Effects of an Abrupt Change in Ration from All Roughage to High Concentrate upon Rumen Microbial Numbers in Sheep

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When three sheep were abruptly changed from a ration of 100% orchardgrass hay to 60% cracked corn-40% orchardgrass hay, fed at equal dry-matter intakes, significant increases in concentration were observed in the rumen microbial population. Bacterial numbers (colony counts) per gram of rumen contents did not appear to have stabilized within 21 days after the ration change; however, protozoan numbers per milliliter plateaued after 5 days. The concentration of cellulose-digesting bacteria varied considerably between animals and decreased in all animals with the change. Changes were observed in total and molar percentages of volatile fatty acids, which were typical for the two types of rations. Although the concentration of protozoa increased after the ration change, only minor differences were observed in their percent generic distribution. A significant decrease in rumen volume was measured in two of the three sheep with the change in ration; however, fluid turnover rates were not significantly affected. Rates of rumen dry-matter turnover were slower with the concentrate ration, although rumen dry-matter digestion was increased. Calculation of total bacterial numbers based on total rumen volume completely negated the effect of ration change in one animal, whereas total numbers in the other two animals were still significantly different between rations and very similar between animals. Adjustment of total protozoa numbers did not alter the trends seen previously with concentration values.

Previous reports have shown that the type and level of ration consumed by an animal affect the numbers and types of bacteria and protozoa in the rumen (1, 4, 15, 17, 22, 23). When the animal is changed from one ration to another, a period of microbial adaptation occurs, which can be defined as that time interval required for the rumen microbial population to stabilize. However, very little information is available on the absolute microbial changes that occur during this adaptation period (9, 18, 23).

Several investigators have estimated the length of this adaptation period by measuring digestibility data as a relative indicator of rumen microbial activity (10, 16, 19). In general, results of these studies have indicated that the length of time required for adaptation depends upon how radical a change is made in the ration.

A recent study conducted in this laboratory with sheep, using daily dry-matter and cellulose digestibility as criteria, investigated the length of the adaptation period when animals were changed from an all-roughage ration to either a corn silage ration, a 60% cracked corn-40% orchardgrass hay ration, or a chopped alfalfa hay ration and back to the roughage ration (20). The most marked changes in daily digestibility coefficients occurred when the sheep were placed on the 60% cracked corn-40% orchardgrass hay ration. However, in almost all cases the digestibility coefficients for all rations showed little if any further change after 5 days.

On the basis of these results, the present study was initiated to investigate the changes in bacterial and protozoan concentrations that occur in the rumen of sheep during this abrupt change from a ration of all orchardgrass hay to one containing 60% cracked corn-40% orchardgrass hay. In addition, concentrations of specific types of rumen bacteria, i.e., cellulose digesters and total volatile fatty acids (VFA), were also measured. Although the sheep were fed at approximately equal dry-matter intakes, the markedly different nature of the rations could affect both rumen volume and rate of passage. Thus, measurement of liquid and dry-matter turnover rates and rumen volume was...
included as a possible help in interpreting any observed microbial changes.

**MATERIALS AND METHODS**

Animals. Three cross-bred wethers, weighing approximately 45 kg, were fed a ration of 800 g of chopped orchardgrass hay per day and then abruptly changed to a ration containing 800 g of 60% cracked corn and 40% chopped orchardgrass hay. The animals were fed once daily at 9:00 a.m. and had free access to water. All three sheep were surgically prepared with rumen fistulae, and composite samples of rumen contents for counting the microbial concentrations were collected from various locations within the rumen just before the daily feeding.

Nine ewes, four fed orchardgrass hay and five fed 60% cracked corn-40% orchardgrass hay, were used to estimate dry-matter turnover. The rations were fed at least 3 weeks before slaughter.

Rumen microbiology: general. The anaerobic culture techniques were similar to those described by Hungate (11). Methods of preparation of media and dilution of rumen ingesta have been described by Dehority (6). Total viable bacterial numbers were determined in roll tubes with 40% rumen fluid-glucose-cellulobiose-starch-agar medium (RGCSA), similar to that described by Bryant and Burkely (3). Total substrate concentration in the RGCSA medium for experiments 1 and 2 was 0.3% carbohydrate, which was divided into 0.125% (wt/vol) each glucose and cellulobiose and 0.05% (wt/vol) soluble starch. For experiment 3, a total substrate concentration of 0.1% carbohydrate was used, 0.025% (wt/vol) each glucose and cellulobiose plus 0.05% (wt/vol) soluble starch. Colonies were counted with a binocular dissecting microscope after 7 days of incubation at 38 C. Bacterial counts were also determined with differential media, in which either 0.3% xylan, 0.3% pectin, 0.3% soluble starch, or 0.75% ball-milled cellulose replaced the glucose, cellulobiose, and starch added to the basal medium. Methods used to solubilize the xylan and pectin were those described by Dehority (6, 7). Total and molar percentages of rumen VFA were determined in all samples from sheep 2 and 3 by gas chromatography (8). Protozoan numbers per milliliter and percent generic distribution were determined according to the procedure of Purser and Moir (21).

Experimental procedures. Before the start of the experimental periods, all sheep were fed the chopped orchardgrass hay for 6 weeks. Sheep 1 was sampled intermittently over a 29-day period from days designated as t<sub>1</sub> to t<sub>29</sub>. From t<sub>1</sub> to t<sub>4</sub>, the sheep was fed chopped orchardgrass hay. After a sample was taken on day t<sub>4</sub>, the animal was changed to the concentrate ration for the remainder of the experiment. With sheep 2, samples were only taken over a 22-day period from t<sub>1</sub> to t<sub>22</sub>. Sheep 3 was sampled over the 28-day period from t<sub>1</sub> to t<sub>28</sub>. Other than the cellulose medium, the only differential counts attempted with sheep 3 were with a 1% soluble starch medium. The starch roll tubes were incubated at 38 C for 24 h, and after the colonies present were counted the rubber stoppers were carefully removed and the tubes were filled with Lugol iodine, which had been diluted 1:8 with distilled water. Within 10 to 15 min the agar medium turned a dark brown to purple color with resultant clear zones appearing where starch colonies had grown. Although considerably more colonies appeared with incubation up to 7 days, after 24 h the clear zones overlapped so much that accurate counts could not be made.

Rumen volume, fluid, and dry-matter turnover rates. Rumen volume and fluid turnover rate were measured by using polyethylene glycol as a marker. Analytical procedures were similar to those of Hyden (14). Preliminary experiments, following a sampling schedule similar to that proposed by Hyden (14), gave abnormally high estimates of rumen volume. Further studies indicated that for animals fed once daily the magnitude of dilution effects upon concentration of a soluble marker was quite large, giving a very steep slope in the first few hours and a corresponding large rumen volume when the line was extrapolated back to zero time. This dilution effect has also been experimentally demonstrated by Warner and Stacy (24). In the present work, polyethylene glycol was added 1 h before feeding, and samples for analysis were taken 1 and 24 h later.

Dry-matter turnover in the ewes was determined at slaughter according to the procedures described by Hungate (12). Acid-detergent lignin (26) was used as a marker to estimate digestibility.

**RESULTS**

Bacterial colony counts. Anaerobic bacterial colony counts per gram of rumen contents for the three sheep during the period when they were abruptly changed from orchardgrass hay to the 60% corn-40% orchardgrass hay ration (day 0) are presented in Fig. 1 and Table 1. Rather marked differences in bacterial concentrations between the three sheep can be seen in Fig. 1. Bacterial concentrations were much lower in sheep 1, and aside from a slight drop on days 1 and 2 after the ration change concentrations gradually increased through day 21. In sheep 2, bacterial concentrations began to increase immediately after the ration change and increased at a much greater rate than for sheep 1. No values were obtained for day 21 in this animal, since it was inadvertently sheared on day 20 and went off feed. Concentrations also began to increase immediately after changing rations for sheep 3; however, a tremendously high peak occurred on day 5, followed by a decrease on days 7 and 14, with an indication of a gradual increase between days 14 and 21. Although the peak observed on day 5 could be the result of a sampling error, concentrations of starch- and cellulose-digesting bacteria (to be presented later) and protozoa all increased in a similar fashion.

In the study reported by Potter and Dehority (20), in which the cellulose and dry-matter di-
Digestibilities of these same rations were estimated over the same period, most of the change occurred between days 1 and 5. Values appeared to be relatively stable from days 6 through 21. On this basis, means of the bacterial concentrations were calculated for those days before the ration change (t<sub>6</sub> to t<sub>9</sub>), the so-called transition period (t<sub>1</sub> to t<sub>3</sub>) and from t<sub>7</sub> on. These data are shown in Table 1, and in all cases concentrations from t<sub>7</sub> on were significantly higher (P < 0.01) than those on the orchardgrass ration (t<sub>6</sub> to t<sub>9</sub>). In sheep 2 and 3, where days t<sub>1</sub> to t<sub>3</sub> were a period of rapid increase, mean values were not different from either of the other two means. In sheep 1, where most of the increase occurred after day 5, the transition period mean did not differ from the value on orchardgrass hay.

Although there were considerable differences between the three sheep, bacterial concentrations increased in all animals after the ration change. Digestibility data (20) had suggested that the transition or change was complete within 5 days; however, this would be in contrast to the present data, where concentrations continued to increase through 21 days and may not even then have reached a plateau.

Selective medium colony counts. In addition to total colony counts for sheep 1 and 2, an attempt was made to estimate the concentrations of bacteria capable of fermenting starch, xylan, pectin, and cellulose. A selective medium was used in which the substrate under study was the only added energy source. With either xylan, pectin, or starch as substrate, 7-day colony counts were made and results were expressed as a percentage of the colony count obtained with RGCSA medium. For the five samples from sheep 1 while on orchardgrass hay, values for the xylan medium ranged from 70 to 113%; for pectin, 70 to 106%; and for starch, 71 to 136%. Similar results were obtained at the other sampling times and also for sheep 2. The probable explanation for this discrepancy is that the 40% rumen fluid basal medium contains enough energy sources to support growth of considerable numbers of colonies (5).

Using the procedure of staining with Lugol iodine, described earlier, the concentration of starch-digesting colonies at 24 h was estimated for sheep 3. The actual number of starch-digesting colonies appearing within 24 h was probably far less than would be obtained at 7 days and represented the faster-growing species. However, the numbers of starch-digesting colonies obtained on days t<sub>5</sub>, t<sub>3</sub>, t<sub>1</sub>, and t<sub>9</sub> were 13, 10, 10, and 18%, respectively, of the colonies observed with RGCSA medium. For days t<sub>1</sub>, t<sub>3</sub>, and t<sub>9</sub>, the percentages rose to 25, 40, and 36%,
respectively. Too many colonies were present to count on days $t_3$, and a value of 47% was obtained for the sample taken on day $t_1$. By $t_1$ and $t_2$, percentages had fallen to 9 and 16%, respectively. These data suggest a temporary marked increase in the starch-digesting species, which grow rapidly enough in artificial media to show colonies in 24 h, during the first week after the change to the high-concentrate ration.

The concentration of cellulose-digesting bacteria can be estimated fairly specifically by counting the clear zones in cellulose-agar roll tubes after incubation for 28 days. Cellulolytic colony counts for the three sheep, over this period of ration change, are shown in Fig. 2. The most obvious difference between animals is that the concentration of cellulolytic bacteria in sheep 3 was about 20 times higher than in sheep 1 and 2. For all animals, decreased concentrations were observed immediately after the change in ration. For sheep 1, colony counts decreased up to 5 days and then increased back to nearly the same level as on the orchardgrass hay ration. In contrast, a marked peak was observed on day 5 in sheep 2 and 3, followed by a decrease to a level lower than the colony counts on all roughage. When mean values on all roughage were compared with mean values for day $t_1$, on the difference was significant only for sheep 3 ($P < 0.01$).

Protozoan concentrations. The concentrations of rumen protozoa determined over the same experimental period are presented in Fig. 3 and Table 2. In general, concentrations were similar in all sheep on the roughage ration, and, as can be noted in Fig. 3, the response to changing from all roughage to concentrate was consistent among the three animals. Concentrations began to increase on day 1 and appeared to reach a plateau about day 5. These data were grouped and the means were calculated in the same manner as for bacterial colony counts (Table 2). For all sheep, a significant increase in protozoan concentration was found between the all-roughage ration and from day 7 on after the change to concentrate. These data agree with the pattern of increased cellulose and dry-matter digestibility reported by Potter and Dehority (20).

With respect to percent generic composition of the protozoa, a significant decrease in Entodinium (94.5 to 88.9%) and increase in Diplodinium (1.6 to 5.7%) ($P < 0.05$) were observed in sheep 1. No changes were found with sheep 2, and the percentage of Entodinium increased significantly ($P < 0.05$) with sheep 3 (92.4 to 95.1%). These data do not suggest any obvious relationships between ration and percent generic composition.

VFA. For sheep 2, a significant increase in

Table 1. Bacterial colony counts per gram of rumen contents during an abrupt change in ration from 100% orchardgrass hay to 60% cracked corn-40% orchardgrass hay

| Day* | Colony count $\times 10^4g$ of rumen contentsb in sheep no.: |
|------|---------------------------------------------------------------|
|      | 1                                                                  | 2                                                                  | 3                                                                  |
| $t_0$ | 32.8 ± 3.7                                                        | 55.0 ± 10.2                                                        | —                                                                  |
| $t_1$ | 42.8 ± 3.4                                                        | 75.4 ± 3.8                                                        | —                                                                  |
| $t_2$ | 33.2 ± 6.0                                                        | —                                                                  | 60.8 ± 1.8                                                        |
| $t_3$ | 45.0 ± 9.3                                                        | 52.4 ± 3.6                                                        | —                                                                  |
| $t_4$ | 37.2 ± 4.7                                                        | 40.6 ± 1.8                                                        | 67.5 ± 7.0                                                        |
| Mean ($t_0-t_4$) | 38.2 ± 2.5c                                                      | 57.1 ± 5.7c                                                      | 74.1 ± 6.8c                                                      |
| $t_5$ | 25.0 ± 4.6                                                        | 70.4 ± 1.8                                                        | 148.2 ± 21.2                                                      |
| $t_6$ | 26.6 ± 6.0                                                        | 94.0 ± 3.0                                                        | 104.5 ± 7.2                                                      |
| $t_7$ | 38.7 ± 2.7                                                        | 127.0 ± 6.4                                                        | 119.5 ± 12.2                                                      |
| $t_8$ | 39.5 ± 5.6                                                        | 178.0 ± 18.0                                                      | 553.0 ± 21.0                                                      |
| Mean ($t_5-t_8$) | 32.4 ± 3.9c                                                      | 117.4 ± 23.3c                                                    | 231.3 ± 107.6c                                                    |
| $t_9$ | 48.5 ± 3.2                                                        | 165.4 ± 22.0                                                      | 310.0 ± 21.2                                                      |
| $t_{10}$ | 55.0 ± 3.4                                                      | 346.4 ± 56.8                                                      | 282.5 ± 22.2                                                      |
| $t_{11}$ | 77.0 ± 9.4                                                      | —                                                                  | 349.5 ± 8.5                                                      |
| Mean ($t_9-t_{11}$) | 60.2 ± 8.6c                                                      | 255.9 ± 90.5c                                                    | 314.0 ± 19.4c                                                    |

* After sampling on day $t_0$, ration was changed from 100% orchardgrass hay to 60% cracked corn-40% orchardgrass hay.

b Mean and standard error of the mean.

c Means in the same column with different superscripts are significantly different at $P < 0.01$. 
total VFA ($P < 0.05$) was observed when the animal was changed to the concentrate ration; mean millimolar values were 158.5 for days $t_{-8}$ to $t_0$ compared with 234.4 for days $t_1$ to $t_{14}$. A significant decrease ($P < 0.05$) in the molar percentage of acetate and an increase in propionate also occurred. Although not significant, the molar percentage of $n$-butyrate showed an increase in the concentrate ration. Changes in total VFA and molar percentages for sheep 3 were almost identical to those for sheep 2; however, in this instance only the decrease in molar percentage of acetate was significant at $P < 0.05$. All other changes were significant at $P < 0.1$. No particular trends in either total VFA or proportions with time after the ration change were apparent. These results agree with the VFA changes reported by Latham et al. (15) when animals are changed to a concentrate ration.

Rumen volume and fluid turnover rate. Using polyethylene glycol as a soluble marker, rumen volume and fluid turnover rates were estimated for the three sheep on both rations (Table 3). These determinations were made after completing the work on microbial changes, and at least 3 weeks were allowed for adjustment of the animal to the ration. A significant decrease ($P < 0.05$) was observed in rumen volume for two of the three sheep; however, rather unexpectedly, fluid turnover rate was not significantly affected by ration. Table 4 presents total bacterial colony and protozoan counts based on rumen volume. Values for the transition period (days $t_1$ to $t_8$) have been omitted, since no estimates of rumen volume were made for that period. These data differ from those in Tables 1 and 2 in two respects: total bacterial numbers in sheep 1 were not significantly affected by the change in ration when rumen volume was considered, and there was a striking similarity between total bacterial and protozoan numbers in sheep 2 and 3.

Possible reasons for the failure of total rumen bacterial numbers to increase in sheep 1 in response to an increased energy intake are not immediately obvious. As can be noted in Fig. 1, bacterial concentrations were still increasing on day 21, and possibly if this animal had been sampled at a later date higher concentrations would have been found. On the other hand, protozoan concentrations did increase, but the increase was less than in the other two sheep, and values were fairly stable from days 7 through 21.

These data suggest that, for studies comparing rumen microbial numbers, colony counts and protozoan numbers based on unit volume
as reported in Tables 1 and 2 can be misleading and more meaningful values can be obtained by using values based on total volume. Also, if marked differences occurred in fluid turnover rates between animals or rations or both, some adjustment of values would be required.

Dry-matter turnover rate. Since both the roughage and concentrate rations were fed at similar dry-matter intakes, it seemed possible that this might explain equal fluid turnover rates. However, because of the readily fermentable nature of the concentrate ration, it would

![Graph](image)

**FIG. 3.** Protozoan concentrations in rumen contents of three sheep during a period of abrupt ration change.

**Table 2.** Protozoan concentrations in the rumen during an abrupt change in ration from 100% orchardgrass hay to 60% cracked corn-40% orchardgrass hay

| Daya | Protozoa × 10^5/cm^3 of rumen contents in sheep no.: |
|------|------------------------------------------------------|
|      | 1          | 2          | 3          |
|      |            |            |            |
| t<sub>-8</sub> | 5.5 | 4.0 | — |
| t<sub>-4</sub> | 5.9 | 4.3 | — |
| t<sub>-2</sub> | — | — | 5.5 |
| t<sub>-2</sub> | 5.7 | — | 4.8 |
| t<sub>-2</sub> | 4.7 | 4.0 | 4.0 |
| t<sub>1</sub> | — | — | — |
| t<sub>1</sub> | 4.5 | 3.5 | 3.5 |
| Mean (t<sub>-8</sub>-t<sub>0</sub>)<sup>b</sup> | 5.3 ± 0.3<sup>c</sup> | 4.0 ± 0.2<sup>c</sup> | 4.4 ± 0.4<sup>c</sup> |
| t<sub>1</sub> | 7.8 | 5.4 | 4.2 |
| t<sub>2</sub> | 7.8 | 8.9 | 7.1 |
| t<sub>3</sub> | 10.5 | 10.2 | 7.2 |
| t<sub>4</sub> | 12.0 | 14.8 | 17.0 |
| Mean (t<sub>1</sub>-t<sub>4</sub>)<sup>d</sup> | 9.5 ± 1.0<sup>d</sup> | 9.8 ± 1.9<sup>c,d</sup> | 8.9 ± 2.8<sup>c,d</sup> |
| t<sub>7</sub> | 10.7 | 12.9 | 16.2 |
| t<sub>14</sub> | 10.0 | 12.2 | 17.2 |
| t<sub>21</sub> | 9.9 | — | 15.6 |
| Mean (t<sub>7</sub>-t<sub>21</sub>)<sup>e,f</sup> | 10.1 ± 0.2<sup>d</sup> | 12.6 ± 0.4<sup>d</sup> | 16.3 ± 0.5<sup>d</sup> |

<sup>a</sup>-<sup>d</sup> See footnotes to Table 1.
TABLE 3. Rumen volume and fluid turnover rates in the same sheep fed either orchardgrass hay or 60% cracked corn-40% orchardgrass hay

| Ration                        | Sheep no. |          |          |          |          |
|-------------------------------|-----------|----------|----------|----------|----------|
|                               | 1         | 2        | 3        |          |          |
|                               | Rumen vol (liters) | Fluid turnover rate/day | Rumen vol (liters) | Fluid turnover rate/day | Rumen vol (liters) | Fluid turnover rate/day |
| Orchardgrass hay              | 6.45 ± 0.47<sup>a</sup> | 1.62 ± 0.08 | 5.96 ± 0.35 | 1.43 ± 0.06 | 5.13 ± 0.17<sup>a</sup> | 1.77 ± 0.10 |
| 60% cracked corn-40% orchardgrass hay | 4.78 ± 0.10<sup>b</sup> | 1.57 ± 0.07 | 5.72 ± 0.25 | 1.18 ± 0.04 | 4.54 ± 0.14<sup>b</sup> | 1.63 ± 0.10 |

<sup>a</sup> For sheep 1 and 3, three determinations were made on orchardgrass hay and four determinations were made on 60% cracked corn-40% orchardgrass. For sheep 2, four and five determinations were made, respectively.

<sup>b</sup> Mean and standard error of the mean.

<sup>c</sup> For each parameter, means within a column followed by different superscripts are significantly different at P < 0.05.

TABLE 4. Comparison of total numbers of bacteria and protozoa in the rumen of sheep when changed from a ration of orchardgrass hay to 60% cracked corn-40% orchardgrass hay

| Ration                        | Sheep no. |          |          |          |          |
|-------------------------------|-----------|----------|----------|----------|----------|
|                               | 1         | 2        | 3        |          |          |
|                               | Total bacteria (× 10<sup>12</sup>) | Total protozoa (× 10<sup>9</sup>) | Total bacteria (× 10<sup>12</sup>) | Total protozoa (× 10<sup>9</sup>) | Total bacteria (× 10<sup>12</sup>) | Total protozoa (× 10<sup>9</sup>) |
| Orchardgrass hay              | 24.6 ± 1.6 | 3.4 ± 0.2<sup>b</sup> | 34.0 ± 3.4<sup>b</sup> | 2.4 ± 0.1<sup>b</sup> | 38.0 ± 3.5<sup>b</sup> | 2.3 ± 0.2<sup>b</sup> |
| 60% cracked corn-40% orchardgrass hay | 28.8 ± 4.1 | 4.9 ± 0.1<sup>c</sup> | 146.4 ± 51.8<sup>d</sup> | 7.2 ± 0.2<sup>c</sup> | 142.5 ± 8.8<sup>d</sup> | 7.4 ± 0.2<sup>c</sup> |

<sup>a</sup> Values are the product of the data in Tables 1 and 2 multiplied by rumen volume, Table 3. Mean and standard error of the mean are presented.

<sup>c</sup> <sup>d</sup> For each parameter, means within a column followed by different superscripts are significantly different at P < 0.01; b, d means are significantly different at P < 0.02.

appear that this ration should have a faster rate of passage. Dry-matter turnover was estimated in four ewes fed orchardgrass hay and five ewes fed 60% cracked corn-40% orchardgrass hay (Table 5). Dry-matter digestibility, as estimated from lignin ratios, was significantly higher (P < 0.01) in the concentrate-fed animals; however, turnover of the dry matter was slower. These data agree with the concept proposed by Hungate (12), that turnover rates based on indigestible feed components will be inversely related to digestibility when feed intake is held constant. Hungate further suggests that with such concentrate rations the fluid turnover rate is probably a better index of rumen function. Although of considerable interest, these values for dry-matter turnover in the rumen do not appear to offer information that can be used to better estimate total daily microbial production.

**DISCUSSION**

The variability between the three sheep used in these experiments agrees with the observations of Warner and Stacy (24) concerning animal differences; however, most of the responses have been noted in various studies by other workers. For example, the increase in bacterial colony counts in sheep 2 and 3 in response to an increased intake of available energy substantiates the results of Bryant and Burkey (4) and Maki and Foster (17). In contrast, Latham et al. (15) obtained results similar to those observed with sheep 1, in which bacterial concentrations did not increase when available energy intake increased. On the other hand, protozoan concentrations increased in all sheep when the available energy intake was raised, agreeing with previously reported results (1, 18, 23).

Warner (23) has reported the changes in ru-
men microbial concentrations of a single sheep when changed from a roughage to a concentrate ration at a fixed level of intake. He observed that about 10 days was required for microbial changes to occur, and concentrations were relatively stable after that time. These findings differ from the present results, in which bacterial concentrations appeared to still be increasing after 14 to 21 days and protozoan concentrations had stabilized after 5 days. When the present observations are considered with respect to the results of Potter and Dehority (20), where digestibility of these two rations had stabilized after 5 days, it would appear that the continuing changes observed in viable bacterial concentrations are not of major significance to overall digestion in the animal.

Maki and Foster (17) have reported that viable counts of bacteria in the rumen contents of cows fed a high-roughage ration represented only 3 to 12% of the number determined by direct count, whereas 57 to 73% of the bacteria from cows fed a ration without roughage (grain plus alfalfa meal) could be cultured. A similar increase in the proportion of viable to direct bacterial count was noted in rumen contents from an all-concentrate-fed animal by Bryant and Burkey (4); however, they also observed that the percentage value of viable counts was similar to that of direct counts, about 8.4%,

when animals were fed all roughage or a mixture of roughage and concentrate. Although direct counts were not made in the present study, the concentrate ration contained 40% orchardgrass hay, and based on the results of Bryant and Burkey (4) it appears probable that viable counts on the two rations represented a similar percentage of the total bacteria. A possible reason proposed for the discrepancy between direct and viable counts is that the direct count measures dead cells and those metabolizing cells which cannot be grown in the artificial medium (12). The differences between roughage and concentrate-fed animals must then be attributed to a lower proportion of these two cell types in the concentrate-fed animal, a shift in the population to more organisms that can be grown, or possibly sequestration of the cells in the fibrous material in hay-fed animals that are not readily disrupted by mixing. One additional point to be considered is that in the studies of Bryant and Burkey (4) and Maki and Foster (17), viable colonies were counted after 3 and 4 days, respectively. More recently, Bryant and Robinson (5) have reported that their 3-day counts averaged only 67% of 7-day counts. Rumen contents for their study were obtained from a cow fed a 79% alfalfa-21% grain ration. A similar increase in colonies has been observed in our laboratory between 5 and 7 days. If those organisms which appear after 3 to 5 days of incubation are specifically associated with roughage digestion, the differences observed between direct and viable counts on roughage- and concentrate-type rations may be less than previously reported.

Adjustment of rumen microbial concentrations for rumen volume in sheep 2 and 3 revealed a striking similarity in the microbial protoplasm supported by a fixed energy intake. In sharp contrast, however, were the low concentrations in sheep 1. After adjustment for rumen volume, total bacterial numbers were the same on the two rations. Obviously other factors must be affecting bacterial concentrations in this animal. The decrease in rumen volume for two of the three sheep, when changed to equal dry-matter intake of the concentrate ration, probably reflects differences in salivary flow and digestibility.

Any attempt to estimate total rumen microbial production per day must take into account the rate of passage of bacteria and protozoa from the rumen. Several investigators have studied this problem; however, because of differences in methods and rations, rather marked differences in results were obtained (13, 25). The primary questions that must be answered

Table 5. Digestion of total dry matter in the rumen and estimation of dry-matter turnover time in sheep fed orchardgrass hay or 60% cracked corn-40% orchardgrass hay

| Determination                      | Ration                          |
|------------------------------------|---------------------------------|
|                                   | Orchardgrass hay*              | 60% cracked corn-40% orchardgrass hay* |
| Intake (g/day)                    | 842 ± 51                        | 881 ± 0.0                               |
| Dry matter in rumen (g)           | 712 ± 31                        | 560 ± 48                                |
| Lignin in feed (%)                | 5.8                             | 2.6                                     |
| Lignin in rumen dry matter (%)    | 12.4 ± 1.0                      | 8.2 ± 0.4                               |
| Digestion of total dry matter in | 52.5 ± 3.5                      | 68.4 ± 1.6†                             |
| the rumen (%)                    | 1.80 ± 0.07*                   | 2.38 ± 0.18†                            |

* Mean and standard error of the mean for four sheep.
† Mean and standard error of the mean for five sheep.

Means within a row followed by different superscripts are significantly different at P < 0.01; c, e means are significantly different at P < 0.05.
are: what is the relationship between fluid flow and microbial passage, and how is this affected by type and amount of ration and frequency of feeding. Since fluid turnover rate did not change between the two rations, we have assumed microbial passage rates to be similar. Further studies may show this assumption to be unwarranted.

Although rumen dry-matter turnover of the roughage and concentrate rations was found to differ, no obvious effect on microbial numbers was evident. A recent report by Akin et al. (2) has confirmed the attachment of rumen bacteria to plant tissue, suggesting that rate of passage for certain species may be influenced by quantity of rumen dry matter. In the present study, rumen dry matter decreased by 21% in animals maintained on the concentrate ration. Concentrations of cellulolytic bacteria were observed to decrease in sheep 1 by 22% and in sheep 2 by 28%, indicating a fairly close relationship between dry matter and cellulolytic bacteria. In contrast, cellulolytic bacterial concentrations in sheep 3 were almost 20 times higher on the roughage ration and decreased by about 77% with the ration change.

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