Observations on the microbiology and biochemistry of the rumen in cattle given different quantities of a pelleted barley ration

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(*Received* 9 *May* 1969—*Accepted* 13 *October* 1969)

1. Three heifers were changed from a diet of equal parts of hay and barley cubes (50:50 diet) to one entirely of barley cubes given in three equal feeds throughout the day. Feed intake was restricted to 80% of calculated appetite at the time of change and this percentage progressively decreased as the live weights of the animals increased.

2. The change of diet had no significant effect on the volume of rumen fluid but the rate of outflow from the rumen was significantly lower on the barley diet than on the 50:50 diet.

3. Animals on the restricted barley diet developed an exceptionally high rumen ciliate population and the bacterial population was shown by Gram films to include a number of organisms typical of roughage-fed animals. In culture, organisms of the genus *Bacteroides* were predominant but these appeared largely as cocco-bacilli in the Gram films. This microbial population was associated with a higher proportion of butyric acid than of propionic acid in the rumen fluid.

4. Occasional fluctuations in ciliate populations occurred in all three heifers. Decreases in ciliate number were paralleled by increases in propionic acid and decreases in butyric acid but not necessarily by a fall in pH. Under these conditions Gram films showed increases in bacteriodes-type rods and in certain curved Gram-negative rods.

5. Rumen ammonia concentrations were on average lower and showed a different diurnal pattern when ciliate numbers were reduced. Lactic acid concentrations were low and were not affected by the size of the ciliate population.

6. When the three heifers were given the barley diet *ad lib.* there was a decrease in rumen pH and a complete loss of rumen ciliates. The rumen bacterial population and the volatile fatty acid proportions were similar to those seen during decreases in ciliate number at the restricted level of intake. These changes also occurred in a fourth heifer which was changed fairly rapidly from the 50:50 diet to a restricted amount of the barley diet.

7. Two steers which had never had access to roughage were changed from *ad lib.* to restricted intake of the barley diet and were later given an inoculum of rumen ciliates. The rumen microbial population and the pattern of fermentation so produced were similar to those found in the heifers on the restricted barley diet.

8. Anomalous values were noted for total counts of rumen bacteria when free starch grains were present in the rumen fluid.

9. It is concluded that large ciliate populations and high proportions of butyric acid can be produced in animals fed exclusively on a barley diet by suitable adjustment of the intake and the method of feeding. It is postulated that the ciliate population may be largely responsible for the high butyric acid concentrations.

The rumen microbial populations of mature steers given *ad lib.* access to a barley diet have been described by Eadie, Hobson & Mann (1967) and have been shown to be similar in many respects to those found in young ruminating calves given large amounts of starchy concentrates (Eadie, 1962). A complete absence of rumen ciliate protozoa was a characteristic finding in both groups of animals and it was found impossible

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to establish these organisms by direct inoculation from donor animals. In the calves, ciliates could be established only when a proportion of roughage was included in the diet (Eadie, Hobson & Mann, 1959; Eadie, 1962). It was concluded that a low rumen pH during all or part of the daily feeding cycle was largely responsible for the failure of ciliates to become established on the all-concentrate diets (Eadie, 1962; Eadie *et al.* 1967).

In the present work a study has been made of the properties of rumen fluid in cattle given a barley diet in amounts below appetite. This restriction of intake has been found to modify conditions within the rumen and to lead to the establishment of very large ciliate populations. Coincident with the increase in ciliate numbers, there were also alterations in the rumen bacterial population and in proportions of volatile fatty acids (VFA) in the rumen fluid. A brief account of these findings has been given elsewhere (Eadie, Hyldgaard-Jensen, Mann, Reid & Whitelaw, 1969).

EXPERIMENTAL

Animals and management. Four Hereford × Ayrshire heifers (794, 795, 832 and 833) fitted with rumen cannulas and two Ayrshire steers (123 and 140) were used. Each animal was kept in a separate pen and was individually fed but was not sufficiently isolated from other members of the group to prevent occasional cross-inoculation with rumen ciliates. Over the experimental period the heifers were kept on wooden-slatted floors and the steers on sawdust.

Heifers 794, 795 and 832 were given a mixture of 50% chopped hay and 50% barley cubes (the 50:50 diet) for 10–14 weeks and were then changed to a ration consisting entirely of barley cubes (the 'barley' diet). The cubes contained 85% barley and 15% protein–mineral–vitamin supplement (Preston, 1963). Heifer 833 remained on the 50:50 diet for a further 4 months. Heifers 794, 832 and 833 were given 5·1 kg each day while 795 was given 4·5 kg each day, these amounts representing 2·4% of live weight at the start of the experiment. This amount of food was fed throughout the experiment except that the ration for 795 was increased to 5·1 kg/day after 14 weeks of barley feeding. The ration was given in three equal feeds at 08.00 h, 14.00 h and 20.00 h. Throughout the text 2·4% of live weight will be referred to as 'calculated appetite' and is considered to be a close approximation to voluntary intake of an all-grain diet. Since the animals were steadily gaining in weight the food given represented a gradually diminishing percentage of possible appetite as the experiment progressed.

The change from the 50:50 diet to the barley diet was made in stages; in 794 and 795 the change took place over 3 weeks, with 1 week on each of the following proportions of barley to hay—65:35, 80:20, 95:5. With 832 and 833 only two intermediate changes were made, 65:35 and 80:20, so that the complete change was made in 2 weeks. In heifer 833 the cannula was broken 7 weeks after this animal was given the barley diet; it was then removed from the experiment.

Following the period of restricted feeding 794, 795 and 832 were changed to an *ad lib.* intake of barley cubes. A rapid change to *ad lib.* feeding was made with 795 but with 794 and 832 the ration was increased gradually until *ad lib.* intakes were reached.

The steers started the experiment on *ad lib.* barley feeding and had never had access to dietary roughage. The daily intake was then restricted and was given in three equal feeds at 07.00 h, 14.00 h and 22.00 h. After 6 weeks an inoculum of mixed rumen ciliates was given and restricted feeding was continued for a further 14 weeks. Rumen fluid samples from the steers were obtained by stomach tube.

Rumen fluid volume and outflow. The volume of fluid in the reticulo-rumen and its rate of passage to the omasum were measured on at least two occasions in each heifer. Polyethylene glycol was used as the reference substance and was injected into the rumen on each of 5 successive days according to the procedures outlined by Hydén (1961). Measurements were made on all four heifers after they had received the 50:50 diet for a period of 10–12 weeks, on 794, 795 and 832 after 6–8 weeks on the barley diet and on 833 after 23 weeks on the 50:50 diet. The second set of measurements on this latter animal coincided in time with the 6–8-week observations on the animals given the barley diet. Further measurements were made on 832 and 794 after 28 weeks and 47 weeks respectively on the barley diet.

Rumen ciliate examination. Rumen fluid samples were taken from the heifers at weekly intervals during the change from the 50:50 diet to the barley diet and at more frequent intervals throughout the periods of restricted and *ad lib.* feeding. Samples were taken from the steers at intervals related to the changes in diet.

Wet preparations of all samples were examined for ciliate identification and the rough assessment of population size. Some of the material was preserved with an equal volume of 10% formalin and ciliate counts were made on chosen samples using the counting chamber described by Boyne, Eadie & Raitt (1957). Suitable dilutions were made with glycerol and buffer solution to give a suspension in 50% glycerol for each count. 'Large', 'medium' and 'small' ciliates were counted separately and where possible a separate record was made of the numbers of the holotrich genus *Isotricha*.

The mixed rumen ciliate inocula were given to each steer by stomach tube and were prepared by concentrating the organisms from 500 ml of rumen fluid obtained from a heifer on a roughage-concentrate ration (Eadie, 1962).

Bacterial examination. Gram films were prepared and examined from all the samples obtained for the study of rumen ciliates and total counts were carried out on a number of these samples using a Coulter Counter (Hobson, Mann & Summers, 1966). The total bacterial counts were carried out after the ciliate counts had been made so that all samples had been subjected to a similar amount of shaking.

Regular counts of viable bacteria were made on samples from heifers 794 and 832 during the change from restricted to *ad lib.* intake of barley and from steers 123 and 140 at each stage of the experiment. Total viable counts were made initially on medium M8 and later on a starch medium (Kurihara, Eadie, Hobson & Mann, 1968). Counts of lactobacilli were obtained on the SL selective medium of Rogosa, Mitchell & Wiseman (1951).

Rumen VFA, lactic acid and ammonia. At chosen stages in the course of restricted feeding a series of rumen fluid samples was taken from the heifers over the period

07.50 h–16.30 h and on a few occasions sampling was continued until 06.00 h on the following day. Total and individual VFA and lactic acid determinations were made on each series of samples and ammonia determinations on some of these series. Single daily samples were taken during the change from restricted to *ad lib.* feeding. In the steers, single samples were taken on two occasions during the course of restricted feeding.

Total VFA were determined by steam distillation in a Markham still and the C_2–C_5 acids separated by gas–liquid chromatography (James & Martin, 1952) with the column packing described by Annison & Pennington (1954). Later samples (those relating to 794 and 832 during *ad lib.* intake and to the steers) were separated by the method of Storry & Millard (1965). Lactic acid was determined by the method of Barker & Summerson (1941) with the modification suggested by Pennington & Sutherland (1956). Ammonia was determined in acidified samples by the method of Conway & O'Malley (1942). The pH of rumen fluid was determined electrometrically soon after withdrawal of samples from the rumen.

RESULTS

Heifers

(A) *Restricted intake of the barley diet*

Feed intake and live-weight gains. The animals ate all the food offered over the period of restricted feeding. The live weights of heifers 794, 795 and 832 covered similar ranges, for 794 rising from 251 to 419 kg in the year of restricted feeding, for 795 from 232 to 370 kg in 6 months and for 832 from 260 to 420 kg in 10 months.

The food consumed by heifer 794 when appetite was taken as 2·4% of body-weight covered the range from 85% of this figure when restricted feeding was started to 50% just before the change to *ad lib.* intake. In heifer 795 the range was from 80 to 65% and in heifer 832 from 80 to 50%. The adjustment of intake in heifer 795 at the 14th week increased the percentage intake from 76 to 82. In heifer 833 the maximum amount offered was only 65% of calculated appetite on account of the greater age, and hence higher live weight, of this animal at the change to barley feeding.

Ciliate populations. Tables 1–3 show the counts for rumen ciliate protozoa and total bacteria along with total VFA concentrations and proportions of individual acids in heifers 794, 795 and 832 on the 50:50 diet and at various stages after the change to the barley diet.

In order to give a comparable time scale the samples are listed under 'weeks on barley diet'; this indicates that the sample was taken during the relevant week after restricted feeding began but samples from consecutive weeks could be up to 13 days apart. All ciliate and bacterial counts were made on samples taken at 10.30 h. Regular microscopic examinations of samples showed that there were no prolonged alterations in ciliate population other than those for which counts are given.

In all three heifers there was a thriving ciliate population on the 50:50 diet at the beginning of the experiment. The organisms present were *Epidinium* spp. and *Isotricha* spp. listed as 'large', *Eremoplastron* spp. and *Dasytricha ruminantium* listed as 'medium' and *Entodinium* spp. making up the 'small'. Because of the variations in the size of the

Table 1. *Rumen microbial populations and volatile fatty acids in heifer 794 when given a diet of equal parts chopped hay and barley cubes (50:50 diet) and at intervals after a change to a restricted intake of a diet consisting entirely of barley cubes*

	Ciliates ($\times 10^{-3}$/ml)					Total bacteria ($\times 10^{-9}$/ml)	Volatile fatty acids*			
								Molar %		
	Large†	Medium	Small	Total	Total unit‡		Total (m-moles/l.)	Acetic	Propionic	Butyric + 'higher'
Diet 50:50	88·8	77·2	426·0	592·0	1235	12·1	97·7	67·3	17·3	15·4
Weeks on restricted barley diet:										
1	108·8	NC	2000·0	2109	2702	15·7	117·6	51·8	29·1	19·1
3	107·2	NC	3232·0	3339	3927	8·34	—	—	—	—
4	8·0	NC	184·0	192	236	22·4	121·2	42·7	35·3	22·0
5	126·4	NC	2360·0	2486	3179	5·98	—	—	—	—
17	176·8	192·0	1404·0	1773	3124	10·8	99·1	64·1	11·8	24·1
23	165·2	223·2	1640·0	2028	3381	—	—	—	—	—
24	183·2	249·6	1248·0	1681	3184	9·12	—	—	—	—
30	146·0	317·6	452·0	916	2352	—	95·1	62·5	8·5	29·0
39	—	—	—	—	—	—	112·5	53·4	12·6	34·0
42	172·8	95·2	964·0	1232	2367	—	—	—	—	—
52	188·0	29·2	684·0	901	2003	8·4	101·5	53·7	16·4	29·9

NC, ciliates either not seen or too few to count.

* With the exception of weeks 39 and 52, which relate to single samples, each VFA value recorded is the mean for five to sixteen samples taken between 07.30 h and 16.30 h on 1 day. Rumen ammonia determinations were made on the samples taken at weeks 4, 17 and 30 (cf. Fig. 1).

† See p. 160 for species included in each group.

‡ A measure of total ciliate volume, see p. 164.

Table 2. *Rumen microbial populations and volatile fatty acids in heifer 795 when given a diet of equal parts chopped hay and barley cubes (50:50 diet) and at intervals after a change to a restricted intake of a diet consisting entirely of barley cubes*

	Ciliates ($\times 10^{-3}$/ml)					Total bacteria	Volatile fatty acids*			
								Molar %		
	Large†	Medium	Small	Total	Total unit‡	($\times 10^{-9}$/ml)	Total (m-moles/l.)	Acetic	Propionic	Butyric+ 'higher'
Diet 50:50	127·4	55·0	358·0	540	1348	3·71	108·2	64·6	17·0	18·4
Weeks on restricted barley diet:										
1	63·6	21·0	149·8	233	620	10·0	110·4	57·2	24·4	18·4
4	51·4	NC	588·0	639	919	—	—	—	—	—
5	—	—	—	—	—	—	113·4	56·5	17·3	26·2
13	191·2	37·3	2156·0	2384	3508	5·84	119·4	62·1	12·4	25·5
15§	47·6	1·6	176·0	225	484	8·5	—	—	—	—
17	23·4	NC	90·0	113	244	14·4	—	—	—	—
18	27·6	NC	283·0	311	458	31·6	—	—	—	—
19	2·0	NC	10·4	12	23	11·6	—	—	—	—
20	21·2	NC	1950·0	1971	2080	15·5	—	—	—	—
22	194·6	NC	1572·0	1767	2833	—	125·3	58·8	18·0	23·2
23	123·2	NC	3144·0	3267	3943	7·86	—	—	—	—

NC, ciliates not seen or too few to count.

* With the exception of week 22, which relates to a single sample, each VFA value recorded is the mean for five to sixteen samples taken between 07.30 h and 16.30 h on 1 day. Rumen ammonia determinations were made on the samples taken at weeks 4 and 13. (cf. Fig. 1).

† See p. 160 for species included in each group.

‡ A measure of total ciliate volume, see p. 164.

§ The ration was increased from 4·5 to 5·1 kg/day at week 14.

Table 3. *Rumen microbial populations and volatile fatty acids in heifer 832 when given a diet of equal parts chopped hay and barley cubes (50:50 diet) and at intervals after a change to a restricted intake of a diet consisting entirely of barley cubes*

	Ciliates ($\times 10^{-3}$/ml)					Total bacteria	Volatile fatty acids*			
								Molar %		
	Large†	Medium	Small	Total	Total unit‡	($\times 10^{-9}$/ml)	Total (m-moles/l.)	Acetic	Propionic	Butyric+ 'higher'
Diet 50:50	73·4	224·8	366·4	665	1512	8·24	91·6	66·4	15·2	18·4
Weeks on restricted barley diet:										
1	84·4	37·6	403·2	525	1060	19·0	128·9	64·3	17·3	18·4
3	13·8	NC	643·2	667	728	—	—	—	—	—
5	1·9	NC	1·5	3	7·8	18·9	123·7	47·3	43·3	9·4
7	NC	NC	NC	NC	NC	—	—	—	—	—
13	7·0	NC	89·7	97	134	7·65	—	—	—	—
17	33·0	NC	237·6	270	452	16·1	116·5	53·0	33·4	13·6
18	86·6	NC	170·0	257	729	10·3	151·0	58·5	17·8	23·7
20	NC	NC	18·0	18	18	21·1	121·0	51·8	40·7	7·5
21	97·0	NC	548·0	645	1178	12·7	130·0	58·5	21·7	19·8
23	186·4	NC	1128·0	1314	2337	7·72	112·0	58·9	13·8	27·3
25	138·0	NC	1200·0	1338	2097	—	—	—	—	—
30	220·8	13·6	2316·0	2550	3785	—	115·1	61·7	15·1	23·2
37	318·4	98·4	2688·0	3105	5049	12·3	—	—	—	—
42	173·6	61·6	1220·0	1455	2527	21·0	144·5	55·9	24·8	19·2

NC, ciliates not seen or too few to count.

* With the exception of weeks 18, 20, 21 and 42, which relate to single samples, each VFA value recorded is the mean for five to sixteen samples taken between 07.30 h and 16.30 h on 1 day. Rumen ammonia determinations were made on the samples taken at weeks, 5 17, 23 and 30 (cf. Fig. 1).

† See p. 160 for species included in each group.

‡ A measure of total ciliate volume, see p. 164.

ciliates and the variations of proportion of each group a 'total unit' figure is given alongside the total number. The total unit is based on ciliate volume and comprises the sum of 'large' × 6·5, 'medium' × 3·0 and 'small' × 1·0. It is given in an attempt to use a figure which may be more representative of effective ciliate activity. Assessments of the average product, length × breadth, determined that factors of 6·5 and 3·0 were appropriate since the size range within each group was not very great. However, these factors assume that the depth of all ciliates is similar.

In all three animals there was a reduction in the numbers of the medium-sized organism *Eremoplastron* on the introduction of the barley diet and by 4 weeks after the change of ration these organisms could not be counted, although they redeveloped at a later stage. *Dasytricha* was not present in any of the animals after the 1st week on barley.

In heifer 794 (Table 1) the change to barley over a 3-week period led to an initial rise in ciliate population despite a reduction in medium-sized organisms. Apart from a sudden decrease in population at the 4th week high ciliate populations were maintained in this animal for almost a year.

In heifer 795 (Table 2) an initial drop in population occurred 1 week after the start of barley feeding. By the 13th week a large population was present but this decreased markedly following the ration increase in the 14th week, and on this occasion the 'medium' ciliate *Eremoplastron* was eliminated. Between the 14th and 22nd weeks a number of rises and falls in ciliate population were observed but by the 23rd week a very high ciliate population had again developed.

In heifer 832 (Table 3) there was a somewhat different picture. In this animal the change to the barley diet was made over only 2 weeks and there was a marked fall in ciliate population following the change of diet. By the 7th week it was impossible to make an accurate count, though *Isotricha* and *Entodinium* spp. were still seen in wet preparations. There then followed a number of fluctuations in ciliate numbers which were not associated with any ration change. The ciliate population in heifer 832 did not become steady or comparable in size with those of the other two heifers until around the 21st week of barley feeding.

In heifer 833, where the change to barley was made over 2 weeks as in 832, the ciliate population had dropped so drastically after 2 weeks on the ration that no ciliate count was possible. Although the food intake in this animal was never greater than 65% of calculated appetite when on the barley diet, ciliates were not seen in samples taken up to 6 weeks after the ration was introduced.

In heifers 794, 795 and 832 the proportion of *Isotricha* spp. in the ciliate population was noted to be a good indication of conditions within the rumen. With high populations *Isotricha* did not exceed 30% of the 'large' organisms but when there was a drop in ciliate numbers the *Isotricha* proportion tended to increase. Thus *Isotricha* made up the whole count of 'large' organisms from 17 to 20 weeks in heifer 795 and in the 5th and 13th weeks in heifer 832 (Tables 2 and 3). Variations in the proportion of entodinia ('small') in the total population were also seen (Tables 1–3) but could not clearly be related to conditions within the rumen. Whenever there was a large active ciliate population the organisms were well filled with starch grains and very few free starch grains were to be seen in the rumen fluid.

Proportions of VFA. The relative proportions of acetic, propionic and butyric plus 'higher' acids are given in Tables 1–3. Later experiments, in which the Storry & Millard (1965) method of gas chromatography was used, have shown that 'higher' acids tend to be low on restricted feeding of barley and that their inclusion with butyric acid would be liable to give erroneously high results for butyric acid only under *ad lib.* conditions (cf. Tables 5 and 7). In view of this the values recorded here for butyric plus 'higher' acids will be considered as butyric acid.

In the tables the VFA values are either the means of a series of samples taken between 07.50 and 16.30 h or are single determinations from samples taken within that period. Since the variation in VFA proportion throughout the day was found to be slight, the few single determinations can be considered to be comparable with the mean values.

The VFA proportions were similar in heifers, 794, 795 and 832 on the 50:50 diet. After the change to barley there was some increase in the proportion of propionic acid and a decrease in acetic acid in heifers 794 and 795 but in neither heifer did the percentage of propionic acid exceed 35. The drop in ciliate population in heifer 794 at the 4th week occurred when propionate was at its highest recorded level in that animal. In later samples from heifer 794 the proportion of butyric acid was high and that of propionic acid very low; these levels persisted throughout the period of restricted feeding and ran parallel to the high ciliate population. The VFA proportions recorded for heifer 795 when ciliate numbers were high were also higher for butyric than for propionic acid. Unfortunately we have no records for VFA during the period following the ration increase at the 14th week when fluctuations in ciliate population occurred. In heifer 832, in which the change to barley was made over 2 weeks, there was a more pronounced increase in the proportion of propionic acid following the change of diet. High values for this acid were recorded at the 5th, 17th and 20th week and each of them was associated with a low ciliate population. Thereafter the ciliate population increased in size and VFA proportions showed a decrease in propionic acid and an increase in butyric acid.

After 2 weeks on the restricted barley diet the VFA proportions in 833 were acetic 38·8%, propionic 49·2% and butyric 12·0% and were similar to values from the other heifers when they were given barley *ad lib.* (Table 5).

Rumen pH. The mean value for rumen pH of weekly 10.30 h samples over the restricted feeding period in heifer 794 was 6·3, with few values below 6·0. In heifer 795 the mean pH was 6·2 for samples having total unit values in excess of 900 and 5·7 for total unit values below this figure. The mean value for heifer 832 from the 4th to 22nd week of restricted barley feeding was 5·6, and from the 23rd week until the end of restricted feeding was 6·0. During the first period the pH was on occasion as low as 5·2 but the lowest value did not always coincide with the lowest ciliate population.

The average pH for thirteen samples taken at 10.30 h from heifer 833 over the 7 weeks in which it was given barley was 5·1.

Rumen lactic acid. Lactic acid could usually be detected only within the 1st h after feeding. The highest values recorded were 72·0 mg/100 ml for heifer 794 after 1 week

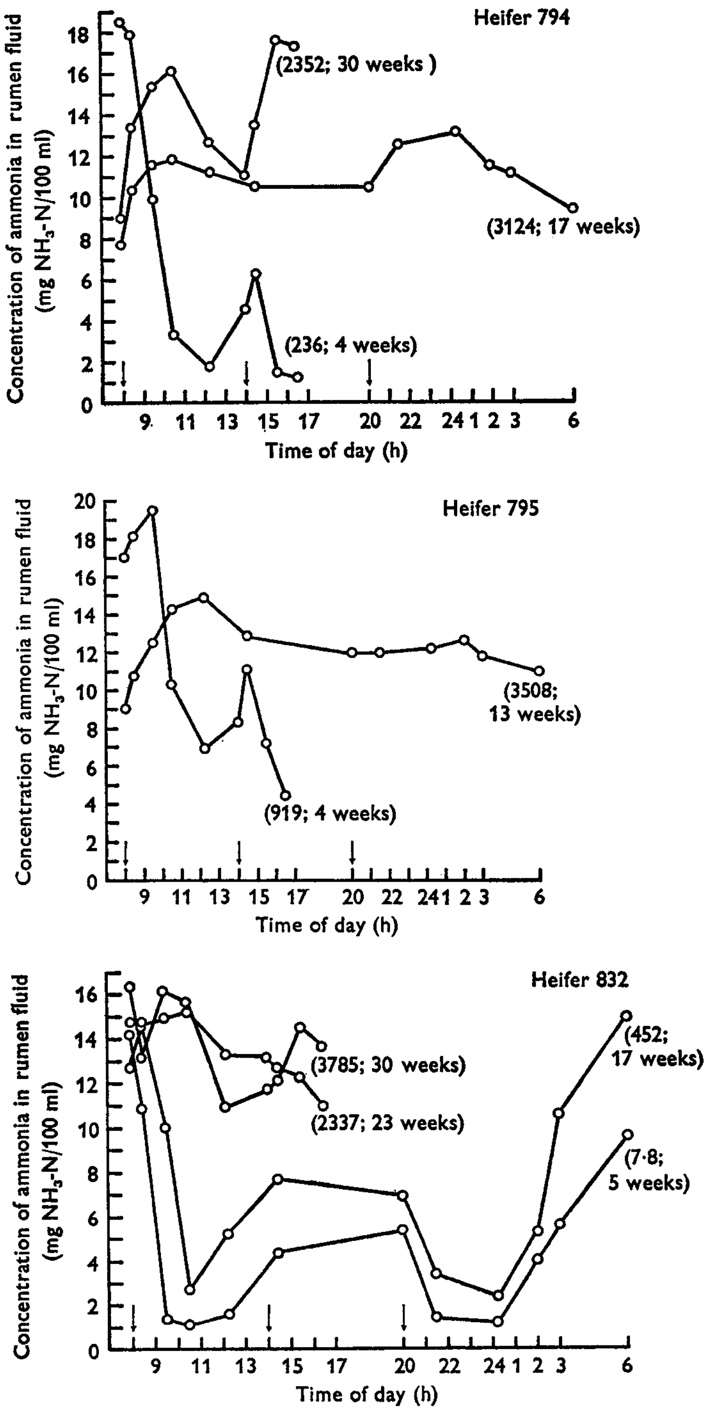


Fig. 1. Rumen ammonia concentrations at intervals throughout the day in three heifers given a barley diet in amounts below appetite. Figures in parentheses indicate rumen ciliate populations (total unit values, see p. 164) and duration of barley feeding. Times of feeding are indicated by arrows.

on barley and 70·0 mg/100 ml for heifer 832 after 17 weeks on barley. There were no records for heifer 795 above 5 mg/100 ml. In general the values obtained bore no relation to the size of the ciliate population.

Rumen ammonia nitrogen. Fig. 1 shows values for rumen ammonia nitrogen concentrations in heifers 794, 795 and 832 during days of high or low ciliate populations. Details of the ciliate populations are listed on Tables 1–3 but only total unit values are given in Fig. 1. The pattern for rumen ammonia concentration differed strikingly between days of high and low ciliate counts; when the ciliate population was low rumen ammonia concentrations dropped markedly after the 08.00 h feed and remained low for up to 16 h, whereas when ciliates were numerous ammonia concentrations remained high throughout the day.

Gram film examination. In heifers 794, 795 and 832 a background of negative coccobacilli on the Gram film was associated with a high and stable ciliate population. Slender, Gram-positive or Gram-variable rods, often in pairs, were also seen in smears from all three animals when ciliates were present; these appeared to be identical with the organism associated with the presence of ciliates in roughage-fed animals (Eadie, 1962). Positive cocci such as *Streptococcus bovis* varied in number in all three heifers, as did large, Gram-negative tetracocci and certain of the large bacteria typical of roughage-fed animals. Typical selenomonads, both large and small, were frequently present and the 'window-pane' sarcina was occasionally seen. *Oscillospira*, an organism not noted before in animals on a high-starch ration, was seen in heifer 794.

In all four heifers a reduction in ciliate numbers was regularly associated with the appearance on Gram films of bacteroides-type rods, together with curved, Gram-negative rods of vibrio, spirillum and atypical selenomonad form. The slender vibrio-like rods (organism A) ranged in length from 1·7 to 2·5 μm with a breadth of 0·3 μm. The spirillum-like Gram-negative rods (organism B) ranged from 2·5 μm × 0·8 μm to 3·7 μm × 0·5 μm. The atypical selenomonads (organism C) were Gram-negative, slightly curved rods which tended to taper at their ends. They varied in length from 2·0 to 6·6 μm with a fairly constant width of about 0·8 μm. Cultures showing this morphological form proved to be *Selenomonas ruminantium* var. *lactilytica*. This organism differed from the typical *Selenomonas* in not being obviously crescent-shaped and in being more uniform in width throughout the cell length.

It was noticeable from regular microscopic examinations of samples that when a drop in ciliate number lasted for only 1–2 days the bacterial changes seen in Gram films were equally short lived. These reductions in ciliate population were not always associated with a lowered rumen pH value and no consistent pattern could be seen in the predominance of organisms A, B or C during successive falls in ciliate number.

Bacterial types from culture. The only cultures made during restricted barley feeding were from samples taken just before a change to *ad lib.* intake. The predominant organisms in the cultures from heifers 794 and 832 after 52 weeks and 42 weeks respectively of restricted barley feeding were curved Gram-negative rods (organism A in 794, organisms A and B in 832) and *Bacteroides* spp. This indicated that the conspicuous coccobacilli in the direct Gram films of rumen contents were most probably *Bacteroides* spp.

Table 4. *Rumen fluid volume and clearance rate in four heifers (nos. 794, 795, 832 and 833) given diets of equal parts chopped hay and barley cubes (50:50 diet), or of barley cubes alone*

		Rumen volume (l.)*					Clearance rate (%/h)*				
Period	Diet	794	795	832	833	SD†	794	795	832	833	SD†
1	50:50: 10–12 weeks	21·1	19·6	23·8	19·8	1·54	10·9	13·8	12·6	11·2	0·89
2	23 weeks	—	—	—	26·3		—	—	—	9·5	
3	Barley: 6–8 weeks	22·1	16·1	19·4	—	3·50	5·8	7·4	5·8	—	0·62
4	28 weeks	—	—	18·7	—	2·37	—	—	5·7	—	0·70
5	47 weeks	27·9	—	—	—	0·96	4·9	—	—	—	0·24

* Each value is the mean of five measurements made on successive days.

† SD, standard deviation: pooled estimates from within-animal comparisons.

Table 5. *Rumen microbial populations, pH and volatile fatty acids in three heifers after an abrupt change (no. 795) or a gradual change (nos. 794 and 832) from 'restricted' to* ad lib. *intake of a barley diet*

		Feed intake					Volatile fatty acids				
								Molar %			
Animal no.	Feeding regime	kg/day	As % appetite*	Total ciliates (× 10^{-3}/ml)	Total bacteria (× 10^{-9}/ml)	pH	Total (m-moles/l.)	Acetic	Propionic	Butyric	'Higher'
795	Restricted†	5·1	65	3267	7·9	6·4	125	58·8	18·0	20·8	2·5
	4 h *ad lib.*	—	—	311	13·6	4·9	—	—	—	—	—
	1 day *ad lib.*	7·66	98	NC	10·3	5·2	158	46·0	50·6	3·4	—
	5 days *ad lib.*	8·50	102	NC	30·9	5·2	197	45·8	51·0	3·2	—
794	Restricted‡	5·1	51	901	8·4	6·4	102	53·7	16·4	22·8	7·1
	2 days after increase	6·9	68	612	43·9	5·5	155	40·1	43·5	12·5	3·9
	1 day *ad lib.*	5·4	54	13	21·7	5·7	123	37·6	43·2	12·4	6·8
	2 days *ad lib.*	8·7	86	NC	25·5	5·1	180	38·3	42·9	10·5	8·3
832	Restricted	5·1	50	1424	6·9	5·8	127	57·6	17·1	21·1	4·3
	2 days after increase	6·9	68	32	19·0	5·8	148	34·3	44·8	11·2	9·8
	1 day *ad lib.*	9·9	98	NC	35·3	5·4	148	32·7	36·3	16·5	14·4
	2 days *ad lib.*	9·9	98	NC	37·5	5·4	148	34·2	44·0	14·6	7·3

* Appetite taken as 2·4% of live weight.

† See Table 2, weeks 22 and 23.

‡ See Table 1, week 52.

Although the curved Gram-negative rods were conspicuous in cultures from both animals they were not obvious in direct Gram films from the same samples. As noted later (p. 170), however, there was an increase in curved Gram-negative rods with the introduction of *ad lib.* feeding and organism A was amongst the predominant species in both animals during the increase in ration. Discrepancies between Gram-film and culture records are discussed by Mann (1970).

Viable bacterial counts. Total anaerobic counts were made from the samples mentioned above. The values obtained were $0{\cdot}18 \times 10^9$/ml for heifer 794 and $1{\cdot}28 \times 10^9$/ml for heifer 832. The ciliate populations were very similar in each instance.

The viable lactobacillus count for heifer 794 after 44 weeks of restricted feeding was $1{\cdot}31 \times 10^6$/ml on a sample of pH 6·4, and for heifer 832 at 36 weeks, on a sample of pH 5·6, was $0{\cdot}52 \times 10^6$/ml. Clearly the lactobacilli were not of great significance in either animal and were not associated with the lower pH in heifer 832.

Total bacterial counts. Total bacterial counts for representative samples from heifers 794, 795 and 832 during restricted feeding are listed in Tables 1–3. There was no obvious correlation between total bacteria and either total ciliate values or total unit values in any of the heifers while the ciliate count remained high, but total bacterial counts usually showed reciprocal changes during large fluctuations in ciliate number.

Rumen fluid volume and outflow. Mean values for rumen volume and fractional clearance rate for each animal over each 5-day period of measurement are given in Table 4. A statistical test on the changes from period 1 to period 3 showed that the difference in change between animal 833, which suffered no alteration in diet, and the other three heifers was just significant for clearance rate ($P = 0{\cdot}05$) but was not significant for rumen volume. The overall mean clearance rate was 11·6%/h for animals receiving the 50:50 diet and 5·9%/h for those receiving the barley diet.

(B) Ad lib. *intake of the barley diet*

Table 5 lists the alterations in rumen fluid components for the heifers which were changed from restricted to *ad lib.* consumption of barley. In heifer 795 this change was made rapidly and the animal increased its intake from 65 to 98% of 'calculated appetite' within 24 h without ill effect. When the same procedure was attempted with heifer 832, 'calculated appetite' was reached within 7 h and the animal became clearly distressed (see Mann, 1970). It was later treated by the addition to the rumen of 10 l. of buffer solution and on two occasions it was given rumen inocula from heifer 794. When the micro-organisms of the rumen of heifer 832 had returned to a restricted feeding pattern the rations of both it and heifer 794 were increased by 900 g/day until they reached *ad lib.* intake.

Ciliate populations. In every heifer there was a dramatic reduction in ciliate population to give the virtually ciliate-free state typical of *ad lib.* feeding of barley (Eadie *et al.* 1967), though in both heifers 832 and 794 a few ciliates (about 20/ml) were seen in samples taken almost 2 weeks after the change. Flagellates were also seen in 794 at this point. Both of these observations followed a day in which food consumption had been low and the rumen pH had risen.

During the gradual increase of ration in heifers 794 and 832 there was a tendency for *Isotricha* to make up a larger proportion of the population. For example, in heifer 794 there was an increase in *Isotricha* from about 6000/ml to 14600/ml during a 24 h period in which the total ciliate population was decreasing.

Rumen pH, VFA and lactic acid. It can be seen from Table 5 that the change to *ad lib.* feeding was associated with a rise in the proportion of propionic acid and a drop in that of butyric acid to give the accepted normal picture for animals fed starchy concentrates *ad lib.* (Eadie *et al.* 1967). At the same time there was a drop in pH and an increase in total VFA. Daily 10·30 h samples taken during the 2 weeks following the change to *ad lib.* feeding showed that the proportion of propionic acid remained well above that of butyric acid except for 2 consecutive days in heifer 832 when the molar proportions of both propionic and butyric acids were above 30%. These were the only occasions on which a high proportion of butyric acid was recorded from a ciliate-free barley-fed animal.

The pH values of the daily 10.30 h samples were generally between 5·0 and 5·5 in all three heifers although values of 6·7 in heifer 794 and 6·0 and 7·0 in heifer 832 were recorded on days following a very low food intake. All values for rumen lactic acid were below 5 mg/100 ml.

Gram film examination. In each heifer, the typical cocco-bacillus picture seen with restricted feeding gave way eventually to a steady state of bacteroides-rod predominance on changing to *ad lib.* intake. In the course of this transition, however, there were temporary but marked increases in the curved Gram-negative rods (organisms A, B and C) previously seen during decreases in ciliate number on the restricted level of intake. These organisms, either singly or in combination, displaced bacteroides rods as the principle bacterial type in each heifer for periods of 1–3 days during the 2 weeks following the change to *ad lib.* feeding. Organism C was predominant in heifer 832 on the 2 days in which high butyric acid levels were recorded.

Bacterial types from culture. The most conspicuous organisms in culture during *ad lib.* feeding were *Bacteroides* and curved Gram-negative rods.

Total viable count. The gradual change to *ad lib.* feeding was associated with a steady increase in total viable count to a value of $19·6 \times 10^9$/ml in heifer 794 and $10·0 \times 10^9$/ml in heifer 832. The lactobacillus count also rose slightly.

Total bacterial count. There was an initial increase in total bacterial count in all three heifers on the introduction of *ad lib.* feeding (see Table 5). This increase, however, was not sustained and total counts during the period of *ad lib.* intake were sometimes lower than the maximum counts observed during restricted feeding. Since viable counts increased steadily during *ad lib.* feeding, viable count as a percentage of total count also increased and on occasions reached exceptionally high values (> 90%). A possible reason for these unusual values is given later (p. 173).

Steers

Feed intake and live-weight gains. When the steers were approximately 45 weeks of age the ration was reduced from *ad lib.* intake of barley to 3·2 kg/day given in three equal feeds. At this time the live weights were 297 and 235 kg for steers 123 and 140

Table 6. *Rumen pH and microbial populations in two steers during* ad lib. *intake of barley cubes and after restriction of intake to amounts below appetite. A rumen inoculum containing mixed ciliate protozoa was given* 6 *weeks after restriction of the ration*

		Steer 123					Steer 140				
				Bacterial counts					Bacterial counts		
Feeding regime	Weeks before (−) or after (+) restriction of intake	Rumen pH	Total ciliates (× 10^{-3}/ml)	Total (× 10^{-9}/ml)	Total viable (× 10^{-9}/ml)	Lacto-bacilli (× 10^{-3}/ml)	Rumen pH	Total ciliates (× 10^{-3}/ml)	Total (× 10^{-9}/ml)	Total viable (× 10^{-9}/ml)	Lacto-bacilli (× 10^{-6}/ml)
Ad lib.	−2	5·3	Nil	27·5	23·1* (84)†	36·6	7·3	Nil	25·1	24·5* (98)†	157
	−1	5·3	Nil	20·3	49·0 (241)	23·5	5·1	Nil	25·9	51·4 (198)	301
Restricted	+4	6·1	Nil	18·1	2·8 (15)	5·5	6·1	Nil	16·9	12·9 (76)	222
Restricted +ciliates	+8‡	6·7	379	6·6	1·3 (20)	< 0·01	7·1	530	1·9	0·02 (1·0)	< 0·01
	+12	7·2	567	4·2	0·04 (1·0)	< 0·01	7·2	1198	3·8	0·07 (1·8)	< 0·01
	+17	7·5	530	1·8	0·03 (1·7)	0·03	7·0	1002	2·7	0·04 (1·5)	< 0·01
	+20	7·1	322	3·6	0·06 (1·7)	—	7·1	198	5·7	0·59 (10·3)	—

* Total viable counts on this sample were made on medium M8; all others on starch medium (see p. 159).
† Values in parentheses indicate the viable count as a percentage of the total count.
‡ Two weeks after ciliate inoculation (cf. Table 7).

Table 7. *Rumen ciliate populations and VFA proportions in two steers given a barley diet in amounts below appetite*

		Ciliates (× 10^{-3}/ml)					Volatile fatty acids (molar %)			
Animal no.	Weeks after inoculum*	Large†	Medium	Small	Total	Total unit‡	Acetic	Propionic	Butyric	'Higher'
123	2	74·2	44·8	260·4	379	873	—	—	—	—
	6	38·2	22·8	506·0	567	819	—	—	—	—
	11	32·6	55·4	442·0	530	815	69·4	10·1	18·9	1·7
	14	19·2	42·4	260·0	322	509	64·6	16·8	16·8	1·8
140	2	54·2	137·6	338·0	530	1110	—	—	—	—
	6	56·8	51·6	1090·0	1198	1607	—	—	—	—
	11	56·8	161·6	784·0	1002	1531	62·7	9·9	27·4	—
	14	95·6	24·4	77·6	198	766	65·8	12·0	22·2	—

* An inoculum of mixed rumen ciliates was given 6 weeks after restriction of intake (see Table 6).
† See p. 172 for species included in each group.
‡ A measure of total ciliate volume, see p. 164.

respectively. A mixed ciliate population was given as an inoculum 6 weeks after the ration had been restricted and 7 weeks later the ration was increased to 4·2 kg/day. Food intake as a percentage of calculated appetite remained between 45 and 65% throughout the period of restricted feeding. Live-weight gains during the 20 weeks of restricted feeding were 61 and 70 kg for steers 123 and 140 respectively.

Bacterial populations. The bacterial populations present in each steer during each stage of the experiment are shown in Table 6. During the *ad lib.* period, rumen samples were thick and difficult to see through microscopically and many free starch grains were present in the rumen fluid. Bacteroides rods and organism C were predominant both in Gram films and in culture. Total bacterial counts and total viable counts were similar to those recorded in the heifers on *ad lib.* intake and, as in the heifers, these gave rise to some impossibly high values for viable count as a percentage of total count. Since these values reached almost 200% in both steers it became evident that the total counts recorded under *ad lib.* conditions were erroneously low (see p. 173).

After the ration was restricted fewer free starch grains were seen in the rumen fluid and there was a marked reduction in total viable count relative to the *ad lib.* period. The Gram film picture showed an increase in coccal forms, a reduction in organism C and the appearance of typical selenomonads. *Bacteroides* spp. and curved Gram-negative rods were the principal types in culture and in both steers organism A made up a large proportion of the curved rods. Typical selenomonads were also obvious in cultures from both animals. Flagellate protozoa were occasionally seen during this ciliate-free period.

When the ciliate population had become established Gram films from the steers showed the same background of negative cocco-bacilli as was seen in the heifers. The slender Gram-variable rod associated with the presence of ciliates was present on every smear but organisms A, B and C were not conspicuous. Examination of the bacteria in culture showed that *Bacteroides* spp. and organism A were the principal types. In addition, typical selenomonads were amongst the predominant organisms in both steers. Total and viable bacterial counts were lower than those recorded in the heifers on restricted barley feeding.

Ciliate populations. The ciliate populations in the steers are given in Tables 6 and 7. The original inoculum included *Epidinium* spp., *Eudiplodinium magii* and *Isotricha* spp. ('large' organisms), *Ostracodinium*, *Eremoplastron* spp. and *Dasytricha ruminantium* ('medium') and *Entodinium* spp. ('small'). All of these species were initially present in both steers after 1 week but *Dasytricha* were few in number and were observed only in samples taken up to 2 weeks after the inoculum. *Isotricha* were present throughout but never made up more than 4% of the large organisms. The large organism *Eudiplodinium maggii* was also present in all samples but was never as numerous as *Epidinium*, the maximum count recorded being 12000/ml. As can be seen from Table 7, there was considerable variation in the proportion of entodinia ('small') so that the total unit digresses considerably from the total ciliate count.

It is clear from Table 7 that the ciliate populations in the steers were lower than those in the heifers. Despite these differences the ciliate populations in the steers would normally be considered large under other dietary regimes.

Rumen pH and VFA proportions. Rumen pH values at each stage of the experiment are given in Table 6 and VFA proportions in the presence of ciliates are given in Table 7. With the exception of one sample in which there had been some salivary dilution, rumen pH was low during the period of *ad lib.* feeding. Restriction of the ration resulted in an increase in rumen pH and a further increase occurred after the establishment of ciliates. The pH values in the steers tended to be higher than those recorded for the heifers but this was probably a reflection of differences in the sampling procedure.

Rumen fluid samples taken 11 and 14 weeks after the original inoculum showed a low proportion of propionic acid and a tendency for the proportion of butyric acid to exceed that of propionic acid (Table 7).

Total bacterial counts and free starch grains. Reference has been made in this work to some unusually high values for viable bacteria as a percentage of total bacteria and it has been suggested that these result from an error in assessing total bacterial counts. A further microscopic examination of preserved samples showed that unexpectedly low total counts were associated with the presence of large numbers of free starch grains in the rumen fluid, and in fresh preparations these grains were seen to be surrounded by an active mass of bacteria. In fixed preparations this mass of bacteria appeared as a 'halo' around each starch grain. These samples also showed the presence of groups of bacteria which appeared to be bound together by a background matrix in much the same way as those forming a halo (see Pl. 1). Additional counts on selected samples showed that the bacteria comprising haloes or groups were not readily separated in formalized samples. One must assume, however, that these bacteria become separated during the preparation of dilutions for culture.

Further experiments are required before a full explanation of the anomalies in total counts can be given but our results suggest that great care must be taken in assessing total counts in samples of rumen fluid containing free dietary starch.

DISCUSSION

Probably the most important finding to emerge from this work is the fact that marked changes in the microbiological and biochemical properties of rumen fluid can be brought about by an alteration in the quantity rather than the quality of an all-concentrate diet. Previous work has shown that diets containing a high proportion of starch usually lead to conditions of low pH, high total VFA concentration and high molar proportions of propionic acid in rumen contents (Balch & Rowland, 1957; Storry & Rook, 1966; Shaw, 1961). On *ad lib.* intake the rumen microbial population is characterized by the absence of ciliate protozoa (Eadie *et al.* 1967). In contrast, the present experiments show that a barley diet given in amounts below appetite favours the development of a large ciliate population and results in an increase in the proportion of butyric acid relative to propionic acid in rumen liquor. Rumen pH and VFA concentrations also differ from those encountered on *ad lib.* intake. In general, the conditions found with restricted intake of the barley diet show features more typical of a roughage diet than of an all-grain diet.

The rumen ciliate populations which developed in the heifers on the restricted barley ration were at times exceptionally large. The highest count recorded was 3.34×10^6 organisms/ml in heifer 794 whereas the highest count quoted by Hungate (1966*a*) in his review of the literature was 3.14×10^6/ml, obtained by Moir & Somers (1957) in sheep given a mixed diet in four equal feeds daily. This latter population, however, was largely composed of the smaller ophryoscolecids of the genus *Entodinium*. In the present work *Entodinium* spp. made up at least 75% of the ciliate populations but exceptionally high counts were recorded also for *Epidinium* spp. and *Eremoplastron* spp.

It should be noted that the ciliate populations observed on the barley diet were achieved in two ways. In the heifers, the populations were established naturally during an initial period of roughage feeding and were then maintained and increased on changing to the restricted barley ration; in the steers, the populations were established by inoculation of animals in which a roughage type of microbial population had never been developed. Thus, contrary to the suggestion of Eadie *et al.* (1967), roughage is not necessary for ciliate development. In this context, it is of interest that the principal ciliate types to develop in the steers were the same as those seen in the heifers despite the fact that the inoculum given to the steers contained a wide range of ciliate species. *Dasytricha*, an organism which does not ingest starch, did not develop in any of the animals and, in agreement with previous findings (Eadie, 1962), organisms of the genus *Isotricha* became more numerous under adverse conditions.

Christiansen, Wood & Burroughs (1964) showed that feeding of certain diets to only 66% of appetite increased the ciliate population and this they attributed to a decrease in the rate of outflow from the rumen. The rate of outflow in our heifers was much lower on the 100% barley ration than on the mixed roughage–barley ration and this could have contributed to the higher ciliate counts. However, this factor alone seems insufficient to account for the total increase in ciliate numbers.

Despite the fact that Gram films showed consistent differences between the restricted and *ad lib.* periods of barley feeding, identification of organisms in culture showed *Bacteroides* spp. to be predominant in both situations. Thus the background of Gram-negative cocco-bacilli generally seen in the presence of ciliates were almost certainly of the same genus as the typical *Bacteroides* rods of *ad lib.* feeding (Eadie *et al.* 1967). Rumen isolations belonging to the genus *Bacteroides* can take on many morphological forms when grown in continuous culture (P. N. Hobson; personal communication). Gram films from animals on restricted barley also showed organisms such as *Oscillospira*, the window-pane sarcina and a slender Gram-variable rod; all of these are commonly seen on roughage diets but have not previously been reported in animals given an all-concentrate diet. Lactobacilli were found only in very limited numbers on restricted feeding but, as shown previously (Eadie *et al.* 1967), they were rather more numerous during *ad lib.* feeding.

Decreases in ciliate number were regularly associated with the appearance in Gram films of certain curved, Gram-negative rods. Since these organisms (A, B and C) also appeared amongst the principal bacterial types in culture from both restricted and *ad lib.* feeding periods they are now being further examined.

The total counts for rumen bacteria on the restricted barley ration were fairly low and similar to counts obtained on roughage-fed animals (Hungate, 1966*b*). The true increase in total bacterial count on changing to *ad lib.* intake is, however, difficult to assess on account of the problem of 'haloes' and linked bacteria. Increases in total bacterial counts in the absence of ciliates have invariably been noted on other rations (Eadie & Hobson, 1962; Kurihara *et al.* 1968; Klopfenstein, Purser & Tyznik, 1966), and the increased viable counts noted in the present work provide confirmation that the bacterial population had indeed risen with each decrease in ciliate numbers.

The total VFA concentrations in the rumen fluid during restricted feeding were generally lower than those recorded on *ad lib.* intake and were not unlike the values quoted by Hungate (1966*c*) for roughage-fed cattle. Similarly, the rumen pH values were higher on restricted intake than on *ad lib.* intake. On the whole, our results confirm the observations of Purser & Moir (1959) and Eadie (1962) that the minimum pH is of particular importance in controlling ciliate numbers. It should be noted, however, that we observed large ciliate populations even when the pH remained below 6·0, and therefore rather lower than the optimum for ciliates in a roughage-fed animal.

In the presence of large ciliate populations there was an increase in the proportion of butyric acid relative to propionic acid in the rumen VFA mixture. The proportion of butyric acid plus higher acids frequently exceeded 25% and the ratio of propionic to butyric acid reached minimum values of 0·37, 0·49 and 0·50 in heifers 794, 795 and 832 respectively. These proportions and ratios of VFA were markedly different from those observed during decreases in ciliate numbers on the restricted level of intake and on changing to *ad lib.* intake. In these circumstances, the proportion of propionic acid and the ratio of propionic to butyric acid both showed a pronounced increase and the values recorded were similar to those encountered on other high-concentrate diets (Balch & Rowland, 1957; Shaw, 1961; Storry & Rook, 1966).

In comparisons of faunated and ciliate-free sheep, Klopfenstein *et al.* (1966) noted an increase in butyric acid relative to propionic acid in the presence of ciliates, but this has not been demonstrated by other workers (Abou Akkada & El Shazly, 1964; Christiansen, Kawashima & Burroughs, 1965). The lowest propionic:butyric ratio reported by Klopfenstein *et al.* (1966) was 0·56. High molar proportions of butyric acid have also occasionally been seen on *ad lib.* barley feeding (F. G. Whitelaw; unpublished observations) but, as in heifer 832 in the present work, these were associated with equally high proportions of propionic acid.

The higher rumen ammonia values noted in the presence of ciliates in the present work are in agreement with previous findings (Abou Akkada & El Shazly, 1964; Klopfenstein *et al.* 1966; Kurihara *et al.* 1968), although a reversal of the general pattern after an overnight, food-free period has not previously been reported.

The relative importance of the bacterial and ciliate populations in producing the alterations in rumen pH and VFA proportions on the restricted level of intake cannot be ascertained directly from these experiments. In the heifers, increases and decreases in ciliate populations tended to be paralleled by changes in the bacterial populations and either or both of these factors could have initiated the changes in rumen conditions. It is of interest, however, that *Entodinium caudatum* has been shown to produce considerably higher molar proportions of butyric acid than of propionic acid when fermenting starch in vitro (Abou Akkada & Howard, 1960). Similarly, *Epidinium ecaudatum* is known to produce mainly acetic and butyric acids, with only traces of propionic acid (Gutierrez & Davis, 1962). Thus, it is at least possible that the alterations in the propionic to butyric ratios noted in the heifers were attributable mainly to the activity of the ciliate populations. Less is known of the bacterial populations but, of the species present, *Bacteroides* spp. are not normally involved in the production of butyric acid (Hungate, 1966*d*).

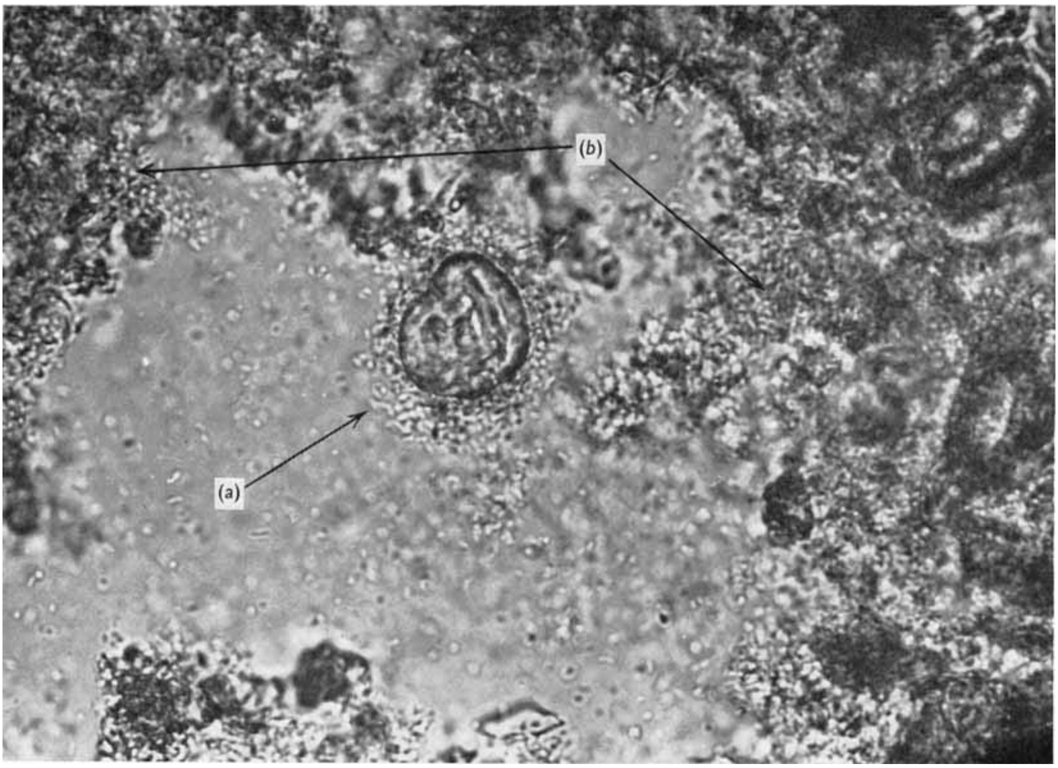
Further evidence of the importance of the ciliate population is contained in the work of Whitelaw, Hyldgaard-Jensen, Reid & Kay (1970) who showed that the rate of VFA production in heifer 832 was considerably lower during a period when ciliates were numerous than during a period in which these organisms were few in number. The presence of ciliates was also associated with substantially lower values of rumen VFA concentration and higher and more stable levels of rumen pH. The authors considered that these effects resulted from the rapid ingestion of starch by the ciliates, which rendered this substrate unavailable to bacterial attack (Whitelaw *et al.* 1970).

The conditions necessary to produce the 'restricted intake' features of the rumen are difficult to define. First, with some variations the amount of barley given has to be 75% or less of *ad lib.* consumption and this should probably be given in at least three equal feeds throughout the day. Secondly, the change from a roughage diet to the barley diet must be made gradually, as in heifers 794 and 795. A more rapid change of diet could lead to prolonged instability of rumen conditions, as in heifer 832, or could result in the immediate development of an *ad lib.* rumen picture as in heifer 833. The advantage of a slow introduction to a high-concentrate diet has previously been mentioned by Hungate, Dougherty, Bryant & Cello (1952) and by Allison, Bucklin & Dougherty (1964).

It should be noted that the establishment of *ad lib.* rumen conditions in heifer 833 on restricted intake and in heifers 794 and 832 during the change to *ad lib.* intake occurred when the quantity of food consumed was well below 75% of calculated appetite. As suggested by Briggs, Hogan & Reid (1957), the rate of change of rumen pH is probably the most important determinant of the final microbial population under these conditions.

Though many questions remain unanswered, clearly a close relationship may exist between diet, method of feeding, rumen microbial populations and fermentation products.

During the course of this work one of us (J. H-J.) was in receipt of a Carlsberg–Wellcome Travelling Research Fellowship. We are most grateful to Miss A. Bostock who carried out the rumen ciliate counts. We thank also Dr R. N. B. Kay for performing the rumen fistulations and Dr P. N. Hobson for the interest he has taken in this work. The valuable technical assistance of Mrs M. Cowie, Mrs M. Kay, Mrs J. Mitchell, Mr W. Shand and Mr F. G. Watson is also gratefully acknowledged.



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EXPLANATION OF PLATE

A 'halo' around a starch granule (*a*) and groups of bound bacteria (*b*) in a formalin-fixed sample of rumen fluid from heifer 794 on *ad lib.* feeding. ×630.

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