The metabolism of formic acid in sheep

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Small amounts of formic acid can be detected in the steam-volatile fatty acids of rumen liquor, but the work of Annison (1954) shows that the formate in the peripheral circulation of ruminants is probably not derived from this source. Blood concentrations of formate, unlike those of acetate, do not rise during feeding, and fall but slightly during a 48 h fast. In this regard Annison & Pennington (1954) showed that formate was not metabolized by rumen epithelium. Experiments in vitro, notably those of Lewis (1951), Beijer (1952), Doetsch, Robinson, Brown & Shaw (1953) and Carroll & Hungate (1955) have shown that formate added to rumen liquor is dissimilated to give carbon dioxide and methane. Short-term experiments with goats with rumen fistulas by Matsumoto (1961) showed that the rate of disappearance of formate was constant, was unaffected by formate concentration and was associated with an increase in the concentration of CH_4 and a decrease in the concentration of CO_2 in the gas phase of the rumen. Such changes, however, do not permit establishment of the relative amounts of CH_4 and CO_2 produced, because CO_2 diffuses from the rumen more rapidly than does CH_4.

Reid (1962) and Bensadoun, Paladines & Reid (1962), however, concluded, on the basis of differences in the concentration of formic acid as between rumen liquor and portal blood, that formic acid may constitute a sizeable proportion of the total steam-volatile acid absorbed. Reid (1962) also reported, but not in detail, that when, in four energy balance trials, formic acid was infused into the rumens of cattle receiving a basal diet of hay, heat production was reduced by an amount equivalent to 92% of the heat of combustion of the formic acid given, and that the formic acid increased the efficiency of energy utilization of the whole diet by 18%.

These conclusions by Reid that considerable amounts of formic acid are absorbed from the rumen do not agree with those of Annison (1954), nor is an effect of formic acid on metabolism consonant with what is known of its rapid breakdown by rumen micro-organisms in vitro. The experiments now presented were undertaken to measure quantitatively the effect of formic acid on the metabolism of the sheep.

EXPERIMENTAL

Preliminary experiments were made to find the amounts of formic acid which could be used continuously without depressing the pH of the rumen by more than 0·5 unit. The maximum amount was 1·5 moles/day which supplied 98 kcal/day. The

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basal diets supplied about 2000 kcal metabolizable energy, so that formic acid could be given to supply about 5% of the total metabolizable energy.

Animals and diets. Five castrated male sheep were used. Four of these sheep (C, R, B and F) had permanent rumen fistulas and one, sheep E, was intact. They were given diets of dried grass containing from 13·4 to 18·8% crude protein on a dry-matter basis.

Five experiments, two with sheep B and one with each of sheep C, R, and B, were made in which formic acid was given by ruminal infusion and the respiratory exchange was measured. In the experiments with sheep C, R and B, after an initial period of 1–2 weeks during which metabolism was measured, increasing amounts of formic acid were infused over a period of 3–5 days until 1·5 moles/day were being infused at constant rates. The total period of infusion lasted 12–17 days, and metabolism was measured continuously during that time. Formic acid infusion was then stopped and measurements continued for 9–14 days. With sheep B, after an initial period of 7 days, formic acid was infused at a rate of 1·0 mole/day for 12 days. The infusion was then stopped for 16 days, measurements being made on the last 9 days. The infusion of formic acid at the rate of 1·0 mole/day was then repeated and continued for 11 days, measurements being made on the last 8 days. The total volume infused was 4 l./day.

Three experiments similar to those above were made with sheep C, B and E kept in respiration chambers in which 0·5 mole sodium formate in 2·0 l. sterile water was infused intravenously through an indwelling catheter.

Two experiments were made in which sheep B and F with rumen fistulas were given constant food for 6 weeks. In the first 2 and last 2 weeks 4 l. saline were infused into the rumen each day. In the 3rd and 4th weeks 1·5 moles formic acid and 4 l. water were infused each day. Faeces were collected over the last 8 days of each 2-week period.

In four short-term experiments with two sheep (B and F) with rumen fistulas, 0·75–2·0 moles sodium formate were given by rapid (30–40 min) infusion into the rumen. The gaseous exchange was measured at 2 h intervals after the infusion began and continued for 8 h. Four control experiments were made with these sheep in which no formate was given.

Analytical and calorimetric methods. The respiration chambers were those of Wainman & Blaxter (1958) and chemical methods those of Graham, Blaxter & Armstrong (1958). Steam-volatile fatty acids were determined by steam distillation by the method of Annison (1954) and separated by gas chromatography (James & Martin, 1952).

<div align="center">RESULTS</div>

Effect of infusion of formic acid or of sodium formate on the apparent digestibility of the basal diet. Table 1 summarizes determinations of the apparent digestibility of the diet before, during and after formic acid infusions. None of the infusions resulted in any significant depression of the digestibility of the energy of the basal diet. The apparent digestibility of the N of the diet was also not significantly affected.

CH₄ production. The records of CH_4 production showed that the day-to-day variation under constant conditions varied slightly from experiment to experiment between the range $\pm 0.6 - \pm 0.7$ l./day (standard deviation). This corresponds to a coefficient of variation of $\pm 1.8 - \pm 2.1 \%$. Fig. 1 shows the results for sheep R and B when 1.5 moles formic acid were infused daily into the rumen, and Table 2 gives the mean results of all experiments in which formic acid was infused into the rumen. The results apply to the last 4 or 5 days of the infusion period. When formic acid was infused into the rumen, CH_4 production increased. The mean increase in these experiments,

Table 1. *Mean apparent digestibility (%) of dietary energy when 1.5 moles formic acid were infused into the rumens or when 0.5 mole sodium formate was infused into the jugular veins of sheep*

Infusion	No. of experiments and code letters of sheep	Mean apparent digestibility of dietary energy (%)			
		Before infusion	During infusion	After infusion	SE of mean
1.5 moles formic acid (rumen)	5 (C, R, B, E and F)	69.4	67.9	68.8	±0.7
1.0 moles formic acid (rumen)	2 (B)	68.6	68.3	67.5	±1.3
0.5 mole sodium formate (vein)	3 (C, B, E)	72.9	73.5	72.9	±1.0

Table 2. *Effect of infusing 1.5 moles or 1.0 mole formic acid into the rumen or 0.5 mole sodium formate into the jugular vein on CH_4 production by sheep*

Sheep	Amount of formic acid or sodium formate infused (moles/24 h)	CH_4 production (l./24 h)				
		Before infusion	During infusion	After infusion	Increase during infusion	SE of increase
C	1.5 (rumen)	28.3	37.3	27.2	9.36	±0.35
R	1.5 (rumen)	32.8	42.1	33.2	9.10	±0.39
B	1.5 (rumen)	31.1	37.1	30.8	6.17	±0.35
Mean		30.7	38.8	30.4	8.21	±1.02*
B	1.0 (rumen)	—	34.0	30.2	3.74	±0.37
B	1.0 (rumen)	30.2	34.5	—	4.30	±0.43
C	0.5 (intravenous	28.0	28.8	28.0	0.82	±0.51
B	0.5 (intravenous)	34.0	35.2	34.1	1.16	±0.51
E	0.5 (intravenous)	29.1†	32.4	33.1	−0.70	±0.69
Mean (intravenous)		31.7	32.1	31.7	0.43	±0.57*

* Between-experiment standard error; others are within-experiment standard errors.
† This value was not used in the analysis because it was thought that the sheep was not fully accustomed to the respiration apparatus during the initial period.

which was 5.1 l. CH_4/mole formic acid, was highly significant statistically. Sheep C and R produced 6.2 and 6.1 l. CH_4/mole, but sheep B produced about 4.0 l. CH_4/mole formic acid in each experiment. When the result of all the experiments in which formic acid was infused into the rumen were calculated as CH_4 production per mole formic acid given, CH_4 production was found to have increased by 4.9 ± 0.52 l./mole formic acid.

When sodium formate was given by intravenous infusion, no significant change

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occurred in CH_4 production (Table 2). This is evident in Fig. 2 which shows the observations made with sheep B.

CO_2 production and O_2 consumption. Fig. 1 also shows the CO_2 and O_2 consumption of sheep R and B when given 1·5 moles formic acid by infusion into the rumen each
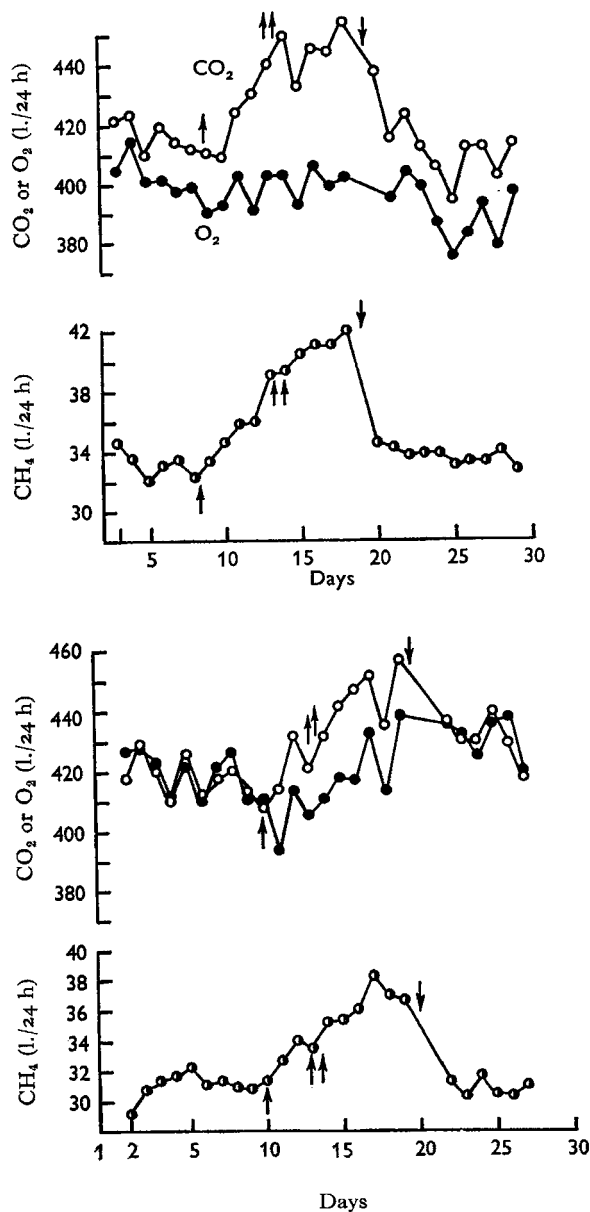

Fig. 1. O_2 consumption, CO_2 production and CH_4 production of sheep R (upper) and of sheep B (lower) when 1·5 moles formic acid/24 h were infused into the rumen: ●—●, O_2 consumption; ○—○, CO_2 production; ◑—◑, CH_4 production. The single arrow ↑ indicates commencement of infusion at the rate of 0·5 mole/24 h, the full amount of 1·5 moles/24 h being achieved at ↑↑. The infusions were stopped at ↓.

day. An increase in CO_2 production occurred with both sheep. With sheep B, O_2 consumption rose during the experimental period and remained elevated during the final period. With sheep R a slight increase occurred throughout the experiment. Fig. 2 shows the effect of intravenous infusion of o·5 mole formate/day on the gaseous exchange of sheep B. No effect on O_2 consumption or on CO_2 production was discerned. The responses to infusion in all the experiments were computed as the difference between the mean values during the last 4–5 days of infusion and the mean values obtained in the last 4–5 days of the two control periods, and these values are given in Table 3.
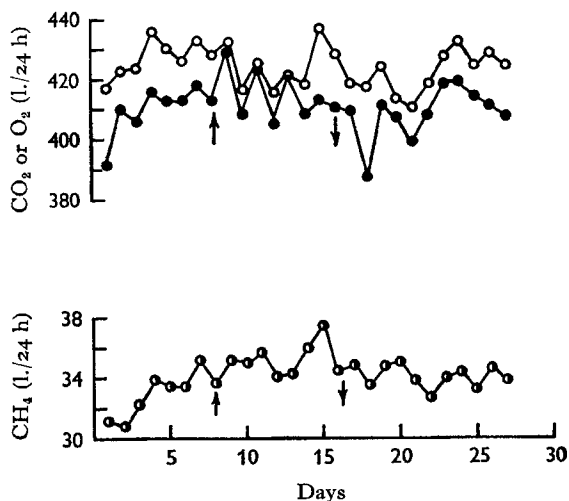

Fig. 2. O_2 consumption, CO_2 production and CH_4 production of sheep B when o·5 mole sodium formate/24 h was infused into the jugular vein: ●—●, O_2 consumption; o—o, CO_2 production; ◑—◑, CH_4 production. The arrows indicate the beginning and end of the infusion.

Steam-volatile acids in blood and urine. No increase in the concentration of steam-volatile acids in peripheral blood was noted in any experiment in which formic acid was infused into the rumen. No increases in the amount of steam-volatile acids in urine were detected in these experiments.

When sheep B was given o·5 mole sodium formate/day by intravenous infusion, the total steam-volatile acids in the urine increased from 37 to 95 m-equiv./day, and when sheep C was given the same amount of formate the excretion of steam-volatile acids rose from 44 to 55 m-equiv./day. Determinations were not made with sheep E. From its behaviour on chromatography, the additional steam-volatile acid was probably formic acid but an absolute identification was not made.

Short-term experiments. When sodium formate was rapidly infused into the rumen of sheep, immediate increases in CH_4 production occurred. With the exception of sheep F given 2·0 moles sodium formate, by 4–6 h CH_4 production had fallen to that noted in control periods. The results given in Table 4 show the increases in CH_4 production that occurred during 8 h. According to Carroll & Hungate (1955) bacterial dissimilation of formic acid results in the formation of 5·6 l. CH_4 and 16·8 l.

CO_2/mole formic acid with no consumption of oxygen. The results in Table 4 show that CH_4 production was 90, 73, 75 and 59% of the theoretical when 0·75, 1·0, 1·5 and 2·0 moles sodium formate were infused. In contrast to the results of the long-term infusions, in only one instance was CO_2 production elevated during the 8 h of infusion. In this sheep the increase was small, however, and the CO_2 production over the whole 24 h was depressed. O_2 consumption was depressed in each experiment. These results suggest that rapid infusion of formate depressed the total metabolism of the sheep, probably owing to an effect on the rumen flora rather than on the animal itself.

Table 3. *Effect of infusion of 1·5 moles or 1·0 mole formic acid into the rumen and mean effect of infusing 0·5 mole sodium formate into the jugular vein on O_2 consumption and CO_2 production of sheep*

Sheep	Amount of formic acid or formate infused (moles/24 h)	O_2 consumption (l./24 h)				CO_2 production (l./24 h)			
		Before infusion	During infusion	After infusion	Increase during infusion	Before infusion	During infusion	After infusion	Increase during infusion
C	1·5 (rumen)	386·9	408·8	384·8	+23·0	390·9	435·5	399·4	40·3
R	1·5 (rumen)	399·4	400·1	387·1	+6·8	413·4	443·6	407·1	33·4
B	1·5 (rumen)	416·1	424·2	430·2	+1·0	414·3	446·8	429·5	24·9
B	1·0 (rumen)	—	389·3	389·1	+0·1	—	413·8	401·5	12·3
B	1·0 (rumen)	389·1	391·5	—	+2·4	401·5	414·0	—	12·5
Mean of three experiments in which 0·5 mole sodium formate was infused into the jugular vein	414·7	414·7	413·6	+0·54±6·5	420·7	419·9	419·6	−0·3±5·2

Table 4. *Changes in 8 h of the gaseous exchange of sheep given 0·75, 1·0, 1·5 or 2·0 moles sodium formate as an intraruminal infusion during 30 min periods*
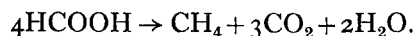
Sheep	Amount of sodium formate infused (moles)	Increase or decrease in 8 h		
		CH_4 production (l.)	CO_2 production (l.)	O_2 consumption (l.)
B	0·75	3·8	−4·6	−14·9
B	1·50	6·1	−14·6	−22·4
F	1·00	4·2	+2·9	−1·3
F	2·00	6·6	−11·6	−9·5

DISCUSSION

The studies of Carroll & Hungate (1955) showed that the dissimilation of methane by methanogenic bacteria of the rumen takes place in two stages:

$$HCOOH \rightarrow CO_2 + H_2, \tag{1}$$

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O, \tag{2}$$

so that $4HCOOH \rightarrow CH_4 + 3CO_2 + 2H_2O.$

Reaction 2 was shown to be considerably faster than reaction 1, which largely accounts for the fact that free hydrogen is very rarely detected in the rumen. It would be expected, therefore, that bacterial dissimilation of 1 mole of formic acid would result

in the formation of 0·25 mole methane and 0·75 mole CO_2; that is, 5·6 l. methane and 16·8 l. CO_2/mole formic acid. If formic acid were completely oxidized to CO_2 and water in the body, 0·5 mole O_2 would be consumed and 1·0 mole CO_2 produced/ mole formic acid.

When small amounts of formic acid were given by intravenous infusion, no significant changes in the respiratory exchange were noted, but a small increase in urinary excretion of a steam-volatile acid occurred. These results indicate that formic acid if absorbed in small amounts is unlikely to cause large changes in metabolism.

The mean values obtained in the long-term experiments in which formic acid was infused into the rumen at constant rates for several days were $4·9 \pm 0·5$ l. CH_4 and $18·1 \pm 2·8$ l. CO_2/mole formic acid given. These mean values do not differ significantly from the values expected from a bacterial dissimilation of formic acid according to the above equations. O_2 consumption, however, rose by a mean of $4·6 \pm 2·8$ l./mole formic acid given, whereas bacterial dissimilation of formic acid involves no consumption of O_2. This mean increase was not statistically significant because variation from animal to animal was large. It is evident from the results for individual sheep that, when an increase in O_2 consumption was noted, CO_2 production was greater than that to be expected from the increase in CH_4 production on the basis that three times as much CO_2 as CH_4 is produced when formic acid is broken down by rumen bacteria. This suggests that the individual high values arose from period-to-period variations in metabolism, unconnected with formic acid infusion. These variations, which reached a maximum of 15 l. O_2, represent maximal variations in metabolism of only 3·5 %.

The depression of metabolism noted when sodium formate was introduced into the rumen in single large doses contrasts markedly with the results found in the long-term experiments. It seems probable, but details are not available, that Reid's (1962) results, suggesting that formic acid improves energy utilization by cattle, were based on the rapid infusion of formate. In the long-term studies with continuous infusion of formic acid, the results above show that, in the living animal, formic acid is quantitatively converted into CO_2 and CH_4 in agreement with studies with rumen microorganisms. This necessarily means that very little is absorbed.

SUMMARY

1. In five experiments with sheep receiving diets of dried grass, formic acid was infused into the rumen at a constant rate for periods of up to 17 days. Methane production increased by $4·9 \pm 0·5$ l./mole and carbon dioxide production by $18·1 \pm 2·8$ l./mole. There was no significant change in oxygen consumption.

2. Intravenous infusion of 0·5 mole sodium formate did not significantly affect CH_4 production, CO_2 production or O_2 consumption but an increase occurred in the urinary excretion of steam-volatile fatty acids.

3. Rapid infusion into the rumen of up to 2·0 moles of sodium formate resulted in relatively smaller increases in CH_4 production with depressions of O_2 consumption and in most experiments with depressions of CO_2 production.

4. It is concluded that formic acid when supplied to the animal at slow constant rates is rapidly dissimilated by rumen bacteria to CH_4 and CO_2 in amounts which agree with those found in in vitro studies with methanogenic organisms. Rapid infusion, however, depresses the total metabolism, probably by interference with micro-organisms other than those concerned with methanogenesis.

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