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The utilization of acetic, propionic and butyric acids by fattening sheep

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Previous work showed that when steam-volatile fatty acids were given to fasting sheep as the sole source of energy, the heat increments of the acids expressed as Cal./100 Cal. metabolized were, acetic acid 41%, propionic acid 13% and *n*-butyric acid 16% (Armstrong & Blaxter, 1957). These measurements represent the inevitable loss of energy as heat which occurs when the acids are utilized instead of body fat and body protein, the two constituents oxidized by fasting animals to meet the energy demands of essential metabolic processes. It was shown subsequently (Armstrong, Blaxter & Graham, 1957) that, although singly the acids showed marked differences in the efficiency with which they were utilized, when they were present in mixtures representing the fairly wide range of molar proportions of acids likely to be formed by the rumen micro-organisms which ferment dietary carbohydrate, they all exhibited closely similar heat increments of 14–15 Cal./100 Cal. metabolized. This high efficiency of utilization appears to manifest itself after entry of the acids into the tricarboxylic-acid cycle (Armstrong & Blaxter, 1957).

Oxidation of the acids to provide the free energy necessary for the maintenance of minimal functional activity is not the sole way in which the acids could be utilized by the animal. Studies with acetic acid labelled with ^{14}C have shown that this acid is incorporated into many compounds. Lipogenesis in mammary tissue of the ruminant results in considerable incorporation of acetic acid into the constituent fatty acids of

milk fat (Folley & French, 1950; Kleiber, Smith, Black, Brown & Tolbert, 1952). Acetate can also act as a precursor of the milk constituents lactose and glycerol (Popják, Glascock & Folley, 1952) and the carbon moiety of the non-essential amino-acids of milk casein (Black, Kleiber, Smith & Stewart, 1957). Acetic acid is used also in the synthesis of liver fat (Rittenberg & Bloch, 1945) and of the lipids of wool fat (Sjöberg, 1956). It is used also as a structural unit in the synthesis of cholesterol (Bucher, 1953). Though it is now generally accepted that propionate is a major precursor of carbohydrate, the metabolic pathways by which *n*-butyrate is utilized are little known (see Armstrong & Blaxter, 1957). Kleiber, Black, Brown, Luick, Baxter & Tolbert (1954) have shown that in the lactating cow *n*-butyrate is concerned in the synthesis of lactose, and like acetate the C_4 acid can provide the carbon moiety of amino-acids of milk casein (Black, Kleiber & Smith, 1952).

The carbon of the steam-volatile fatty acids is thus undoubtedly incorporated into new compounds synthesized by the animal, but the efficiency of the process is not known. It is not known, for instance, whether in effecting synthesis they are used with the same high efficiency as they are in meeting minimal demands for energy.

This paper deals with the utilization of the energy of the three acids by sheep receiving a ration sufficient or more than sufficient to meet maintenance needs. The results show that the efficiency with which the acids are used to effect net synthesis is considerably less than that found to occur when they are completely oxidized. It must be pointed out, however, that efficiency in this context is a 'calorimetric efficiency' not a 'machine efficiency' (see Hutchens, 1951). Data that would permit calculation of machine efficiency, entailing detailed knowledge of the metabolic pathways of both dissimilation and synthesis and their equilibria together with the entropies and free energy changes involved, are not yet available.

METHODS

Animals. Three adult castrated male sheep, L, P and O, each equipped with a cannula passing through a fistula into the dorsal sac of the rumen, were used as experimental animals.

Rations. Each sheep was given 1000 g of dried grass daily in four feeds at 6 h intervals throughout the experiments. Sheep L was given the grass in the long form and the others were given it coarsely chopped. The composition of the dried grass is given in Table 1.

Experimental procedure. The effect of the acids on metabolism was assessed by giving them by continuous infusion by means of a slow-speed pump (Armstrong & Blaxter, 1957) for 7 days, measuring the metabolism and comparing the results with those obtained during a period of similiar duration in which the animal received daily the same volume of water. Sheep L received water followed by acetic acid. Sheep P received the sequence of treatment, water–acetic acid–propionic acid–*n*-butyric acid–water–*n*-butyric acid. Sheep O received the sequence water–acetic acid–propionic acid–water, in a first series of experiments and then the sequence acetic acid–water–*n*-butyric acid in a second series. Four experiments with acetic acid, two with pro-

pionic acid and three with *n*-butyric acid were thus made. The acid solutions supplied approximately 400 Cal. combustible energy of each acid/24 h, equivalent to 1·9 moles acetic acid, 1·1 moles propionic acid and 0·8 mole *n*-butyric acid. One experiment was made with 800 Cal. *n*-butyric acid (1·5 moles). The volume of solution infused was 2·0 l./24 h.

Table 1. *Percentage composition of the artificially dried grass used as the basal ration*

Conventional analysis		Carbohydrate fractions	
Ash	10·6	Sucrose	0·0
Crude protein	17·9	Hexose	1·6
Ether extractives	2·2	Fructosan	0·0
Crude fibre	25·0	Cellulose	30·2
N-free extractives	44·3	Cellulosic pentosans	6·2
		Non-cellulosic pentosan	2·7

Calorific value (Cal./g) 4·45

Samples taken. The food was weighed to the nearest g and sampled. Samples of ingesta were taken from the rumen once daily. Samples of blood were taken from the jugular vein once weekly after 7 days of continuous infusion. Blood was not analysed in the experiment with the 800 Cal. level of *n*-butyric acid. Measurements of gaseous exchange were made during the final 4 or 5 days of each experimental period. Faeces and urine were collected quantitatively throughout the whole period. In the experiments with sheep L no measurements of the gaseous exchange were made.

Analytical methods. Methods for the determination of pH and concentration of steam-volatile fatty acids in rumen contents and methods used to determine sugar, steam-volatile fatty acids, ketones and CO_2-combining capacity in blood have been given in an earlier paper (Armstrong & Blaxter, 1957). Calorimetric methods were those routinely adopted in this laboratory for the determination of the energy exchange and the retention of C and N in the body (Blaxter, Graham & Rook, 1954; Blaxter & Graham, 1955).

RESULTS

Effect of acid administration on the utilization of the grass. The volume and quantity of acid given were purposely kept low, because there was a possibility that its presence might interfere with the microbiological processes involved in the digestion of the basal ration. Also, to avoid any possible disturbance of digestion and rumen emptying by administration of liquid, comparisons were always made between periods in which the sheep received the acid under study and one or more in which it received water alone. An indication of the extent of any change in the fermentation or other digestive processes of the animals receiving an infusion of water after the introduction of acid into the rumen can be assessed in three ways. First, by direct comparison of conditions within the rumen during infusions of water or acid, secondly, by measuring any changes in the methane production of the animals and lastly by measuring any changes in the faecal energy losses.

Table 2 summarizes the observations made on the pH of the rumen on the 6th day after infusion began. The mean values show that there was a very slight fall of 0·17 unit in the pH of the rumen when acetic acid was given but this fall was not statistically significant. The mean pH 24 h after the beginning of the acid infusion was 6·22, showing that negligible adjustments took place during the whole period. The concentration of steam-volatile fatty acids in rumen liquor was highly correlated with rumen pH as shown in Fig. 1, which summarizes all the observations made. The figure suggests that the pH was lower and the concentration of steam-volatile acids higher when acetic acid was infused. There were, however, no statistically significant changes in the concentration of steam-volatile acids in the rumen liquor when the acids were infused.

Table 2. *Mean* pH *of rumen contents of three sheep receiving infusions of water or of solutions of acetic acid, propionic acid and* n-*butyric acid into the rumen, recorded on the 6th day after the beginning of the infusion*

	Solution infused			
Sheep	Water	Acetic acid	Propionic acid	*n*-Butyric acid
O (first series of experiments)	6·55	6·36	6·45	—
	6·06	—	—	—
O (second series of experiments)	6·43	6·14	—	6·24
P	6·35	6·24	6·39	6·50
	6·55	—	—	6·35*
L	6·55	6·34	—	—
Weighted mean	6·45	6·28	6·42	6·33

* 819 Cal./24 h given; in the other experiments about 400 Cal./24 h were given.

Table 3 gives the mean amounts of methane excreted in the experiments. Sheep P habitually excreted more methane than sheep O, but there was little change in excretion to be ascribed to an effect of the acids in changing the pattern of fermentation. The mean reduction in methane excretion of 17 ± 8·2 Cal./24 h, which represents 0·4% of the energy of the basal ration, was not statistically significant.

Table 4 gives the mean losses of energy in faeces by the sheep and the mean apparent digestibility of the energy of dietary constituents. There was no change in

Table 3. *Mean losses of methane (Cal./24 h) by two sheep receiving infusions of water or of solutions of acetic, propionic and* n-*butyric acids into the rumen*

	Solution infused			
Sheep	Water	Acetic acid	Propionic acid	*n*-Butyric acid
O (first series of experiments)	248	258	258	—
	270	—	—	—
O (second series of experiments)	295	269	—	263
P	305	278	294	307
	301	—	—	293*
Weighted mean	287	270	276	281

* 819 Cal./24 h given; in the other experiments about 400 Cal./24 h were given.

the faecal loss of energy when the acids were given, and the digestibility of the basal ration was unchanged. Furthermore, since approximately 400 and, in one instance, 800 Cal. energy were supplied as acid in addition to this basal ration, it would appear that the acids were completely absorbed from the gut.

The effect on the composition of the blood. Table 5 summarizes the analytical results relating to the composition of peripheral (jugular) blood. Administration of the acids

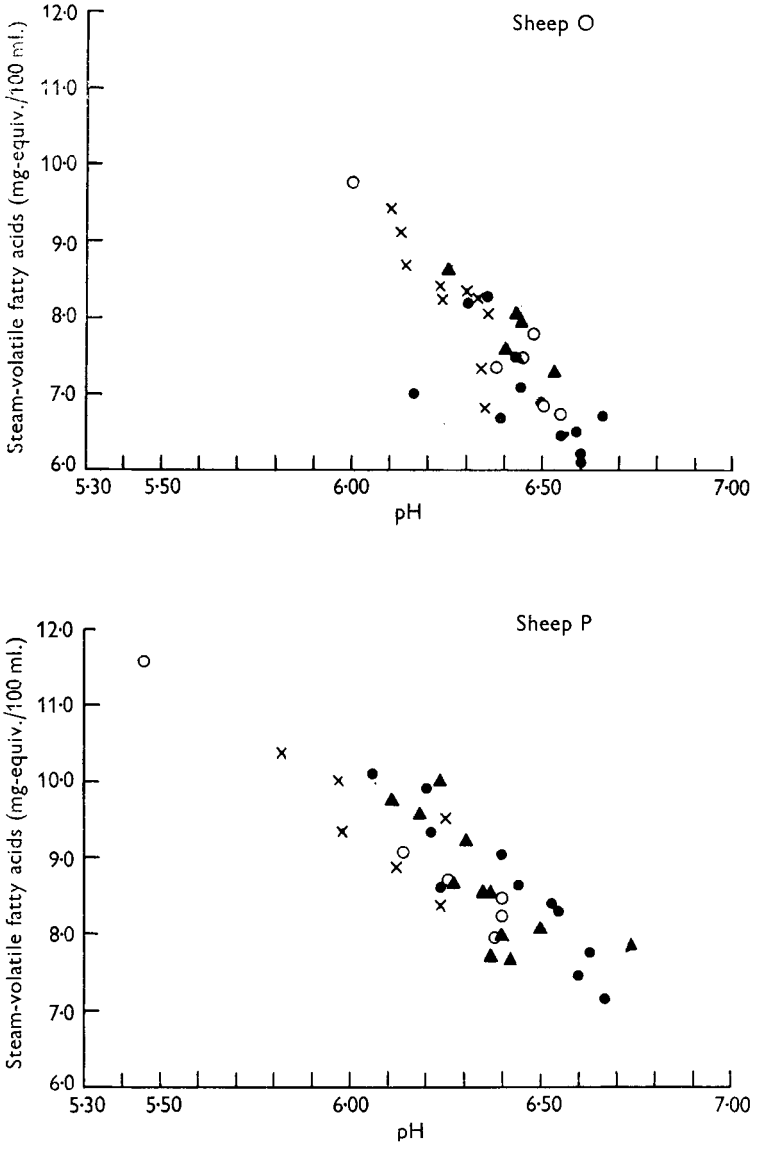

Fig. 1. Relation between the pH and the concentration of steam-volatile fatty acids in the rumen liquor of two sheep (O and P) given a basal ration of dried grass and in addition infusions into the rumen of steam-volatile fatty acids or of water. ●, water; ×, acetic acid; ○, propionic acid; ▲, *n*-butyric acid.

caused a very small increase in blood-sugar content. In agreement with results obtained in the fasting animal (Armstrong & Blaxter, 1957) an increase in blood ketones occurred when *n*-butyric acid was administered. Falls in the CO_2-combining capacity of the plasma took place when acetic acid and *n*-butyric acids were given. The changes in all constituents were small, much smaller than the changes observed when the acids were given to fasting sheep. It may be concluded that the analyses did not reveal any gross or abnormal changes in the metabolism of the sheep resulting from infusion of the acids.

Table 4. *Mean intake of energy as grass (Cal./24 h), loss in the faeces (Cal./24 h), and mean apparent absorption of dietary energy (Cal./100 Cal. ingested) by three sheep receiving infusions of water or of solutions of acetic, propionic and* n-*butyric acids into the rumen*

Solution given	Mean energy intake as grass	Faecal loss of energy	Apparent absorption of dietary energy*
Water	3848	1065	72·3
Acetic acid	3821	1070	71·9
Propionic acid	3973	1077	72·8
n-Butyric acid	3968	1109	72·0

* Assuming complete absorption of the steam-volatile fatty acids infused.

Table 5. *Mean concentrations of sugar, ketones and steam-volatile acids in the peripheral blood and the CO_2-combining capacity of blood plasma of sheep receiving infusions of water or of solutions of acetic, propionic and* n-*butyric acids into the rumen to supply approximately 400 Cal./24 h*

	Solution given			
	Water	Acetic acid	Propionic acid	*n*-Butyric acid
Sugar (mg/100 ml.)	38·5	39·2	40·0	40·0
Ketones (mg acetone/100 ml.)	1·92	1·72	0·91	3·05
Steam-volatile fatty acids (m-equiv./100 ml.)	0·065	0·081	0·062	Not determined
CO_2-combining capacity (vol. %)	51·6	49·8	51·8	45·2
No. of sheep	3	3	2	2

The effect on heat production. The heat production of the sheep when given water or the acids was estimated from the values obtained in the respiration apparatus in two ways: first, from the non-protein respiratory quotient (R.Q.), urinary N excretion, methane production and oxygen consumption; and secondly, as the difference between the energy intake less that in faeces, urine and methane, and the energy stored in the body as fat and protein, these latter being determined from the retention of C and N. In the second method, the final figure depends on fourteen analytical determinations of C, N or heat of combustion and sampling errors are attached to the analysis of food and excreta. The first method utilizes four analytical determinations only and errors would be expected to be lower for this method. The respiratory-quotient method, however, involves the assumption that the non-protein R.Q., cor-

rected for methane production, can be used to measure the proportion of fat and carbohydrate being dissimilated, and that these two sources of energy are the only ones involved. In our experiments the steam-volatile fatty acids were also present and, as pointed out previously (Armstrong & Blaxter, 1957), the calorific equivalent of O_2 for a given R.Q. when a short-chain fatty acid is being oxidized differs from that expected if fat and carbohydrate were being dissimilated. An error of assumption (bias) might appear to be involved in using the R.Q. method. On the other hand, the non-protein R.Q. can be interpreted in terms of fat and carbohydrate only, if it is assumed that the acids are incorporated into fat without themselves undergoing oxidation. Clearly, the bias involved in direct application of the non-protein R.Q. to the values will not be as large as might appear at first consideration. In support of this assumption is the fact that when the acids were given, the non-protein R.Q.'s increased, as shown in Table 6. If the acids had been oxidized completely the non-protein R.Q. would have fallen. Fat formation from the acids would have no effect or only a very slight positive effect on the non-protein R.Q. These considerations suggest that the use of the R.Q. method is justified. The estimation of the heat production from the C and N retention and calorimetric measurements on food and excreta is free from such errors of assumption but, as pointed out above, is likely to be less precise owing to errors of measurement.

Table 6. *Mean non-protein respiratory quotients, corrected for methane production, of sheep receiving infusions of water or of solutions of acetic, propionic and* n-*butyric acids into the rumen*

Solution given	No. of determinations	Mean respiratory quotient
Water	21	0·947
Acetic acid	15	0·985
Propionic acid	10	0·988
n-Butyric acid	13	0·989

Since measurement of metabolism was begun after 2 or 3 days' infusion, and the amounts of acid involved were small, it can be assumed that the measurements represent a 'steady state' and not an approach to an equilibrium.

In each of the three series of experiments the daily determinations of heat production were analysed statistically to provide estimates of the errors attached to each heat increment. An example of the calculation of these errors is given in Table 7, which refers to sheep O given the sequence of treatment water–acetic acid–propionic acid–water. It will be noted that the standard error of the increase in metabolism was about $\pm 10\%$ of the amount determined. Similar errors occurred in the other two series of experiments. Such large percentage errors are understandable because increments of heat were small, indeed the errors attached to the mean estimates of heat production were all less than $\pm 1\%$. No estimate of the error attached to estimates of heat increments based on estimates of heat production calculated from C and N retention in individual experiments can be made since excreta were pooled for analysis. It is likely that it exceeded $\pm 10\%$ of the value obtained.

Table 8 shows the increments of heat determined by the two methods. Analysis of variance of the determinations of heat increment by each method showed that the error variance calculated from the C- and N-retention estimate exceeded, by a factor of 2·8, that calculated from the R.Q. method. This difference was expected for the reasons already given. There was, however, no significant difference between the means of the heat increments determined by the two methods. In combining the two estimates each was weighted according to its invariance. The weighting given to the estimate by the R.Q. method was 0·71 and that given to the estimate by the C- and N-retention method 0·29.

Table 7. *Analysis of variance of mean daily heat production estimated by the R.Q. method (Cal./24 h) of sheep O receiving into the rumen infusions of water in two experiments, acetic acid in one experiment and propionic acid in another, together with the calculation of the heat increment*

Component of variance	Degrees of freedom	Estimated mean square	Variance ratio	Significance
Total determinations	19	—	—	—
Between two determinations with water	1	302·5	4·5	N.S. (error variance the greater)
Between determinations with water, acetic acid and propionic acid	2	126,418·0	91·6	***
Error (within experiments)	16	1,379·9	—	—

Mean heat production, mean heat increments and their standard errors

No. of observations	Solution given	Amount given (Cal./24 h)	Heat production (Cal./24 h)	Heat increment	
				Cal./24 h	Cal./100 Cal. acid given
10	Water	—	2213·9 ± 11·7	—	—
5	Acetic acid	415	2472·4 ± 16·6	258·5 ± 20·3	62·3 ± 4·9
5	Propionic acid	425	2390·1 ± 16·6	176·2 ± 20·3	41·4 ± 4·9

*** Significant when $P < 0.001$.
N.S., not significant.

The mean values given at the foot of Table 8 show that the metabolism of acetic acid under the conditions of these experiments was associated with a loss of 67% of its energy as heat. When propionic acid and butyric acid were given the losses were much lower at 33 Cal./100 Cal. and 38 Cal./100 Cal. respectively. All these values are significantly greater than the values for the fasting animal previously recorded (Armstrong & Blaxter, 1957; Armstrong *et al.* 1957). Calculated as Cal./mole acid metabolized, the heat losses increased with increasing chain length of the acid.

Synthesis of fat and protein. When the sheep received an infusion of water into the rumen they were all storing energy and protein in their bodies. The mean energy retention was 360 Cal./24 h, of which protein storage accounted for 113 Cal./24 h. The 'plane of nutrition' as defined by Blaxter & Graham (1955) was 0·3.

That part of the energy of the acids not liberated as heat was retained in the body. The increases in the daily retention of N when the acids were infused are given in Table 9. Errors were high, but the mean effect of acid infusion was to increase

Table 8. *Heat increments (Cal./24 h and Cal./100 Cal. acid given) of acetic, propionic and* n*-butyric acids in fattening sheep calculated from the heat production determined by the non-protein respiratory-quotient method and by the C- and N-retention method*

Sheep	Acetic acid			Propionic acid			*n*-Butyric acid		
	R.Q. method	C- and N-retention method	Weighted mean*	R.Q. method	C- and N-retention method	Weighted mean*	R.Q. method	C- and N-retention method	Weighted mean*
				Cal./24 h					
O (first series of experiments)	259	238	—	176	219	—	—	—	—
O (second series of experiments)	306	374	—	—	—	—	170	169	—
P	270	289	—	176	249	—	136	157	—
							332†	264	
				Cal./100 Cal. acid given					
O (first series of experiments)	62·4	57·3	61·7	41·4	51·5	42·8	—	—	—
O (second series of experiments)	72·1	88·2	74·3	—	—	—	40·4	40·3	40·4
P	64·6	69·1	65·2	42·2	59·7	44·6	33·8	39·0	34·5
							40·5†	32·3	39·3
Mean heat increment (Cal./100 Cal. acid given)	—	—	67·1 ± 2·6	—	—	43·7 ± 3·2	—	—	38·1 ± 2·6
Mean heat increment (Cal./mole acid given)	—	—	140·5 ± 5·4	—	—	160·5 ± 11·8	—	—	199·7 ± 13·6

* Weighted according to the error invariances.

† 819 Cal./24 h given; in the other experiments about 400 Cal./24 h were given.

N retention by about 400 mg with each acid. The largest increase in N retention occurred in the experiment in which 819 Cal. as *n*-butyric acid were given to sheep P. The energy equivalent of the additional protein retained in this experiment was 75 Cal./24 h, and the total additional energy retained was 508 Cal. Even in this experiment, fat deposition accounted for the major proportion of the energy retained. Protein deposition thus contributed little to the energy retained by the sheep when the acids were given. As shown in the last line of Table 9, about 90% of the energy was stored as fat.

Table 9. *Mean increase in the daily retention of N (g/24 h) by three fattening sheep when receiving infusions of solutions of acetic, propionic and n-butyric acids into the rumen*

	Solution given		
Sheep	Acetic acid	Propionic acid	*n*-Butyric acid
O (first series of experiments)	−0·96	−0·46	—
O (second series of experiments)	+0·31	—	−1·56
P	+0·85	+1·28	+0·69 +2·02*
L	+1·76	—	—
Mean	+0·49	+0·41	+0·38
Mean energy equivalent of protein retained (Cal./24 h)	17	17	17
Mean energy retention (Cal./24 h)	130	216	334
Proportion of energy retained as fat (%)	87	92	95

* 819 Cal./24 h given; in the other experiments about 400 Cal./24 h were given.

DISCUSSION

The technique of giving relatively small amounts of the steam-volatile acids to sheep fed on rations which permitted storage of energy in the body was successful in avoiding gross upsets in conditions within the rumen, and had but minor effects on the composition of the blood. The experiments were thus physiologically acceptable in that comparison of the effects of the acids was not vitiated by large changes in the metabolism of the basal ration. On the other hand, the use of but small additions necessarily increases the errors with which their effects expressed as Cal./100 Cal. metabolized can be measured. The accuracy of measurement of the mean heat increments is thus less than that obtained in similar experiments with fasting sheep (Armstrong & Blaxter, 1957; Armstrong *et al.* 1957) which, on occasion, received nearly three times the quantity of acid supplied in the present experiments. Even so, the mean heat increments and their standard errors of $67{\cdot}1 \pm 2{\cdot}6$, $43{\cdot}7 \pm 3{\cdot}2$ and $38{\cdot}1 \pm 2{\cdot}6$ Cal./100 Cal. for acetic acid, propionic acid and *n*-butyric acid respectively indicate that the errors were kept down to about a tenth of the values determined.

The simultaneous changes in N and energy retention show that the acids were primarily used to synthesize fat in the body, and the high increments of heat indicate that the efficiency of calorie conversion in this process was low. Synthesis of fat from a short-chain steam-volatile fatty acid is an endergonic process, since it can be calculated from thermal data that about 100 Cal. energy/mole have to be supplied to effect

every C-to-C condensation in lengthening the fatty-acid chain. Presumably the heat increments arise from the fact that considerable amounts of the acids have to be oxidized in order to provide the energy to effect these condensations. In contrast, when mixtures of the acids are oxidized in the body to supply the energy necessary for the essential cellular activity which occurs in the fasting animal, and thereby spare body fat from oxidation, they are utilized extremely efficiently (Armstrong *et al.* 1957).

These determinations of the heat increments of steam-volatile fatty acids may be compared with those of heat increments of carbohydrate (starch) measured in animals with simple alimentary tracts in which the carbohydrate is absorbed as hexose (Table 10). In rats and pigs carbohydrate used as a source of energy in lipogenesis

Table 10. *Published data on the heat increments associated with lipogenesis from carbohydrate (a) in animals in which the fermentation of carbohydrate to steam-volatile fatty acids is negligible, (b) in ruminants given the steam-volatile fatty acids, and (c) in ruminants given carbohydrate which undergoes fermentation*

Species	Source of energy	Heat increment (Cal./100 Cal. metabolized)	Reference
Non-ruminants:			
Pig	Starch	17	Fingerling (1914, 1932*a*, *b*)
Rat	Starch	22	Kriss, Forbes & Miller (1934)
Ruminants:			
Sheep	Acetic acid	67	Present work
Sheep	Propionic acid	44	
Sheep	Butyric acid	38	
Ox	Starch (involving some fermentation to steam-volatile fatty acids)	44	Kellner (1900)
Sheep	Starch (involving some fermentation to steam-volatile fatty acids)	40	Jucker (1948)
Ox	Wheat straw (utilized as steam-volatile fatty acids)	77	Kellner (1900)
		75	Fingerling (1936)

results in heat increments of 17–22 Cal./100 Cal. metabolized. This value is much higher than the heat increments of sugar, 5–8 Cal./100 Cal. metabolized (see Lusk, 1931), in the fasting simple-stomached animal and tends to confirm the view that lipogenesis is energetically an expensive process compared with maintenance of the integrity of cellular metabolism during fasting.

The results presented in this and an earlier paper (Armstrong & Blaxter, 1957) show that heat increments of the products of carbohydrate digestion in sheep are higher than the heat increments of the products of carbohydrate digestion in non-ruminants both above and below the maintenance datum.

The most reliable value for the heat increment of dietary fat in lipogenesis in simple-stomached animals is that of Fingerling (1938) of 9 Cal./100 Cal. metabolized, obtained by feeding arachis oil to pigs. This heat increment reflects not only the energy liberated as heat on hydrolysis of the fat, the energy expended in translocation of the fatty acids and their incorporation in depot fat but also the metabolism of the constituent glycerol. The fact that the heat increment of fat given above the maintenance datum is lower than that of carbohydrate in the simple-stomached animal is again

consonant with the suggestion that heat increments in lipogenesis largely depend on the thermodynamic cost of the synthesis.

When carbohydrate is given to ruminants, part is fermented in the rumen to give the lower steam-volatile acids with the production of heat assessed as about 6 Cal./100 Cal. substrate (Marston, 1948). The free energy liberated in this oxidation is used to effect synthesis of bacterial polysaccharide, some of which can possibly be digested at a lower level in the digestive tract. The heat increment of carbohydrate in the ruminant can thus be regarded as consisting of four fractions. One part arises during the bacterial oxidation (the heat of fermentation), a second part arises from the metabolism of the fatty acids in the tissues, a third part arises from metabolism of energy absorbed as hexose, this being derived either from any fraction of the original substrate which escapes bacterial action or from bacterial polysaccharide synthesized, and a final part can be attributed to the physical work done in ingesting and chewing the food and propelling it through the gut. The values given in Table 10 for the heat increments of starch and wheat straw, even when corrected for the heat of fermentation by deducting 6 Cal./100 Cal., are both higher than the value obtained for the heat increment of starch in simple-stomached species. The fact that the value obtained in ruminants for wheat straw is higher than that for starch could arise in several ways: first, the physical work done to obtain 100 Cal. metabolizable energy from straw is undoubtedly higher than it is from starch; secondly, there can be no contribution from preformed hexose capable of hydrolysis by enzymes secreted by the wall of the digestive tract when wheat straw is given, whereas some starch could be hydrolysed and absorbed as hexose because straw contains virtually no simple carbohydrate; thirdly, during the more protracted fermentation of straw more energy may be lost as heat than in the more rapid fermentation of starch; and lastly, as indicated earlier, the proportion of acetic acid in the mixture of acids formed in the rumen would be greater with straw than with starch and increments of heat due to fatty-acid dissimilation would consequently be greater. The present experiments do not enable separation of these effects: they do, however, suggest that differences in the heat increment of carbohydrate foods in ruminants as large as those observed with starch and wheat straw could arise even if very little or no muscular work was involved in consuming equal quantities of metabolizable energy of the materials.

SUMMARY

1. Seventeen calorimetric experiments were made with two fistulated sheep given rations of dried grass sufficient to cause positive energy retention. Two experiments with a further sheep were made outside the respiration apparatus. The experiments were designed to measure the heat losses and other metabolic changes which occurred when steam-volatile fatty acids were given by constant slow infusion over long (7-day) periods.

2. Administration of about 400 Cal. energy as acetic, propionic or *n*-butyric acid or 800 Cal. as *n*-butyric acid had slight effects on rumen pH, rumen steam-volatile fatty-acid concentration, no effect on faecal losses of energy or on methane production and

resulted in but small changes in the concentration of sugar, ketones and steam-volatile fatty acids in blood and in the CO_2-combining capacity of the blood plasma. These results are taken to show that acid administration did not interfere with the normal process of rumen fermentation or impose non-physiological conditions upon the animals.

3. The heat increments of the steam-volatile acids were: acetic acid $67{\cdot}1 \pm 2{\cdot}6$, propionic acid $43{\cdot}7 \pm 3{\cdot}2$ and butyric acid $38{\cdot}1 \pm 2{\cdot}6$ Cal./100 Cal. acid metabolized. These values in fattening sheep greatly exceed those previously noted in fasting sheep (Armstrong *et al.* 1957).

4. The energy retained when the acids were given was partly stored as protein, a small nitrogen-sparing effect similar to that noted in experiments in which fatty-acid mixtures were given to fasting sheep being detected. The energy was, however, largely retained as fat.

5. The results are discussed in relation to the relative nutritive value of roughages and concentrated foods in ruminant animals.

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