The heat increment of steam-volatile fatty acids in fasting sheep

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The heat increment of feeding represents the additional heat produced by an animal consequent upon the ingestion of food. In man and dog it is usually called the specific dynamic effect (S.D.E.), or less correctly the specific dynamic action (S.D.A.), and is measured by giving the fasting subject an amount of food containing sufficient energy to meet the basal requirement and by determining the increase in heat production which results. This procedure entails the continuation of measurements until such time when metabolism is again basal. It was shown by Glickman, Mitchell, Lambert & Keeton (1948) that often insufficient time was allowed in such studies to permit the complete metabolism of the food, hence many estimates of the S.D.E. of food by this technique are too low. In farm animals the technique employed by Kellner & Köhler (1900) and by Fingerling (1914) to estimate heat increments was to give individual foods or nutrients as additions to a ration which already permitted small positive retentions of energy and to measure the increase in the 24 h heat production which resulted. The two methods do not give identical results since heat increment is smaller when fasting and not maintenance is the base-line, as has been shown in many experiments with a variety of species. Between-species comparisons of the heat increment of feeding must clearly be made at similar nutritional planes.

In Table 1, the results of published experiments are presented which allow an estimate to be made of species differences in the heat increments of feeding. It will be noted that data are not available for man at maintenance levels of feeding.

Table 1. *Heat increment* (*Cal.*/100 *Cal. metabolizable energy*) *of feeding in different species evaluated with fasting as the base-line* (S.D.E.) *and with maintenance as the base-line*

	Starvation base-line			Maintenance base-line				
Nutrient	Man[1]	Rat[2]	Sheep	Man	Rat[2]	Pig[3]	Steer[4]	Sheep[5]
Fat	4	6	—	—	17	9	35	29
Carbohydrate	6	9	7[6]	—	23	17	37	32
Protein	30	25	—	—	31	26	52	54
Mixed rations	10–17[7]	~17[8]	~22[9]	—	~31[8]	10–40[10]	35–70[10]	35–70[11]

(1) Lusk (1931).
(2) Kriss, Forbes & Miller (1932).
(3) Fingerling (1914).
(4) Kellner & Köhler (1900).
(5) Heim (1946).
(6) Armstrong & Blaxter (1956).
(7) Glickman *et al.* (1948).
(8) Forbes, Kriss & Miller (1934).
(9) Blaxter & Graham (1955).
(10) Werner & Francke (1953).
(11) Blaxter & Graham (1956).

The data of Table 1 show that above maintenance the ruminant animal loses approximately twice as much heat in metabolizing the carbohydrate of its diet as does the non-ruminant rat and pig. With mixed diets the increments of heat range from 35 to 70% in the ruminant but from only 10 to 40% in the pig. Comparisons of the S.D.E. in the fasting animal are difficult to make, but it appears that here also the ruminant animal loses more energy as heat compared with the non-ruminant rat and man. The large effect of nutritional plane is very evident.

There have been several attempts to explain the high heat increment of the ruminant. Kellner (1920), following the work of Zuntz, ascribed it to the work of digestion (*Verdaungsarbeit*). He took it to include not only intestinal work in the sense originally used by von Mering & Zuntz (1877), but the mechanical work of prehension and mastication of food. The extent of this energy expenditure has not been measured directly. Earlier, the high heat increment had been ascribed to a stimulating effect of the steam-volatile fatty acids on metabolism (Grouven, 1864). This theory was revived by Ritzman & Benedict (1938) only to be rejected since they could not reconcile the high heat increments found in the non-ruminant but herbivorous horse with a stimulation of metabolism by the lower steam-volatile fatty acids, not realizing, perhaps, the massive nature of microbial fermentation in the large intestine of that species.

More recently, attempts have been made to explain the high heat increments of ruminants in terms of the thermodynamics of the intermediary metabolism of the end-products of ruminant digestion. Marston (1948*a*) suggested that the transfer of at least some of the absorbed fatty acids into the cycles of intermediary metabolism involved considerable thermodynamic losses. He showed also (Marston, 1948*b*) that the heat of fermentation, that is the free energy of the microbial fermentation, could not account for more than 15% of the total heat increment in the ruminant. He later stated that heat loss occurring in the dissimilation of acetic acid 'is probably responsible for a major part of the relatively high rate of heat production of the fed ruminant' (Marston, 1951).

McClymont (1952) expanded Marston's contention that inefficiency of free-energy capture in the dissimilation of acetic acid was the main reason for the high heat increment of the ruminant, and suggested that a high heat increment of acetic acid and of butyric acid might be inferred from known facts about their intermediary metabolism. He suggested that very rapid oxidation in non-lipogenic tissue was the main reason for the high heat increment, and that two-carbon units arising from acetic acid or butyric acid undergo a continuous partition between oxidation and lipogenesis, the former being, thermodynamically, a wasteful process.

To arrive at this hypothesis, McClymont had perforce to make certain assumptions. The first one was that the metabolism of the steam-volatile fatty acids in the ruminant, particularly of acetic and butyric acids, is indeed associated with very large losses of heat. He quoted unpublished results of Marston's which suggested that the heat increment of acetic acid was 70%, and of propionic acid 30%, and adduced the rather unsatisfactory experiments of Lusk (1921) in which an increase in the heat production of a dog was noted on the giving of 3 g acetic acid. He quoted also the results of the experiments of Dye & Marsters (1943) with nephrectomized dogs, who had found that

when sodium acetate was injected intravenously the excess oxygen consumption was equivalent to 49% of the theoretical amount needed to oxidize the acetate completely. A lower value of 39% was obtained in the nephrectomized eviscerated animal. With butyric acid the value was 26% in both types of animal preparation. McClymont (1952) concluded from this work that the S.D.E. of acetic and butyric acids could be placed at 50–70% and that the resulting heat increment due to their metabolism would account for a major proportion of the total heat increment, reported as 30–60% of the metabolizable energy. This conclusion, however, is difficult to reconcile with the results of calorimetric experiments with rats by McManus, Bender & Garrett (1943) in which acetic acid fed in the form of triacetin constituting 20% of the diet was used as efficiently as a source of energy as a mixture of glycerol and glucose.

With the possible exception of Marston's work, details of which are not available, none of the above experiments appeared to us to provide an unequivocal proof that the metabolism of the end-products of rumen fermentation is in fact associated with large losses of heat under normal conditions, whereas the experiments of McManus *et al.* (1943) suggest the contrary. For this reason the contribution of the steam-volatile acids to the heat increment of sheep has been studied in some detail by indirect methods of calorimetry. This paper primarily deals with the heat increments found in starved sheep when acetic, propionic and *n*-butyric acids were given singly. Subsequent papers will deal with the more physiological experiments in which a range of steam-volatile acid mixtures was given to fasted and fed sheep and in which marked associative effects were found.

EXPERIMENTAL

Animals. Three adult castrated male sheep (Halfbred × Down) were used as experimental animals. A fistula into the rumen was made by the technique of Phillipson & Innes (1939) and of Stoddard, Allen, Hale, Pope, Sorenson & Winchester (1951), and the fistula closed with a cannula of Perspex. These animals were kept in pens and fed *ad lib.* until 10 days before an experiment began. They were then placed in cages and given a ration sufficient to maintain their body-weight.

Plan of experiments. Each of the three sheep was given acetic, propionic or butyric acid to supply approximately 700 Cal. combustible energy daily. Each was also given a mixture of acetic, propionic and butyric acids in the molar proportions of 5:3:2 to supply 1100 Cal. Four further experiments were made. Two were with acetic acid, one with a mixture of the acids to supply 600 Cal. and one with a glucose solution supplying 600 Cal. combustible energy. With the exception of the last two experiments with acetic acid the experiments all conformed to one plan. The sheep was starved for 4 days during which dilute saline was dripped into the rumen usually at the rate of 6·5 l./day. During the next 2 days a fatty-acid solution containing the same amount of saline was given under identical conditions. The sheep then reverted to the dilute-saline regimen for 2 days. A second fatty-acid solution was then given for 2 days and the experiment ended with a 2-day period in which the dilute saline was again given. In one experiment the second fatty-acid solution was replaced by a glucose solution containing penicillin. The antibiotic was added to reduce possible rumen bacterial

activity. The total period without solid food was thus 12 days, the whole period being spent within a respiration chamber under controlled ambient conditions. In the last two acetic-acid runs the animals received dilute saline for the first 4 and last 3 days of a 10-day experiment and acetic acid during the intervening 3 days. At the end of each experiment the rumen of the sheep was inoculated with ingesta from a normal sheep's rumen and the animal allowed limited food for a few days before being given unlimited access to food. Recovery was usually uneventful. At least 4 weeks were allowed between experiments with any one individual. Under this system of management of the animals there was no cumulative loss of condition in the series of experiments. The average loss of body-weight during a 12-day experiment was 6·6 kg with individual losses ranging from 5·3 to 10·0 kg. Part of this loss must be accounted for as loss of ingesta from the gut.

Steam-volatile fatty-acid mixtures. In much of the previous work on the metabolism of the steam-volatile fatty-acids, the acids have been given in buffered solutions as the sodium salts. If continued, such administration gives rise to a marked systemic alkalosis (cf. Johnson, 1955) and is clearly not a suitable method to use if CO_2 production is to be measured over long periods. Elsden & Phillipson (1948) have mentioned that acetic acid could be dripped into the rumen at the rate of 4–5 g/h (up to 2 g-equiv./24 h) with no change in the alkali reserve of the blood. Preliminary experiments with other fistulated sheep showed that the mixed acids themselves could be infused into the rumen without producing any gross acidosis, in amounts which supplied up to 1100 Cal. gross energy daily, and that there was no necessity to buffer the solutions provided they were dilute. Accordingly, all steam-volatile acid mixtures were given in dilute solution, to which was added an equal volume of a neutral physiological saline solution, containing per l., 6 g NaCl, 0·4 g KCl, 0·2 g $MgCl_2$, 0·2 g $CaCl_2$ and 0·45 g NaH_2PO_4.

Table 2 summarizes the data relating to the steam-volatile fatty-acid solutions used.

Continuous-drip feed pump. For such relatively long-term experiments it was thought necessary to administer the solution to the animals at a steady rate throughout rather

Table 2. *Characteristics of solutions of steam-volatile fatty acids and glucose used in the experiments**

Substance	Approx. volume of solution supplied/24 h (l.)	Normality	Moles supplied/24 h	Energy supplied/24 h (Cal.)
Acids:				
Acetic	6·5	0·515	3·35	700
Propionic	6·5	0·293	1·91	700
Butyric	6·5	0·206	1·34	700
Mixture of the three in the molar proportions 5:3:2	6·5	0·529	3·44	1100
Mixture (same)	4·2	0·448	1·88	600
Glucose	4·2	38·2 g/l.	0·89	600

* The values for the number of moles and the energy supplied varied slightly from experiment to experiment, depending upon the output of the pumps used to introduce the solutions into the rumen.

than in large amounts at intervals. To introduce the acids a pump was found necessary since gravity-flow systems were not found to be satisfactory. It consisted of a slow-speed motor (Drayton Regulator and Instrument Co. Ltd), geared to operate the plungers of two Record syringes as pistons. The liquid flow from the reservoir to the chamber via the syringes was controlled by two sets of Bunsen valves made from plastic tubing (Vinyl 'Portex', laboratory grade 6c, Portland Plastics Ltd). Rubber tubing was unsatisfactory since it lost its elasticity in the presence of the acids. By alteration of the size of the syringes, the stroke of the pistons or the reduction gearing, a very wide range (1–30 l./24 h) of fluid output could be obtained. The outflow from the pump was connected to a tube leading into the rumen by drainage tubing (Vinyl 'Portex' drainage tubing 6HD, Portland Plastics Ltd) which first passed through a seal in the side of the respiration chamber and then over a series of pulleys and counterpoise weights that allowed the animal to lie and stand at will. The tube into the rumen was protected by an outer sheath of Polythene tubing and was so constructed that the acid outlet was unlikely to be restricted and the acid leaving it unlikely to come into direct contact with the rumen wall without prior dilution by rumen contents.

The output of the pump was very constant from day to day but in practice the 24 h input was always measured gravimetrically. Two such pumps have been in operation for nearly 2 years without any mishap. The valves are renewed periodically and the pistons greased but otherwise maintenance is negligible. Pl. 1, 1, shows a fistulated sheep in the respiration chamber, Pl. 1, 2, a photograph of the pump and Pl. 1, 3, the internal lead into the sheep's rumen.

Calorimetric methods. Two respiration apparatuses were used to determine the CO_2 production, O_2 consumption and CH_4 production of the sheep. Measurements were made at 12 h intervals throughout except in the final two experiments with acetic acid, when the interval was 24 h. Urine was collected quantitatively at the same intervals. The analytical and other methods employed in the respiration experiments were those previously described (Blaxter, Graham & Rook, 1954). The computations involved are discussed in the text.

Sampling of blood and rumen liquor. Blood samples from the jugular vein were withdrawn daily, heparin being used as anticoagulant. Rumen-liquor samples were also taken daily via the fistula every 24 h.

Analysis of rumen liquor. After filtration through glass wool the total steam-volatile acid content was determined by the method of Elsden, Hitchcock, Marshall & Phillipson (1946). A further sample collected under liquid paraffin was used for pH estimation with the glass electrode. In some experiments samples were examined qualitatively by direct microscopy by Dr Constance Higginbottom.

Analysis of blood. Blood sugar was determined by the method of Nelson (1944) with the copper reagent of Somogyi (1945). The CO_2-combining capacity of plasma was determined after equilibration with a 5% CO_2-air mixture by the method given by Peters & Van Slyke (1932). Ketone bodies were determined by a modification of the method of Thin & Robertson (1952). Proteins were first precipitated with trichloroacetic acid as suggested by El Hawary & Thompson (1954). The colour reagent

comprised 0·1 ml. salicylic aldehyde in 8 ml. 4N-KOH. After incubation at room temperature overnight, 0·5 ml. of the coloured solution was added to 4 ml. water and the resulting solution read in a Unicam Spectrophotometer at a wavelength of 540 mμ, in 1 cm cells. The total steam-volatile acid content was determined by steam distillation of the whole blood by the method of Scarisbrick (1952); 0·1 ml. silicone (M.S. Antifoam A, Hopkin & Williams Ltd, Chadwell Heath, Essex) in carbon tetrachloride, 4% (w/v) was used as an antifrothing agent, 4 ml. blood were used in each estimation and three 40 ml. volumes of distillate collected.

Analysis of urine. N was determined by Kjeldahl's method with $CuSO_4$ and Se as catalysts. Ketones were determined initially by the method of Greenberg & Lester (1944) as modified by Pugh (1954) and later by that used for the determination of ketone bodies in blood.

RESULTS

The results are presented first for the twelve experiments with the three acids given separately and as a mixture. Results for the remaining four experiments are given in less detail.

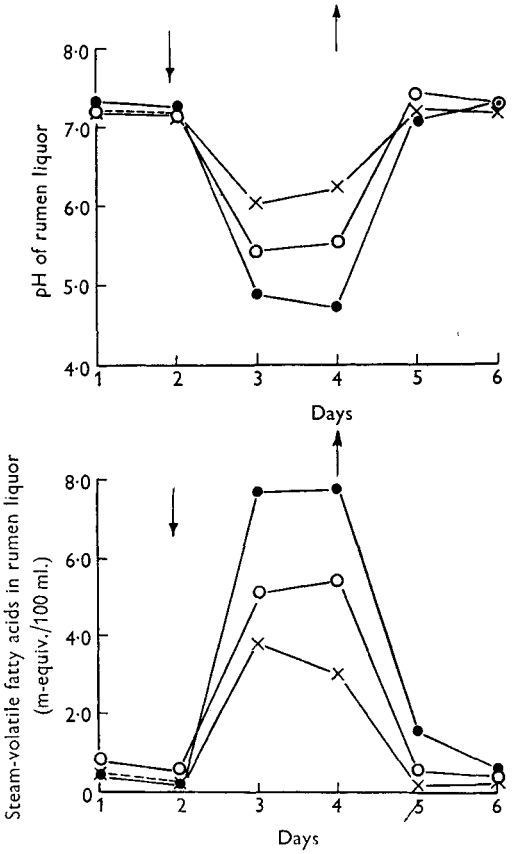

Fig. 1. Mean pH and mean concentration of steam-volatile fatty acids in rumen liquor of fasting sheep as affected by the infusion of 700 Cal. energy as acetic, propionic or *n*-butyric acid. ●—●, acetic acid; ○—○, propionic acid; ×—×, butyric acid; ↓, beginning of infusion; ↑, end of infusion.

Conditions within the rumen. As was to be expected from the large fluid intake used in these experiments the character of the rumen contents changed markedly as starvation proceeded. By the end of the 4th day the contents, although still green, contained relatively little vegetable debris and samples drawn at later stages were colourless and contained many epithelial cells. The bacterial count showed a steady decline throughout and the flora was a much simplified one, being largely composed of short rods and cocci. The reduced activity of the rumen flora by the end of the 4th day of starvation was indicated by the fact that during the previous 24 h the mean total methane production per animal amounted to 2·03 l. as compared with the 26–28 l. produced by a fed animal. The mean production of methane during the 12th day of starvation was 0·58 l.

In Fig. 1, the mean results for the pH and steam-volatile acid concentration in rumen liquor are given. It will be noted that for equal calorie intakes as steam-volatile acid the depression of the rumen pH and the elevation of its steam-volatile acid content was greatest for acetic acid and smallest for butyric acid, that is, in proportion to the number of hydrogen ions supplied. The mean values obtained at the end of 24 and 48 h for the rumen concentration of steam-volatile acid and the pH at 48 h are shown in Table 3.

Table 3. *Concentration of steam-volatile fatty acids in rumen liquor of fasting sheep, receiving infusions of steam-volatile fatty acids into the rumen, 24 and 48 h after the beginning of infusion, and rumen pH at 48 h*

(Mean values for three sheep)

Acid infused	Actual amount supplied (moles/24 h)*	Concentration of steam-volatile fatty acids in rumen (m-equiv./100 ml.) After 24 h	After 48 h	Rumen pH after 48 h
Acetic	3·32	7·67	7·73	4·73
Propionic	1·94	5·41	5·44	5·54
Butyric	1·32	3·85	3·03	6·24
Mixture of the three in the molar proportions 5:3:2	3·49	6·80	6·72	5·37
None	None	—	0·01–0·04	6·9–7·4
Standard error of means†	—	±0·39	±0·39	±0·12

* Values in this column are actual intakes. They differ slightly from those given in Table 2 owing to the variable output of the infusion pumps.

† The standard error of differences between the concentration of the acids at the 24th and 48th h obtained from the acids × days component in the analysis of variance was ±0·36.

There were no significant differences between values for steam-volatile acid concentration obtained at the 24th and 48th hour. The acids must, therefore, have been absorbed, and the concentration noted at the 48th hour represented a steady-state relationship in which the amount leaving the rumen was equal to the amount infused. The differences in concentration of the acids and the pH ascribable to the effects of the acid infused were highly significant statistically. Even the highest values observed for concentration of steam-volatile fatty acids are not abnormal and are in fact lower

than many values recorded in the literature for sheep on natural foods (Phillipson, 1942; El-Shazly, 1952; Johns, 1955).

Prestarvation pH values in these sheep were in the region of 6·0–6·5. The increase in rumen pH to 6·9–7·4 on starvation is in agreement with the results of Phillipson (1942). A rumen pH of 4·7 is low but values as low have been recorded in sheep in which fermentations giving rise to a fair proportion of lactic acid occurred (Phillipson, 1952; Hungate, Dougherty, Bryant & Cello, 1952). It is remarkable, however, that in the absence of the natural buffers of food and in the absence of the stimulus of solid food on the secretion of bicarbonate into the rumen via the saliva, the pH was not more seriously depressed by the acid infusion. It may be concluded that the steam-volatile acids may be given to supply 700–1100 Cal./day without any serious accumulation in the rumen.

Fermentation of acids. Despite the fact that the sheep were starved, the rumen still contained micro-organisms and some methane was produced. Methane production is a good indication of the extent to which bacterial fermentation is proceeding within the rumen and the experiments thus provide information on whether or not the acids are further degraded with the production of methane. In Fig. 2, the mean production of methane by the sheep receiving the individual acids is shown. It is clear that in none was there an increase in the production of methane, which provides support for the finding of Beijer (1952) that solutions of the sodium salts of these acids are not fermented in vitro by rumen bacteria.

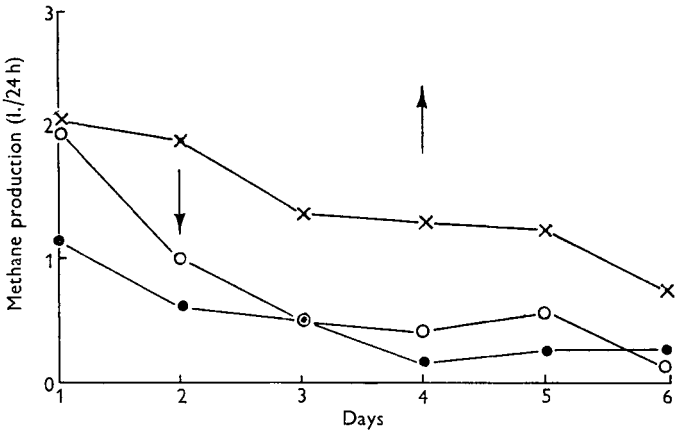

Fig. 2. Methane production of fasting sheep as affected by the infusion of 700 Cal. energy as acetic, propionic, or *n*-butyric acid. ●—●, acetic acid; ○—○, propionic acid; ×—×, butyric acid; ↓, beginning of infusion; ↑, end of infusion.

Composition of the blood. Fig. 3 summarizes the observations made on the concentration of glucose, steam-volatile fatty acids and ketone bodies in the whole blood, and the CO_2-combining capacity of the plasma. The mean values obtained 48 h after the beginning of the infusion are shown in Table 4, together with their standard errors.

From the concentration of steam-volatile acids given in Table 4 it is clear that the infusion of acetic acid resulted in a very considerable accumulation in peripheral blood. There were small, but nevertheless statistically significant, increases with butyric acid and with the mixture but no change at all with propionic acid. Annison (1954) found the steam-volatile acid content of venous (jugular) blood from fed sheep to range from 0·056 to 0·143 m-equiv./100 ml. After the sheep had fasted for 48 h

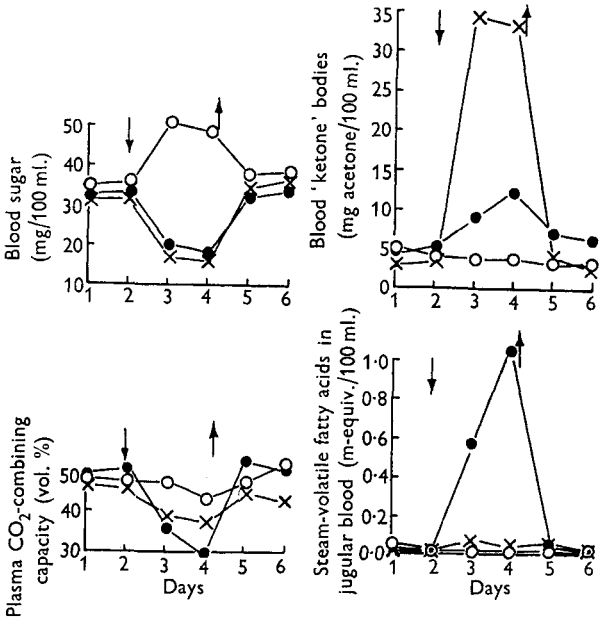

Fig. 3. Concentration of sugar, 'ketone' bodies and steam-volatile fatty acids in whole blood, and the CO_2-combining capacity of the blood plasma, of fasting sheep as affected by the infusion of 700 Cal. energy as acetic, propionic or *n*-butyric acid. ●—●, acetic acid; ○—○, propionic acid; ×—×, butyric acid; ↓, beginning of infusion; ↑, end of infusion.

Table 4. *Concentration of sugar, ketone bodies and steam-volatile acids in the jugular blood, and the CO_2-combining capacity of the blood plasma of fasting sheep receiving infusions of steam-volatile fatty acids into the rumen*

(Mean values for three sheep 48 h after the beginning of infusion)

Acid	Steam-volatile fatty acids* (m-equiv./100 ml.)	Sugar (mg/100 ml.)	Ketone bodies (expressed as acetone) (mg/100 ml.)	CO_2-combining capacity of plasma (vol. %)
None (starvation values before infusion)	0·014 ± 0·002	33·5	4·1	47·4
Acetic	1·043 ± 0·031	17·7	12·1	29·6
Propionic	0·013 ± 0·002	48·1	3·6	42·3
n-Butyric	0·049 ± 0·004	16·1	33·2	37·0
Mixture of the three in the molar proportions 5:3:2	0·056 ± 0·003	45·1	3·6	41·0
Standard error of means	—	± 2·12	± 1·51	± 1·18

* Separate standard errors are given for the steam-volatile fatty acids since the mean concentration when acetic acid was given was almost 100-fold that observed when the others were supplied.

the values had fallen to below 0·03 m-equiv./100 ml. Reid (1950*b*) reported arterial (carotid) blood values from fed sheep ranging from 0·102 to 0·195 m-equiv./100 ml., and when allowance is made for the considerable arterio-venous differences that exist (these may be of the order of 50% in individual animals (Reid, 1950*b*; Annison, 1954), it can be seen that the venous concentrations are similar to those obtained by Annison. There is little doubt that when acetic acid was given alone the very high level of steam-volatile acid in jugular blood represented a considerable departure from normality and was hardly compatible with McClymont's (1952) contention that acetic acid is very rapidly oxidized. On the assumption that the acid is distributed throughout the whole of the body water and that the venous concentration of steam-volatile acid represents the concentration of acetic acid in this water, some 18 g acetic acid are present in the tissues at any one time. This is clearly a minimal estimate. No comparable accumulation of propionic acid was found and with *n*-butyric acid the amount in the tissues, on a similar assumption, was approximately 150 mg.

These results suggest that when acetic acid is given alone it is not metabolized particularly rapidly, whereas *n*-butyric acid and propionic acids are. When the mixture containing 1·72 g-equiv. acetic acid was given, no accumulation of volatile fatty acid in the blood occurred, which suggests that the presence of either propionic or butyric acids or both, acting synergistically, facilitates the metabolism of acetic acid. From Table 3 it can be seen that administration of acetic or *n*-butyric acid resulted in a highly significant depression of the blood glucose, whereas propionic acid and the mixture produced a rise in sugar levels to normal and above. The implications of these results are dealt with in the discussion.

From the blood-ketone values reported in Table 3, it is apparent that *n*-butyric acid and, to a lesser extent, acetic acid, caused a highly significant increase in ketone bodies. Jarrett, Potter & Filsell (1952), Johnson (1955) and Clark & Malan (1956), among others, have demonstrated in vivo the ketogenic nature of the C_4 acid for ruminants. The experiments of Pennington (1952) and of Seto, Tsuda & Umezu (1955) have shown that the rumen epithelium is a major site for the conversion of butyric acid to ketone bodies. The finding of a smaller, but nevertheless significant, rise in blood ketones with acetic acid is in agreement with the results of Jarrett & Potter (1950), Jarrett *et al.* (1952) and Clark & Malan (1956). All the above-mentioned workers have demonstrated the antiketogenic nature of propionic acid. The absence of an increase in blood ketones after the infusion of the mixed steam-volatile acids is further confirmation of the antiketogenic nature of the C_3 acid.

As might be expected, the CO_2-combining capacity of the venous plasma was reduced when acetic acid was given. The reduction was from 50 vol. % CO_2 to 29·6. It has already been noted that the rise in concentration of steam-volatile fatty acids in the blood was 1 mg-equiv./100 ml. and, since 1 mg-equiv. $CO_2 = 22{\cdot}4$ ml. CO_2 (the carbon dioxide is held as the bicarbonate), it would appear that the major cause of the acidosis was the peripheral accumulation of acetic acid. There was, however, concomitantly an increase in the ketones of the blood, and clearly these would account in part for the depression of the CO_2-combining capacity. With butyric acid a slightly less severe acidosis developed. Analogous reasoning suggests that the major cause

was accumulation of ketones, presumably β-hydroxybutyric acid and acetoacetic acid (Clark & Malan, 1956), in the blood stream rather than the accumulation of the unoxidized acid. The considerable production of acetoacetic acid from *n*-butyric acid in rumen epithelium was shown by Pennington (1952). With propionic acid there was no ketosis and no accumulation of the acid in the blood. The small drop in the CO_2-combining capacity of the plasma that occurred during the 2nd day of acid infusion may have been due to the accumulation of lactic or pyruvic acids which were not estimated in these experiments. A rapid rise in the blood levels of these two acids consequent upon the injection of propionate into the blood stream of sheep was noted by Jarrett & Potter (1950), and Pennington & Sutherland (1954) have found that when propionate is metabolized alone by sections of sheep-rumen epithelium, lactic acid is

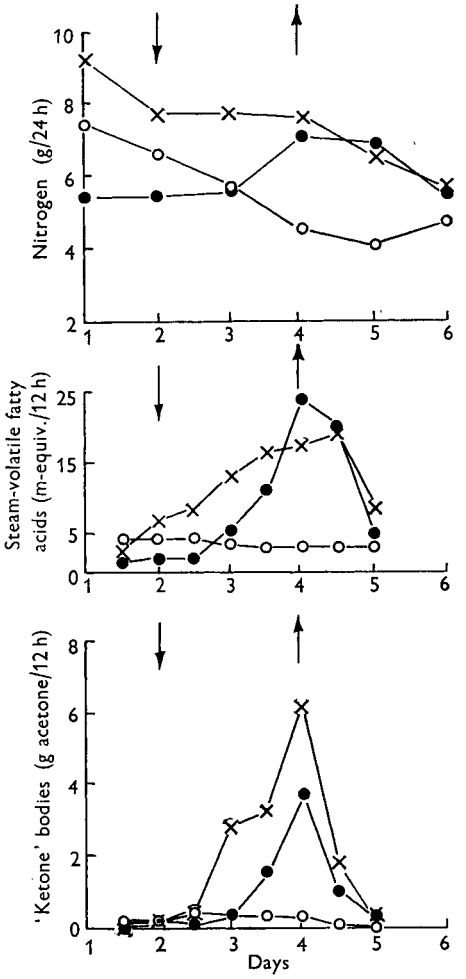

Fig. 4. Quantities of nitrogen, 'ketone' bodies and steam-volatile fatty acids excreted in the urine of fasting sheep as affected by the infusion of 700 Cal. energy as acetic, propionic or *n*-butyric acid. ●—●, acetic acid; ○—○, propionic acid; ×—×, butyric acid; ↓, beginning of infusion; ↑, end of infusion.

produced in amounts which account for between 30 and 50% of the propionate disappearing.

Composition of the urine. In Fig. 4 the mean values are shown for the nitrogen and steam-volatile acid content of urine for the three sheep, and of urine 'ketone' bodies for sheep P, when the single acids were infused into the rumen.

The excretion of nitrogen in the urine rose markedly when acetic acid was given and fell when the C_3 acid was supplied. The summarized data for N excretion given in Table 5 show that the difference between the two acids was statistically significant. With *n*-butyric acid no significant change in nitrogen excretion occurred. These results indicate a marked protein-sparing effect of propionic acid but a considerable increase in protein katabolism with acetic acid. The administration of the C_2 and C_4 acids resulted in a significant increase in urine content of the steam-volatile acids. The loss of these acids from the body via the kidney represented a very small proportion of the acid intake. Thus with acetic acid some 1670 m-equiv. of acid were introduced into the rumen each 12 h and yet the maximal excretion did not exceed 25 m-equiv., or 1·5%.

Table 5. *Changes in excretion of nitrogen (g/24 h) by fasting sheep receiving infusions of steam-volatile fatty acids into the rumen*

(Mean values for three sheep during the last 24 h of infusion)

Acid	Mean increase (+) or decrease (−) in N excretion (g/24 h)	Odds that change significantly different from zero
Acetic	+1·74	13:1
Propionic	−1·09	5:1
n-Butyric	+0·08	—
Mixture of the three in the molar proportions 5:3:2	−1·02	3:1
Standard error of means	±0·50	—
Significant difference, $P=0·05$	±1·97	—

The amounts of ketones contained in the urine reflect the changes that occurred in the ketone content of the blood as a result of the acid infusions. Thus the ketonuria was more pronounced with the C_4 than the C_2 acid and with the C_3 acid there was no change.

Respiratory metabolism. In Fig. 5 the O_2 consumption and CO_2 production of the three sheep given the three steam-volatile fatty acids are given, together with the mean respiratory quotients. It is clear that ingestion of acetic acid to supply 700 Cal./day resulted in a greater increase in O_2 consumption and in CO_2 production than did the ingestion of either propionic acid or *n*-butyric acid.

In order to determine the heat increments of food by indirect calorimetry the usual procedure is to use the non-protein respiratory quotient to arrive at that proportion of the O_2 consumed needed for the complete dissimilation of fat and that needed for dissimilation of carbohydrate. In the present experiments, when the infusions of acids were begun after 4 days of starvation following feeding at a maintenance level, the non-protein respiratory quotients were all close to 0·7, indicating that metabolism

of glycogen reserves and any carbohydrate reaching the tissues from the gut was negligible, and that the sheep were using body fat and protein as the sole sources of energy. The calorific value of O_2 under these conditions was taken to be 4·69 Cal./l. An increase in the R.Q. during an experiment, in which a steam-volatile acid is given,

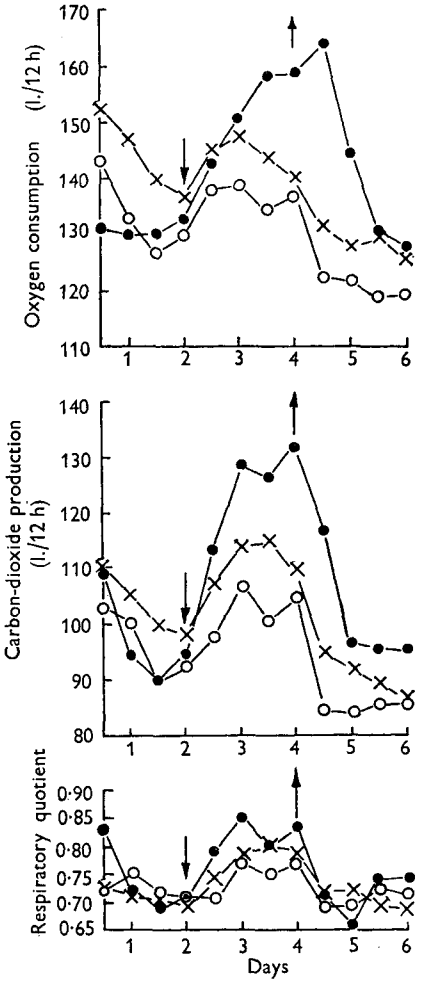

Fig. 5. O_2 consumption, CO_2 production and uncorrected respiratory quotients of fasting sheep as affected by the infusion of 700 Cal. energy as acetic, propionic or *n*-butyric acid. ●—●, acetic acid; ○—○, propionic acid; ×—×, butyric acid; ↓, beginning of infusion; ↑, end of infusion.

to a value approaching 0·8 does not, however, for several reasons necessarily mean that the calorific value of O_2 increases by 2·5% to a value of 4·80 as might be inferred from the Zuntz-Schumberg tables as modified by Lusk (1931). Firstly, as shown in Table 6, for complete dissimilation of acetic acid, although the R.Q. is 1·00, the same as for carbohydrate, the calorific value of O_2 is not 5·047 but 4·674 Cal./l., that is 7% less. Secondly, it has been shown that acetic acid by accumulating in the tissues leads to acidosis and a loss of bicarbonate in the plasma. This additional CO_2 is not

to be considered in computing a non-protein respiratory quotient. The same arguments apply to the C_3 and C_4 acids. Finally, when butyric acid is administered any urinary excretion of ketone bodies, implying only a partial oxidation of the C_4 acid means that oxygen has been consumed without a concomitant loss of CO_2 and the calorific value of this oxygen is not known though it is obviously small. A calculation of the calorific value of the oxygen so used can be made by first assuming complete combustion of butyric acid and then deducting the heat of combustion of the acetoacetic acid formed from it.

Table 6. *Calorific value of O_2 in the dissimilation of fat, carbohydrate and the steam-volatile fatty acids, and overall O_2 consumption and CO_2 production associated with other reactions in which the steam-volatile acids may participate*

Variable	Heat of combustion or of reaction (Cal./mole)	O_2 consumed (l./mole)	CO_2 produced (l./mole)	R.Q.	Calorific value of O_2 (Cal./l.)	Reference
			Complete dissimilation			
Tripalmitin	7657	1623·5	1143·1	0·703	4·716	Lusk (1931)
Mixed fat	—	—	—	0·707	4·686	Lusk (1931)
Starch	680·4	134·4	134·4	1·000	5·063	Swift & French (1954)
Mixed carbohydrate	—	—	—	1·000	5·047	Lusk (1931)
Acetic acid	209·4	44·8	44·8	1·000	4·674	Calculated from heat of combustion and chemical composition
Propionic acid	367·2	78·4	67·2	0·857	4·683	
n-Butyric acid	524·3	112·0	89·6	0·800	4·681	
			Other reactions			
Release of CO_2 from bicarbonate due to systemic acidosis	None	None	22·4	—	—	—
Oxidation of *n*-butyric acid to acetoacetic acid	?	22·4	None	—	?	—
Glucogenesis from propionic acid	?	11·2	None	—	?	—

The method finally adopted to calculate heat productions during the acid runs was to first compute the O_2 consumption, CO_2 production and urinary-N excretion to be expected in the experimental periods. This computation was done by fitting quadratic regressions to the results obtained on the 3rd, 4th, 8th and 12th days of starvation, when no acids were given (eight points) and interpolating to obtain values for the 5th, 6th, 9th and 10th days when the acids were supplied. From these interpolated values heat production was computed by the use of the non-protein R.Q. and the tables of Zuntz & Schumberg (Lusk, 1931).

The actual O_2 consumption and CO_2 production during the periods when the acids were infused were corrected for protein metabolism in the usual way. The non-protein R.Q.'s were not, however, calculated but, instead, each l. of O_2 consumed was assumed to be associated with the production of 4·7 Cal. This method is justified since the non-protein R.Q.'s obtained during the periods of acid infusion result from the oxidation of mixtures of fat and fatty acids, and the calorific values/l. of O_2 for fat and the three

fatty acids differ by less than 0·5% (see Table 6). The errors resulting from a neglect of the respiratory quotients are negligible.

This method of calculation of heat production when the acids were given, by ignoring the CO_2 production, effectively minimizes errors that might arise owing to loss of bicarbonate from the tissues during the acidosis. Results for sheep P with this method are given in Table 7.

Table 7. *Heat production in experiments with sheep P when acetic, propionic or butyric acids were given separately, or their mixture in the molar proportions* 5:3:2, *by infusion into the rumen*

Acid given	Twelve-hour period of experiment	Base-line of heat production* (Cal./24 h)	Heat production in experimental period (Cal./24 h)	Increase in heat production (Cal./24 h)
Acetic	1	1269	1345	76
	2	1271	1572	301
	3	1276	1514	238
	4	1282	1514	232
Propionic	1	1160	1214	54
	2	1151	1263	112
	3	1143	1245	102
	4	1136	1263	127
Butyric	1	1276	1444	168
	2	1256	1437	181
	3	1238	1316	78
	4	1221	1331	110
Mixture	1	1354	1527	173
	2	1326	1518	192
	3	1310	1410	100
	4	1297	1411	114

* Calculated by interpolation of CO_2 production, O_2 consumption and urinary N excretion in starvation periods when no acids were given.

In Table 8, the mean increments of heat in Cal./24 h from each of the three sheep are given. The values obtained for the first 12 h period of acid infusion were not used in computing the mean increments. With butyric acid allowance was made for the oxygen used in oxidizing the acid to acetoacetic (see footnote 1, Table 8). In order to compute the calorie equivalent of the acids completely oxidized within the body, the calorie equivalent of the weight of acid infused was corrected for the amount of acid accumulated in the tissues (see footnote 2, Table 8), the amount of acid excreted in the urine (see footnote 3, Table 8) and the calorie equivalent of the quantity of ketone bodies excreted (see footnote 4, Table 8).

The results given in Table 8 show that acetic acid had the highest heat increment and that propionic acid and *n*-butyric acid had significantly lower ones. The last line of Table 8 shows that the increment of heat as a percentage of the calories metabolized was 41% for acetic acid, 13% for propionic acid and 16% for *n*-butyric acid. The value obtained for acetic acid is considerably lower than the value reported by McClymont (1952) from the unpublished results of Marston in which the introduction of 70 g

acetic acid into a sheep resulted in a rise in heat production approximately equivalent to two-thirds of the energy in the acetic acid. It is, however, similar to that obtained by Dye & Marsters (1943) from the increase in O_2 consumption when sodium acetate was injected into nephrectomized eviscerated dogs. The value for butyric acid is considerably lower than that obtained by the last-mentioned workers.

A mixture of acetic, propionic and butyric acids in the molar proportions of 5:3:2 would be expected to give a heat increment of 27%. The observed value was 17%, which is considerably lower. Furthermore, the level of intake of the steam-

Table 8. *Mean heat increments (Cal./24 h) of each of three sheep receiving infusions of steam-volatile fatty acids into the rumen, and calculation of heat increment in terms of metabolized fatty acids*

	Acetic acid (Cal./24 h)	Propionic acid (Cal./24 h)	Butyric acid (Cal./24 h)	Mixture of the three in the molar proportions 5:3:2 (Cal./24 h)	Standard error of means
Sheep P	257	114	123	135	±27·6
Sheep T	220	83	95	222	
Sheep S	288	90	112	209	
Mean	255·1	95·7	110·4	188·7	±33·3*
Heat attributed to O_2 used in oxidizing butyrate to acetoacetate†	0	0	11·8	0	—
Corrected heat increment	255·1	95·7	98·6	188·7	—
Calorific value of acid infused	694	711	691	1115	—
Correction:					
Tissue accumulation‡	35	0	0	0	—
Urinary excretion of fatty acids§	7	0	18	0	—
Unoxidized 'ketone' bodies in urine‖	27	0	52	0	—
Calorific value of acid metabolized	625	711	621	1115	—
Heat increment as percentage of acid metabolized	40·8±5·3	13·5±4·7	15·9±5·4	16·9±3·0	—

* The standard error for the mean values in line 4 (±33·3) was obtained from the sheep × acid interaction term in the analysis of variance. The error applying to results for individual sheep was obtained from the 12 h period × acids × sheep interaction term.

† Calculated from the observed increase in ketone excretion by sheep given butyric acid. Excretion of 0·125 mole acetoacetic acid would be associated with the consumption of 2·8 l. O_2. This quantity of O_2 if full calorific value for dissimilation of butyrate is assumed would give rise to 13·2 Cal. If the heat associated with the oxidation of *n*-butyric acid to acetoacetic acid is taken as 11 Cal./mole, the difference, 13·2 − 1·4 = 11·8 Cal., represents the overestimation of the heat production due to neglect of the ketosis.

‡ Calculated from the increase in the steam-volatile acids in the tissues from the 24th h to the 48th h. It was assumed that the jugular-blood concentration represented the concentration of acid in the whole body water of the sheep which was taken to be 60% of the body-weight. The mean body-weight was 59·0 kg. No correction was necessary for ketone concentration in the tissues as there was no change during the 24 h concerned.

§ The mean increase in the excretion of steam-volatile acid in the urine of sheep given acetic and *n*-butyric acids was 34·0 m-equiv./24 h in both instances.

‖ The mean increases in excretion of ketone bodies were for acetic acid 3·7 g and for butyric acid 7·0 g acetone/24 h.

volatile acid mixture was higher and, as previously pointed out, higher intakes might be expected to result in higher heat increments.

Table 9 shows that there were no significant differences between sheep, though it was thought, in the course of the experiments, that the smallest sheep (S) showed the most pronounced effects. There was no significant variation in the results from 12 h period to 12 h period during the final 36 h of experiment for any of the acids, though here it was suspected that metabolism did not reach a steady state when acetic acid

Table 9. *Analysis of variance of the individual heat increments (Cal./24 h) of each of three sheep receiving infusions of steam-volatile fatty acids into the rumen*

Component	Degrees of freedom	Mean square	Variance ratio	Significance
Total	35	—	—	—
Sheep	2	1465·5	—	NS
Acids	3	49313·9	14·81	**
Sheep × acids	6	3328·2	—	—
Total experiments	11	—	—	—
Within individual experiments	24	—	—	—
12 h periods	2	649·7	—	NS
12 h periods × acids	6	3012·4	1·31	NS
12 h periods × sheep	4	2857·3	1·25	NS
12 h periods × acids × sheep	12	2283·8	—	—

NS = not significant statistically, $P > 0{\cdot}05$.
** = significant statistically, $0{\cdot}01 > P > 0{\cdot}001$.

was given. The continued accumulation of acetic acid in peripheral blood suggests that the metabolism was not, in fact, constant, and it was invariably found that in the first 12 h after the infusion of acetic acid the heat production and oxygen consumption remained elevated. This was not so apparent with propionic and butyric acids, as may be seen in Fig. 5. Presumably, this continued metabolism reflected the gradual reduction of the accumulated acetic acid in the tissues.

Effect of sugar. In a previous paper (Armstrong & Blaxter, 1956) the metabolic effects were presented of a solution of sugar dripped into the rumen of sheep P starved for 8 days and given antibiotics. These results showed that the heat increment due to glucose was 6·4%, a value similar to that found in man and in the rat. Some fermentation of the sugar took place despite precautions to avoid it, but was of no great magnitude.

Further experiments with acetic acid. Two further experiments with sheep S and T were carried out in which acetic acid was given to supply about 700 Cal. (approx. 3·3 moles)/24 h, and, in these, the period of infusion was prolonged to 72 h. Metabolism measurements were made at 24 h intervals instead of 12 h intervals. The results showing the composition of the blood and rumen contents are given in Table 10. The values were similar to those obtained in the earlier series, but the degree of acidosis judged by the alkali reserve was considerably greater in sheep T than it was in the previous experiment with this sheep. With sheep S a metabolic equilibrium of acid accumulation in the blood appeared to have been reached by 24 h when the CO_2-combining capacity of the plasma was 20–25 vol. %. Sheep T had reached an equilibrium of 12–14 vol. % at 48 h.

Table 10. *pH and concentration of steam-volatile fatty acids in rumen liquor, CO_2-combining capacity of plasma, concentration of steam-volatile fatty acids, ketones and sugar in whole blood of two sheep given 3·3 moles acetic acid/24 h for 72 h by infusion into the rumen*

		Preliminary period (days before infusion)		Period of infusion. End of day			Recovery period. End of day		
Variable	Sheep	2	1	1	2	3	1	2	3
pH of rumen liquor	S	7·13	7·44	4·78	4·66	4·44	7·54	7·54	7·45
	T	7·01	7·05	5·00	4·86	4·52	7·41	7·57	7·41
Acids in rumen liquor	S	0·73	0·94	7·10	8·76	9·98	0·82	0·76	0·63
(m-equiv./100 ml.)	T	0·44	0·50	7·54	7·29	8·74	1·13	0·84	0·69
Plasma CO_2-combining capacity (vol. %)	S	41·5	39·0	23·0	20·0	25·0	37·0	36·0	41·0
	T	43·0	43·0	25·0	14·0	12·5	31·0	—	34·0
Blood sugar	S	27·3	28·6	13·1	25·1	29·7	46·2	36·9	36·4
(mg/100 ml.)	T	31·5	28·4	22·2	18·6	26·4	55·9	—	39·5
Total ketones in	S	6·73	5·89	14·80	9·75	31·95	10·76	3·70	9·57
blood (mg acetone/ 100 ml.)	T	6·22	5·55	14·46	14·63	12·11	11·77	—	8·41
Acids in blood	S	0·010	0·013	1·237	1·110	1·210	0·010	0·003	0·010
(m-equiv./100 ml.)	T	0·003	0·003	0·963	1·340	1·710	0·010	—	0·003

The O_2 consumption and urinary N excretion were used to compute the increase in heat production by the method previously outlined, and the calorie equivalent of O_2 was taken to be 4·70 Cal./l. irrespective of the non-protein R.Q. The results are given in Table 11.

Table 11. *Heat production in experiments with sheep S and T when about 700 Cal. acetic acid were given by infusion into the rumen*

Sheep and calorific value of acid given	Period of measurement (h)	Base-line of heat production (Cal./24 h)	Heat production in experimental period (Cal./24 h)	Increase in heat production (Cal./24 h)
Sheep S (705 Cal.)	0–24	1296	1322	26
	24–48	1284	1497	213
	48–72	1255	1544	289
Sheep T (732 Cal.)	0–24	1330	1364	34
	24–48	1330	1586	256
	48–72	1336	1659	323

Table 12 shows that the mean heat increment expressed as a percentage of the acid metabolized in these experiments was 41·1% in excellent agreement with the previous result of 40·8%. Inclusion of the two values obtained in these experiments with the previous two narrows the margin of error attached to the mean estimate of heat increment to about ±4%.

Carbon retention. The estimate of the heat increment for acetic acid may be checked independently from the carbon retention of the sheep. In the calculation of the heat increments, given in Tables 8 and 12, O_2 consumption only was used since the calorific value of O_2 consumed in the complete dissimilation of acetic acid is very close to the

Table 12. *Mean heat increments (Cal./24 h) of sheep S and T when receiving infusions of acetic acid into the rumen, and calculation of heat increment in terms of acetic acid metabolized*

(Values for final 48 h of infusion)*

	Sheep S	Sheep T
Mean increase in heat production	251	289
Calorific value of acetic acid	705	732
Correction:		
Tissue accumulation of fatty-acid and 'ketone' bodies†	18·4	27·3
Urinary excretion of fatty acid‡	8·0	13·0
Unoxidized urine 'ketone' bodies§	42·7	16·6
Calorific value of acetic acid metabolized	635·9	675·1
Therefore, heat increment as percentage of acid metabolized	39·5	42·8

* For methods of computation see footnotes ‡, § and ||, Table 8.
† Mean body-weight of sheep S 51·0 kg, of sheep T 65·6 kg.
‡ Mean increase in urinary excretion of fatty acid (m-equiv./24 h): sheep S 39·7, sheep T 61·9.
§ Mean increase in 'ketone' bodies in urine (g acetone/24 h): sheep S 5·93, sheep T 2·27.

Table 13. *Computation of heat increment of acetic acid given by infusion into the rumen in sheep S and T from their metabolism of carbon and nitrogen**

Component	Sheep S	Sheep T
Carbon balance (g/24 h)		
C supplied as acetic acid	+80·79	+83·91
C excreted by lungs as CO_2	−42·96	−39·89
C excreted in urine as acetone	−3·68	−3·96
C excreted in urine as acetic acid	−0·95	−1·49
C released from bicarbonate in tissues owing to acidosis†	−0·17	+1·39
C of acetic acid accumulated in tissues	+0·10	−3·68
C of 'ketone' bodies accumulated in tissues	−1·62	+0·28
C of body spared from oxidation	+31·51	+36·56
Carbon and nitrogen balance (g/24 h)		
N of body spared from oxidation	−1·61	−0·33
C spared as protein	−5·15	−1·05
C spared as fat‡	+36·66	+37·61
Energy balance (Cal./24 h)		
Energy of depot fat spared from oxidation	+460	+472
Energy of body protein spared from oxidation	−56	−11
Energy retained as acetic acid in tissues§	−1	+30
Energy retained as 'ketone' bodies in tissues§	+19	−3
Total energy balance	+422	+488
Energy intake as acetic acid	+705	+732
Therefore heat increment	+283	+244
Heat increment from oxygen consumption (see Table 12)	+251	+289

* *To avoid confusion the signs attached to the values represent the algebraic operation of the terms.* Thus C excreted in respiratory CO_2 is a loss of C. It, however, contains some CO_2 arising from the fall in alkali reserve, so for sheep T in which the alkali reserve fell markedly the sign becomes positive. The C of acetic acid accumulated in the tissues does not contribute to the retention of fat or protein and therefore has a negative sign. Since sheep T showed a decrease in blood ketone content during the experiment the C of the ketones is given a positive sign.

† Calculated from the body-weight of sheep, on the assumption that the total body water is 60% of the weight and that the reduction in the CO_2 content and increase in the acetic-acid content of the plasma and whole blood applies to the whole body water.

‡ The total carbon retained as fat and protein less the carbon retained as protein. The factors of Blaxter & Rook (1953) were used in making the computation.

§ The accumulation of acetic acid and of ketones in the body represents a gain of energy to the animal and is not therefore included in the heat increment.

calorie value of O_2 consumed in the combustion of body fat. The respiratory CO_2 was ignored. In Table 13, the carbon retention of sheep S and T in the second series of experiments has been computed. The components to be subtracted from the intake of C are the respiratory C in the CO_2 exhaled and the C of the urine. For simplicity of presentation the values are expressed as the mean changes in C excretion over the base values, in the 24th–72nd h of experiment.

The carbon retained in the body when acetic acid is given represents the carbon from body tissue spared by oxidation of the acid. From this carbon balance and that for nitrogen it is possible to compute the amounts of body fat and protein that have been spared when 700 Cal. energy as acetic acid were given. Details of the computation are given in Table 13, and footnotes explain the logic of the steps taken.

The results given in Table 13 show that heat increments computed from a knowledge of the increase in O_2 consumption are in agreement with those calculated independently from the carbon and nitrogen retention of the animal within the range of analytical error to be expected in this type of work.

DISCUSSION

Blood sugar of the ruminant. The findings about the effect of the steam-volatile fatty acids on the blood sugar of the fasting sheep are of some interest. The mean value for the blood sugar in the fed sheep in Britain has been given by Reid (1950*a*) as 39·1 mg/100 ml., and in fasting it falls to values ranging from 26 to 35 mg/100 ml. The values we have obtained in fasting sheep are similar to these, and the few for sheep fed at the maintenance level range from 40 to 43 mg/100 ml.

The rise in blood sugar when propionic acid was given is in agreement with the observations of others with ruminants (Johnson, 1955; Clark & Malan, 1956) and is in agreement with the fact that propionic acid is glucogenic (Ringer, 1912; Deuel, Butts, Hallman & Cutler, 1935–6).

With acetic acid the fall in blood sugar is in agreement with the classical work of Ringer & Lusk (1910), which showed that it is not converted into glucose. The critical experiments of Reid (1951) showed that acetic acid will not relieve insulin coma in the sheep, whereas propionic acid is as effective as glucose. There are metabolic pathways whereby acetate carbon could be incorporated into the glucose molecule (Lorber, Lifson & Wood, 1945; Potter & Heidelberger, 1950) notably through entry of acetyl coenzyme A into the tricarboxylic-acid cycle but such pathways do not result in any net increase in carbohydrate.

Considerable uncertainty exists as to the effect of *n*-butyric acid on the blood sugar of ruminants. Jarrett *et al.* (1952) found a marked rise in the blood glucose of sheep after intravenous injection of the sodium salt, but pointed out that, since the injections were followed by marked respiratory distress and partial collapse, the response in blood sugar may have been due to glycogenolysis stimulated by the sympatho-adrenal system. Potter (1952) reported that intravenously injected sodium butyrate relieved insulin coma in sheep and caused a rise in blood glucose. On the other hand, in experiments in which butyric acid was orally administered to goats, Schultz & Smith (1951) found that after a brief initial rise in blood sugar there was a marked decline

to one-half the initial value. Clark & Malan (1956) found a significant drop in blood sugar in most of the sheep they dosed with sodium butyrate. They point out that the results obtained by injecting the acid into the blood stream may be misleading since the normal route of absorption of butyric acid is via the epithelium of the rumen, where a large-scale conversion to ketones takes place (Pennington, 1952). The results of our experiments would suggest that in the fasting sheep, and over considerable periods of time, butyric acid is not glucogenic. It will be noted from Fig. 3 that the levels of sugar were depressed after 24 h and remained so until the acid infusion was stopped.

The fact that the blood-sugar levels rose when the three acids were administered together is in agreement with the finding by Clark & Malan (1956) of a glucogenic effect for mixtures of acetic–propionic and propionic–butyric acids in sheep.

Metabolism of acetic acid. The results obtained in the five experiments with acetic acid show that when given as the sole source of energy it has a high heat increment. The four experiments with mixtures of steam-volatile acids, however, suggest that when it is given with propionic acid and *n*-butyric acid in the molar proportions 5:3:2 the increment of heat is very considerably less. The magnitude of the heat increment of mixtures of steam-volatile acids of different composition, particularly those more closely resembling the products of rumenal fermentation will be discussed in a subsequent paper. The present results suggest very strongly, however, that it is not correct to consider the metabolism of each acid separately in order to find the cause of the high heat increment of the ruminant animal.

It is of considerable interest, to see to what extent the metabolic changes and high heat increment of acetic acid given as the sole source of energy can be explained in terms of the biochemistry and thermodynamics of intermediary metabolism, as elucidated in studies on tissues and isolated enzyme systems in vitro. The fact that the blood sugar falls when acetic acid is given suggests that an excessive demand is placed on glycolysis. The increase in urinary N excretion further suggests gluconeogenesis from protein or an excessive demand for the intermediaries of carbohydrate metabolism. This, in turn, suggests that very little glucose is being formed from acetic acid.

The known main pathways of acetic-acid metabolism, apart from special systems such as cholesterol synthesis, are entry into the tricarboxylic-acid cycle, and entry into the cyclic process of lipogenesis. The first step in the entry of acetic acid in both instances is the formation of acetyl coenzyme A, which takes place at the expense of the pyrophosphate bond of adenosine triphosphate, generated in the tricarboxylic-acid cycle or in glycolysis. Condensation of acetyl coenzyme A with oxaloacetic acid would allow entry of acetic acid into the tricarboxylic-acid cycle, where it would be oxidized or, since the steps of glycolysis are reversible, could form glucose. The factor limiting its entry would be the supply of oxaloacetate, a product of carbohydrate metabolism.

For lipogenesis from acetyl coenzyme A, the first stage would be the condensation of two molecules of acetyl coenzyme A, to form acetoacetyl coenzyme A. For the synthesis of the higher homologues, hydrogen equivalents in the form of reduced diphosphopyridine nucleotide and reduced flavin adenine dinucleotide must be

available. The supply of both can come only from the oxidation of sugar. Their absence would undoubtedly lead to hydrolysis of acetoacetyl coenzyme A and hence to the ketosis observed.

The biochemical findings in the in vivo experiments suggest that in the sheep given acetic acid as the sole source of energy, metabolic blocks occurred at three points, the supply of oxaloacetic acid, the supply of the reduced coenzymes for lipogenesis, and possibly the supply of adenosine triphosphate for the initial acetylation of coenzyme A. Understandably, acetic acid accumulated as shown by the rise in the blood steam-volatile fatty-acid content, the fall in the alkali reserve and the rise in the urinary excretion of steam-volatile acid. These three blocks probably resulted from the low supply of glucose in the fasting animal. The provision of carbohydrate or a substance that would enter with ease the tricarboxylic-acid cycle would clearly facilitate the metabolism of acetic acid. Undoubtedly, the low heat increment of the mixture of steam-volatile fatty acids, in which propionic acid was present, can be explained in this way. Furthermore, the low heat increment of diets containing triacetin as a source of part of the energy can be explained by the concomitant presence of carbohydrate in the diet together with glycerol (McManus *et al.* 1943).

Obviously, since a considerable portion of the carbon of the acetic acid was retained in the body, the lack of the products of glycolysis was not absolute. In fact, it was noticeable that the increase in urinary N excretion was greater when acetic acid was given on the 9th and 10th days of starvation than when it was given on the 5th and 6th, when probably glycogen reserves were less exhausted.

The reason for the observed high increment of heat is not clear. Once a two-carbon unit has entered the tricarboxylic-acid cycle or the lipogenetic cycle through gluconeogenesis from protein it is difficult to see why there should be excessive losses of heat apart from those attributable to hydrolysis of peptide bonds of tissue proteins and deamination of amino-acids. There are two possible explanations. The first is that there is an alternative metabolic pathway for the complete dissimilation of acetic acid to carbon dioxide and water in which the efficiency of free-energy capture is considerably less than in oxidation in the tricarboxylic-acid cycle. The second is that the accumulation of acetic acid and the metabolic acidosis which occurs produce spontaneous hydrolysis of the pyrophosphate bond of adenosine triphosphate or of the thioester bond of acyl derivatives of coenzyme A.

That severe acidosis due to mineral acid may increase the heat increment of food in the ruminant and that neutralization of the mineral acid can abolish it has been shown experimentally by Møllgaard & Thorbek (1941). Working with milking cows in the respiration apparatus at Copenhagen, they found that the heat increment of A.I.V. silage before neutralization of the mineral acid was 84% of the metabolizable energy. After neutralization with $NaHCO_3$, the heat increment fell to 24%. It is of interest that in our experiments the heat increments of the acids and of glucose were inversely related to the CO_2-combining capacity of the plasma at the end of each experiment, as shown in Fig. 6. The regression was significant statistically. The hypothesis that the high heat increment of acetic acid may be due to hydrolysis of high-energy linkages under conditions of acidosis is clearly an extension of the acid-stimulus theory of the

specific dynamic effect advanced by Benedict & Ritzman (1927). Further work in vivo and in vitro is needed to establish or refute it.

Metabolism of propionic and butyric acids. The low heat increment of propionic acid is not surprising in view of its glucogenic nature. The oxidation of glucose, besides yielding adenosine triphosphate and acetyl coenzyme A and the hydrogen equivalents in the form of diphosphopyridine and flavin adenine dinucleotide necessary for fatty-acid synthesis provides a source of glycerol in the free or phosphorylated form necessary for the synthesis of fat or of phosphatides.

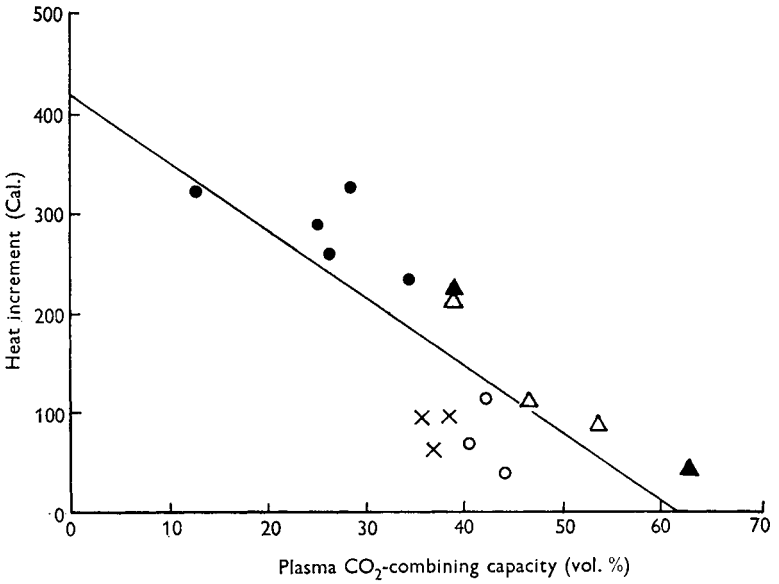

Fig. 6. Relation between the heat increment (H.I.) of the steam-volatile fatty acids and the CO_2-combining capacity of the plasma (P) in individual experiments with fasting sheep. H.I. = 420 − 6·85 P. ●, acetic acid; ○, propionic acid; ×, butyric acid; △, steam-volatile fatty-acid mixture; ▲, glucose.

The low heat increment for butyric acid is by no means easy to explain. Butyric acid like acetic acid finally undergoes oxidation in the form of two-carbon fragments. Hence one would expect it to have a similar effect on tissue metabolism. The fact that considerable amounts of butyrate absorbed from the rumen are metabolized in the epithelial wall lining the rumen, primarily to acetoacetate (Pennington, 1952) and that the oxidation of acetoacetate by extrahepatic tissues is considered by many (see Stadie, 1945) to be the pathway for the utilization of a considerable portion of fat metabolized in normal, fed animals may have some bearing on the point. In our experiments two factors are noteworthy. Firstly, there was no peripheral accumulation of acid such as occurred with acetic acid, and consequently a considerably less severe acidosis. Secondly, there appeared to be no increase in N katabolism. The need for further study of the metabolism of butyric acid has been pointed out by Phillipson & Cuthbertson (1956).

Heat increment of the ruminant. The single experiment with sugar giving a value for

the heat increment of 6·6%, together with the values obtained for the heat increment of steam-volatile acid mixtures, might be taken to suggest that the heat increment associated with the metabolism of the end-products of ruminant carbohydrate digestion is possibly about double that of carbohydrate digestion in simple-stomached animals. This estimate is based on a single experiment with glucose and, if errors in this experiment are similar to those observed in the series reported in detail, the true value for the heat increment of glucose may lie in the region of 10%. Indeed, the value for the S.D.E. of glucose in man is often quoted as 10%, and the calculations of Soskin & Levine (1946) from in vitro studies suggest that since the oxidation of 1 mole of glucose provides the energy for the phosphorylative synthesis of 6–12 moles of glycogen, the S.D.E. might be predicted to lie within a range of 6–16%. Further experiments with ruminants are clearly necessary.

These considerations suggest that the heat increments of absorbed mixtures of steam-volatile acids in the fasting ruminant may not greatly exceed those of absorbed glucose.

SUMMARY

1. Experiments in which three fistulated sheep were starved for periods of 12 days were carried out to study the heat production associated with the dissimilation of acetic, propionic and *n*-butyric acids when given to supply about 700 Cal. The metabolism of a mixture of these acids in the molar proportions 5:3:2 was also studied as well as the metabolism of glucose.

2. Infusion of acetic acid into the rumen for 48 or 72 h caused in five experiments a rise in the concentration of steam-volatile acids in the rumen, a fall in the pH of the rumen to 4·6, a considerable rise in the concentration of steam-volatile acids in the blood, acidosis, ketosis and a fall in the blood sugar. There was a rise in the urinary excretion of nitrogen, ketones and steam-volatile acids.

3. The increment of heat was computed from the oxygen consumption and N metabolism, and the value found to be 40·8% in three experiments of 48 h duration, measurements being made in the final 36 h. In two experiments of 72 h duration, the heat increment during the 24th–72nd h was 41·1%.

4. In the latter two experiments, the increment of heat was also computed independently from the carbon and nitrogen retention and almost identical results were obtained.

5. Administration of propionic acid in three experiments of 48 h duration resulted in a marked increase in blood sugar and a heat increment of 13%. There was no ketosis or acidosis and there was a reduction in the urinary N excretion, that is an N-sparing effect.

6. Administration of butyric acid in three experiments of 48 h duration resulted in marked ketosis, very slight accumulation of the acid in peripheral blood with a consequent slight decline in the plasma carbon dioxide-combining capacity. The increment of heat was 15·9%.

7. A mixture of the acids gave a heat increment of 16·9%, which was considerably lower than what would be expected from summation of the heat increments of the separate components of the mixture. Glucose given under conditions in which minimal fermentation took place in the rumen had a heat increment of 6·6%.

8. The results are discussed in relation to knowledge of the metabolic pathways whereby the acids might be dissimilated, and it is suggested that the reason for the metabolic findings with acetic acid is linked with the shortage of intermediaries and reduced coenzymes which arise in carbohydrate dissimilation. The high heat increment is not to be explained in this way and the hypothesis is advanced that an increase in the rate of non-enzymic hydrolysis of the pyrophosphate bond of adenosine triphosphate and thioester linkage of coenzyme A occurs because of the resulting metabolic acidosis.

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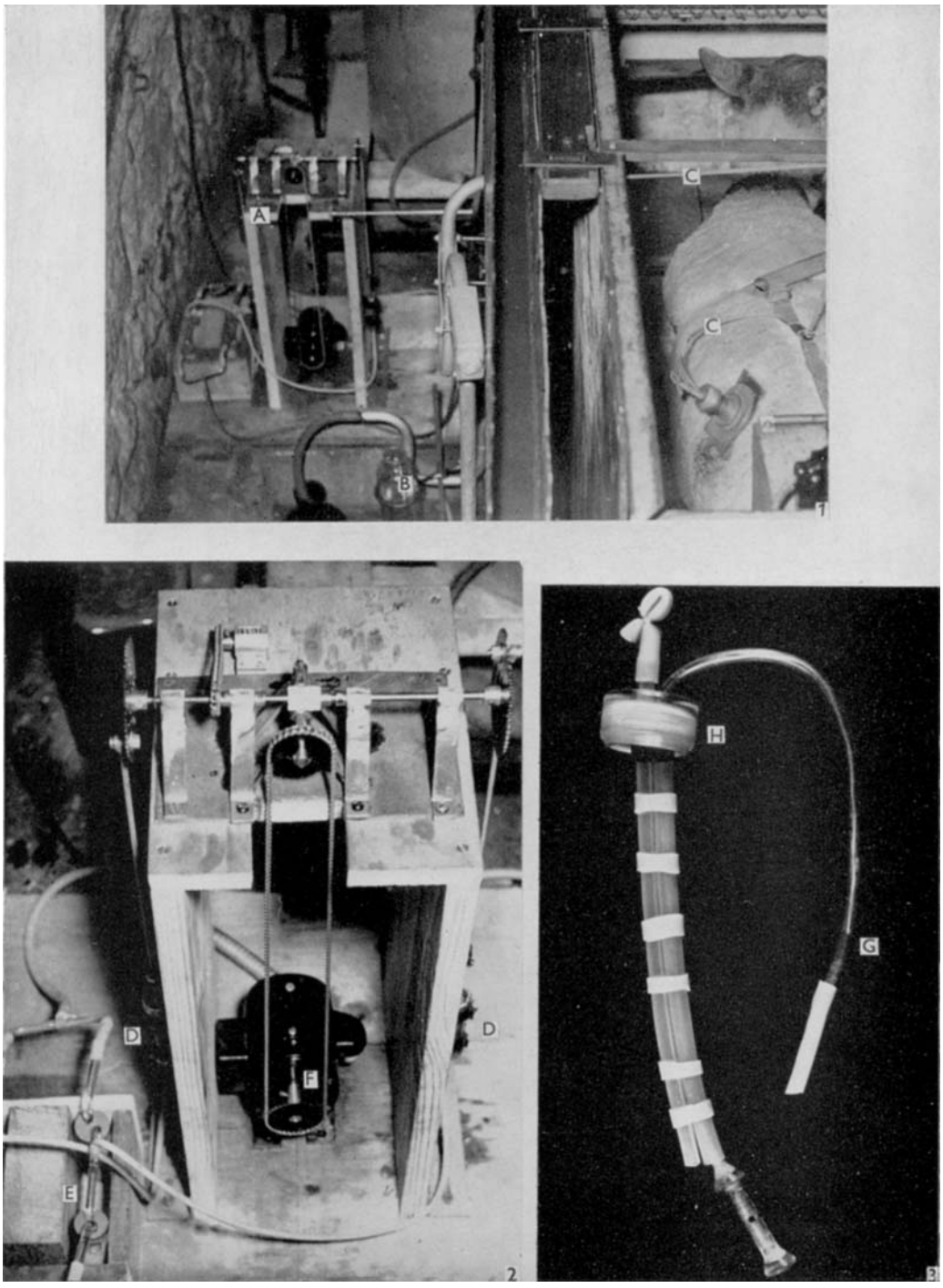
EXPLANATION OF PLATE

1. View from above of the respiration chamber showing the infusion pump (A), the acid reservoir (B) and the lead into the rumen of the sheep passing through the counterpoise system (C).
2. Infusion pump showing the Record syringes and plungers (D), the Bunsen valves (E) and the slow-speed motor (F).
3. Internal cannula used to introduce the acids. The acid lead (G) has a piece of stainless steel wire inside, which together with an outer sheath of Polythene tubing prevents blockage. The screw cap (H) holds the cannula in place.

Note added in proof. In subsequent experiments in which *n*-butyric acid was given to fasting sheep for periods longer than 48 h considerable day-to-day variation in the heat increment was found. Under these conditions the mean value was 23·6 Cal./100 Cal. acid metabolized, which is higher than the values given in this paper.



(*Facing p.* 272)