The heat increments of mixtures of steam-volatile fatty acids in fasting sheep

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Kellner (1920), in his calorimetric studies with cattle, showed that, as the fibre content of the ration increased, the net availability of its metabolizable energy fell. He attributed this fall to the additional physical work with consequent loss of heat in comminuting and digesting the more fibrous food. This cause may not be the sole contributor. Data in the literature, as summarized in Table 1, suggest that the molar composition of the fatty-acid mixtures formed in the rumen differs from ration to ration, and that roughages may be associated with the formation of mixtures with higher proportions of acetic acid and lower proportions of propionic acid than those arising from concentrates. This table must, however, be interpreted with caution because of differences in technique adopted by the different workers. It is thus possible that the differences in utilization observed by Kellner reside in part in differences in the composition of the energy-yielding constituents absorbed from the gut when foods high and low in fibre content are given. In this regard, it has been shown that acetic acid when given to fasting sheep is less efficiently utilized than either propionic or *n*-butyric acids; these in turn are less efficiently utilized than glucose (Armstrong & Blaxter, 1957).

Table 1. *Percentage composition (on a molar basis) of the steam-volatile fatty acids found in the rumen liquor of cattle and sheep given different rations*

Ration given	Species	m-equiv. of individual acid/100 m-equiv. total steam-volatile acid: Acetic	Propionic	Butyric*	Higher†	Reference
Concentrates and hay: 24 lb. + 2 lb.	Cow	40·6	37·8	9·2	12·4	Balch, Balch, Bartlett, Bartrum, Johnson, Rowland & Turner (1956)
20 lb. + 16 lb.	Cow	57·9	23·8	12·7	5·6	Balch *et al.* (1956)
Flaked maize with a little hay	Lamb	55·5	32·7	7·7	3·8	Phillipson (1952)
Fresh grass	Sheep	50–62	21–30	12–17	3–10	Johns (1955)
Dried grass	Sheep	58–64	22–26	9–12	Not recorded	el-Shazly (1952)
Lucerne hay	Sheep	70	19	11	Not recorded	Gray & Pilgrim (1951)

* Includes *n*- and *iso*-butyric.
† As valeric acid.

The present experiments were designed to determine whether the proportion of acetic acid in mixtures of steam-volatile fatty acids is related to the size of the heat increment which occurs when the mixtures are given as the sole source of energy to fasting sheep. The results showed that within the range of composition of fatty-acid mixtures likely to be found in the rumen, the heat increments changed very little.

EXPERIMENTAL

Animals. Four adult castrated male sheep, each equipped with a Perspex cannula passing through a permament rumen fistula were used. Their management was as previously given (Armstrong & Blaxter, 1957).

Experimental plan. Table 2 gives details of the fifteen experiments carried out. Exps. 14 and 15 with acetic acid alone and Exps. 6–8 with a mixture of acetic, propionic and *n*-butyric acids in the molar proportions of 5:3:2 were reported previously (Armstrong & Blaxter, 1957) and are included here for comparative purposes. The remaining ten experiments all lasted 11 days and conformed to the same general plan. The sheep, confined in a respiration chamber, was starved for 4 days during which

Table 2. *Details of procedure in the fifteen experiments with sheep*

Exp. no.	Sheep	Mixture of acids given (m-equiv. of individual acid/100 m-equiv. total steam-volatile acid)			Amount of mixture supplied/24 h* (moles)	Approximate energy supplied/24 h* (Cal.)	Period of administration of acids (h)
		Acetic	Propionic	Butyric			
1	T	0	60	40	2·6	1100	96
2	S	0	60	40	2·6	1100	96
3	T	25	45	30	2·9	1100	96
4	S	25	45	30	2·9	1100	96
5	Pa	25	45	30	2·9	1100	96
6	T	50	30	20	3·4	1100	48
7	S	50	30	20	3·4	1100	48
8	Pa	50	30	20	3·4	1100	48
9	T	75	15	10	3·2	850	96
10	S	75	15	10	3·2	850	96
11	Pa	75	15	10	4·2	1100	96
12	Pe	90	6	4	3·0	700	96
13	Pa	90	6	4	3·0	700	96
14	T	100	0	0	3·3	700	72
15	S	100	0	0	3·3	700	72

* Actual intakes of acid (and hence of energy) for the individual experiments differed slightly from the values quoted above owing to variations in the volume delivered by the pumps.

a dilute saline solution was infused into the rumen. The animal then received one of the steam-volatile fatty-acid mixtures for 4 days and, finally, the dilute saline for a further 3 days. Samples of jugular blood and rumen contents were withdrawn daily. Details of the methods of infusing the saline and acid solutions, of the composition of the saline solution and of the methods of sampling rumen contents and blood have already been described (Armstrong & Blaxter, 1957). In all mixtures of steam-volatile fatty acids, propionic acid and *n*-butyric acid were in the molar proportions 3:2. For

ease of presentation the steam-volatile fatty-acid mixtures are referred to in the text as 100, 90, 75, 50, 25 and 0% acetic-acid mixtures.

Calorimetric and analytical methods. The experiments were all carried out at temperatures in the respiration chamber between 20 and 22°. The measurements of oxygen consumption, carbon-dioxide and methane production, and of excretion in the urine of nitrogen, ketones and steam-volatile acids were made at 24 h intervals. The analytical methods used to determine the concentrations of steam-volatile fatty acids, ketones and sugar in blood, the CO_2-combining capacity of the plasma and the pH and steam-volatile fatty-acid concentration in rumen liquor were those previously employed (Armstrong & Blaxter, 1957). Partition of the steam-volatile fatty acids in rumen liquor was carried out by gas–liquid chromatography with the apparatus and technique of James & Martin (1952). The rumen-liquor samples were prepared for gas–liquid chromatography as follows. Rumen liquor (15 ml.), previously strained through muslin, was added to an equal volume of saturated magnesium-sulphate solution containing 2·5% (w/v) sulphuric acid. After mixing the two liquids the mixture was allowed to stand for 10 min. It was then centrifuged for 10 min at 3000 rev./min (2100 ***g***), and subsequently filtered through Whatman no. 41 filter-paper. A 20 ml. portion of the filtrate was vigorously steam-distilled in a Markham distillation apparatus (Flaig and Co. Ltd, 39 Waterloo Road, London, N.W. 2) and the first 160 ml. of distillate were collected and made just alkaline to litmus paper with a few drops of about normal NaOH. The distillate was evaporated to dryness in vacuo, 1 ml. water added, the flask stoppered and shaken, and 0·025 ml. quantities were transferred to the column of the gas-chromatography apparatus by the technique of James & Martin (1952). The final titration was with 0·01 N-alkali.

RESULTS

Conditions within the rumen. Tables 3 and 4 show the mean pH in rumen liquor and the mean concentration of steam-volatile acids before, during and after infusion of the acid mixtures. When 100% acetic acid was given, the pH fell throughout the infusion period to a value of 4·48 after 72 h. The content of steam-volatile fatty acids

Table 3. *Mean pH of rumen liquor of sheep receiving infusions of steam-volatile fatty acids into the rumen*

m-equiv. acetic acid/ 100 m-equiv. total steam-volatile fatty-acid mixture infused	No. of sheep	Approximate amount of energy supplied (Cal./24 h)	Preliminary period. Day before infusion		Period of infusion. End of day				Recovery period. End of day		
			2	1	1	2	3	4	1	2	3
100	2	700	7·07	7·25	4·89	4·76	4·48	—	7·48	7·56	7·43
90	2	700	7·10	7·17	5·06	4·97	5·39	5·67	7·16	7·46	7·48
75	2	850	7·05	7·10	5·13	5·18	5·32	5·66	7·56	7·53	7·53
	1	1100	7·04	7·01	5·07	5·13	5·29	5·41	7·34	7·32	7·34
50	3	1100	7·15	7·20	5·26	5·36	—	—	7·22	7·49	—
25	3	1100	7·07	7·11	5·48	5·87	6·39	6·76	7·38	7·28	7·34
0	2	1100	7·06	7·10	5·90	6·31	6·53	7·03	7·59	7·46	7·46

showed a corresponding rise, and at 72 h had reached a value of 9·36 m-equiv./100 ml. With the 90% acetic-acid mixture the lowest pH recorded occurred at 48 h, after which there was a slow but steady rise. With the mixtures containing 75% acetic acid or less the pH was lowest after 24 h of infusion and thereafter rose. In general, the rise in pH reflected a fall in the concentration of steam-volatile acids. The decline in acid concentration and consequent rise in pH with continued time of infusion was very marked for the 25% and 0% acetic-acid mixtures.

Table 4. *Mean concentration of steam-volatile fatty acids (m-equiv./100 ml.) in rumen liquor of sheep receiving infusions of steam-volatile fatty acids into the rumen*

m-equiv. acetic acid/100 m-equiv. total steam-volatile fatty-acid mixture infused	Preliminary period. Day before infusion		Period of infusion. End of day				Recovery period. End of day		
	2	1	1	2	3	4	1	2	3
100	0·59	0·72	7·32	8·02	9·36	—	0·97	0·80	0·66
90	0·45	0·47	7·53	7·72	6·95	6·59	0·25	0·28	0·28
75	0·50	0·47	7·47	7·70	7·72	6·47	0·30	0·29	0·36
	0·53	0·69	7·52	8·15	7·94	6·94	0·34	0·23	0·25
50	0·54	0·52	6·80	6·72	—	—	0·21	0·31	—
25	0·66	0·60	7·20	6·13	4·62	3·39	0·19	0·33	0·43
0	0·58	0·52	6·29	6·11	5·22	3·95	0·14	0·16	0·26

No. of sheep and approximate amount of energy supplied are given in Table 3.

In Fig. 1, the changes in pH and content of steam-volatile fatty acids of rumen liquor for the 100% acetic-acid and 25% acetic-acid solutions are shown graphically. The accumulation of acid, presumably acetic, that gradually developed in the rumen of the sheep receiving the C_2 acid only must reflect a failure to maintain equilibrium with acid input by absorption from the rumen. It is extremely unlikely that the fall in concentration of steam-volatile fatty acids occurring when the mixtures low in acetic acid were infused was due to a cumulative retention of water in the rumen as the experiments proceeded. The amount of water necessary to cause such dilution would in some experiments be considerable. Not only would such dilution cause noticeable distension of the rumen and be detected from changes in the body-weight of the sheep, but it would be reflected in a considerable decrease in the volume of urine secreted. Computations of the water retention in experiments with 25% acetic acid are given in Table 5. They show that there was no increase in water retention during the later part of the period of acid infusion, indeed, the sheep lost about 100 g more water from its tissues or gut contents during the second 48 h than it did in the first 48 h. If the volume of rumen contents at the beginning of the infusion had been 3 l., to account for the fall in concentration of steam-volatile fatty acids from 7·2 to 3·4 m-equiv./100 ml. in terms of dilution, the volume of rumen contents would have had to increase to 6·4 l., and 3·4 l. of fluid would have been retained. The fall in concentration of acids and rise in pH thus represent a real increase in rate of removal of the acids from the gut, and were not due to dilution of gut contents.

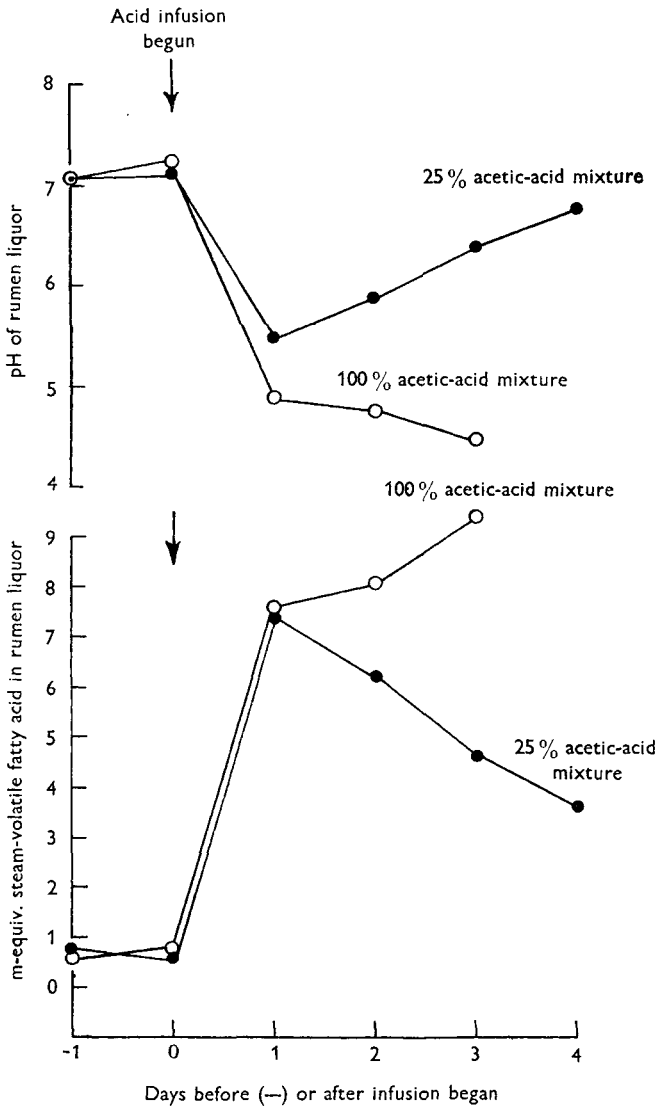

Fig. 1. Effect of continued infusion of acetic acid, o—o, and of a mixture of acetic acid, propionic acid and *n*-butyric acid of molar composition 2·5:4·5:3, ●—●, on the pH of, and on the content of steam-volatile acids in, the rumen liquor of fasting sheep. Means for two animals for acetic acid and three for the mixture.

The cause of the increase in rate of absorption of mixtures containing large amounts of propionic and *n*-butyric acids with continued infusion is not easy to understand. It is possible that starvation reduces the ability of the rumen epithelium to absorb the acids. In this regard, it has been shown that rumen epithelium metabolizes *n*-butyric acid (Pennington, 1952) and propionic acid (Pennington & Sutherland, 1956). This would suggest that acid absorption is a process which necessitates an actively metabolizing rumen epithelium.

In Table 6, the proportions are given of the steam-volatile fatty acids found in the rumen during the periods of acid infusion in the two experiments with the 0% acetic-acid mixture. In view of the calculations above, the changes in proportions can be interpreted in terms of changes in absorption rates. Initially, propionic acid was absorbed from the rumen more slowly than butyric acid but, as the experiments proceeded, the rate of absorption of butyric acid fell and that of propionic acid increased. The changes in rumen pH and the rates of absorption of the two acids agree with the findings of Danielli, Hitchcock, Marshall & Phillipson (1945) and of

Table 5. *Mean retention of water (g/24 h) in three sheep during the first 2 and last 2 days of infusing into the rumen a mixture of acetic, propionic and* n*-butyric acids in the molar proportions 2·5:4·5:3 (the 25% acetic-acid mixture)**

	Fluid intake				Fluid excretion			
Time (h)	Solution infused	Water consumed	Metabolic water	Total	Urine	Water vapour	Total	Fluid gain or loss
0–48	6449	0	160	6609	5778	1012	6790	−181
48–96	6368	80	151	6599	5872	1013	6885	−286

* The values are approximate since the infused volumes have not been corrected for the weight of acid they contain, or for the weight of solid material dissolved in the urine. The corrections would, however, be small.

Table 6. *Composition of the mixture of steam-volatile fatty acids in the rumen liquor of sheep S and sheep T when receiving infusions of a mixture of propionic and* n*-butyric acids in the molar proportions 3:2*

			m-equiv. of individual acid/100 m-equiv. total steam-volatile fatty acid		
Sheep	Days of acid infusion completed	pH of rumen liquor	Acetic acid	Propionic acid	*n*-Butyric acid
S	1	5·84	0·0	72·7	27·3
	2	6·12	0·0	71·7	28·3
	3	6·39	0·0	64·1	35·9
	4	7·05	0·0	55·3	49·7
T	1	5·96	0·0	69·1	30·9
	2	6·49	0·0	63·5	36·5
	3	6·66	0·0	56·1	43·9
	4	7·01	0·0	56·5	43·5

Pfander & Phillipson (1953) who found that as the pH of the rumen fell, so the rate of absorption of butyric acid increased. These results are in agreement with the hypothesis advanced above as, if the initial preferential absorption of butyric acid represents an initial preferential demand for this acid by the cells of the rumen epithelium (Pennington, 1952), then it would appear that the absorption of propionic acid is facilitated by an actively metabolizing epithelium. Changes in pH would be secondary to changes in the metabolism of the epithelium.

Composition of the blood. Table 7, which summarizes data on the concentration of steam-volatile fatty acids in peripheral (jugular) blood, shows that detectable increases in concentration occurred in all experiments. The increases were broadly related to the

proportion of acetic acid in the mixture supplied, but with 100% acetic acid the increase was almost ten times that noted with 90% acetic acid.

There was no consistent tendency for the concentration of the acids in peripheral blood to fall as the experiment progressed. Thus, in the sheep given the 25% acetic-acid mixture, although the concentration of steam-volatile acids in the rumen fell from 7·20 to 3·39 m-equiv. owing to increased absorption (Table 4), the concentration in peripheral blood fell from 0·17 to only 0·12 m-equiv. The increased absorption of acids was associated, therefore, with an increase in the ability of the sheep to use them.

Table 7. *Mean concentration of steam-volatile fatty acids (m-equiv./100 ml.) in whole blood of sheep receiving infusions of steam-volatile fatty acids into the rumen*

m-equiv. acetic acid/100 m-equiv. total steam-volatile fatty-acid mixture infused	Preliminary period. Day before infusion		Period of infusion. End of day				Recovery period. End of day		
	2	1	1	2	3	4	1	2	3
100	0·006	0·008	1·100	1·225	1·460	—	0·010	0·003	0·006
90	0·002	0·002	0·187	0·225	0·185	0·155	0·030	0·010	0·025
75	0·000	0·004	0·094	0·049	0·028	0·056	0·010	0·010	0·009
	0·013	0·003	0·083	—	0·095	0·088	0·010	0·010	0·010
50	0·048	0·021	0·059	0·056	—	—	0·033	0·021	—
25	0·004	0·003	0·017	0·010	0·015	0·012	0·011	0·007	0·008
0	0·025	0·007	0·013	0·015	0·013	0·019	0·013	0·000	0·014

No. of sheep and approximate amount of energy supplied are given in Table 3.

Table 8. *Mean CO_2-combining capacity (vol. %) in blood plasma of sheep receiving infusions of steam-volatile fatty acids into the rumen*

m-equiv. acetic acid/100 m-equiv. total steam-volatile fatty-acid mixture infused	Preliminary period. Day before infusion		Period of infusion. End of day				Recovery period. End of day		
	2	1	1	2	3	4	1	2	3
100	42·3	41·0	24·0	16·0	18·7	—	34·0	36·0	37·5
90	49·2	47·7	37·3	37·8	41·3	42·7	50·2	50·2	48·0
75	47·8	44·3	33·0	32·8	34·3	36·5	48·0	44·0	45·0
	45·0	46·0	42·0	—	45·6	51·0	53·5	52·0	49·0
50	46·5	46·3	38·2	41·0	—	—	50·7	48·8	—
25	48·8	46·5	41·1	41·8	45·0	46·8	50·2	41·5	41·3
0	42·0	40·8	30·0	31·8	35·0	39·8	46·8	46·3	44·8

No. of sheep and approximate amount of energy supplied are given in Table 3.

Table 8 summarizes the results obtained for the CO_2-combining capacity of the blood. Marked acidosis occurred with the 100% acetic-acid mixture only, but there was a slight depression with the other mixtures. As with rumen pH, there appeared to be a gradual adaptation to the infused mixtures, for the CO_2-combining capacity increased as the infusion continued.

Table 9 shows mean values for the concentration of reducing sugar in the blood throughout the experiments. Immediate increases in blood sugar occurred in all experiments in which the mixture contained 75% acetic acid or less. With 100% acetic acid and with 90% acetic acid there was a marked initial fall. With the 90%

acetic-acid mixture the sugar content of the blood rose as the infusion period increased, and on stopping the infusion a fall in concentration occurred. This suggests that adaptations of metabolic processes were occurring throughout the infusion period.

Table 10 shows that marked ketosis occurred when the 100% and 90% acetic-acid mixtures were given. The 75 and 25% acetic-acid mixtures reduced the mild ketosis of starvation. A considerable ketosis occurred when infusion of the 0% acetic-acid mixture was stopped. The sheep in this experiment showed a more marked ketosis in the preliminary period than did the sheep in the other experiments. The sheep were fatter

Table 9. *Mean concentration of sugar (mg/100 ml.) in whole blood of sheep receiving infusions of steam-volatile fatty acids into the rumen*

m-equiv. acetic acid/100 m-equiv. total steam-volatile fatty-acid mixture infused	Preliminary period. Days before infusion		Period of infusion. End of day				Recovery period. End of day		
	2	1	1	2	3	4	1	2	3
100	29·4	28·5	17·7	21·8	28·0	—	51·0	36·9	37·8
90	26·7	36·2	26·3	26·5	36·3	43·4	32·8	31·2	34·9
75	29·2	32·5	44·3	49·1	51·5	56·6	42·0	41·8	36·5
	35·4	39·9	44·6	—	47·9	45·7	36·5	36·8	28·9
50	33·4	33·3	43·1	45·1	—	—	35·1	30·1	—
25	29·5	27·8	39·3	40·2	39·3	39·3	40·3	33·9	29·8
0	33·0	27·5	49·2	50·8	51·8	55·8	34·4	41·5	44·5

No. of sheep and approximate amount of energy supplied are given in Table 3.

Table 10. *Mean concentration of total ketone bodies (mg acetone/100 ml.) in whole blood of sheep receiving infusions of steam-volatile fatty acids into the rumen*

m-equiv. acetic acid/100 m-equiv. total steam-volatile fatty-acid mixture infused	Preliminary period. Days before infusion		Period of infusion. End of day				Recovery period. End of day		
	2	1	1	2	3	4	1	2	3
100	6·47	5·72	14·60	12·19	22·03	—	11·26	3·70	8·99
90	3·45	3·45	6·40	13·02	9·08	5·47	5·38	3·95	5·29
75	2·44	4·45	9·08	4·96	3·03	1·60	2·19	6·98	5·88
	5·38	4·03	6·77	—	3·87	1·17	4·37	4·53	4·87
50	Not determined		Not determined				Not determined		
25	3·53	5·25	2·69	3·87	3·31	2·44	2·36	6·50	3·98
0	6·65	24·05	16·14	27·75	4·71	—	75·77	39·52	64·41

No. of sheep and approximate amount of energy supplied are given in Table 3.

in the experiments with the 0% acetic-acid mixture than in the others. The state of the fat reserves may have been responsible for this effect.

The increments of heat. Table 11 summarizes the heat increments obtained in each experiment. They are shown graphically in Fig. 2. The method used to obtain them was described in a previous paper, together with the methods of computing the metabolizable energy (Armstrong & Blaxter, 1957). With the exception of Exps. 14 and 15, in which 100% acetic acid was infused, the corrections were small. When 100% acetic acid was infused there was a considerable accumulation of steam-volatile constituents

Table 11. *Mean heat increments (Cal./24 h) of sheep receiving infusions of steam-volatile fatty acids into the rumen, and calculation of heat increment in terms of acid metabolized*

(Values for Exps. 1–5 and 9–13 relate to the last 72 h of infusion, for Exps. 14 and 15 to the last 48 h of of infusion and for Exps. 6–8 to the last 24 h of infusion)

Sheep	Exp. no.	m-equiv. acetic acid/100 m-equiv. total steam-volatile fatty-acid mixture infused	Heat increment as measured (Cal./24 h)	Acid infused (Cal./24 h)	Corrections applied*: Tissue accumulation S.V.A. (Cal./24 h)	Tissue accumulation Ketones (Cal./24 h)	Urine loss S.V.A. (Cal./24 h)	Urine loss Ketones (Cal./24 h)	Corrected intake as metabolizable energy (Cal./24 h)	Heat increment as percentage of metabolizable energy	
S	15	100	251·0	705	−0·8	+19·3	+8·3	+42·7	635·5	39·5	41·2
T	14	100	289·5	732	+30·7	−3·4	+12·9	+16·6	675·2	42·9	
Pa	13	90	124·4	705	−0·4	+0·1	−0·2	−0·4	705·9	17·6	15·3
Pe	12	90	97·2	748	−1·2	−2·9	−0·1	+1·0	751·2	12·9	
Pa	11	75	128·4	1099	+1·3	−5·0	−1·3	−2·7	1106·7	11·6	14·4
S	10	75	133·1	889	−1·5	−7·0	−0·7	0·0	898·2	14·8	
T	9	75	142·3	845	+0·3	−3·0	−1·3	0·0	849·0	16·8	
Pa	8	50	135·0	1117	0·0	+9·8	−0·2	−0·7	1108·1	12·2	16·9
S	7	50	209·0	1132	+4·4	0·0	0·0	−0·8	1128·4	18·5	
T	6	50	222·0	1097	−6·0	0·0	−0·8	+2·8	1101·0	20·2	
Pa	5	25	128·4	1052	−0·3	−2·1	−1·0	+0·3	1055·1	12·2	12·8
S	4	25	119·0	1109	0·0	+0·5	0·0	+1·6	1106·9	10·8	
T	3	25	169·6	1109	−0·1	+1·0	−0·3	−3·4	1111·8	15·3	
S	2	0	93·4	1075	0·0	+2·7	−0·5	−0·2	1073·0	8·7	9·3
T	1	0	109·5	1122	+0·4	+7·5	−1·5	−1·4	1117·0	9·8	

S.V.A. = steam-volatile fatty acids.

* To obtain the corrected intake the calories accumulated in the tissues and excreted in the urine were deducted from the calories in acid infused; where there was a loss from the tissues or a fall in excretion, the values were added.

in the blood and excretion of acid in the urine. In addition, the excretion of ketone bodies during the acid infusion was considerably in excess of that occurring in the preliminary or final periods when the saline solution was infused. With the 0% acetic-acid mixture, although ketone bodies accumulated in blood and a considerable increase occurred in excretion of these constituents in the urine during the acid infusion, these changes were maintained in the subsequent period of water administration. Hence the calorie corrections were small.

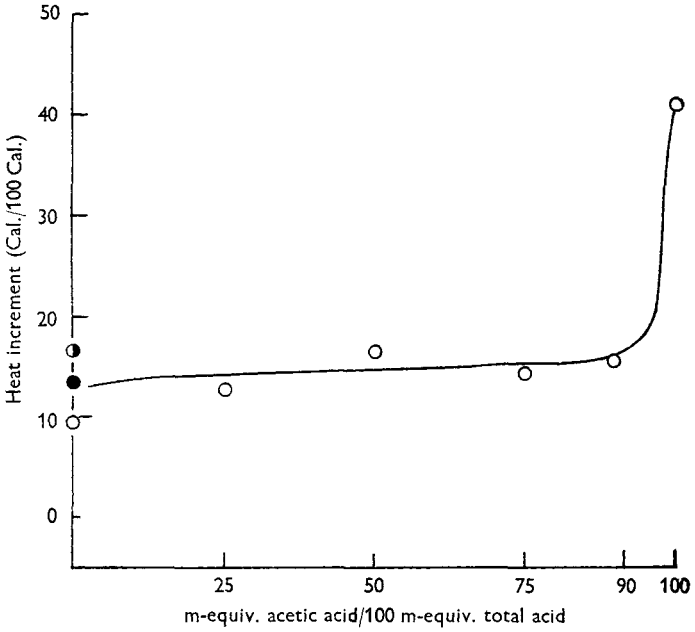

Fig. 2. Increments of heat, expressed as Cal./100 Cal. metabolized, associated with the metabolism of mixtures containing different amounts of acetic acid. ○, acetic acid with a mixture of propionic acid and *n*-butyric acid in the molar proportions 3:2; ◑, *n*-butyric acid alone; ●, propionic acid alone.

Statistical analysis of the values in the last column in Table 11 was carried out to assess the significance of the differences between the observed mean heat increments when mixtures were given and those predicted from linear interpolation between the observations with the 100 and 0% acetic-acid mixtures. These differences allow one to assess whether the heat increment increased in direct proportion to the amount of acetic acid present. The results given in Table 12 show that this was not so. Thus when the 90% acetic-acid mixture was given, the heat increment observed was $15{\cdot}3 \pm 2{\cdot}36$ and the expected value was $38{\cdot}0 \pm 1{\cdot}14$, the difference being highly significant statistically. Only very small quantities of propionic and *n*-butyric acids were able to facilitate the dissimilation of acetic acid and allow about 85% of its energy to be used by the fasting animal to save body fat and protein from being oxidized to provide energy. There were no statistically significant differences between the mean heat increments of mixtures containing 25, 50, 75 or 90% acetic acid.

In Table 13, the increases in the retention of carbon and nitrogen in the body when

Table 12. *Statistical assessment of the departure from direct proportionality of the relationship between heat increment expressed as a percentage of metabolizable energy and the proportion of acetic acid in the steam-volatile acid mixture given*

m-equiv. acetic acid/100 m-equiv. total steam-volatile fatty-acid mixture infused	Expected heat increment based on direct proportionality of response to amount of acetic acid given* (%)	Observed mean heat increment† (%)	Difference (%)	Odds against a difference of the same magnitude arising by chance
100	41·2 ± 1·26	—	—	—
90	38·0 ± 1·14	15·3 ± 2·36	22·7 ± 2·64	277:1
75	33·2 ± 1·00	14·4 ± 1·82	16·2 ± 2·08	332:1
50	25·2 ± 0·89	16·9 ± 1·83	8·3 ± 2·03	37:1
25	17·2 ± 1·00	12·8 ± 1·83	4·4 ± 2·08	7:1
0	9·3 ± 1·26	—	—	—

* Obtained from the linear regression of heat increment on percentage of acetic acid for the four observations at 100% and 0% acetic acid (Exps. 1, 2, 14 and 15). The errors are standard errors of expected values.

† The errors were obtained from analysis of variance of the eleven observations obtained when mixtures containing 25, 50, 75 and 90% acetic acid were given (Exps. 3–13 inclusive).

Table 13. *Computation from the metabolism of carbon and nitrogen* of heat increments of three steam-volatile fatty-acid mixtures given by infusion into the rumen of sheep T*

Component	m-equiv. acetic acid/100 m-equiv. total steam-volatile fatty-acid mixture infused: 75	25	0
Carbon balance (g/24 h)			
C supplied as acid	+90·05	+108·20	+106·54
C excreted by lungs as CO_2	−30·50	−27·09	−21·27
C excreted in urine as acetone and steam-volatile fatty acid	+0·37	+0·38	+0·29
C released from bicarbonate in tissues owing to acidosis	−0·22	−0·58	−0·70
C accumulated in tissues as 'ketone' bodies and steam-volatile fatty acid	+0·15	+0·09	−1·29
C of body fat and body protein spared from oxidation	+59·85	+81·00	+83·57
Carbon and nitrogen balance (g/24 h)			
N of body spared from oxidation	+1·15	+1·19	+2·02
C spared as protein	+3·68	+3·81	+6·46
C spared as fat	+56·17	+77·19	+77·11
Energy balance (Cal./24 h)			
Energy of body fat spared from oxidation	+705	+969	+968
Energy of body protein spared from oxidation	+6	+6	+11
Energy retained in tissues as 'ketone' bodies and steam-volatile fatty acid	−3	−1	+8
Total energy spared or retained	+708	+974	+987
Energy intake as steam-volatile fatty acid	+845	+1109	+1122
Therefore heat increment	137	135	135
Heat increment estimated from measurements of O_2 consumption	142	170	110

* The values for retention of C and N refer to the increments above the base-line of fasting. The signs indicate the algebraic operation of the terms. The factors used are those given by Armstrong & Blaxter (1957).

three acid mixtures were given to sheep T are presented. Similar data for the same sheep given acetic acid alone were given in a previous paper (Armstrong & Blaxter, 1957), where the detailed method of computation is described. The results show that the increment of heat arising in the dissimilation of these mixtures, calculated from the carbon and nitrogen metabolism of the sheep, was in agreement with that calculated from its oxygen consumption, the discrepancies being within the bounds of experimental error. Similar computations for the other experiments gave similar agreement. It may be pointed out that a difference in heat increment of 20 Cal./24 h is equivalent to an error of 4 l. O_2 or 3 l. CO_2 in 24 h, or an error of less than 2% in the determination of the gaseous exchange.

Nitrogen metabolism. The increase or decrease in the excretion of N in the urine when the acid mixtures were given is shown in Table 14. The amount excreted when no acid was given was calculated from the quadratic regression of N excretion on time during the 4 days before, and the 2nd and 3rd days after, the acids were given. The amount excreted during the period of acid infusion refers to that excreted from the 24th hour onwards.

Table 14. *Mean increase (+) or decrease (−) from starvation values in excretion of nitrogen (g/24 h) by sheep given infusions of steam-volatile fatty acids into the rumen*

Exp. no.	Sheep	m-equiv. acetic acid/100 m-equiv. steam-volatile fatty-acid mixture infused	Metabolizable energy* (Cal./24 h)	Change in urinary N excretion (g/24 h)	Change in N excretion/Cal. energy metabolized (mg/Cal.)	
1	T	0	1117	−2·02	−1·81	−2·26
2	S	0	1073	−2·91	−2·71	
3	T	25	1112	−1·19	−1·07	−1·94
4	S	25	1107	−2·32	−2·10	
5	Pa	25	1055	−2·82	−2·67	
6	T	50	1101	−0·15	−0·14	−0·91
7	S	50	1128	−1·59	−1·41	
8	Pa	50	1108	−1·31	−1·18	
9	T	75	849	−1·15	−1·35	−2·07
10	S	75	898	−2·33	−2·59	
11	Pa	75	1107	−2·53	−2·29	
12	Pe	90	751	−1·01	−1·34	−0·86
13	Pa	90	706	−0·27	−0·38	
14	T	100	675	+0·32	+0·47	+1·50
15	S	100	636	+1·61	+2·53	
Values (Armstrong & Blaxter, 1957) for acids given singly to fasting sheep (mean for three sheep)						
	Acetic acid		625	+1·74	+2·78	
	Propionic acid		711	−1·09	−1·53	
	n-Butyric acid		621	+0·08	+0·13	

* See Table 11.

The table shows that all the mixtures had an effect in sparing body protein from destruction (a N-sparing effect), but that acetic acid given by itself caused, in agreement with previous work, an increase in the excretion of N. The difference between the effect of mixtures and the effect of acetic acid alone was highly significant statistically. The spread of individual values was large, and the mean effects of

increasing the acetic-acid concentration in the mixtures up to 90% had little systematic effect on the increase in excretion/Cal. energy metabolized. The mean value of $-1{\cdot}61 \pm 0{\cdot}30$ mg N/Cal., obtained with the mixtures, is of the same order as that noted by many workers who have given carbohydrate to fasting animals of several species as summarized by Munro (1951).

It is known that the administration of carbohydrate to fasting animals exerts a marked N-sparing effect, whereas the administration of fat does not (see Munro, 1951). This effect is a specific effect of carbohydrate and carbohydrate intermediaries. It would appear from the results given in Table 13 that the N-sparing effect of the mixtures of steam-volatile acids, measured as mg N/Cal. energy metabolized, is independent of the composition of the mixture. Armstrong & Blaxter (1957) have shown that acetic acid, when given alone, caused an increase in N excretion, and that *n*-butyric acid had no effect whereas propionic acid exerted a marked N-sparing effect. If the effects of mixtures of steam-volatile fatty acids on N metabolism were additive properties of their effects when given alone, the N-sparing action of the 75% acetic-acid mixture would not have been $-2{\cdot}07$ mg N but $+1{\cdot}0$ mg N/Cal. energy metabolized. That of the 25% acetic-acid mixture would not have been $-1{\cdot}94$ mg. N but $+0{\cdot}1$ mg N/Cal. energy metabolized. The observations on N metabolism suggest that once the cycles of carbohydrate metabolism of the fasting animal are primed by carbohydrate in small amount, then all sources of energy can be used to spare body protein. This suggestion provides further support for the conclusion of Munro (1951) that though carbohydrate and fat can substitute for one another in sparing body protein during undernutrition, in starvation only carbohydrate exerts a N-sparing effect.

DISCUSSION

Previous work showed that when acetic acid was given to fasting sheep as the sole source of energy, its heat increment was 41% of the energy metabolized: with propionic acid a value of 13% was obtained and with *n*-butyric acid the heat increment was 16% (Armstrong & Blaxter, 1957). The present work shows that with a mixture of propionic and *n*-butyric acids in the molar proportions 3:2 the heat increment is reduced to 9%. Subsequent addition of acetic acid to this mixture results in a small rise in heat increment until the molar proportion of acetic acid is 90% and the heat increment in the region of 15%. Thus the metabolism of each of the acids is considerably modified by the presence of one or more of the others.

The reason for these marked synergistic effects, most noticeable in the metabolism of acetic acid, is probably related to the entry of two-carbon and four-carbon compounds into the tricarboxylic-acid cycle. It was previously pointed out (Armstrong & Blaxter, 1957) that when acetic acid was given to fasting sheep its metabolism caused a heavy drain on blood sugar and stimulated gluconeogenesis from protein. It was suggested that a supply of oxaloacetic acid and of the reduced coenzymes normally arising from metabolism of carbohydrate were necessary for the dissimilation of acetic acid by way of the tricarboxylic-acid cycle. It now appears that very small quantities of propionic acid, constituting not more than one molecule for every fifteen molecules

acetic acid metabolized, are a sufficient supply of carbohydrate intermediary to facilitate oxidation of the acetic acid, and prevent breakdown of body protein to furnish the necessary glucogenic amino-acids.

There is little possibility that differences in the composition of the mixture of steam-volatile acids produced by fermentation of different types of ration could contribute very much to differences in the increments of heat of these rations. Within the physiological range of composition of steam-volatile fatty-acid mixtures present in the rumen (see Table 1) the mean increase in heat increment with increasing proportion of acetic acid in the mixture is best computed from the values obtained when the 25 and 75% acetic-acid mixtures were given. The increase in heat increment per unit rise in the percentage of acetic acid in the mixture was $0{\cdot}03 \pm 0{\cdot}05$ Cal./100 Cal. energy metabolized. This mean value is not only numerically trivial but is not significant statistically.

Differences in the heat increments of submaintenance rations must be due to causes other than differences in composition of the steam-volatile fatty-acid mixtures produced by fermentation in the rumen. There are four possible ways in which such differences in heat increment could arise. First, differences in the contribution of protein to the metabolized energy might be responsible because, in the utilization of the energy of protein, considerable quantities of energy are lost in the urine as urea and other N-containing compounds, and the thermodynamic cost of urea synthesis is high. Thus, the whole of the energy of protein is not available to meet other demands. Secondly, differences in the proportion of the carbohydrate absorbed as simple sugar or as steam-volatile fatty acids would cause variation in the heat increment within a range of 7–15% of the carbohydrate dissimilated. The lower limit represents the value of the heat increment of glucose in the fasting sheep (Armstrong & Blaxter, 1956). Thirdly, the heat produced incidental to the fermentation of food in the rumen could vary from ration to ration, and, finally, differences could occur in the amount of physical work done by the animal in prehending, masticating and ruminating the foods concerned.

Table 15, which embodies unpublished details of experiments already published (Blaxter & Graham, 1955, 1956), shows the heat increments of four dried grasses given to sheep at the maintenance level of nutrition and the partition of the increments of heat into their component parts. Two of the dried grasses, one high in protein content and one low, were given in the long form; the other two, similarly differentiated, were given as cubes. The heat increments ranged from 21 to 38%. The heat arising from oxidation of protein, assessed from the difference in the N excretion of the sheep when fed and when fasted, was largely responsible for the high heat increments on rations containing large amounts of protein. The heat of fermentation was computed from the results of Marston's (1948) in vitro fermentation studies in which he found that for every Cal. of CH_4 produced 0·9 Cal. of heat was evolved. The heats of fermentation so determined varied slightly as between rations.

The difference term in Table 15 represents the work of digestion together with the heat evolved in the metabolism of the end-products of carbohydrate dissimilation. As pointed out above, the latter can vary between limits of about 7–15% of the energy

absorbed. The values obtained for cubes which necessitate little muscular effort to prehend and chew, and which did not cause the sheep to ruminate, gave heat increments due to these two causes of 16%. The additional work involved in masticating chopped dried grass appears to be in the region of 5% of the non-protein calories metabolized or, on a dry-matter basis, 120 Cal./kg grass consumed. This figure is regarded as a minimal one, since it predicates that the ratio of steam-volatile acids to hexose absorbed when cubes and chaff are given remains the same.

Table 15. *Mean heat increments of dried grass determined with sheep at the maintenance level of nutrition, and their partition**

	Dried grass, cubed		Dried grass, long	
	High-protein diet, sheep no. 3	Medium-protein diet, sheep nos. 16–18	High-protein diet, sheep no. 3	Medium-protein diet, sheep nos. 16–18
Energy retained (Cal./24 h)	+66	+109	−12	+78
Energy absorbed† (Cal./24 h)	1613	1577	1782	1597
Containing:				
Protein energy (Cal./24 h)‡	445	264	399	283
Carbohydrate energy (Cal./24 h)§	1168	1313	1383	1314
Heat increment from fasting level (Cal./24 h)	497	325	675	408
Heat increment as a percentage of energy absorbed	30·8	20·6	37·9	25·5
Partition of heat increment:				
Increase or decrease in protein oxidation from fasting level (Cal./24 h)‖	162	−64	203	−56
Heat of fermentation (Cal./24 h)¶	146	174	197	177
Difference, representing heat of work done in mastication and heat increment of products of carbohydrate digestion and fermentation (Cal.)	189	215	275	287
Difference as a percentage of carbohydrate absorbed	16·2	16·3 ± 1·28	19·9	21·8 ± 1·28

* The values were calculated from the experiments of Blaxter & Graham (1955, 1956).
† Energy of food less energy of faeces and methane.
‡ g protein apparently digested × 5·4.
§ The difference between energy absorbed and protein energy absorbed. It includes a small amount of energy as lipid which has been ignored.
‖ Calculated as the increase or decrease in urinary N excretion in g × 26·5.
¶ Calculated as Cal. CH_4 excreted × 0·9 (Marston, 1948).

What is of interest in Table 15 is that the experimental results with steam-volatile fatty-acid mixtures are shown to be consonant with observations on sheep given natural foods. It appears reasonably certain that, in animals given food sufficient to meet their requirement for maintenance, the whole of the carbohydrate dissimilated could be absorbed as fatty acids and yet the heat increment would be low. It would be higher than that observed in man given a comparable amount of digestible carbohydrate, since the heat increment of the acids is 15% in the sheep, whereas in man the heat increment of hexose is about 8%. Furthermore, in man no loss of heat by fermentation takes place.

Whether, above the maintenance datum, when the animal is using the energy arising from carbohydrate dissimilation to synthesize body fat, the composition of the fatty-acid mixture absorbed has any effect on the magnitude of the increment of heat will be the subject of further study. The present study shows that when the energy of mixtures of steam-volatile fatty acids is used to prevent loss of energy from the tissues, composition of the mixture has very little effect and their utilization for this purpose is very efficient.

SUMMARY

1. Ten experiments were carried out in which four fistulated sheep were starved for periods of 4 days and then given mixtures of steam-volatile fatty acids to study the heat production associated with the dissimilation of mixtures of acetic, propionic and *n*-butyric acids, and of propionic and *n*-butyric acids when given to supply between 850 Cal. and 1100 Cal./24 h. The results were compared with those of five experiments previously reported (Armstrong & Blaxter, 1957).

2. With mixtures of volatile fatty acids containing little or no acetic acid, the rate of absorption of the acids from the rumen increased as the experiment progressed.

3. When a mixture of propionic and butyric acids in the molar ratio 3:2 was given, butyric acid initially left the rumen more quickly than propionic acid. Later the reverse was true. This change was associated with a rise in the pH of the rumen.

4. Administration of the acid mixtures gave rise to small increases in the content of steam-volatile fatty acids in peripheral blood and slight depression of the plasma CO_2-combining capacity. The blood sugar increased with administration of all mixtures except with that containing, on a molar basis, 90% acetic acid, 6% propionic acid and 4% butyric acid. In this instance, an initial fall was followed by a rise, and slight ketosis was apparent. Acetic acid alone caused a marked reduction in blood sugar during the first 2 days.

5. The mean increments of heat for the mixture containing propionic acid and *n*-butyric acid in the molar ratio 3:2 was $9{\cdot}3 \pm 1{\cdot}26$ Cal./100 Cal. acid metabolized. For mixtures obtained by addition of acetic acid to this mixture of propionic acid and *n*-butyric acid, heat increments ranged from $12{\cdot}8 \pm 1{\cdot}83$ Cal./100 Cal. metabolized for a mixture with the molar composition 25% acetic acid, 45% propionic acid and 30% butyric acid to $15{\cdot}3 \pm 2{\cdot}36$ Cal./100 Cal. metabolized for the mixture containing 90% acetic acid.

6. Since previous work had shown that acetic acid alone has a heat increment of $41{\cdot}2 \pm 1{\cdot}26$ Cal./100 Cal. metabolized, it was clear that the metabolism of acetic acid was considerably modified by the addition of small quantities of the mixture of propionic acid and *n*-butyric acid.

7. Each of the mixtures caused a reduction in the excretion of urinary N and the reduction was much the same for each mixture, the mean N-sparing effect being $1{\cdot}6 \pm 0{\cdot}3$ mg N/Cal. metabolized. Since acetic acid given alone caused a rise in urinary N excretion, marked synergistic effects in the metabolism of the acids were apparent.

8. The results are discussed in relation to previous studies of food utilization by

sheep, and it is shown that the low increments of heat obtained in the experiments with mixtures of steam-volatile fatty acids are in agreement with the increments of heat found in sheep given natural foods in sufficient quantities to maintain them.

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