1. Distribution of nitrogen in the rumen of sheep at different times after feeding on (A) the wheaten hay ration and (B) the lucerne hay ration. ○, total N; ●, microbial-N; ▲, plant-N; ×, soluble-N. Each value represents one sheep.

Coincident with the loss of plant-N there was an increase in the microbial-N and soluble-N fractions. With the wheaten hay diet microbial-N increased from about 3 g before feeding to about 9 g 6 h later, and with lucerne hay the increase was from 4–5 g to about 12 g in 3 h.

Thus, though a little of the loss of plant-N in these periods may have been due to onward passage of digesta to the omasum, and no doubt a part was also due to removal of soluble nitrogenous compounds, yet obviously by far the largest part of the loss was due to conversion of plant-N into microbial-N. We must conclude that the microbial attack on the plant nitrogenous compounds was very rapid indeed in the earliest stages of the fermentation, and that the numbers of micro-organisms increased with like rapidity.

Bacteria and protozoa

The concentrations of the different forms of N in the rumen are listed in Table 2 and the proportions of bacterial-N and protozoal-N in the total microbial-N fraction are given in Table 3. It is clear that, whereas protozoa did not at any time form more than a small part of the microbial population in sheep fed on the wheaten hay diet, yet they accounted for 25–40% of the microbial-N in the latter half of the feeding cycle with lucerne hay. It will be noted, however, that the amounts of N involved at this time were relatively small.

Table 2. *Distribution of nitrogen (mg/100 g rumen contents) in the rumen of sheep*

Time after beginning of feeding (h)	Wheaten hay ration				Lucerne hay ration			
	Plant-N	Bacterial-N	Protozoal-N	Soluble-N	Plant-N	Bacterial-N	Protozoal-N	Soluble-N
3	92	126	7	29	141	185	6	69
6	56	133	8	17	136	169	35	45
10	42	170	12	7	120	189	62	26
16	39	107	22	12	54	117	28	30
24	16	63	9	11	60	69	45	20

Nitrogen in the omasum

The purpose of examining the omasal contents of some of the animals was to see whether this material showed the same sorts of changes in composition, after feeding, as were found in the rumen. It has been reported (Gray *et al.* 1958*a*) that the digestion coefficients for cellulose and hemicellulose in the omasal contents of sheep fed on these roughage diets did not change in response to feeding, as they did, of course, in the rumen itself. These earlier findings were interpreted to mean that the material reaching the omasum was thoroughly digested—in respect of cellulose and hemicellulose—even in the period immediately after the intake of fresh food. Table 4 compares the N:lignin (N:L) ratios in the rumen and omasum of the sheep fed on the wheaten hay ration.

Since the plant N:L ratios in the omasum were very uniform throughout the day it has been assumed that the samples from the omasum were representative of the

Table 3. *Percentages of bacterial and protozoal nitrogen in the microbial nitrogen of the rumen of sheep*

Time after beginning of feeding (h)	Wheaten hay ration		Lucerne hay ration	
	Bacterial-N	Protozoal-N	Bacterial-N	Protozoal-N
3	95	5	97	3
6	94	6	83	17
10	93	7	75	25
16	83	17	81	19
24	87	13	61	39

Table 4. *Ratios of plant-N and microbial-N to lignin in the rumen and omasum of sheep fed on wheaten hay ration*

Time after beginning of feeding (h)	Plant-N (mg):lignin (g)		Microbial-N (mg):lignin (g)	
	Rumen	Omasum	Rumen	Omasum
3	62	26	90	107
6	43	25	108	132
10	30	15	130	153
16	29	26	96	77
24	19	18	84	79

digesta passing through the compartment during the whole feeding cycle. The ratios did not alter in response to the eating of foodstuffs in which the ratio was much higher, but remained similar to those found in the rumen 10–24 h after feeding. This observation suggests that very little undigested material from the new fodder reached the omasum during and immediately after feeding. On the other hand, it appears that newly formed microbial-N was able to pass to the omasum during this period since the microbial-N:L ratio remained much the same in the rumen and omasum at all sampling times.

Table 5. *Microbial-N (as percentage of total solids) in the rumen and omasum of sheep fed on wheaten hay ration*

Time after beginning of feeding (h)	Rumen	Omasum
3	59	80
6	71	84
10	81	91
16	77	75
24	82	81

Extent of conversion of plant-N into microbial-N in the rumen

The need for further knowledge of the extent of this conversion was indicated in a previous report (Weller *et al.* 1958). The proportions of microbial-N in the total N of the solids present in the rumen and omasum of sheep fed on the wheaten hay diet are given in Table 5. The extent of conversion of plant-N into microbial-N in the rumen must be indicated by the highest values of the range (60–82%) found there throughout the day, since the material in the omasum exhibited this level of conversion at all times. It should be remembered, however, that with this diet there was probably a small but nevertheless significant quantity of soluble nitrogenous compounds formed and absorbed in the rumen. Consequently the 80% conversion shown in Table 5 refers only to that part of the plant-N not converted into soluble-N.

Passage of lignin and polyethylene glycol from the rumen

The findings from this experiment are presented in Fig. 2 in which the ratio of polyethylene glycol to lignin has been given the arbitrary value of 100 in the rumen at 4 h after feeding, and the ratios for subsequent times have been calculated accordingly. Each ratio was calculated from the mean values given by three sheep. From the decline in the ratio during the day it is evident that the glycol left the rumen at a greater rate than lignin.

DISCUSSION

The results of these experiments clearly illustrate the extremely rapid attack on nitrogenous substances in the wheaten and lucerne hay roughages; they demonstrate differences in the make-up of the microbial fractions of the N in the rumen of sheep fed on these two diets; and they indicate the procedure needed to obtain a more

precise estimate of the extent of conversion of plant-N into microbial-N than that previously determined from the rumen composition alone. They also provide additional evidence that only well-digested residues reached the omasum, so that the evidence now comprises information about the N, cellulose, pentosans, and total solids present in the digesta.

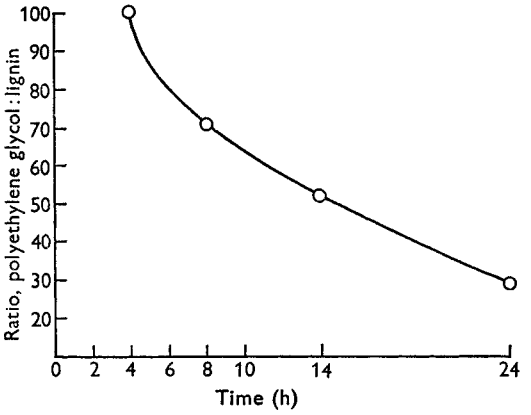

Fig. 2. Ratio of polyethylene glycol to lignin in the rumen of sheep at different times after feeding. Each value is a mean for three sheep.

The passage of digesta from the rumen to the omasum in sheep fed on the roughage diets we have been considering can be described as an intermittent flow of a 'fluid stream' through the reticulo-omasal orifice. This fluid stream is a suspension of fine plant particles which have broken away from coarser particles or fibres of the fodder during its fermentation in the rumen. The fine particles are usually covered with adherent bacteria and their sizes range from the smallest residues up to the maximum size found in the omasum or abomasum. In addition the stream carries many 'free-living' protozoa and bacteria. The latter have either become detached from fibres they had been attacking or else are normally free-living in the rumen fluid. The concept of a fluid stream, circulating in the reticulo-rumen and continually receiving fine particles during its passage through the coarse mass of fermenting roughage, was put forward clearly by Schalk & Amadon (1928). The stream carries the fine particles to the reticulum and from there they may pass, intermittently, to the omasum. There seems to be no reason to doubt that particles beyond a certain small size do not normally pass through the reticulo-omasal orifice, and one would not therefore expect to find much freshly consumed roughage in the omasum as time must elapse before significant numbers of fine particles are formed. Support for this view was given by Schalk & Amadon (1928) who noticed that the bolus of rumination did not contain recently eaten food, but rather the residues of the previous meal. They considered that this was the material which passed to the omasum in the period immediately after feeding.

Direct evidence in favour of the suggestion that only well-digested residues reach the omasum was reported by us earlier (Gray *et al.* 1958*a*) and the experiments

described here offer further support for it. The earlier evidence was that in sheep fed on wheaten hay or lucerne hay the coefficients of digestion of cellulose, pentosans and solids in the omasal contents were always as high as the highest coefficients found in the rumen during the day—there was no lowering of the coefficients after feeding as was necessarily so in the rumen itself. In the experiments now described the plant-N has been examined, and here again it has been shown that after feeding only thoroughly digested material was present in the omasum.

The microbial-N fraction of the N increases during fermentation and we have shown that its concentration relative to lignin was much the same in the omasum as in the rumen at all times of the day. In the rumen the ratio between microbial-N and lignin must have tended to decline sharply when feeding began but it showed no decline at 3 h, because of the very rapid bacterial growth and attack on the new fodder.

Since the fibres of the new fodder quickly become encrusted with developing bacteria during their rapid attack on the plant substances, it would be expected that the microbial-N:L ratio in the rumen would increase after feeding. Further, one might expect that the presence of some readily soluble nitrogenous compounds in the diet would lead to an even higher microbial-N:L ratio in the fluid stream passing to the omasum. The limited results available (Table 4) agree with these expectations.

It now seems clear that digesta reaching the omasum throughout the day would consist of plant residues in which the original cellulose, pentosans and plant-N are all well digested, and that these residues would be accompanied by the corresponding lignin fraction of the plant tissues together with micro-organisms containing that part of the digested N which does not remain as soluble-N. In addition, of course, the material must include other substances synthesized by the micro-organisms, and all the soluble products of the fermentation not absorbed through the rumen wall. At no time does a detectable excess of unattacked plant material reach the omasum in sheep fed on these roughages. Although the first 3 h after the beginning of feeding were not accounted for in our experiments, one would expect this to be the time when unattacked fodder would be least likely to have reached the particle size necessary for passage to the omasum. Some unattacked particles might be expected to pass on immediately, however, if the food were given in a finely ground form.

The relative losses of lignin and polyethylene glycol from the rumen also fit in readily with the concept of a fluid stream discussed above. Within about $1\frac{1}{2}$ h after injection, the soluble polyethylene glycol would be distributed evenly through the whole of the water in the fluid stream (Gray, Jones & Pilgrim, 1960)—but the lignin present in the coarse plant fibres would only gradually be added to the stream and would therefore pass more slowly to the omasum.

SUMMARY

1. The amounts of nitrogen in the form of plant-N, microbial-N and soluble-N in the rumen of five sheep fed on wheaten hay and of five sheep fed on lucerne hay indicate an extremely rapid attack on plant-N, and a correspondingly rapid growth of micro-organisms.

2. The two roughage diets gave rise to somewhat different proportions of protozoal-N and bacterial-N in the microbial-N fraction.

3. Comparison of the contents of the rumen and omasum indicated that material entering the omasum was well digested, in respect to plant-N, even in the period immediately after feeding.

4. Polyethylene glycol was shown to pass out of the rumen more rapidly than lignin.

5. The nature of the passage of digesta from rumen to omasum is discussed.

REFERENCES

Gray, F. V., Jones, G. B. & Pilgrim, A. F. (1960). *Aust. J. agric. Res.* **11**, 383.
Gray, F. V., Pilgrim, A. F. & Weller, R. A. (1958*a*). *Brit. J. Nutr.* **12**, 404.
Gray, F. V., Pilgrim, A. F. & Weller, R. A. (1958*b*). *Brit. J. Nutr.* **12**, 413.
Schalk, A. F. & Amadon, A. S. (1928). *Bull. N. Dak. agric. Exp. Sta.* no. 216.
Shaffer, C. B. & Critchfield, F. H. (1947). *Analyt. Chem.* **19**, 32.
Sperber, I., Hydén, S. & Ekman, J. (1953). *LantbrHögsk. Ann.* **20**, 337.
Weller, R. A., Gray, F. V. & Pilgrim, A. F. (1958). *Brit. J. Nutr.* **12**, 421.

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