Kinetics of Microbial Activity in the Bovine Rumen

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Rates of microbial processes in the rumen are rapid, and many can be measured manometrically. Rates during in vitro incubation can be extrapolated to the rumen if experimental procedures are suitably selected. Microbial activity in the intact animal can be estimated from total methane, if the ratio of methane to other products is determined in parallel in vitro experiments. The carbon dioxide from the rumen fermentation can be calculated and the observed R.Q. of the animal corrected by this amount. The volatile acids supplied the host can also be estimated in the intact animal. On the assumption that the rate of fermentation is roughly proportional to the total microbial protoplasm, the rates of fermentation before and after incubation can be used to estimate rumen synthesis. The efficacies of added feed constituents in promoting synthesis can be compared.

THE SIGNIFICANCE TO THE HOST of the microbial fermentation in the rumen can be evaluated by isolating important organisms, studying their activities in pure culture, and inferring their action in the rumen. Many technical operations are required and because the procedures are time-consuming, their use is often impractical.

The rumen organisms may also be studied as a natural mixture, without distinguishing individual types. The methods used are relatively simple and rapid, and in suitably designed experiments can give results directly applicable to the rumen. Mixtures have been employed chiefly in studies of ruminant nutrition. The techniques include in vitro incubation of whole or fractionated rumen contents (*13*, *15*), and manometric experiments with washed cell suspensions (*5*, *11*, *17*) or rumen contents (*14*, *18*). Although relative rates of fermentation with different treatments have been measured, in most investigations the absolute rates of processes have not been obtained. Since the rates in the rumen itself are most pertinent to host nutrition, their measurement merits attention.

Measurement of Rates

Manometric. Two methods have been used to determine rates in the rumen. In the first, weighed rumen contents are incubated for short periods in large manometer vessels under conditions similar to those of the rumen (*9*). Vessels of 160-ml. capacity accommodate the samples, which must be large to reduce sampling error. The high gas pressures necessitate use of mercury in the manometers. The following experiments, measuring the fermentation rates in animals on different rations, illustrate a use of this method.

Ten grams of rumen contents from numbered steers and 30 ml. of inorganic salt solution (*7*) were placed in each

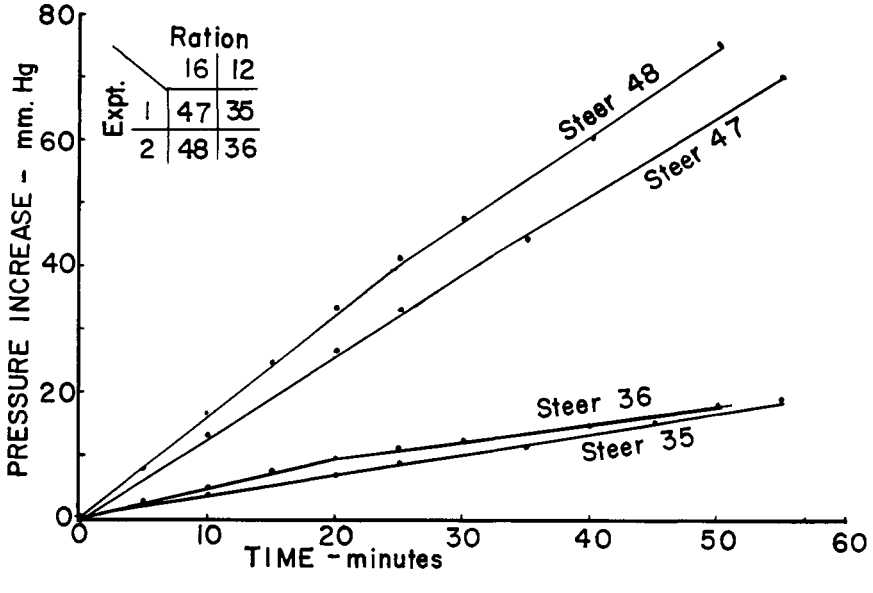

Figure 1. Comparison of fermentation rates in steers receiving two different rations

Table I. Composition of Rations and Average Daily Gain

Ration No.	Composition, %[a] Wheat straw	Fat	Molasses	Barley	Milo	Oats	Beet pulp	Salt	Urea	Average Gain, Lb.
12	82	7	0	4.5	1.5	0.73	1.47	1	1.8	−0.03
16	50	0	10	18	6	4.67	9.32	1	1	+1.06

[a] To improve palatability, 5% of molasses and 114 mg. of Stoc-Joy AM/lb. of ration were added to each ration after pelleting.

vessel. Oxygen was displaced with carbon dioxide which, with sodium bicarbonate, maintained a suitable acidity. Pressure increases due to carbon dioxide and methane production, including liberation of carbon dioxide from bicarbonate by acid, are shown in Figure 1. As the vessels were of approximately the same size, the pressure changes were directly comparable without conversion into amounts of individual products. The proportions of the products in each vessel were assumed to be identical.

From Figure 1 it is evident that the fermentation from ration 16 was significantly greater than from ration 12. Agreement between the two experiments is very close.

The composition of the rations and the average gain in weight of the steers after 183 days (*6*) are shown in Table I. The ration showing the greater fermentation rate gave a significantly greater weight increase.

The manometric method has been used to compare fermentation rates in bloated and normal cattle (*9*), to determine the fate of formate in rumen contents (*4*), and to demonstrate an influence of low levels of antibiotics on the rumen microbiota (*10*).

Chemical Analytical. The second procedure for measuring rates in the rumen is the chemical analytical (*3*). Rumen contents are removed at the start of an experiment and analyzed immediately, and again after various intervals of incubation, or, if substrates were added directly to the rumen, additional samples of rumen contents are periodically removed and analyzed (*8*, *16*, *17*). A curve showing concentration at various times is constructed, and the slope of the curve at zero time—i.e., at the start of the experiment—gives the conversion rate in the rumen itself. Rates of product formation and substrate utilization can be measured.

For estimating rumen fermentation rates, the analytical method is theoretically more accurate than the manometric. In manometry the rumen contents must be transferred to the flasks and the gas and liquid phases equilibrated before the first readings. The first measured rates are not precisely those of the rumen. However, because rates of many processes do not diminish rapidly when rumen contents are removed, the initial manometric rate usually approximates the rumen rate. For comparing rates in two rumens, from which samples were simultaneously removed, the manometric method is very accurate.

The analytical procedure was used (*3*) to establish rates of production of the individual volatile acids in the rumen of hay-fed steers. Acetic, propionic, and butyric acids were produced at average rates of 8.6, 3.0, and 2.6 micromoles per gram-hour, respectively.

Much of the propionic acid in the rumen is believed to arise through decarboxylation of succinate (*12*). Sijpesteijn and Elsden (*17*) found that added succinate disappeared from a sheep rumen at a rate of 6.8 μmoles per gram-hour. In one experiment with a hay-fed steer, the results showed a disappearance rate of 17.2 μmoles of succinate per gram-hour. Both these rates are more than sufficient to account for the propionic acid formation (3.0 μmoles per gram-hour) in the hay-fed animals. This does not prove that succinic decarboxylation is the chief mechanism for formation of propionic acid in the rumen, but the rates are in agreement with this interpretation. The low concentration of succinate in rumen contents, even though succinic acid is an important fermentation product of several types of rumen bacteria, is explained.

Integration of Rates

The rate per unit of rumen contents has been used in the above illustrations. This rate varies, and the total volume of rumen contents varies as ingesta move along the alimentary tract. A means of integrating the various rates and volumes to obtain an estimate of the total fermentation in the intact animal is needed. The methane from the rumen is not further metabolized. It appears in the excreted gases and can be recovered quantitatively with suitable respiration apparatus, without disturbing the animal. Because the rumen is the chief source of methane, the quantity formed represents the integrated product of the variable rates and volumes. It can serve as an index to the total quantities of the other fermentation products, provided the ratios in which they are formed are known and relatively constant. Average ratios can be obtained from several short-time manometric experiments and, on the assumption that the ratios in vitro are the same as in the rumen, the total amount of each product can be calculated from the total methane.

Rumen contents from a steer on a ration with the percentage composition of alfalfa hay 82, fat 7, grain 8, beet pulp 2, and salt 1, were tested manometrically and the amounts of acid, carbon dioxide, and methane measured. The molar ratios of these products were acid 58, methane 16, and carbon dioxide 26. In the previously reported experiments (*9*), using contents from a steer fed on grass hay, the ratios were 38,. 26, and 36, respectively, and for animals on Ladino clover pasture, 35, 15, and 50. The ratios of the products varied with the feed. In the Ladino clover studies, the number of experiments was sufficient to disclose variation even on constant feed, but the average from several independent in vitro measurements showed a standard error of less than 10%.

Armsby and Moulton (*1*) found 142 grams of methane produced daily in a timothy hay–fed steer which digested 3.5 kg. of carbohydrates. The methane-acid ratio obtained with the 82% alfalfa–fed steer would indicate a production of 4.75 pounds of volatile acids per day for this amount of methane. The ratio for the grass hay–fed animal would give a production of 1.92 pounds of volatile acid, and that for the Ladino clover steers 3.1 pounds. These calculated values are not accurate because they are derived from comparisons of different animals on different feeds. But they are reasonable in magnitude and suggest that determinations of product ratios and total methane on the same animal would permit fairly accurate estimates of total volatile acid production.

The production of some methane in the large intestine introduces an error which becomes larger as the ratio of intestinal methane to rumen methane increases. The greater size of the rumen and its higher fermentation rate make the error relatively small.

The total methane and the ratio of methane to carbon dioxide from in vitro fermentations also permit estimates of the total fermentation carbon dioxide. The calculated carbon dioxide from fermentation can be subtracted from the total obtained in respiration experiments, the remainder being the metabolic carbon dioxide from the ruminant itself. With the corrected metabolic carbon dioxide, accurate respiratory quotients can be calculated.

These examples suggest that not only rates of rumen activity but also the

Table II. Estimates of Rumen Synthesis Based on Changes in Fermentation Rates

Expt. No.	Ration	Substrate Added	Length of Incubation, Min.	Pressure Increase during Incubation, Mm./Min.		Rate with Added Sugars, Mm./Min.		Change in Rate	
				Total	Av.	Initial	Final	Mm./min.	%
1	Hay	None	120	19.2	0.16	0.87	0.70	−0.17	−19
2	Hay	1 g. hay	142	95	0.67	0.67	1.13	+0.46	+69
3	Hay and grain	1 g. ration	120	126	1.05	1.10	1.95	+0.85	+77
4	Hay and grain	1 g. ration	245	193	0.79	1.42	2.73	+1.31	+92
5	No. 12 (Table I)	None	40	14	0.35	3.86 (hay)	2.40	−1.46	−38
6	No. 16 (Table I)	None	40	64	1.60	4.80 (hay)	7.00	+2.20	+46

integrated products of the intact animal can be measured.

Rumen Synthesis

Kinetics can be applied to the estimation of rumen synthesis. Let it be assumed that, with substrate in excess, the fermentation rate is roughly proportional to the quantity of active microbial protoplasm or protein. If, at the start of an experiment, excess soluble carbohydrates (100 mg. each of glucose, sucrose, and maltose) are added to rumen contents in one manometric vessel, the rate of pressure increase measures what may be called the potential fermentation rate, proportional to the viable microbial population. A duplicate sample in a second vessel, incubated a short time (1 to 4 hours) and then similarly dosed with excess sugar, gives a potential fermentation rate characteristic of the final population. The difference measures the growth. Results from experiments of this sort are shown in Table II.

In experiments 1 and 5, incubation caused no increase in the potential fermentation. No substrate was added and the low rates during incubation indicate that little food was available. Death exceeded growth and the viable population diminished. In the other experiments the fermentation rate during incubation was appreciably greater. The final potential rate exceeded the initial; protoplasm was synthesized.

In experiments 5 and 6, powdered hay instead of sugar was used when the initial potential fermentation rate was measured. This may have introduced an error. However, since in preliminary experiments with contents from hay-fed steers, added sugars gave almost the same fermentation rate as added hay, the initial rates in experiments 5 and 6 are probably valid. The relative changes in rates are in reasonable accord with the other runs.

The high initial rate in experiments 5 and 6 suggests a greater concentration of viable organisms than in the rumen contents of the other experiments. In partial agreement with this unexpected result is the observation of Bryant and Burkey (*2*) that microscopic and culture counts for straw-fed animals were about as high as for animals fed alfalfa hay or hay plus concentrate.

In spite of the high initial potential for fermentation in experiment 5, the actual rate during incubation was very low, and the final potential rate was less than the initial. The high initial activity, with a substrate supporting so little growth, is unexplained. In experiment 6 the grain in the ration accounts for the active fermentation and growth.

This method of estimating growth is most valid when no microbial deaths occur. If growth and death occur simultaneously, the change in potential fermentation rate will reflect the total increase in viable protoplasm but not the proportion of cells that died. If the dead cells serve as protein for the host, comparisons of metabolic rate will not give a true measure of the microbial contribution. For this reason rate studies for estimating microbial synthesis are probably most useful during intervals when the microorganisms are well nourished.

Although additional evidence is needed to establish whether the kinetic method is accurate for quantitative measurements of growth, these preliminary experiments suggest that it may provide qualitative information difficult to obtain in other ways.

Conclusion

The practical aim of rumen microbiology is to manipulate the factors influencing rumen function in order to obtain faster growth, cheaper feed, and healthier animals. For successful manipulation, a thorough understanding of rumen processes, their interactions, and their relationship to the host, is essential. It is not sufficient to study pure cultures or in vitro mixtures unless the results are pertinent also to the rumen itself. It may be difficult to identify rumen processes and determine their rates and the organisms responsible, but it is not an impossible task, and with the current interest in the rumen, it is a feasible goal. Because thorough understanding often precedes important practical applications, the long-term development of rumen microbiology calls for many well devised and carefully executed basic experiments. Some of these should be designed to explain more completely the kinetics of the rumen fermentation.

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