Metabolism of propane-1:2-diol infused into the rumen of sheep

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1. Propane-1:2-diol (100 g/d) was infused through a cannula into the rumen of sheep receiving a ration of hay and dried grass. The concentration of volatile fatty acids, propanediol, lactic acid and of added polyethylene glycol, and the pH of the rumen contents were measured. The energy metabolism of the sheep was also determined.

2. Most of the propanediol disappeared from the rumen within 4 h of its infusion. The infusion of propanediol resulted in a 10% decrease in the concentration of total volatile acids; the concentration of acetic acid decreased by about 30%, that of propionic acid increased by up to 60% and there was no change in the concentration of butyric acid.

3. The methane production of the sheep decreased by about 9% after the infusion of propanediol and there were increases in the oxgyen consumption, carbon dioxide production and heat production of the animals; each of these increases was equivalent to about 40% of the theoretical value for the complete metabolism of 100 g propanediol.

4. It is concluded that, when propanediol is introduced into the rumen, a proportion is metabolized in the rumen and a large proportion is absorbed directly.

Gupta & Robinson (1960), Huff (1961), and other workers, have shown that propane-1:2-diol is converted into lactaldehyde and subsequently into lactic acid in various mammalian tissues, including the liver. Thus, propanediol may enter the normal pathways of carbohydrate metabolism within the body and may ultimately be converted into glucose, and could be a useful additive in the treatment of ketosis (Johnson, 1954). The glucogenic effect of propanediol has been amply demonstrated (Waldo & Schultz, 1960), and the observed changes in the composition of the rumen fluid implied partial fermentation of this substance in the rumen.

Recently, Czerkawski & Breckenridge (1972) showed that propane-1:2-diol is fermented by mixed rumen micro-organisms in vitro. The fermentation was accompanied by the production of acetic and propionic acids, temporary accumulation of lactic acid and no additional methane was produced. In fact, in the presence of propanediol, the endogenous methane production from the rumen contents used in 'the artificial rumen' was depressed by 20–30%.

With these results in mind, it was decided to investigate further the metabolism of propanediol by sheep, with special reference to their methane and heat production, and to study the metabolism of propanediol in the rumen.

EXPERIMENTAL

Sheep. Two Down-Cross castrated male sheep were used. Each animal was fitted with a permanent rumen cannula.

Food. The animals were offered food twice daily at 10.00 and 22.00 hours. Each meal consisted of 250 g hay and 250 g high-quality long dried grass, and the total dry-matter intake of the sheep was 863·6 g/d. The ration was given to the animals for 14 d before the experiments were begun. The mixed ration offered to the sheep contained 11·6% crude protein, 6·6% ash, 39·0% acid-detergent fibre, 5·37% lignin and its heat of combustion was 4·41 kcal (18·43 kJ)/g.

Determination of energy exchanges. The energy exchanges were measured in a respiration calorimeter (Wainman & Blaxter, 1958) where the carbon dioxide production, oxygen consumption and methane production of the sheep during successive periods of 24 h were measured. There was an initial control period of 5 d followed by an infusion period which lasted for 10 d during which 50 g propanediol dissolved in 450 ml water were infused throughout each meal, by means of a peristaltic pump (Watson-Marlow Ltd, Marlow, Bucks.), into the rumen through the cannula. Thus the total quantity of propanediol given to the animal was 100 g/d. The infusion period was followed by the second control period lasting 7–8 d. Each respiration chamber experiment, therefore, lasted for 22 or 23 d. Faeces and urine were collected throughout the experiment and weighed samples were prepared for each period for the determination of dry matter, organic matter, energy and nitrogen in the faeces, and nitrogen and energy in the urine, by the methods described by Graham, Blaxter & Armstrong (1958). Acid-detergent fibre and lignin in the faeces and foods were measured by the method of Van Soest (1963).

Determination of the effect of an infusion of propane-1:2-diol on the composition of rumen liquor. In these experiments, the sheep were kept in metabolism cages. The lengths of the control and infusion periods were the same as those in the calorimetric experiments. As before, solutions of propanediol were infused while the animals were eating. Faeces were collected and analysed as before. On certain days, a dose of 12 g polyethylene glycol (PEG) dissolved in 300 ml water was injected into the rumen through the cannula at 06.00 hours and samples of the rumen liquor were withdrawn through the cannula at 10.30, 11.00, 11.30, 12.00, 13.00, 14.00, 17.00 and 22.00 hours. The pH of these samples was determined at once. After centrifuging for 5 min at 1000 *g* the concentrations of the steam volatile fatty acids in the samples were determined by gas–liquid chromatography by the method of Cottyn & Boucque (1968) using crotonic acid as an internal standard; lactic acid concentrations were determined by the method of Conway (1962) and, during and immediately after the infusion periods, the concentrations of propanediol in the rumen samples were determined by the method of Jones & Riddick (1957). The concentration of PEG was determined by the method of Hydén (1961) and of soluble sugar by that of Smith & Montgomery (1956).

RESULTS

The effect of the infusion of propanediol upon the apparent digestibility of various components of the diet is shown in Table 1. There were no statistically significant changes in the apparent digestibilities of any of the components measured.

Figs. 1 and 2 summarize the effect of infusing 100 g propanediol/d upon the concentration of various components in the rumen liquor of the sheep. In general, the pH values were slightly lower after the infusion of propanediol but these differences

Table 1. *Effect of infusion of propane-1:2-diol into the rumen of sheep upon the apparent digestibilities (%) of various food components*

(Each value is the mean with its standard error and the number of determinations is given in parentheses)

Food component	Control (8)	Infusion (6)
Dry matter	60·4 ± 1·9	60·3 ± 1·6
Organic matter	61·8 ± 1·8	61·6 ± 1·5
Nitrogen	62·9 ± 2·5	63·2 ± 3·3
Energy	58·2 ± 2·2	58·3 ± 1·3
Acid-detergent fibre	53·1 ± 4·9	54·5 ± 1·4
Lignin	12·6 ± 4·9	10·7 ± 5·2

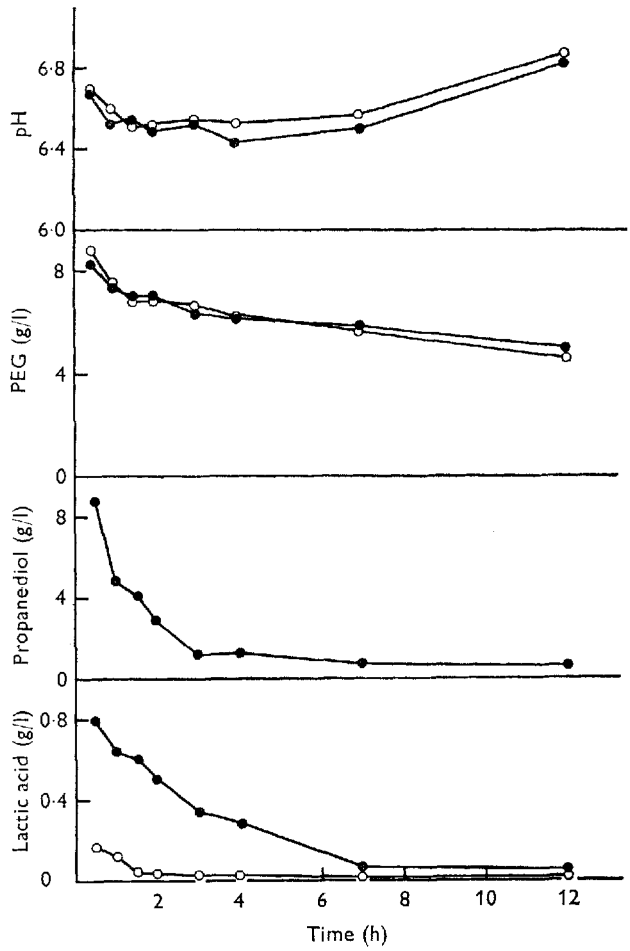

Fig. 1. Effect of infusing propane-1:2-diol into the rumen of sheep upon the rumen pH, the concentration of polyethylene glycol (PEG), propanediol and lactic acid in the rumen liquor. ○, control; ●, infusion.

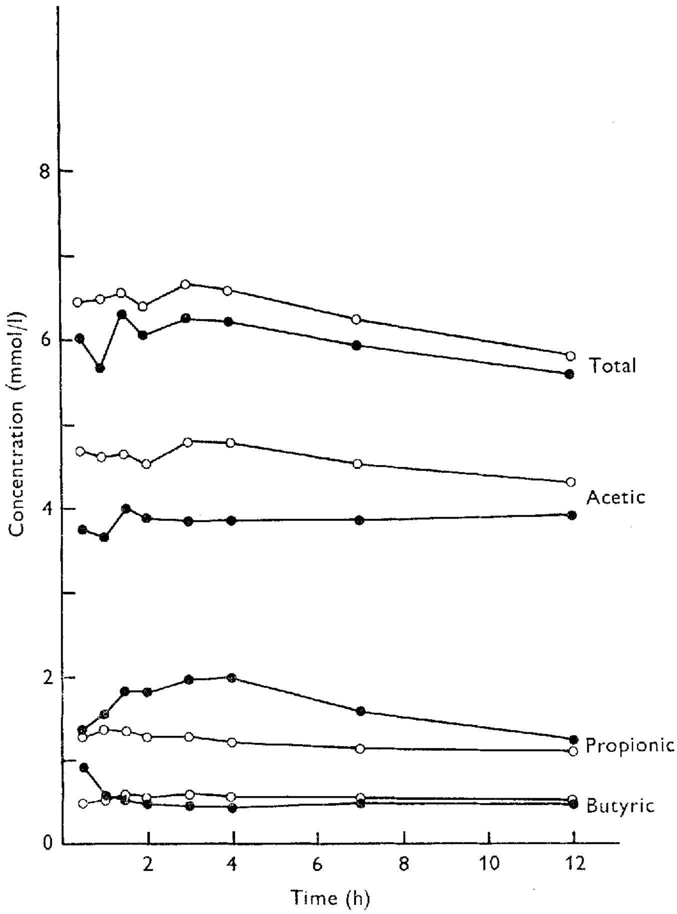

Fig. 2. Effect of propane-1:2-diol infused into the rumen of sheep on the concentration of volatile fatty acids in the rumen liquor. ○, control; ●, infusion.

were not statistically significant. The infusion had no significant effect upon the concentration of PEG in the rumen liquor and thus apparently had no effect on the rate of passage of the liquid digesta from the rumen. In order to ensure thorough mixing, the PEG was injected into the rumen 4 h before feeding and, as a result, it was difficult to arrive at a reasonable estimate of the volume of the rumen contents. For purposes of comparison, therefore, the rumen is assumed to be about 6 l.

The concentration of propanediol in the rumen liquor was high immediately after the infusion and it decreased rapidly for 3 h. Thereafter, the concentration decreased slowly for the remainder of the period before the next meal. Multiplying the initial concentration of propanediol, 8·8 g/l, by the assumed rumen volume of 6 l showed that there were some 53 g of propanediol in the rumen immediately after the infusion, which agreed with the amount infused at each meal, namely 50 g.

During the control periods, the concentration of lactic acid in the rumen liquor was negligible except immediately after feeding; even then, the concentration was less than 0·2 g/l. When propanediol was infused, the concentration of lactic acid in the

Table 2. *Effect of a daily infusion of* 100 g *propane*-1:2-*diol upon the carbon dioxide production, oxygen consumption, methane production and heat production of sheep*

(Each value quoted is the mean of 10 d with its standard error)

	Control	Infusion	Control
CO_2 production (l/d)	369·3 ± 4·4	399·1 ± 4·1	360·2 ± 0·3
O_2 consumption (l/d)	364·3 ± 7·9	408·1 ± 7·8	353·3 ± 2·3
CH_4 production (l/d)	24·57 ± 0·39	22·05 ± 0·23	24·26 ± 0·14
Heat production (kcal/d)	1832 ± 10	2032 ± 24	1771 ± 9
Heat production (kJ/d)	7658 ± 42	8492 ± 100	7403 ± 37

rumen fluid increased rapidly to 0·8 g/l immediately after the infusion; it then decreased until, just before the next feed, the value was the same as that in the control periods.

Fig. 2 shows the total concentration of the steam volatile fatty acids in the rumen liquor and the concentration of acetic, propionic and butyric acids. The total concentration of the acids was reduced by about 10% after the infusion of propanediol and this persisted throughout most of the inter-feeding period, although the extent of the depression was reduced to about 4% of the control value just before the next meal. Immediately after the infusion, the concentration of acetic acid was about 30% lower than the control value and this difference gradually became less throughout the inter-feeding period. With propionic acid, the pattern was different. The largest difference between the infusion and control values occurred at 3–4 h after the infusion, when the increase was 57% of the control value. Immediately after the infusion the apparent concentration of butyric acid was high but later there were no significant changes. This was probably due to transient accumulation of acrylic acid when the concentration of lactic acid was high. Acrylic acid is an intermediate in one of the pathways of formation of propionic acid from lactic acid and when measured by gas–liquid chromatography its retention volume is the same as that of butyric acid. When the mean values for all the samples are considered, the molar proportion of acetic acid in the rumen liquor changed from the control value of 72·1 to 64·1% after the infusion of propanediol ($P < 0·001$), that of propionic acid changed from 19·6 to 26·9% ($P < 0·01$) and that of butyric acid from 8·5 to 9·0%.

The results of measurements of the energy metabolism/d of the sheep are shown in Table 2. There was an increase of 34 l in the carbon dioxide production, and an increase of 49 l in the oxygen consumption, with a reduction of 2·4 l (or 9% of the control value) in the methane production and an increase of 231 kcal (966 kJ) in the heat production after the infusion of propanediol.

DISCUSSION

A comparison of the rate of change of the concentration of propanediol with that of PEG in the rumen liquor (Fig. 1) shows that the former fell much more rapidly than the latter. If the propanediol were merely removed from the rumen with the liquid portion of the rumen contents, then the two should fall at the same rate. Therefore,

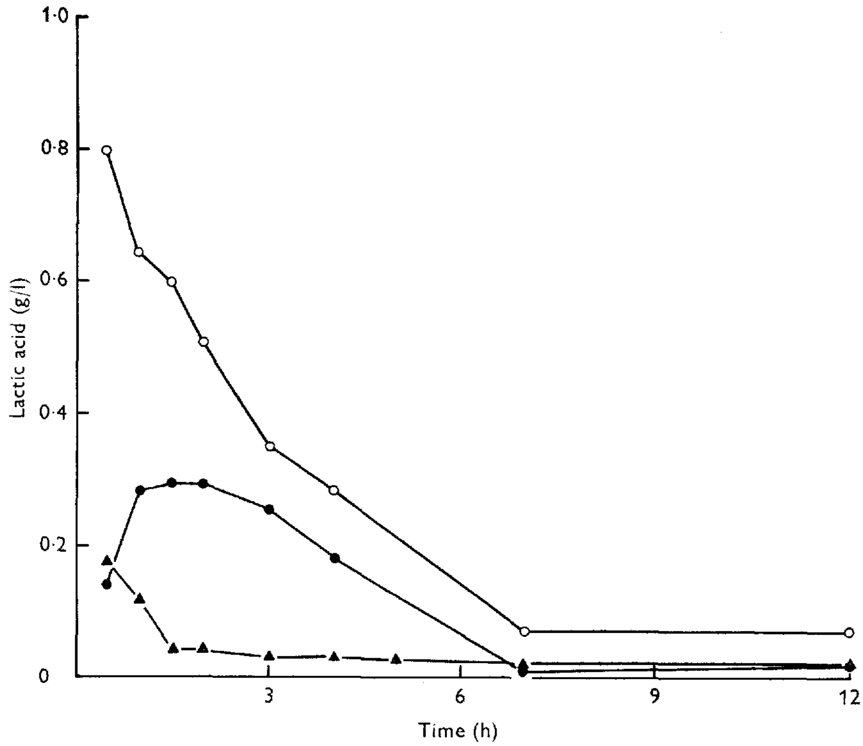

Fig. 3. Effect of propane-1:2-diol infused into the rumen of sheep upon the apparent concentration of lactic acid in the rumen liquor. ▲, lactic acid, control period; ○, lactic acid, infusion period (uncorrected); ●, lactic acid, infusion period (corrected for propanediol).

the propanediol must have been absorbed unchanged through the rumen wall, or metabolized within the rumen, or both.

Within the rumen, it is likely that one of the intermediate compounds formed in the metabolism of propanediol is lactaldehyde that is later converted into lactic acid which is, in turn, further metabolized. In Fig. 1, the lactic acid concentration, though much lower than that of the propanediol, apparently decreased in a similar manner. In these experiments, the lactic acid was determined by the method of Conway (1962). This involves the oxidation of the lactic acid in the rumen liquor with ceric sulphate in acid solution, the resulting acetaldehyde being trapped in semi-carbazide solution and the concentration of the complex is then determined colorimetrically. After the experiments were completed, it was realized that propanediol might be partly oxidized to acetaldehyde. Further experiments showed that this was so, 100 μg propanediol giving a response equal to 7·6 μg lactic acid. This was not due to any free lactic acid in the sample of propanediol used. When the lactic acid values shown in Fig. 1 were corrected for this, using the concentrations of propanediol found in the experiments, the values shown in Fig. 3 were obtained. The lactic acid concentration rose to a peak value between 1 and 2 h after feeding and then fell until, at 7 h after feeding, it was indistinguishable from the value obtained in the control periods. This was consistent with the hypothesis that lactic acid is an intermediate in the metabolism of propanediol in the rumen.

There was no change in the concentration of butyric acid in the rumen liquor after the infusion of propanediol but the concentration of propionic acid increased and that of acetic acid fell. Wood (1961) and Ladd & Walker (1959) have shown that, under the actively reducing conditions found in the rumen, lactate may be converted into acrylate and finally, into propionate. Thus propionic acid may be regarded as an end-product of the metabolism of propanediol. The fact that the peak concentration of lactic acid occurs at about 1·5 h, and that of propionic acid at about 3 h, after the infusion of propanediol is consistent with a multistep reaction, probably involving several micro-organisms.

The decrease in the concentration of acetic acid is more difficult to explain. It was shown by Czerkawski & Breckenridge (1971) that, when propanediol was metabolized in vitro in an artificial rumen, there was a lag-phase in the production of acetic acid followed by a short period during which there was vigorous production of the acid. In the rumen, where the substrate is continuously removed by absorption and by passage to the lower gut, the propanediol added might not give rise to any acetic acid at all. The results, however, imply either that the production of acetic acid from the food is inhibited by propanediol (or by some product of its metabolism) or that the rate of absorption through the rumen epithelium is increased in the presence of propanediol in the rumen fluid.

Huff (1961) has shown that DL-propane-1:2-diol can be converted into lactaldehyde in the liver of the rabbit, and this reaction is common to many mammalian species (West, Todd, Mason & Van Bruggen, 1965). Thus, any propanediol absorbed directly from the rumen can be metabolized in the liver and enter the normal carbohydrate metabolism.

In the present experiments, the reduction in the methane production of the sheep by propanediol was only 9 % compared with the values of 20–30 % inhibition found in experiments in vitro by Czerkawski & Breckenridge (1971). This difference is probably due to the fact that, in vivo, propanediol or its products are rapidly removed from the rumen by absorption or by passage to the lower gut whereas, in vitro, they are retained within the artificial rumen.

If all the propanediol infused were completely metabolized to carbon dioxide and water, then 89 l carbon dioxide would be produced, 118 l oxygen would be consumed and 578 kcal (2416 kJ) heat would be produced. In these experiments, the additional carbon dioxide production was 34 l (41 % of the maximum) the additional oxygen consumption was 49 l (41 % of the maximum) and the additional heat production was 231 kcal (956 kJ) (40 % of the maximum). Over-all, therefore, about 40 % of the propanediol infused into the rumen seems to have been oxidatively metabolized and the equivalent of 60 % appears to have been converted into various products and stored in the body.

It is concluded that, when 100 g propane-1:2-diol is infused into the rumen of sheep eating a ration of hay and dried grass, some of the propanediol is metabolized in the rumen via lactic acid and 60 % of the total amount infused is converted into products that are ultimately stored within the body.

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