

Effect of glucose on fermentation heat in sheep rumen fluid in vitro

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(Received 8 January 1986 - Accepted 14 March 1986)

1. Heat production rate (\dot{H}) of rumen fluid was measured in a direct calorimeter. Basal \dot{H} of samples of 15 ml rumen fluid mixed with 45 ml buffer was 0.4 mW/ml rumen fluid.
2. Addition of glucose (0.4-6.4 mg/sample) was followed by a dose-dependent increase in \dot{H} . Maximal \dot{H} was 1.1 mW/ml and lasted up to 5 min, returning thereafter to the basal level.
3. Expression of fermentation heat (H_f ; kJ/mol substrate added) against glucose dose indicated an asymptotic dose response.
4. Maximal H_f (at infinite dilution) agreed with stoichiometric calculations whereas minimal H_f suggested a partial fermentation of the substrate at a high-glucose dose in the rumen environment.

Carbohydrates are fermented within the anaerobic environment of the rumen, giving rise mainly to volatile fatty acids (VFA), methane, CO₂, water, heat and microbial matter. According to stoichiometric calculations the fermentation heat (H_f) is 180 kJ/mol when hexoses are fermented in the rumen. This value is almost constant, regardless of the proportions of the end-products (Hungate, 1966). However, experimental determinations of H_f vary from 140 kJ/mol (Hershberger & Hartstook, 1970) to 360 kJ/mol (Menke & Ehresvard, 1974).

The large variation in experimental results may be related to the determination procedure. H_f was measured indirectly by difference from carbon balance of in vitro fermentation (Marston, 1948; Menke & Ehresvard, 1974), or estimated from the difference between total visceral metabolism and the aerobic metabolism of the tissues of the gut wall (Webster *et al.* 1975). Some of the direct determinations of H_f in rumen contents are within the range of stoichiometrically calculated values (Haupt, 1968; Hershberger & Hartstook, 1970). In these latter studies H_f was measured over relatively long periods of several hours over which microbial mass could vary and thereby affect H_f .

Information on H_f of rumen micro-organisms over short periods is not available. Discrepancies between rumen H_f values from expected stoichiometric calculations may also be explained by the direct incorporation of substrates into cell microbial matter (Forrest, 1969; Seeley *et al.* 1969), which is influenced by energy availability (Bauchop & Elsdon, 1960). Hence, it might be assumed that with low inputs of substrate the proportion of substrate energy converted to heat is relatively high, whereas these proportions decrease when substrate is rechannelled from fermentation to polysaccharide formation. Such a hypothesis was examined in the present study by assessing the effect of glucose dose on rumen H_f over short periods.

MATERIALS AND METHODS

Prefeed samples of rumen fluid were removed from sheep fed on a hay - concentrate diet (1:2 w/w) by suction via a stomach tube. Samples were transferred to the laboratory in a prewarmed vacuum flask and bubbled with carbon dioxide. Ingesta were homogenized for six periods of 10 s over 2 min to dislodge some of the micro-organisms from feed particles. The homogenized ingesta were strained to yield the inocula.

Strained fluid samples were transferred to 100 ml flasks, mixed with McDougall (1948) artificial saliva and flushed with CO₂. The extent of dilution of ruminal fluid with buffer is given below. Flasks were plugged with Bunsen stoppers and incubated in a thermostatic water-bath at 39.2° for at least 2 h for stabilization. Typical pH values of mixed rumen fluid samples were within the range 6.7–6.9; they decreased towards the end of incubation by 0.1–0.2 units.

Heat production (\dot{H}) was measured in a semiadiabatic titration calorimeter (model 450; Tronac, Orem, Utah). The calorimeter was equipped with a 100 ml rapid-response silvered-glass Dewar reaction vessel. The Dewar assembly comprises stirrer, heater, thermistor and titrant tubes. The heater facilitated an electrical calibration and enabled the setting of temperature to the desired level. The heat change and the rate of heat flow was calculated from changes in the temperature of a medium with a known heat capacity with the thermistor output recorded. The medium was stirred by a glass stirrer at 600 rev/min.

Continuous thermograms with a precision of 40 mJ, were recorded using a Yokogawa recorder (Model 3407) with a sensitivity of 0.5–1 mV full-scale deflection range and a chart speed of 10–20 mm/min. The calorimeter-bath temperature was set at 39.0°.

Electrical calibration was performed by filling the reaction vessel with 60 ml water and measuring the stirrer (blank) effect *v.* the heat evolved by a 100 (SE 0.01) ohm resistor set to produce 21.7 mJ/s. Calibration was performed at least twice a day resulting in a mean calibration factor of 1 μ V = 11.7 mJ (SE 0.12).

The \dot{H} in rumen fluid samples was calculated with the aid of the recorder trace. The heat produced by a subsequent reaction above the steady-state reading (as the heat evolved due to addition of substrate) was determined as the difference between the extrapolated lines relating the quantity of heat produced to time (see Fig. 1).

Samples were rapidly introduced into the reaction vessel under a stream of CO₂ and inserted into the calorimeter bath. Thermal perturbation decayed within minutes. Due to the similarity of the water-bath and calorimeter temperatures and to rapid transition of samples, only minor heating (20–80 mJ/s for a few minutes) of the reaction vessel contents was needed in order to bring them up to set temperature.

After reaching a steady rate of \dot{H} , a single dose of glucose was added to the reaction vessel. The glucose solution was prewarmed in the calorimeter bath in a burette and transferred to the reaction vessel at a rate of 0.4 ml/min (SE 0.1%). The calorimetric measurement was terminated after \dot{H} returned to basal values. A typical single run lasted for about 30 min.

Three experiments were performed to determine the effect of glucose addition on \dot{H} of rumen fluid *in vitro*.

Expt 1. The effect of dilution of rumen fluid with buffer on basal \dot{H} and H_t of glucose was studied using three dilutions (ml rumen fluid: ml buffer; 10:50 15:45 and 20:40) together with single glucose doses (mg; 1.6, 3.2 and 6.4). Subsequent experiments were conducted with a dilution of 15 ml rumen fluid: 45 ml buffer.

Expt 2. The effect of the duration of glucose infusion on \dot{H} was studied using single doses of glucose; 1.6 mg/min were infused into 15 ml rumen fluid plus 45 ml buffer for 1, 2 or 4 min but 1 min infusion of glucose was used in the subsequent experiment.

Expt 3. The final experiment in the series was designed to study the effects of glucose dose on H_t and on \dot{H} in rumen fluid. This experiment was designed to reduce variation between inocula. On each experimental day measurements of \dot{H} were made in fifteen rumen fluid samples (five doses times three determinations). Thus 7 h lapsed between the first and last determination from the same inoculum. The mean \dot{H} by rumen fluid samples was not significantly affected by the period of incubation in the water-bath.

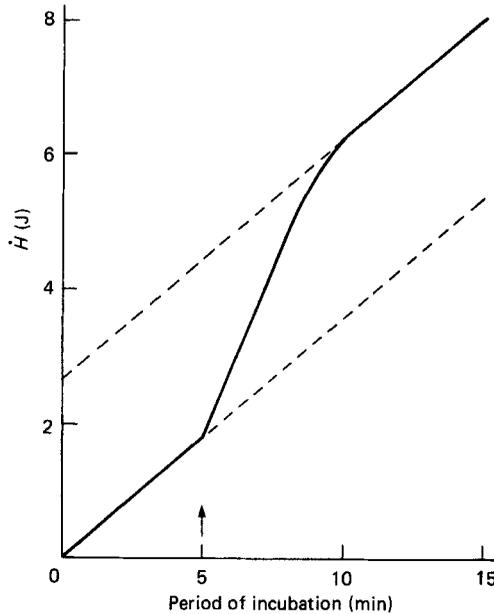


Fig. 1. Effect of a single glucose dose (6.4 mg) on heat production rate (\dot{H}) in rumen samples (15 ml rumen fluid plus 45 ml buffer). \uparrow , Time of administration.

Calculations

Results for \dot{H} were expressed as mW/ml rumen fluid and H_f as kJ dissipated as heat/mol substrate added. Results are given as means with their standard errors; comparisons between treatments were analysed by Student's *t* test and analysis of variance. An asymptotic curve of the form $y = a + b \cdot e^{cx}$ was fitted to the values from Expt 3, where x is glucose dose (mg), y is H_f and a , b and c are constants. Values of these parameters were solved by an iteration procedure based on Newton's approximation.

RESULTS

When a rumen fluid inoculum was incubated without exogenous substrate, a minimal \dot{H} was observed. \dot{H} of rumen micro-organisms was stable for several hours, having a mean coefficient of variation of 12%.

Fig. 1 depicts a typical incubation, 1.80 J were dissipated by 15 ml rumen fluid and 45 ml buffer, during 5 min before glucose infusion. This is indicated by the basic slope which is equivalent to a value for \dot{H} of 0.36 mW/ml rumen fluid. When 6.4 mg glucose was added (during a 1 min infusion), \dot{H} increased immediately, the steeper slope demonstrates an increase to 1.05 mW/ml rumen fluid. Thereafter \dot{H} declined and 5 min after the introduction of the substrate, the value for \dot{H} was similar to the basal value. The difference between the two parallel lines represents H_f which in Fig. 1 is 75 kJ/mol.

Expt 1

An increase in rumen fluid concentration in the reaction vessel was associated with a decrease in basal \dot{H} from 0.52 mW/ml rumen fluid at a dilution of 1:5 to 0.31 mW/ml rumen fluid at a dilution of 1:2 ($P < 0.001$; Table 1).

Increases in rumen fluid concentration were usually followed by increases in stimulated

Table 1. *Expt 1. Effect of dilution with buffer on basal rate of heat production (\dot{H}) and on glucose induced fermentation heat (H_f) in rumen fluid*

(Mean values with their standard errors; no. of determinations in parentheses)

Rumen fluid:buffer (ml:ml)	\dot{H} (18) (mW/ml rumen fluid)		H_f (6) (kJ/mol)					
			1.6*		3.2*		6.4*	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
10:50	0.52 ^a	0.01	120	8	102	6	74 ^a	3
15:45	0.39 ^b	0.01	108	6	82	5	72 ^a	2
20:40	0.31 ^c	0.01	117	6	88	2	63 ^b	1

^{a, b, c} Mean values with different superscript letters differed significantly ($P < 0.05$).

* Glucose (mg) added to the reaction vessel.

Table 2. *Expt 2. Effect of duration of glucose infusion on rate of heat production (\dot{H}) and on fermentation heat (H_f) in rumen fluid*

(Mean values with their standard errors for six determinations)

Duration of infusion† (min)	Amount of glucose infused (mg)	\dot{H} (mW/ml rumen fluid)		H_f (kJ/mol)	
		Mean	SE	Mean	SE
		1	1.6	0.78	0.01
2	3.2	0.74	0.01	94 ^b	1
4	6.4	0.82	0.01	86 ^c	2

^{a, b, c} Mean values with different superscript letters differed significantly ($P < 0.05$).

† Glucose infused at 1.6 mg/min.

\dot{H} and by a faster return of \dot{H} to basal values (not shown). Dilution had no consistent effect on H_f when 1.6 or 3.2 mg glucose were added. Lower H_f values were found with the higher concentration of rumen fluid (1:2) when 6.4 mg glucose were added to the reaction vessel. With all dilutions H_f decreased when the amount of glucose added was increased.

Expt 2

Table 2 shows the effect of the duration of glucose infusion on stimulated \dot{H} and on H_f . The mean preinfusion \dot{H} of eighteen samples (15 ml rumen fluid plus 45 ml buffer) was 0.4 (SE 0.02) mW/ml rumen fluid. Increasing the duration of glucose infusion (1.6 mg/min) from 1 to 4 min was associated with a sustained \dot{H} . However, an increase in amount of substrate was followed by a significant decrease in H_f from 111 to 86 kJ/mol ($P < 0.001$). When a constant amount of glucose was added over different periods of time, e.g. 3.2 mg over 1, 2 or 4 min, similar H_f were obtained (not shown).

Expt 3

Fig. 2 depicts a single dose-response effect of infusion of 0.4 to 6.4 mg glucose for 1 min on stimulated \dot{H} on rumen fluid. Basal \dot{H} was 0.36 (SE 0.01) mW/ml rumen fluid (n 120). The rate and duration of the stimulated \dot{H} were dose-dependent. As shown in Fig. 2, at the highest glucose dose maximal \dot{H} was 1.1 mW/ml rumen fluid. This \dot{H} level was maintained for 3–5 min.

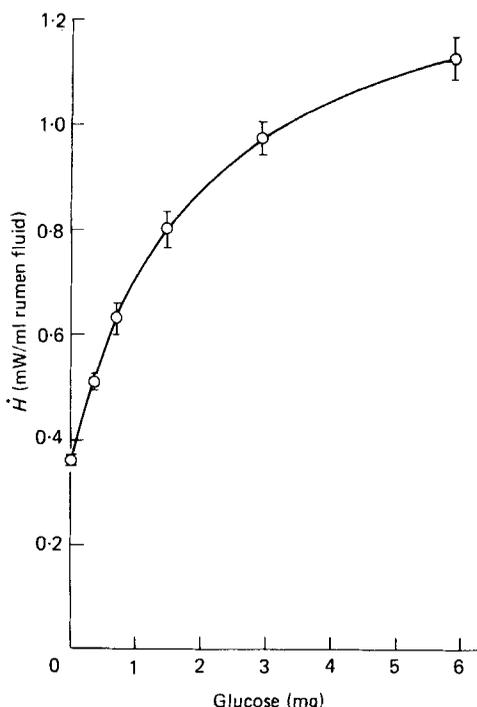


Fig. 2. Expt 3. Effect of glucose on heat production rate (mW/ml; \dot{H}) in rumen fluid. Points are mean values with their standard errors represented by vertical bars (n 120 for incubation without glucose, twenty-four replicates for incubations in the presence of glucose).

Table 3 summarizes H_f values obtained from different doses of glucose in the treated samples. A sixteenfold increase in glucose dose was accompanied by an eightfold increase in H_f . Hence an increase in glucose dose from 0.4 to 6.4 mg per sample resulted in a decrease of H_f from 149 to 71 kJ/mol ($P < 0.001$).

The response relation for H_f v. glucose dose could be described by the equation:

$$y = 69.2 + 107.4 e^{-0.667x} \quad (1)$$

where y is H_f (kJ/mol) and x is glucose dose (mg).

The correlation coefficient between observed mean values and the values calculated by this equation was 0.9996 (n 5, $P < 0.001$).

DISCUSSION

There is little information on the heat evolved during fermentation of available carbohydrates in the rumen (Walker & Forrest, 1964). Principles of stoichiometry provide a framework for calculating the amount of substrate energy dissipated as heat when a certain amount of substrate is completely fermented. However, when substrate is added to rumen fluid a variable amount of its energy is not fermented but is incorporated into bacterial cells (Ørskov, 1975).

The basal rate of \dot{H} in the present study was constant throughout the incubation period of 2–9 h. Therefore, it is possible that the population of micro-organisms within the samples remained constant. Hence basal \dot{H} may represent the basal energy expenditure for non-growth functions of rumen micro-organisms such as ion balance, protein turnover, etc. (Russell & Hespel, 1981).

Table 3. *Expt 3. Effect of glucose on heat production in rumen fluid samples*
(Mean values with their standard errors for twenty-four determinations)

Glucose added (mg)	Heat production (kJ/mol)	
	Mean	SE
0.4	149 ^a	5
0.8	134 ^b	5
1.6	106 ^c	2
3.2	83 ^d	2
6.4	71 ^e	1

a, b, c, d, e Mean values with different superscript letters differed significantly ($P < 0.05$).

The \dot{H} values obtained in the present study were higher than those reported for unbuffered rumen fluid samples from fasting ruminants (0.07 mW/ml and 0.12 mW/g; Walker & Forrest, 1964; Houpt, 1968). The finding that the rate of basal \dot{H} in rumen fluid is decreased with increasing concentration of rumen fluid in the medium (Table 1) suggests uptake of substrate from the rumen fluid by the micro-organisms, as substrate flux to micro-organisms is inversely related to population density (El-Shazly & Hungate, 1965; Button, 1985), whereas the rate of \dot{H} is proportional to cell concentration when endogenous energy sources are used (Nunomura & Fujita, 1981).

In the present study, the effect of glucose was rapid and of short duration: \dot{H} values returned to basal levels 3–5 min after the introduction of substrate. No measurements of disappearance of glucose and formation of VFA were made. Such measurements would be difficult over the short intervals of time used. However, when a second glucose dose (1.6 or 6.4 mg) was added, \dot{H} increased to levels similar to those obtained after the first dose, suggesting that the return of \dot{H} to basal level was due to the exhaustion of substrate in the medium. This conclusion is compatible with the rate of glucose metabolism in rumen contents, which has a turnover time of 2.5 min (Hungate, 1966).

The maximal \dot{H} of rumen samples in the present study, 1.1 mW/ml rumen fluid, was similar to \dot{H} in rumen fluid samples taken from animals fed on concentrates (Houpt, 1968).

Glucose-induced \dot{H} was related to glucose dose (Fig. 2) but was unaffected by the duration of infusion (Table 2). This may be due to the effect of the initial substrate concentration on \dot{H} . The initial glucose concentration in the present study was within the physiological range. Low glucose concentrations (e.g. 0.4 mmol/l) are found in animals fed on a mixed roughage – concentrate diet (Takahashi & Nakamura, 1969), whereas a short time after giving a concentrate diet, glucose concentrations as high as 9.3 mmol/l rumen content were reported (Mackie *et al.* 1984).

In the earlier studies, \dot{H} was obtained over fermentation periods of several hours. As the microbial population of the rumen grows at the rate of 8% /h (El-Shazly & Hungate, 1965), it may be presumed that only limited changes occurred in the size of microbial population during the fermentation of glucose in the present study. Hence, the \dot{H} values obtained probably represent direct fermentation of substrates unbiased by variation in population size, which may occur over long fermentation periods.

The main finding of the present study is that under *in vitro* conditions, direct H_t of glucose by rumen micro-organisms is related to the amount of added substrate. An increase in glucose dose by varying the infusion time (Table 2) or the amount of glucose added (Table 3) resulted in a decrease in H_t . It is possible that in the presence of large amounts of glucose, maintenance requirements of the rumen microbes are met and a shift from catabolic to

anabolic processes is likely, resulting in intracellular accumulation of substrates or synthesis of storage polysaccharides (Walker & Monk, 1971).

The fitted extrapolation equation relating H_f to glucose dose (eqn (1)) predicts that at very small amounts of glucose, about 177 kJ/mol substrate energy, could be converted into heat; this is close to the value obtained from stoichiometric calculations (Hungate, 1966). This is probably true when apparently all the substrate is catabolized. On the other hand, in the presence of a large glucose dose (e.g. 6.4 mg glucose/sample), H_f decreased to 70 kJ/mol substrate energy.

It is noteworthy that H_f of about 117 kJ/mol cellobiose was found when a very large amount (2 mmol) was added to rumen contents (Forrest, 1969). In that study \dot{H} in rumen contents was stoichiometrically related to VFA production in the reaction mixture.

The results presented in Table 3 are compatible with the hypothesis that an increase in glucose input to rumen micro-organisms is associated with a decrease in the proportion of substrate energy converted to heat. Assuming that 177 kJ/mol glucose is dissipated as heat when the substrate is completely fermented (eqn (1)), then with high glucose availability about 60% of substrate may escape catabolism.

The apparent storage efficiency of available carbohydrates suggested by the present findings and by the results of Forrest (1969) appears to be higher than the average values reported in the literature (Isaacson *et al.* 1975). This difference might be related to the short-term fermentation, in which efficiency determination does not include deterioration and lysis of cells as compared with the reported efficiency determination. Nevertheless, such calculations are within the percentage range of fermentable organic matter entering cells in the rumen (Leng, 1983). Further studies are, however, required to clarify the conditions influencing the distribution of substrate energy between cells and fermentation.

The author thanks Mr U. Aharoni for use of the exponential iteration program. The financial assistance of the Peter Haas Foundation is gratefully acknowledged.

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