Methane production and soluble carbohydrates in the rumen of sheep in relation to the time of feeding and the effects of short-term intraruminal infusions of unsaturated fatty acids

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1. The daily methane production of sheep given sugar-beet pulp was greater than that of sheep given hay. The rates of methane production on both diets increased during feeding and then decreased to an apparently steady value. When no food was given the rates of methane production continued to fall exponentially with a half-life of about 24 h.

2. Mixtures of unsaturated long-chain fatty acids infused into the rumen during feeding inhibited the production of methane. The inhibition was greater when the sheep were given hay than when they were given sugar-beet pulp.

3. In general the concentration of soluble carbohydrates in the rumen increased during feeding and fell rapidly to the values found before feeding as soon as the sheep finished eating. The infusion of unsaturated fatty acids during feeding did not result in an accumulation of soluble carbohydrate in the rumen.

4. It is suggested that the rapid methane production during feeding was associated with fermentation of the more soluble part of the diet and that the fermentation of carbohydrate was not inhibited by the infused fatty acids. The results are consistent with specific inhibition of methanogenesis by unsaturated fatty acids.

When long-chain unsaturated fatty acids are added to the diet of sheep there is a significant reduction in the amounts of methane produced (Czerkawski, Blaxter & Wainman, 1966*a*). The reduction is greater when the acids are given with food than when the same amounts are infused continuously into the rumen. Little is known at present about the mechanism of this inhibition, and a plausible explanation seems to be that the metabolic hydrogen in the rumen is diverted from the production of methane to the hydrogenation of unsaturated fatty acids. However, the incorporation of saturated fatty acids into the diet also results in considerable inhibition of methane production (Czerkawski, Blaxter & Wainman 1966*b*), a finding that is inconsistent with this simple explanation.

Although the addition of unsaturated fatty acids to the diet of sheep results in a decrease in methane production, there is no marked decrease in the apparent digestibility of the rations. Therefore, it seems that these compounds may have a specific effect on methanogenic bacteria or on some particular metabolic process of these micro-organisms. The methanogenic bacteria in the rumen can utilize relatively simple substrates, such as formate or $CO_2 + H_2$ (Czerkawski, 1969), but it has never been proved unequivocally that they cannot use more complex substrates for growth.

It has been known for some time that the rates of methane production by sheep are highest during and immediately after feeding and that they fall to lower values 4–5 h after feeding (Pilgrim, 1948; Blaxter, 1966; Graham, 1967). Thus, the maximum rates of methane production occur soon after the soluble components of food are released in the rumen. Therefore, it may be postulated that the high rate of methane production is associated with the fermentation of the soluble carbohydrate of the diet and that the lower steady rates may be associated with the fermentation of the insoluble part including cellulose.

The simple soluble sugars are fermented rapidly in the rumen and it is normally assumed that only negligible proportions of these can leave the rumen and enter the lower parts of the intestinal tract (Ryan, 1964). The plant carbohydrates include, apart from the simple sugars such as sucrose, a number of polysaccharides, some of which are soluble or moderately soluble in water (Waite & Boyd, 1953; Bailey, 1967); these too may contribute to the initial rapid formation of methane.

Initially, the object of this study was to define the changes in the rates of methane production by sheep and in the concentration of soluble carbohydrate in the rumen with respect to time of feeding and type of diet, and to investigate whether there is any correlation between the changes in the concentrations of sugars and the changes in the rates of production of methane. Later a study was made of the effect of a short-term infusion of unsaturated fatty acids upon rates of methanogenesis and concentrations of soluble sugar in the rumen. Unfortunately, it is difficult to take frequent samples of rumen contents in sheep confined to respiration chambers, and the two types of experiments in which methane was measured and in which samples were taken from the rumen were conducted separately. Some of these results have been reported briefly by Clapperton & Czerkawski (1967).

EXPERIMENTAL AND RESULTS

Animals and diets

Five wether sheep, each with a rumen fistula, were used for the experiments in which the rates of methane production were measured in the respiration chambers. An additional four sheep, also provided with rumen fistulas, were kept in metabolism cages. They were all given water *ad lib.*

The sheep were given single meals daily at 09.00 h. Some of them were given chopped hay and others were given molassed sugar-beet pulp moistened with an equal weight of water. As a rule, 1·0 kg of hay was given each day and this was consumed in about 2 h. The ration of sugar-beet pulp was also 1·0 kg/day, but in some instances it had to be reduced to 0·7 or 0·8 kg/day. The sugar-beet rations were usually consumed in 20–40 min.

Measurement of methane production

The sheep, after suitable training periods, were confined to the respiration chambers for at least 4 days and, during that time, the chambers were opened at 08.50 h in order that various manipulations associated with respiration calorimetry could be carried out (Wainman & Blaxter, 1958). The chambers were then closed, the animals were given their food, and samples of the air in the chamber were taken, first at 09.00 h and then every 2 h until 19.00 h. Finally, samples were taken at 23.00 and at 06.00 h the next day. The methane concentration was measured with an Automatic

Analyser (Cambridge Instrument Co. Ltd, Cambridge) and the amount of methane produced was calculated.

In some experiments the frequency of sampling of the air was increased during the first 9 h after feeding (one sample every 1·5 h).

Sampling procedure

Samples were withdrawn from the rumen by means of a probe similar to that described by Czerkawski (1966). It was constructed of stainless steel and could withdraw 6–8 g of rumen contents at one time. At any given time the probe was thrust into the fore, hind and middle parts of the rumen and the three samples were pooled. The pooled samples were then strained through four layers of muslin, subsampled and mixed as soon as possible with suitable reagents.

Infusion of fatty acids

Two types of fatty acid mixtures were used: (1) the technical grade of linoleic acid containing 80% linoleic acid, 13% oleic acid and 7% saturated acids and (2) a mixture of this acid and linseed oil acids containing 60% linolenic acid, 15% linoleic acid, 16% oleic acid and 9% saturated acids (both preparations from British Drug Houses Ltd, Poole, Dorset). In each experiment, the emulsion was prepared by half neutralizing the acids with dilute NaOH solution and homogenizing the mixture. On specified days the emulsions were infused into the rumen while the animals were eating their rations. The volume of emulsion infused was approximately 500 ml.

Chemical analysis

Steam-volatile acids. The concentrations of the steam-volatile acids were determined by the micro-diffusion method of Conway (1962) as described by Czerkawski (1967).

Concentration of carbohydrates. The concentrations of carbohydrates in the rumen were determined by the method of Smith (1956). In this method 1 ml of 5% (w/v) phenol was mixed with 1 ml of test solution. To this mixture 5 ml concentrated H_2SO_4 were added, and after 30 min the optical density was determined at 490 μm. Preliminary experiments showed that glucose and starch gave absorption maxima at 490 μm, pentosan and xylan gave maxima at 483 μm and fructosan at 488 μm. The extinction coefficients were higher with pentosan than with glucose; the extinction coefficient with sucrose was almost the same as with glucose. Several samples of rumen liquor diluted 20 to 100 times gave absorption maxima close to 490 μm. Hence, all the measurements were made at 490 μm and the results were expressed in terms of glucose.

Fractionation of carbohydrate

Some samples of rumen contents from sheep given hay or sugar-beet pulp were fractionated by the method of Bailey (1967). With the hay rations the increase in the concentration of carbohydrate in the rumen was almost wholly confined to the moderately soluble substances, such as certain pentosans, pectin and part of the hemicellulose. These substances were soluble in hot trichloroacetic acid (TCA).

With the sugar-beet pulp, the major increase was in the simple sugar fraction accompanied by small increases in the pentosan and pectin fractions.

In most of the experiments the samples of strained rumen contents were mixed with equal volumes of 10% (w/v) aqueous solution of TCA. They were centrifuged for 10 min at 1000 **g**, the supernatant fluids were decanted and the residues re-extracted with small volumes of 5% TCA. The extracts were pooled and concentrations of carbohydrates determined. The residues were extracted further with 5% TCA for 10 min at 100° and the concentrations of the extracted carbohydrates determined as before.

The samples of food used in these experiments were extracted three times with about ten times their weight of hot water (100°) and the carbohydrate content of the extract was determined as described above, with glucose as standard. The results are given in Table 1.

Table 1. *Gross analysis of constituents in the rations*

(% of air-dry weight)

Ration	Dry matter	Cellulose*	Soluble sugar†
Chopped hay	84·7	30·2	12·5
Sugar-beet pulp	94·5	16·6	26·8

*Determined by the method of Crampton & Maynard (1938).

†Extracted three times for 30 min with water at 100°, and carbohydrate determined by the method of Smith (1956) with glucose as standard.

Methane production

Methane production in relation to the time of feeding. When the sheep were apparently adapted to the rations, that is to say when the daily methane production did not change significantly on 4 consecutive days after 2 weeks on the ration, the mean daily methane production in those given hay was 23·2 ± 0·8 l., whereas in those given sugar-beet pulp it was 33·4 ± 3·9 l. Thus, as expected, the amounts of methane produced when sheep were given sugar-beet pulp were considerably greater than when they were given hay, although the values with sugar-beet pulp showed greater variability.

On both rations the rates of methane production were high during and soon after feeding and they fell rapidly at first and then more slowly to a steady value. In general, these changes in methane production rates can be conveniently described by a purely empirical equation:

$$C = C_0 - \frac{\alpha t}{\beta + t}, \qquad (1)$$

where C is the rate of methane production at time t after feeding, C_0 is the rate at the time of feeding and α and β are constants for any given conditions. The equation can be re-arranged in a linear form in which the plot of $t/(C_0 - C)$ against t gives a straight line with slope $1/\alpha$ and the intercept is β/α; from these the values of α and β can be calculated. By this procedure the values of α and β in these experiments were determined by regression analyses. The constant α represented the difference between the rate of methane production when $t = 0$ (time of feeding) and the approximate rate at the end of the 24 h period. The value of α was characteristic of the diet and in many experiments was about five times larger with sugar-beet pulp than with hay. The other constant, β, had dimensions of time and may be regarded as an inverse measure of the initial fall in the rates of methane production. In other words, when β was small the curves were steep. As a rule, the values of β were about four times greater with hay than with sugar-beet pulp.

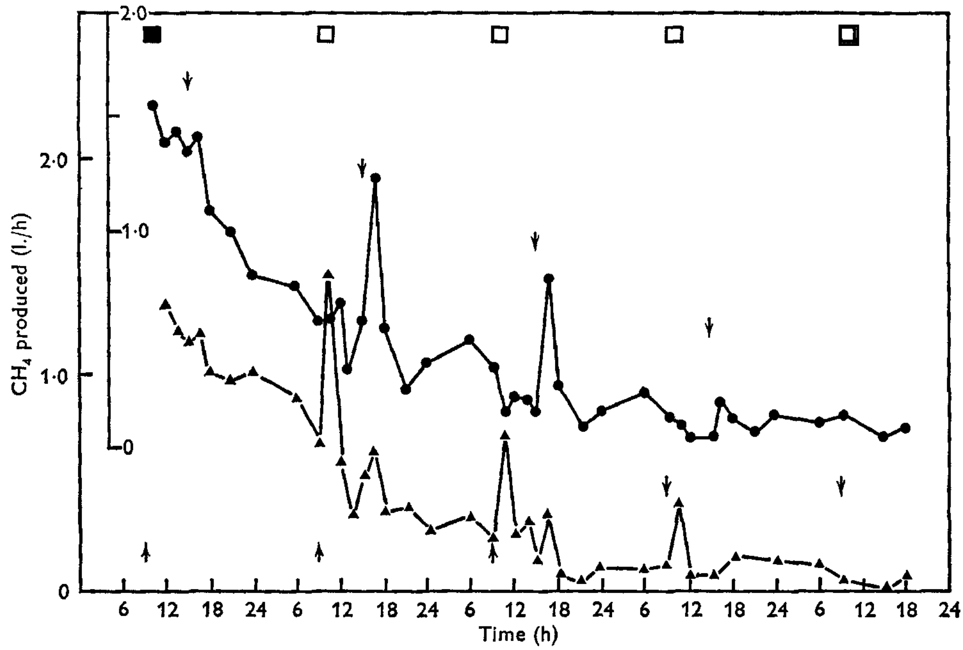

Fig. 1. Variation in the rate of production of methane when, in two experiments (●, ▲), sheep were given 1·0 kg of hay at 09.00 h on the 1st day and when no food was offered on the subsequent 4 days. The arrows indicate the time when the respiration chamber was opened. The open rectangles indicate the normal feeding times and the solid one indicates the time when food was actually given.

Since the rates of methane production were still considerable just before the next feed, it was of interest to observe the effect of starvation on methane production. Two experiments were performed and, in each, the sheep was given 1·0 kg of hay on the 1st day of the experiment, but not on the subsequent 3 days. In one experiment, the chamber was opened at 09.00 h and in the other it was opened every day at 15.00 h, although in both experiments the sheep were initially fed at 09.00 h. The results (Fig. 1) show that, although there was a general decline in the rate of methane production, there was always a peak associated with the opening and closing of the chamber. The second experiment showed that much smaller peaks were associated with the normal feeding time when, in fact, no food was offered. When these peaks were ignored the rate of methane production conformed to an exponential curve with a half-life of 24·5 h.

Effect of short infusion of fatty acids. In one series of experiments two sheep, A and B, were placed in the respiration chambers and given the hay rations at 09.00 h. The chambers were closed and methane production was measured. On the 2nd day an emulsion of a mixture of fatty acids (17 g linseed oil acids + 23 g linoleic acid) was infused into the rumen while the sheep were eating. On the subsequent 2 days the animals were given normal rations but no fatty acids were infused. Methane production was measured as before on these 2 recovery days. In a similar series of experiments, sheep C and D were given 0·7 an 1·0 kg of sugar-beet pulp respectively. The emulsion infused on the 2nd day of this trial contained technical linoleic acid only (40 g).

Table 2. *Methane production (l./24 h) of sheep given 1·0 kg of hay or sugar-beet pulp and the effect of the infusion of unsaturated fatty acids during feeding*

(When hay was given to the sheep 40 g of a mixture of linoleic acid (17 g) and linseed oil acids (23 g) were infused. When sugar-beet pulp was given, 40 g of technical linoleic acid were infused)

Food	Sheep	Expt	Day 1 (control)	Day 2 (infusion)	Day 3 (recovery)	Day 4 (recovery)
Hay	A	1	23·6	14·3	20·3	23·9
		2	25·1	18·6	23·4	24·0
	B	1	22·8	12·7	18·2	21·8
		2	21·1	10·7	20·0	22·7
Sugar-beet pulp	C*	1	23·3	21·7	24·2	25·2
		2	41·9	37·3	40·8	41·7
	D	1	35·7	33·5	36·3	36·1
		2	36·2	33·5	35·7	36·1

*Only 0·7 kg of sugar-beet pulp was given to this sheep.

The results for the total daily methane production in these trials are shown in Table 2. When sheep were given hay the short-term infusion of fatty acids reduced methane production on that day by about 40%, but by the 4th day of the experiment methane production had returned to normal. On the other hand, when sheep were given sugar-beet pulp the infusion of linoleic acid depressed the total methane production by only 8%. The recovery seemed complete on the next day.

The results of these experiments are shown in Fig. 2*a* and *b*. Clearly, when the sheep were given hay the short-term infusion of acids had some effect on the steady rate of methane production but did not alter the value of α which is the difference between the initial and steady value. The initial slope of the curve increased however, that is to say there was a considerable decrease in the value of β, which reverted to a more normal value on days 3 and 4 of the experiment (Table 3). If the initial high rate of methane production is associated with the fermentation of the more soluble carbohydrate of the diet and a large proportion of methane produced at the steady rate is associated with fermentation of the less soluble part, these results seem to imply that methane production associated with soluble carbohydrate is inhibited by fatty acids, whereas that associated with less-soluble carbohydrate is not inhibited.

When the sheep were given sugar-beet pulp containing large amounts of soluble carbohydrate, the curves were steep and invariably gave low terminal rates of methane production (large α, small β, Table 3). The effect of the infusion of fatty acids is not readily apparent in Fig. 2*b* but, as can be seen in Table 3, only the constant β was affected.

The experiments with sugar-beet pulp were extended. On this occasion, sheep D and E were given 0·8 kg of sugar-beet pulp once a day at 09.00 h. The fatty acids were infused during feeding on the 2nd day and, although at least 5 days were allowed to

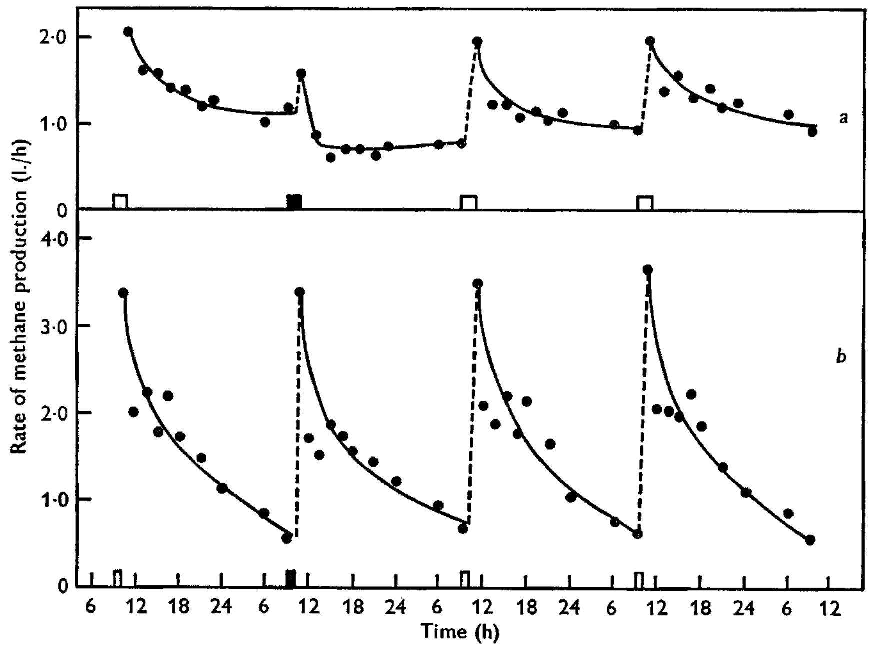

Fig. 2. Effect of infusion of fatty acids during feeding on the rates of methane production in sheep given (*a*) hay or (*b*) sugar-beet pulp. The open rectangles show the feeding times and the solid rectangles indicate the infusion of fatty acid during feeding.

Table 3. *Mean values of α and β, the constants in the equation* $C = C_0 - [\alpha t/(\beta+t)]$ *obtained by regression analysis of results of two experiments each with two sheep given* 1·0 *kg of hay/day and two sheep given* 1·0 *kg of sugar-beet pulp/day*

(*C* is the rate of methane production at time *t* in h, C_0 is the rate when $t = 0$. An infusion of linoleic acid was given into the rumen during feeding on day 2)

Food	Day no.	α(l./h)	β(h)
Hay	1	1·26	6·55
	2	1·24	−0·17
	3	1·07	1·21
	4	1·18	3·39
	Mean	1·19	
Sugar-beet pulp	1	5·44	1·40
	2	5·08	0·68
	3	6·59	0·67
	4	5·77	0·69
	Mean	5·72	—

elapse before the next period, no measurements of methane production were made during the last 3 days. The measurements were confined to the first 2 days, i.e. control day and infusion day. On two occasions with each sheep, an emulsion containing 40 g of linoleic acid was infused as before. On two other occasions a mixture containing 23 g of technical linoleic acid and 17 g of linseed oil fatty acids was infused. This preparation had already given considerable inhibition of methane production when it was infused into the rumen of sheep which had been given hay. The results are summarized in Table 4. The depression in methane production was again small when linoleic acid was infused, but when acids containing large proportions of linolenic acid

Table 4. *Effect of two types of unsaturated fatty acids on the methane production of sheep given 0·8 kg of sugar-beet pulp*

	Methane produced (l./24 h)		Value of the constants			
			Control day		Infusion day	
Type of fatty acids infused	Control day	Infusion day	α	β	α	β
Technical linoleic (40 g)	26·2 ± 0·9	22·4 ± 1·9	3·1	3·9	3·2	2·7
Mixture of linoleic (17 g) and linseed oil acids (23 g)	26·3 ± 2·4	19·8 ± 1·3	3·3	3·9	3·2	1·5

were infused the daily methane production was inhibited by about 25%. Also, the inhibition of methane production was almost wholly confined to the period soon after feeding and was very slight during the remainder of the experimental day. The inhibition of methane production using the mixture of acids was smaller when the animals were given sugar-beet pulp than when they were given hay, although again it was mainly confined to the period of maximum fermentation of the soluble sugar of the diet. This is surprising in view of the fact that sugar-beet pulp contains much larger proportions of soluble sugar.

Changes in the concentrations of soluble carbohydrate and steam-volatile acids in relation to feeding

Exploratory experiments showed that the concentration of soluble carbohydrates in the rumen increased during feeding and tended to decrease rapidly as soon as the animals had finished eating their food. This occurred fairly consistently during any 4–5 consecutive days, but when the experiments were repeated some weeks later the height of the concentration peaks tended to decrease. This progressive decrease in the maxima was apparent with both hay and sugar-beet pulp. When sheep were first given hay rations the maximal concentration of total sugar soluble in hot 5% TCA was about 4 mg/ml but later this decreased to slightly under 2 mg/ml. This progressive decrease in concentration peak in sheep on a constant ration was more spectacular when sheep were given sugar-beet pulp. Some of the representative results are summarized in Table 5, where each value is an average of results obtained on 3 consecutive days.

The results of an experiment in which 1·0 kg of hay was given to sheep are shown in Fig. 3 (days 1 and 2). The concentration of sugar in both the hot and cold TCA extracts increased during feeding, but the increase was not very great in the more soluble, cold TCA fraction. This finding was the reverse of that of the earlier one in this series. When no food was given on days 3 and 4 the concentration of the more soluble sugar fraction decreased steadily. The concentration of the components soluble in hot TCA solution showed an overall decrease but small increases were apparent at the time that the animal should have been fed (cf. Fig. 1). The changes were most interesting when the animal was fed again on day 5. The maximum concentration of

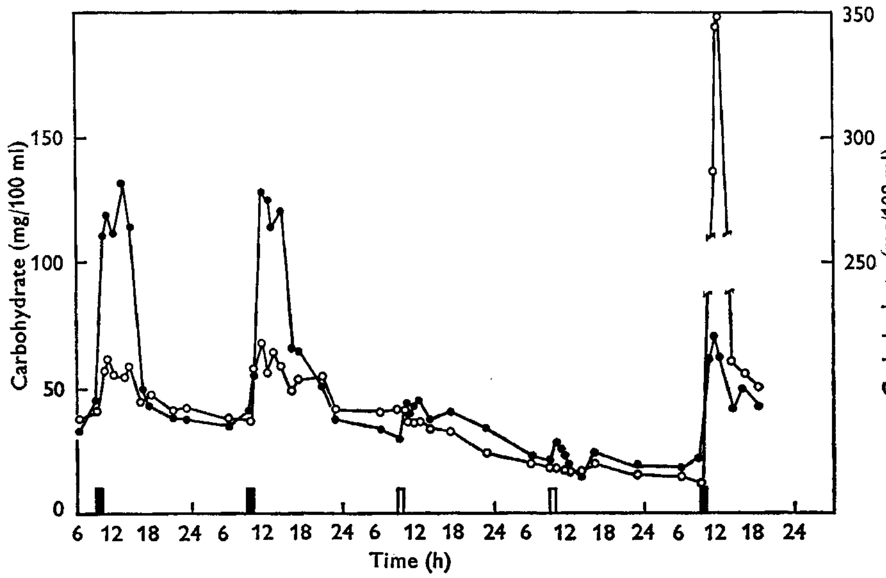

Fig. 3. Changes in the concentrations of carbohydrate fractions in the rumen fluid of sheep given 1·0 kg of hay on 2 days, given no food on the subsequent 2 days and given 1·0 kg of hay on the 5th day. The rectangles indicate normal feeding times (solid when food was given). ○, carbohydrate soluble in cold 5% trichloroacetic acid; ●, carbohydrate soluble in hot 5% trichloroacetic acid.

Table 5. *Long-term changes in maximum and minimum concentrations of soluble sugar in the rumen fluid of two sheep given sugar-beet pulp*

		Concentration of sugar extracted with cold 5% TCA (mg/100 ml)	
Date of last of the 3 days	Sheep	Maximum value soon after feeding	Value before feeding and during most of the day
17.iv.68	F	1210	100
18.iv.68	G	1250	109
1.v.68	G	1050	65
4.v.68	G	860	45
19.v.68	F	607	55
19.vi.68	G	128	61
27.vi.68	G	109	54

the more soluble component increased to almost six times the corresponding pre-starvation value. There was also an increase in the concentration of the fraction soluble in hot TCA but the maximum value attained was always significantly smaller than the value before the period of starvation.

When sheep were given sugar-beet pulp (1·0 kg at 09.00 h) the increase in the concentration of sugar soluble in cold 5% TCA solution was considerable, as shown in Fig. 4, where the results of sampling on 2 consecutive days are given. The concentrations decreased almost to the pre-feed value in less than 3 h after the sheep were given food that was consumed rapidly. The concentration of the less-soluble fraction also rose after feeding, but to a much smaller extent. It decreased subsequently to a level that was higher than just before feeding and that persisted late into the evening. The results of analyses of the concentration of steam-volatile acids are also shown in

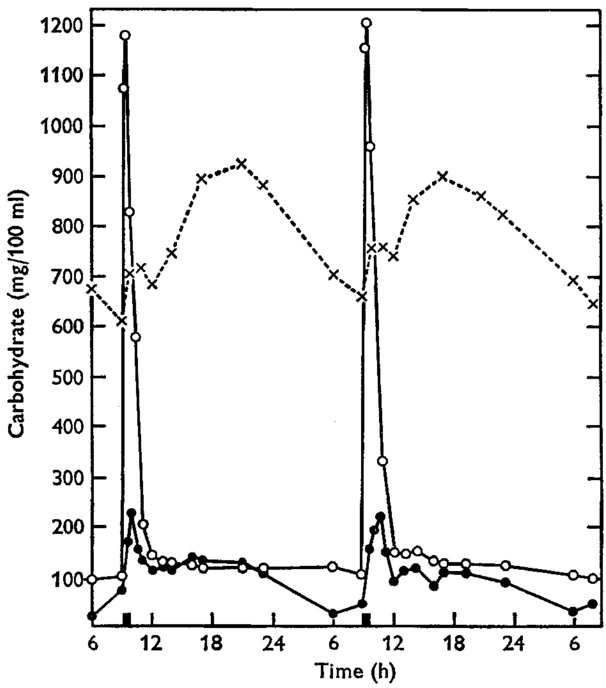

Fig. 4. Changes in the concentrations of carbohydrate fractions and of total steam-volatile acids in the rumen fluid of sheep given sugar-beet pulp on 2 consecutive days. The feeding times are indicated by solid rectangles. ○, carbohydrates soluble in cold 5% trichloroacetic acid; ●, carbohydrates soluble in hot 5% trichloroacetic acid; ×, steam-volatile acids.

Fig. 4. Invariably, the concentration rose to a small peak that occurred at the time of the maximum removal of soluble carbohydrate. This was always followed by a further rise to a broad maximum that occurred in the late afternoon. Presumably the steam-volatile acids in the first peak were derived from fermentation of soluble sugar and those in the main peak were end-products of fermentation of the less-soluble carbohydrates —such as cellulose or hemicellulose (Waite & Wilson 1968). When hay was given to the sheep, analyses of the concentrations of steam-volatile acids gave essentially similar results, but often the small initial peak observed with sugar-beet pulp appeared only as a shoulder on the main curve.

It was not possible to correlate the changes in the rates of methane production with the changes in the concentrations of the carbohydrate or steam-volatile acids in the rumen. In fact, the rates of methane production on the two diets seemed to be associated with the changes in different fractions of the soluble carbohydrate. When hay was given to sheep methane production appeared to be more closely related to the less-soluble fraction (Fig. 3), and when sugar-beet pulp was given the methane production was better correlated with the more soluble fraction (Fig. 4). Nevertheless, the sugar concentration decreased and reached a low value more rapidly than the rate of methanogenesis.

Effect of short infusions of long-chain fatty acids on the concentrations of carbohydrates in the rumen

Each of these experiments lasted 5 days. The sheep received 1·0 kg of hay or sugar-beet pulp at 09.00 h and samples were taken throughout each day as before. Usually, 2 consecutive control days were followed by 1 infusion day and 2 recovery days. The fatty acids were infused while the animals were eating.

It was argued that if the long-chain fatty acids interfered with the fermentation of soluble carbohydrate in the rumen, the sharp concentration peak that was observed soon after feeding should broaden considerably. Eventually, the concentration would be expected to fall more slowly owing to the passage of digesta and the dilution of rumen contents. If there was no fermentation and no dilution, the concentration of carbohydrate in the rumen fluid would be approximately 2500 mg/100 ml in sheep given hay and 5400 mg/100 ml in sheep given sugar-beet pulp (calculated by assuming that the volume of rumen contents was 5 l.). These values are considerably greater than the maximum values measured at any time, even at the beginning of the experiments (400 and 1250 mg/100 ml on hay and sugar-beet pulp respectively). Therefore, if the fatty acids interfered with the fermentation of soluble carbohydrate, not only should the peaks have broadened but they should have become much higher.

Table 6. *Effect of infusion of a mixture of linoleic and linseed oil fatty acids into the rumen of sheep given 1·0 kg hay on the maximum concentration of total soluble carbohydrate extracted with cold and hot 5 % trichloroacetic acid and of total steam-volatile acids (SVA) in rumen fluid*

	Maximum concentration	
Day	Sugar (mg/100 ml)	SVA (m-equiv./100 ml)
1	484	8·2
2 (infusion)	121	8·8
3	211	7·2
4	224	8·0

Hay diet. Mixtures of linseed oil fatty acids (23 parts) and linoleic acid (17 parts) were used in these experiments. On one occasion 32 g of these mixed acids were infused and the results are given in Table 6. There was no rise in the concentration of soluble sugar after feeding and infusion: on the contrary, there was a considerable decrease and partial recovery on subsequent days. There was no significant change in the concentrations of steam-volatile acids. When attempts were made to infuse greater amounts of these acids, (50–60 g), the sheep temporarily refused to eat their rations. This made the interpretation of results difficult and no further attempts were made to infuse large amounts of fatty acids.

Sugar-beet pulp diet. Two levels of linoleic acid emulsion were infused initially when sugar-beet pulp was given to sheep. On one occasion 15·1 g of technical linoleic acid were infused and on another the amount was increased to 29 g. When the smaller amount was infused there was no significant change in the concentration patterns of sugar or steam-volatile acid. When the larger amounts were infused the concentration maximum of carbohydrate in the rumen decreased (Table 7) and there was also a small decrease in the maximum concentration of steam-volatile acids on the day of infusion. These changes were part of a continuing trend.

Table 7. *Effects of infusion of linoleic acid and a mixture of linoleic and linseed oil acids into the rumen of sheep given 1·0 kg of sugar-beet pulp upon the maximum concentration of carbohydrate soluble in cold 5% trichloroacetic acid and of steam-volatile acids (SVA) in rumen fluid*

(The concentrations of SVA are the maxima reached in the latter part of each day. The figures in parentheses are the concentrations at the small peaks that occurred soon after the ingestion of food)

		Maximum concentration	
Type of fatty acids infused	Day no.	Sugar (mg/100 ml)	SVA (m-equiv./100 ml)
Technical linoleic (29 g)	1	1050	8·5 (4·5)
	2	995	8·6 (5·3)
	3 (infusion)	840	7·0 (5·0)
	4	875	7·6 (5·6)
	5	790	8·9 (6·6)
Mixture of linoleic acid and linseed oil acids (29 g)	1	603	8·2 (5·0)
	2	645	8·2 (5·2)
	3 (infusion)	923	7·0 (4·0)
	4	651	7·9 (5·1)
	5	750	7·7 (4·6)

When an emulsion of the mixture of linoleic and linseed oil fatty acids (29 g) was infused, the peak of soluble sugar concentration increased on the day of infusion and became a little broader, but this increase was small compared with the total sugar intake (268 g) (Table 7). Again, when higher levels of these acids were infused (50 g), there was a temporary refusal of food.

DISCUSSION

As a rule the total daily methane production of sheep given sugar-beet pulp was higher than that of sheep given the same amount of hay. Moreover, the rates of methane production soon after feeding were higher and dropped to lower values towards the end of the day when sugar-beet pulp was given instead of hay. This was consistent with the relative content of readily fermented carbohydrate in these two foods and it suggested that the methanogenic bacteria utilized relatively simple products of sugar fermentation. When long-chain fatty acids were infused during feeding, the inhibition of methane production was greatest soon after the sheep had consumed their rations. This was the time when the amounts of the products of fermentation of the soluble carbohydrate would be expected to be maximal. This could be clearly demonstrated when the sheep were given hay when, after the initial period (during which the emulsion was still diffusing throughout the rumen contents and during which there was no inhibition), the rates of methane production fell sharply to the values associated with the fermentation of the less soluble components of the diet.

The increase in the rate of methane production associated with opening and closing the chamber was surprising and difficult to explain. An experimental artifact would be expected to cause a reduction in the rate of methane production. It must be concluded tentatively that the peak was due to a response of the animal to a sudden environmental change associated with opening and closing the chamber.

When sugar-beet pulp was given to sheep the soluble carbohydrates were apparently metabolized very rapidly, as judged by the rapid decrease in their concentration in the rumen and by the fact that the maximum concentrations never reached the calculated potential values. The high rate of methane production was consistent with this, but it is difficult to explain the low inhibition of methanogenesis with the acid mixtures rich in linoleic and linolenic acids when sugar-beet pulp was given to sheep. If fatty acids inhibit the methane production associated with fermentation of soluble carbohydrate, as has been demonstrated with hay rations, the inhibition of methane production with sugar-beet pulp should have been greater both proportionally and absolutely than with the hay diet. It is possible that the sugar-beet pulp offers a large area for absorption of fatty acids and thus exerts a certain measure of protection.

Although methane production was inhibited soon after feeding, there was little or no evidence of inhibition of the fermentation of the soluble or the insoluble part of carbohydrate of the ration, as judged by no accumulation of such carbohydrate following infusion of fatty acids. This agrees with the previous finding in vivo (Czerkawski *et al.* 1966*b*) where it was shown that the inhibition of methane production by unsaturated fatty acids was not accompanied by any marked reduction in the apparent digestibility of the basal ration. Recent work in vitro (Czerkawski & Breckenridge, 1969) also indicated that under specified conditions unsaturated fatty acids inhibited methane production when sugar-beet pulp or sucrose was fermented. Therefore, it must be concluded that the fatty acids act at some later stage of fermentation or act specifically on the methane bacteria.

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