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# The effect of functional groups other than carboxyl on the metabolism of $C_{18}$ and $C_{12}$ alkyl compounds by sheep

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1. Sixteen experiments were made with eight sheep to determine the effects of various compounds on methane production, digestion and metabolism. 2. Two experiments with two sheep given stearic acid incorporated in the diet showed that it depressed $CH_4$ production by 2·7 moles/mole stearic acid. The digestion of the basal diet was also depressed, the apparent digestibility of dietary cellulose falling from 61·7 to 55·0%. 3. Three experiments with two sheep given liquid paraffin (mainly the $C_{18}$ hydrocarbon) by infusion into the rumen showed that it had no effect on $CH_4$ production and that it increased the excretion of fatty acids in the faeces and the non-lipid energy of the faeces. 4. Oleyl alcohol infused into the rumen of one sheep had no effect on $CH_4$ production. It was excreted unchanged in large amounts in the faeces and increased the faecal excretion of fatty acids. 5. A preparation of sulphated $C_{18}$ and $C_{16}$ alcohols infused into the rumen of one sheep reduced $CH_4$ production by 11·4 moles/mole sulphated alcohol. The preparation also caused a large increase in the excretion of non-lipid calories in the faeces and in the heat production of the animal. A larger amount of sulphated alcohols, given by infusion into the rumen of another sheep, reduced $CH_4$ production to 41% of the initial values in 5 days, but caused refusal of food. 6. Two experiments were made with two sheep in which lauric acid was given by infusion into the rumen. Food refusals occurred within 48 h of commencing the infusion. 7. Sodium lauryl sulphate given to two sheep by infusion into the rumen reduced $CH_4$ production by 8·4 moles/mole. It greatly increased the faecal loss of non-lipid energy and also increased the heat production of the animals. When two further sheep were given sodium lauryl sylphate incorporated in the diet, cellulose digestion was depressed. Evidence of hydrolysis of the sodium lauryl sulphate was obtained. 8. Lauryl alcohol had no effect on the $CH_4$ production of one sheep, but increased the faecal excretion of lipid. Inorganic sulphate had no effect on metabolism in an experiment with a further sheep. 9. The results, together with previous work with oleic, linoleic and linolenic acid (Czerkawski, Blaxter & Wainman, 1966*a*), suggest that depression of $CH_4$ production is a function of molecules with both polar and non-polar activities, that is with surface-active properties, and that such compounds when given to ruminants have a greater affect on the $CH_4$-producing organisms than on organisms concerned in cellulose digestion.

In a previous paper (Czerkawski, Blaxter & Wainman, 1966*a*) it was found that when oleic, linoleic and linolenic acids were infused into the rumens of sheep, methane production was depressed, the magnitude of the depression increasing slightly with increasing unsaturation of the fatty acid. A single experiment with palmitic acid confirmed that the depression of $CH_4$ production was not due entirely to the presence of double bonds in the fatty acid molecule, and that saturated long-chain fatty acids would produce this effect.

The present paper describes experiments undertaken to examine the nature of this effect of the long-chain fatty acids on $CH_4$ production.

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## EXPERIMENTAL

### *Sheep*

Four adult wether sheep (sheep J, L, M and R), and four adult wether sheep with cannulas permanently inserted in their rumens (sheep B, W, D and A) were used.

### *Experiments*

Table 1 lists the sixteen experiments that were made. Two methods of administration of the compounds were used: first, the compound was incorporated in a mixture of ground foods which was then pelleted and given as part of the diet, and secondly the compounds were given as supplements to a diet of dried grass by continuous infusion of them into the rumen. They were given in these latter experiments as emulsions in 2 l. water or in solution in 2 l. water or as the pure compound. In the two experiments with sheep J and R in which stearic acid was given, one sheep received the control diet followed by the diet containing stearic acid and the other sheep received the diet containing stearic acid followed by the control diet. The sheep spent the last 10 days of each 28-day period in a respiration chamber. The same change-over was used in the experiments with sheep L and M given sodium lauryl sulphate, but the measurements made were limited to quantitative analysis of food and faeces. In the experiments in which sheep B and W received saturated hydrocarbons in the form of liquid paraffin, oleyl alcohol or the sulphated alcohols (Paste 'O', see Table 2) the sheep were confined throughout in a respiration chamber and preliminary and final control measurements were made. In the first two experiments with sodium lauryl sulphate with sheep B listed in Table 1, the experiment with the larger amount of sodium lauryl sulphate (18·0 g) followed immediately that with the smaller amount (5·8 g) with no intermediate control period. In the last three experiments, the amounts of sodium lauryl sulphate, $NaHSO_4$ and lauryl alcohol given were designed to supply 0·064 moles/day.

### *Diets*

In the experiments in which materials were infused into the rumen, the diet was 1000 g dried grass daily given in two equal meals. In the experiments with stearic acid, the control diet consisted of 300 g/day of dried grass and 900 g/day of a control pellet which had the following percentage composition: sugar-beet pulp 8·4, groundnut meal 20·0, barley meal 30·0, oatmeal 30·0, oat husk 10·0, NaCl 1·0, $CaHPO_4$ 0·5 and MgO 0·1. The basal mixture used to make the stearic acid diet had the same composition as the control diet and to it were added 6·06 kg stearic acid/100 kg. The amount of the pellet made from the final mixture given each day was 945 g together with 300 g dried grass. In the experiments in which sodium lauryl sulphate was given in the diet, the control diet was made to the same formula as that used in the experiments with stearic acid, and the diet containing sodium lauryl sulphate was the same meal mixture to which 2·0 kg sodium lauryl sulphate/100 kg were added before pelleting, 918 g of the resultant mixture being given each day.

Table 1. *Description of experiments*

| Preparation given* | Method of administration | Amount given daily (g) | Sheep | Length of periods (days) Control | Experimental | Experimental design |
|---|---|---|---|---|---|---|
| Stearic acid | Incorporated in diet | 54·0 | J | 28 | 28 | Change-over design, measurements on last 10 days of each period |
| Stearic acid | Incorporated in diet | 54·0 | R | 28 | 28 | |
| Saturated hydrocarbons | Infusion as emulsion | About 20 | B | 12 | 12 | Continuous measurement |
| Saturated hydrocarbons | Infusion neat | 58·0 | B | 12+12 | 12 | |
| Saturated hydrocarbons | Infusion neat | 55·5 | W | 21+12 | 14 | |
| Oleyl alcohol | Infusion as emulsion | 66·0 | W | 16+12 | 12 | Continuous measurement |
| Fatty sulphate paste | Infusion as emulsion | 24·3 (fresh weight) | B | 21+16 | 16 | Continuous measurement |
| Fatty sulphate paste | Infusion as emulsion | 50·0 (fresh weight) | W | See text | | |
| Lauric acid | Infusion as emulsion | 60 | D | See text | | — |
| Sodium lauryl sulphate | Infusion as emulsion | 5·8 | B | 12 | 8 | Continuous measurement |
| Sodium lauryl sulphate | Infusion as emulsion | 18·0 | B | 14 | 16 | |
| Sodium lauryl sulphate | Incorporated in diet | 18·0 | L | 21 | 21 | Change-over design, faeces collection only |
| Sodium lauryl sulphate | Incorporated in diet | 18·0 | M | 21 | 21 | |
| Sodium lauryl sulphate | Infusion as emulsion | 18·2 | A | 21 | 21 | Measurements on last 6 days only of each period |
| $NaHSO_4$ | Infusion in solution | 7·7 | D | 21 | 21 | |
| Lauryl alcohol | Infusion as emulsion | 12·9 | B | 21 | 21 | |

* Further descriptions of the preparations and their purity are given in Table 2 and on p. 498.

Table 2. *Percentage composition of the fatty acid or fatty alcohol preparations* and of the fatty alcohols derived from hydrolysis of the fatty sulphates*

| Acids or alcohols | Stearic acid | Oleyl alcohol | Fatty sulphate paste (alcohols) | Lauric acid | Lauryl alcohol | Sodium lauryl sulphate (alcohols) |
|---|---|---|---|---|---|---|
| 10:0 | — | — | — | — | 6·6 | 1·2 |
| 12:0 | — | — | — | 95·2 | 63·4 | 58·8 |
| 14:0 | — | — | 6·7 | 4·8 | 25·1 | 21·4 |
| 14:1 | — | 3·0 | 2·8 | — | — | — |
| 15:0 | — | Trace | 2·0 | — | — | — |
| 16:0 | 7·2 | 9·2 | 44·0 | — | 5·0 | 8·9 |
| 16:1 | — | 14·9 | 4·7 | — | — | — |
| 17:0 | 3·3 | 3·4 | — | — | — | — |
| 18:0 | 89·5 | Trace | 11·1 | — | — | 9·7 |
| 18:1 | — | 60·0 | 28·8 | — | — | — |
| 18:2 | — | — | — | — | — | — |
| 18:3 | — | — | — | — | — | — |
| 20:0 | — | — | — | — | — | — |
| Unidentified | — | 9·6 | — | — | — | — |
| Calorific value (kcal/g dry weight) | 9·57 | 10·25 | 6·80* | 8·89 | 10·12 | 6·38† |

* Further descriptions of the preparations are given on p. 498.
† Calorific value of total mixture of alkyl sulphates and free alcohols.

### *Composition of materials given*

The $NaHSO_4$ was pure. None of the other materials given was pure; the chemical composition and calorific values of six of the additives are given in Table 2.

*Stearic acid* (*Hopkins & Williams*). This was fairly pure being contaminated with palmitic acid and the $C_{17}$ saturated acid.

*$C_{18}$ hydrocarbon.* This was liquid paraffin BP (Evans Medical Ltd). Inquiries of the manufacturers indicated that it consisted almost entirely of the $C_{18}$ hydrocarbon. No confirmation of its composition by gas–liquid chromatography was achieved by us. Its calorific value was 11·16 kcal/g. No information on the calorific value of the $C_{18}$ hydrocarbon was found in the literature, but hexadecane has a heat of combustion of 11·2 kcal/g and eicosane a heat of combustion of 11·3 kcal/g (Kharasch, 1929).

*Oleyl alcohol.* Obtained from Glovers Ltd, Leeds, the sample of oleyl alcohol had as its largest contaminant a $C_{16}$ mono-unsaturated alcohol. It contained in addition to the $C_{17}$ alcohol an unidentified alcohol with a retention time between those of the $C_{17}$ and $C_{18}$ alcohols.

*Fatty sulphate paste.* This material has the trade name Paste 'O' and was given to us by A.B.M. Industrial Products Ltd, Stockport, Cheshire; it was a water-based paste which our analysis showed to contain 52% water, 3% free fatty alcohols, 6% sodium sulphate and 37% of sodium alkyl sulphates. The predominant fatty alcohols isolated after hydrolysis were cetyl and oleyl alcohols.

*Lauric acid.* Obtained from Hopkins & Williams, this material was 95% pure.

*Lauryl alcohol.* Obtained from Hopkins & Williams, the major contaminant of this material was myristyl alcohol.

*Sodium lauryl sulphate.* The composition of the alcohols obtained after hydrolysis of this material was very similar to that of the lauryl alcohol which was used. It was obtained from Marchon Products Ltd, Whitehaven, Cumberland.

### *Methods*

Determinations of $CH_4$ and carbon dioxide production and oxygen consumption and of the composition of the faeces and urine were made by methods described previously (Czerkawski *et al.* 1966*a*). Fatty acids were esterified by the method of Kates (1964) and analysed using the Pye Argon gas chromatograph with polyethylene glycol adipate as the stationary phase (Farquhar, Insull, Rosen, Stoffel & Ahrens, 1959; cf. Czerkawski *et al.* 1966*a*). The fatty alcohols were dried and applied to the column of the gas chromatograph directly.

## RESULTS

*Stearic acid.* When stearic acid, incorporated in pellets, was given to sheep R and J the results shown in Table 3 were obtained. The stearic acid resulted in a considerable increase in faecal energy. This was associated with an increase in faecal lipid, which if assumed to be stearic acid and given the calorific value of 9·57 kcal/g accounted for 75% of the increase in the heat of combustion of the faeces. Faecal cellulose and

Table 3. *Mean effect of stearic acid when added to the diet of two sheep*

| Diet | Value/24 h: Energy intake (kcal) | Faecal energy (kcal) | Faecal cellulose* (kcal) | Faecal lipid (g) | Faecal lipid calories† | Faecal N‡ (g) | Urine energy (kcal) | $CH_4$ energy (kcal) | Heat production (kcal) | Energy retained (kcal) |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 4591 | 975 | 318 | 17·8 | — | 153 | 203 | 400 | 2449 | +564 |
| Containing added stearic acid | 5152 | 1318 | 375 | 44·5 | — | 186 | 175 | 285 | 2442 | +932 |
| Increase due to stearic acid | 561 | 343 | 57 | 26·7 | 256 | 33 | −28 | −115 | −7 | +368 |

* 4·2 kcal/g cellulose.
† The increment was taken to have the same calorific value as stearic acid (9·57 kcal/g).
‡ 30 kcal/g N.

Table 4. *Effect of infusing liquid paraffin into the rumen on the daily metabolism of two sheep given two different diets of dried grass*

| | No. of days of measurement | Energy intake (kcal) | Faecal energy (kcal) | Faecal lipid (g) | Faecal lipid* (kcal) | Faecal N† (kcal) | Urine energy (kcal) | $CH_4$ energy (kcal) | Heat production (kcal) | Energy retained (kcal) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Sheep B:** | | | | | | | | | | |
| Control period | 12 | 3365 | 727 | 24·0 | — | 151 | 213 | 287 | 1808 | +330 |
| Infusion of 58·0 g liquid paraffin | 8 | 4012 | 1336 | 72·0 | — | 155 | 203 | 293 | 1802 | +378 |
| Change due to liquid paraffin | — | 647 | 609 | 48·0 | 536 | 4 | −10 | +5 | −6 | +48 |
| Sheep W: | | | | | | | | | | |
| Control period | 12 | 3795 | 1168 | 33·9 | — | 192 | 195 | 275 | 2189 | −32 |
| Infusion of 55·5 g liquid paraffin | 6 | 4414 | 1768 | 73·6 | — | 197 | 190 | 276 | 2135 | +45 |
| Change due to liquid paraffin | — | 619 | 600 | 39·7 | 439 | +5 | −5 | +1 | −54 | +77 |

* Increment given the calorific value of liquid paraffin 11·16 kcal/g.
† 30 kcal/g N.

faecal N also increased, the increase in faecal cellulose accounting for 17% of the increase in the heat of combustion of the faeces and the faecal 'protein' for 10%. The apparent digestibility of dietary cellulose fell from 61·7 to 55·0%. The fall represented 11% of the amount of cellulose digested; $CH_4$ production, however, fell by 29%.

Heat production was unaffected by the addition of the stearic acid, and energy retention rose by 368 kcal, or 66% of the additional energy supplied as stearic acid. In these experiments as, in the single experiment in which palmitic acid was infused

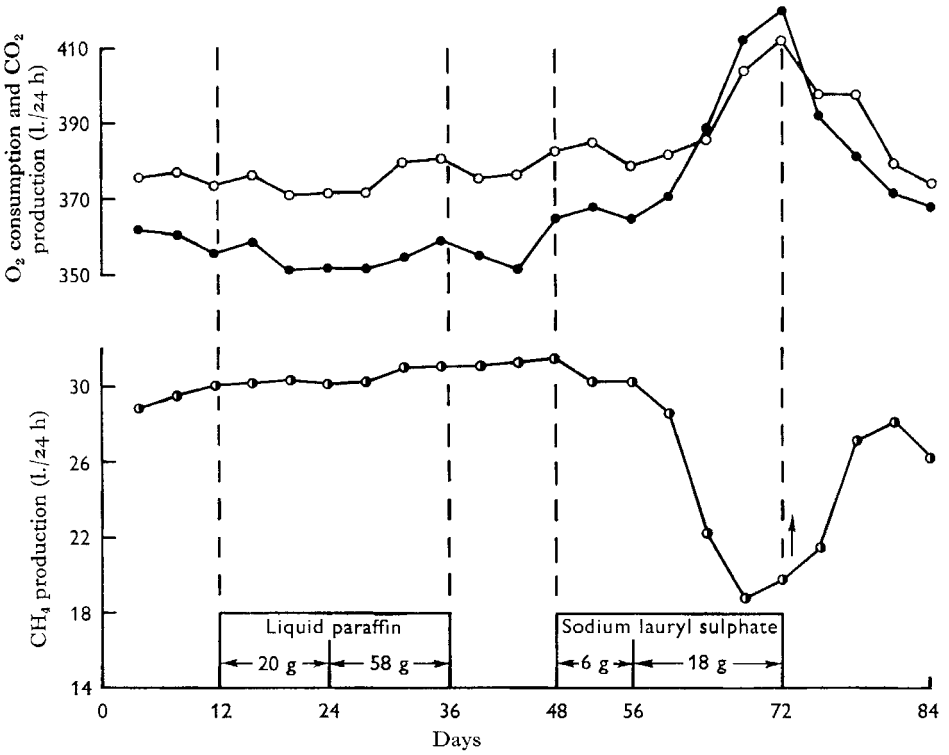


Fig. 1. Effect of daily infusion of 20 or 58 g liquid paraffin or 6 or 18 g sodium lauryl sulphate into the rumen of sheep B on its $CO_2$ production, $O_2$ consumption and $CH_4$ production. ○, $CO_2$ production; ●, $O_2$ consumption; ◑, $CH_4$ production.

into the rumen of a sheep (Czerkawski *et al.* 1966*a*) a saturated long-chain fatty acid depressed $CH_4$ production. At the same time, however, it depressed cellulose digestion, but much less markedly.

*Saturated hydrocarbons.* The results obtained in the two experiments with sheep B given two amounts of liquid paraffin by infusion into the rumen are shown in Fig. 1, and Table 4 summarizes the results of the experiment with this sheep given 58·0 g/day liquid paraffin and that with sheep W given 55·5 g/day. The experiment with sheep B, in which about 20 g liquid paraffin were infused into the rumen, was quantitatively unsatisfactory. The liquid paraffin was given as an emulsion which did not prove stable and this resulted in some uncertainty about the precise amount infused. Nominally 25 g were given but the amount may have been as low as 20 g on some days.

In the experiments in which 58·0 or 55·5 g liquid paraffin were introduced, it was infused undiluted.

Fig. 1 shows that neither amount of liquid paraffin given to sheep B affected the production of $CH_4$ by the sheep, its consumption of $O_2$ or its production of $CO_2$. Table 4 shows that the faecal loss of energy increased considerably when liquid paraffin was given, but that not the whole of the additional energy (647 and 619 kcal) given as liquid paraffin was recovered, the discrepancy being 38 kcal in sheep B and 19 kcal in sheep W. This was not due to failure to achieve equilibrium with respect to passage of the paraffin through the gut. Thus with sheep B, the mean faecal excretions of energy were 1332 and 1341 kcal/day for the last two 4-day periods of the 12-day infusions. On stopping the infusion, faecal excretion in subsequent 4-day periods was 1345, 920, 843 kcal/day and then 766 kcal/day.

The amounts of fatty acids found in the faeces, after hydrolysis of any soaps, increased when the paraffin was given by about 1 g in each sheep; the amounts of the major acids excreted are summarized in Table 5. Outstanding was the large increase in the excretion of stearic acid by both sheep when given liquid paraffin, with smaller increases in the excretion of palmitic, oleic and linoleic acids and a reduction in the excretion of linolenic acid. This suggests either an entrainment by the paraffin of dietary fatty acids or of fatty acids secreted into the gut, or bacterial oxidation of some of the paraffin which was given. In any event, the increment in lipid in the faeces was not entirely paraffin, suggesting that some paraffin was absorbed. Faecal N excretion did not change significantly in either sheep and $CH_4$ production was unaffected by the infusion of paraffin.

Table 5. *Weights of fatty acids (mg/day) excreted in the faeces of two sheep given liquid paraffin by infusion into the rumen*

| | Sheep B given 58·0 g liquid paraffin | | Sheep W given 55·5 g liquid paraffin | |
|---|---|---|---|---|
| Fatty acid | Control period | Infusion period | Control period | Infusion period |
| Total | 940 | 2090 | 1220 | 2292 |
| C 16:0 | 182 | 215 | 225 | 243 |
| C 18:0 | 194 | 979 | 346 | 781 |
| C 18:1 | 66 | 479 | 143 | 593 |
| C 18:2 | 48 | 126 | 79 | 112 |
| C 18:3 | 134 | 86 | 151 | 103 |

*Oleyl alcohol.* The results of experiments with sheep W given oleyl alcohol are summarized in Table 6. The alcohol resulted in a considerable increase in the faecal excretion of energy and the whole of this could be accounted for by an increase in the excretion of lipid. Of the increase in total lipid, fatty acids accounted for 9·0 g/day and unsaponifiable material for 30·0 g/day. Of the unsaponifiable material 43 % was oleyl alcohol. The major part of the increase in lipid excretion was thus unchanged oleyl alcohol. The amounts of fatty acids excreted/day are given in Table 7. The distribution of individual acids excreted in the control period was similar to those in the control periods of the experiments with liquid paraffin, as shown in Table 5, and

Table 6. *Effect on the daily metabolism of sheep of infusing oleyl alcohol and a fatty sulphate into the rumen*

| | No. of days of measurement | Energy intake (kcal) | Faecal energy (kcal) | Faecal lipid (g) | Faecal lipid (kcal) | Faecal N (kcal) | Urine energy (kcal) | $CH_4$ energy (kcal) | Heat production (kcal) | Energy retention (kcal) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Sheep W given 66 g oleyl alcohol | | | | | | |
| Control period | 8 | 3516 | 1097 | 27·4 | — | 193 | 197 | 259 | 2052 | −89 |
| Infusion period | 8 | 4193 | 1426 | 66·2 | — | 175 | 186 | 258 | 2127 | +196 |
| Increase due to oleyl alcohol | — | 677 | 329 | 38·8 | 349 | −18 | −11 | −1 | +75 | +285 |
| | | | | Sheep B given fatty acid sulphate paste (10·2 g organic material) | | | | | | |
| Control period | 12 | 3516 | 1032 | 31·4 | — | 161 | 184 | 266 | 1805 | +229 |
| Infusion period | 8 | 3599 | 1190 | 33·8 | — | 182 | 183 | 199 | 1984 | +43 |
| Increase due to fatty sulphate | — | 83 | 158 | 2·4 | 22 | 21 | −1 | −67 | +179 | −186 |

Calculations were made using the factors given in the footnote to Table 3.

the relative increases in fatty acid excretion on infusion of oleyl alcohol were also similar to those noted when liquid paraffin was infused; the major increases were in stearic and oleic acids. When oleyl alcohol was infused the smallest increase was in the linolenic acid excretion; with liquid paraffin a fall occurred. These results suggest that fatty acids derived from food or from secretions into the tract were dissolved in the oleyl alcohol which was not absorbed.

Table 7. *Daily excretion (mg/day) of fatty acids in the faeces of sheep W given oleyl alcohol by infusion into the rumen*

| Acid | Control period | During oleyl alcohol infusion |
|---|---|---|
| **Total** | 833 | 9859 |
| C 15:0 | 48 | 89 |
| C 15:0 branched | 38 | None |
| C 16:0 | 159 | 1044 |
| C 18:0 | 160 | 5319 |
| C 18:1 | 84 | 1290 |
| C 18:2 | 56 | 571 |
| C 18:3 | 117 | 364 |

Oleyl alcohol infusion had no effect either on $CH_4$ production or on the heat production of the animal.

*Sulphated $C_{18}$ and $C_{16}$ alcohols.* The first experiment with the fatty sulphates was made with sheep W. After a control period which established that 26·8 l. $CH_4$ (254 kcal) were produced daily, 50 g of the fatty sulphate paste was infused into the rumen. On the subsequent 5 days, $CH_4$ production was 25·4, 18·1, 16·1, 13·4 and 11·1 l./day. The sheep left part of the food uneaten on the 3rd day and amounts left uneaten rose rapidly. The amount of fatty sulphate given was clearly too great and in the experiment with sheep B, the results of which are summarized in Table 6, 25·4 g of the fatty sulphate paste were given. This supplied 9·4 g of alkyl sulphates and 0·8 g of fatty alcohols which the experiment described above showed to be inactive in reducing $CH_4$ production. The material supplying only 83 kcal resulted in an increase in the faecal loss of energy which far exceeded the reduction in $CH_4$ production. It also caused a marked increase in the heat production of the sheep. No clinical signs of abnormality were associated with this increase.

*Lauric acid.* Two experiments were attempted in which emulsions of lauric acid were infused into the rumens of sheep. These sheep had been confined in the respiration chamber to estimate the initial $CH_4$ production. They were well trained to experimental conditions and had received infusions before. After 48 h of infusion, each sheep left part of the basal diet uneaten. In one sheep the infusion was continued for a further 48 h, by which time the sheep had ceased to eat. The infusion was stopped, but it took 10 days to re-establish normal food intake in this animal.

*Sodium lauryl sulphate.* Fig. 1 shows the effect of two amounts of sodium lauryl sulphate on the $CH_4$ and $CO_2$ production and $O_2$ consumption of sheep B, and the results for the higher amount are summarized in Table 8 together with results for

Table 8. *Effect of infusing sodium lauryl sulphate, lauryl alcohol and $NaHSO_4$ into the rumen on the daily metabolism of individual sheep given a diet of dried grass*

| | No. of days of measurement | Energy intake (kcal) | Faeces energy (kcal) | Faeces lipid (g) | Faeces lipid (kcal) | Faeces cellulose (kcal) | Faeces N (kcal) | Urine energy (kcal) | Methane energy (kcal) | Heat production (kcal) | Energy retention (kcal) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sheep B given lauryl sulphate: | | | | | | | | | | | |
| Control period | 6 | 3365 | 727 | 24·0 | — | — | 151 | 213 | 287 | 1808 | +330 |
| Infusion period | 6 | 3477 | 1033 | 36·5 | — | — | 201 | 261 | 183 | 2075 | −75 |
| Change due to sodium lauryl sulphate | — | 112 | +306 | +12·5 | +112 | — | +50 | +48 | −104 | +267 | −405 |
| Sheep A given lauryl sulphate: | | | | | | | | | | | |
| Control period | 6 | 3516 | 943 | 31·7 | — | 212 | 170 | 221 | 267 | 2099 | −14 |
| Infusion period | 6 | 3632 | 1304 | 41·0 | — | 347 | 197 | 199 | 161 | 2189 | −221 |
| Change due to sodium lauryl sulphate | — | 116 | +361 | +9·3 | +84 | +135 | +27 | −22 | −106 | +90 | −207 |
| Sheep B given lauryl alcohol: | | | | | | | | | | | |
| Control period | 6 | 3516 | 1052 | 29·1 | — | 243 | 185 | 169 | 265 | 2195 | −165 |
| Infusion period (10·8 g) | 6 | 3625 | 1123 | 47·1 | — | 247 | 197 | 166 | 261 | 2263 | −188 |
| Change due to lauryl alcohol | — | 109 | +71 | +18·0 | 162 | +6 | +12 | −3 | −4 | +68 | −23 |
| Sheep D given sodium bisulphate: | | | | | | | | | | | |
| Control period | 6 | 3516 | 1025 | 36·1 | — | 234 | 172 | 186 | 259 | 1900 | +146 |
| Infusion period | 6 | 3516 | 1036 | 34·0 | — | 249 | 175 | 187 | 253 | 1932 | +108 |
| Change due to $NaHSO_4$ | — | 0 | +11 | −2·1 | −18 | +15 | +3 | +1 | −6 | +32 | −38 |

Calculations were made using the factors given in the footnote to Table 3.

sheep A also given sodium lauryl sulphate. Table 8 shows that 18 g sodium lauryl sulphate increased the loss of energy in the faeces by 306 and 361 kcal and reduced $CH_4$ production by 104 and 106 kcal in sheep B and A respectively. The increase in the faecal loss of energy was in part due to an increase in lipid excretion, but cellulose excretion also increased. This was confirmed in two further experiments with sheep L and M given 20 g sodium lauryl sulphate incorporated in their diet. The diet supplied 251 g cellulose daily and the faecal excretion of cellulose by sheep L increased from 83·7 to 105·7 g/day and by sheep M from 73·7 to 100·9 g/day on addition of sodium lauryl sulphate.

Table 8 also shows that the N content of the faeces was increased when sodium lauryl sulphate was given, but urine energy increased with one sheep and fell with the other. In both sheep, and this is clearly evident from the results for sheep B in Fig. 1 where $O_2$ consumption and $CO_2$ production are shown, heat production increased. The net effect of adding 18 g sodium lauryl sulphate was to depress energy retention in both sheep A and B.

Determinations of the amount of inorganic sulphate excreted in the urine by sheep A showed that in the control period it excreted 4·9 g $SO_4$ per day and when given sodium lauryl sulphate to supply 6·1 g sulphate, it excreted 8·6 g $SO_4$ per day. This shows that at least 61 % of the sodium lauryl sulphate was hydrolysed in either the tissues or the gut. In control experiments in which 6·1 g sulphate were given as $NaHSO_4$ urinary excretion of $SO_4$ rose from 4·4 to 9·7 g, a recovery of 71 %, which suggests that hydrolysis of the sodium lauryl sulphate may have been greater than the value of 61 % suggests.

*Lauryl alcohol.* As shown in Table 8, infusion of lauryl alcohol had no effect on $CH_4$ production or on cellulose digestion. The heat of combustion of the faeces increased, but the increase was less than that accounted for by excretion of lipid, suggesting some enhancement of absorption of non-lipid material. Heat production increased slightly and during the infusion of lauryl alcohol the energy retention of the animal was depressed by only 23 kcal; this cannot be regarded as a significant effect.

*Sodium bisulphate.* As shown in Table 8, infusion of $NaHSO_4$ had no effect on cellulose digestion, on $CH_4$ production or on heat production. The end-products of hydrolysis of sodium lauryl sulphate, namely inorganic sulphate and lauryl alcohol, thus had no effect on metabolism, whereas the fatty sulphate had a large effect.

## DISCUSSION

The effects of the various compounds on $CH_4$ production and on the excretion of non-lipid materials in the faeces have been summarized in Table 9 which also includes results from previous experiments (Czerkawski *et al.* 1966*a*, *b*) with unsaturated fatty acids, palmitic acid and linseed oil glycerides. Previous work (Czerkawski *et al.* 1966*b*) showed that giving unsaturated fatty acids twice daily with the food had a greater effect on $CH_4$ production and on cellulose digestion than giving the acids by continuous infusion into the rumen. The effects noted when stearic acid was incorporated in the diet may not therefore be fully comparable with those obtained with infused fatty acids.

Table 9. *Summary of the results of the experiments and those of Czerkawski, Blaxter & Wainman* (1966*a*, *b*) *in which alphatic compounds with* 18 *or* 12 *C atoms were given to sheep in the diet or by infusion into the rumen*

| | Compound given | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Stearic acid in diet | Oleic acid infused | Linoleic acid infused | Linolenic acid infused | Linolenic acid in diet | Linseed glycerides in diet | Liquid paraffin infused | Oleyl alcohol infused | $C_{16}$ and $C_{18}$ alkyl sulphates infused | Palmitic acid infused | Lauryl alcohol infused | Sodium lauryl sulphate infused | $NaHSO_4$ infused |
| Amount given daily (moles fatty acid or equivalent) | | 0·11–0·26 | 0·10–0·28 | 0·11–0·46 | 0·11–0·22 | 0·10–0·20 | 0·22 | 0·24 | 0·03 | 0·20 | 0·06 | 0·06 | 0·07 |
| Depression of $CH_4$ production/mole fatty acid or equivalent | 2·67 | 1·70 | 1·79 | 2·05 | 3·47 | 3·21 | 0·06 | 0·02 | 11·42 | 2·72 | 0·42 | 8·43 | 0·39 |
| Values/100 kcal compounds given: | | | | | | | | | | | | | |
| Depression of $CH_4$ production (kcal) | 20·5 | 13·8 | 14·2 | 16·4 | 28·9 | 25·6 | 0·5 | 0 | 80·7 | 23·6 | 3·7 | 92·9 | Indeterminate, no effect found |
| Increase in non-lipid material in faeces (kcal) | 15·5 | 6·3 | 4·6 | −2·9 | 11·8 | 11·8 | 18·5 | −2·9 | 163·9 | N.D. | −83·0 | 200·0 | — |

Calculations were made using the factors given in the footnote to Table 3.

The results show that substitution of a methyl or a hydroxyl group for the carboxyl group of stearic or oleic acids abolished their effects on $CH_4$ production. The hydrocarbon was largely but not entirely excreted unchanged, and when excreted it entrained some lipid of endogenous or food origin. About 50% of the alcohol was also excreted in the faeces again entraining considerable amounts of fatty acids of body or food origin. The hydrocarbon increased the excretion in the faeces of non-lipid material, a small part of which contained N. The alcohol had no effect on the excretion of non-lipid material.

When the sulphated $C_{16}$ and $C_{18}$ alcohols were given, their effect on $CH_4$ production on a molar basis was about four times as great as that noted when the corresponding saturated acids were given. In addition, the fatty sulphates increased the faecal loss of of non-lipid material, and, despite the decline in total energy absorbed, markedly increased both the oxygen consumption and $CO_2$ production of the sheep.

These results suggest that depression of $CH_4$ production is a function of molecules which have both polar and non-polar characteristics, that is molecules with surface-active properties. The experiments with sodium lauryl sulphate were in fact undertaken with this hypothesis in mind. In the first experiment with sheep B given 5·8 g sodium lauryl sulphate, the amount given was decided by experimental determination of the relative effects of oleic acid and sodium lauryl sulphate on the surface tension of rumen liquor. The same reduction of surface tension was produced by 1 mg of sodium lauryl sulphate as was produced by 10 mg sodium oleate. As shown in Fig. 1, 5·8 g sodium lauryl sulphate had virtually no effect on $CH_4$ production, whereas Table 9 shows that 60 g oleic acid caused a very marked reduction. This result suggests that the simple measurement of surface activity adopted did not reflect quantitatively the properties of these two molecules as far as their effects on $CH_4$ production are concerned.

The experiments with the large amounts of sodium lauryl sulphate showed that it was active in reducing $CH_4$ production but it also increased the loss of non-lipid material in the faeces. This was due to the fatty sulphate itself because, although evidence of its hydrolysis was obtained, lauryl alcohol and inorganic sulphate were without effect on $CH_4$ production. As with the sulphated $C_{16}$ and $C_{18}$ alcohols, sulphated lauryl alcohol increased $O_2$ consumption and $CO_2$ production. No reason for this effect can be given. The abortive experiments with lauric acid suggest that it has effects on metabolism of the rumen micro-organisms, and possibly on that of the sheep considerably greater than the effects of the $C_{18}$ fatty acids.

All the compounds tested that reduced $CH_4$ production affected the faecal loss of non-lipid material. The number of calories by which $CH_4$ production fell was, with the exception of the experiments with alkyl sulphates, greater than the increase in the non-lipid calories in the faeces. A net gain of energy from the basal diet thus occurred. From Table 9 it appears that the greatest net gain occurred with linolenic acid given by infusion into the rumen closely followed by the intake of linolenic acid incorporated in the diet.

Comparison of the effects of the compounds in this way, however, minimizes the separation which was achieved between cellulolysis and methanogenesis. Thus when

stearic acid was given, cellulose digestion fell by 11% whereas $CH_4$ production fell by 29%. When oleic acid was given in previous experiments (Czerkawski *et al.* 1966*a*) cellulose digestion fell by 2·4% and $CH_4$ production fell by 39%. With linoleic acid, cellulose digestion fell by 2·1% and $CH_4$ production fell by 43%. The relative depression of $CH_4$ production when fatty acids were given exceeded considerably the depression of cellulolysis. In this regard those methanogenic bacteria which have been isolated in pure culture cannot ferment carbohydrates or amino acids, and they obtain their energy from the end-products of metabolism of cellulolytic and amylolytic organisms, notably formic acid, methanol and ethanol. If the reason for the depression of $CH_4$ production had been a reduction in the amount of fermentable substrate which the methanogenic organisms could use, that is a reduced rate of cellulolysis, then $CH_4$ production and cellulose digestion should have fallen by equal relative amounts.

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