must be stressed that these latter findings are of a preliminary nature and are as yet unsupported by any *in vivo* work, so that although we have evidence of an association of pseudo-cholinesterase activity with the white fibre tracts of the central nervous system we are at present unable to reach any conclusions as to its physiological function in these areas.

SUMMARY

1. The relative rates of hydrolysis of a number of different choline esters by preparations from different areas of human and other mammalian brains have been determined.

2. In all the areas studied, a measurable degree of

hydrolysis of benzoylcholine and butyrylcholine was observed.

3. The benzoylcholine-hydrolysing enzyme present in human nervous tissue appears to be a 'butyro-cholinesterase' closely resembling the pseudo-cholinesterase present in human plasma.

4. The white fibre tracts of the human cerebrum were found to contain significantly more of this pseudo-cholinesterase than the grey matter.

Our thanks are due to the Chief Superintendent, Experimental Station, Porton, for providing us with DFP, to Dr V. P. Whittaker for a sample of propionylcholine perchlorate, and to Mr L. C. Beaney and Mr D. H. Burchett for technical assistance. We also wish to thank the Medical Research Council for a grant to one of us (M.G.O.).

REFERENCES

- Adams, D. H. (1949). *Biochim. Biophys. Acta*, **3**, 1.
 Adams, D. H. & Thompson, R. H. S. (1948). *Biochem. J.* **42**, 170.
 Adams, D. H. & Whittaker, V. P. (1949). *Biochim. Biophys. Acta*, **3**, 358.
 Augustinsson, K.-B. (1948). *Acta physiol. scand.* **15**, Suppl. 52.
 Birkhäuser, H. (1940). *Helv. chim. Acta*, **23**, 1071.
 Bloch, H. (1941). *Helv. med. Acta*, **8**, Suppl. 7, 15.
 Boell, E. J. (1945). *J. cell. comp. Physiol.* **25**, 75.
 Burgen, A. S. V. & Chipman, L. M. (1951). *J. Physiol.* **114**, 296.
 Earl, C. J. & Thompson, R. H. S. (1952). *Brit. J. Pharmacol.* (in the Press).
 Hawkins, R. D. & Mendel, B. (1949). *Biochem. J.* **44**, 260.
 Little, J. M. (1948). *Amer. J. Physiol.* **153**, 436.
 Mendel, B., Mundell, D. B. & Rudney, H. (1943). *Biochem. J.* **37**, 473.
 Mendel, B. & Rudney, H. (1943*a*). *Biochem. J.* **37**, 59.
 Mendel, B. & Rudney, H. (1943*b*). *Science*, **98**, 201.
 Nachmansohn, D. (1939). *Bull. Soc. Chim. biol., Paris*, **21**, 761.
 Nachmansohn, D. & Rothenberg, M. A. (1944). *Science*, **100**, 454.
 Nachmansohn, D. & Rothenberg, M. A. (1945). *J. biol. Chem.* **158**, 653.
 Ord, M. G. & Thompson, R. H. S. (1950). *Biochem. J.* **46**, 346.
 Ord, M. G. & Thompson, R. H. S. (1951). *Biochem. J.* **49**, 191.
 Sawyer, C. H. (1946). *Amer. J. Physiol.* **146**, 246.
 Smith, M. I. & Lillie, R. D. (1931). *Arch. Neurol. Psychiat., Chicago*, **26**, 976.
 Sturge, L. M. & Whittaker, V. P. (1950). *Biochem. J.* **47**, 518.
 Zeller, E. A. (1949). *Helv. chim. Acta*, **32**, 484.

The Metabolism of Short-Chain Fatty Acids in the Sheep

1. FATTY ACID UTILIZATION AND KETONE BODY PRODUCTION BY RUMEN EPITHELIUM AND OTHER TISSUES

By R. J. PENNINGTON

Rowett Research Institute, Bucksburn, Aberdeenshire

(Received 5 November 1951)

Large quantities of volatile fatty acids, chiefly acetic, propionic and butyric acids, are produced by the bacterial decomposition of the foodstuffs in the rumen of the sheep and other ruminants and are absorbed directly from this organ (see review by Elsdon & Phillipson, 1948). The problem of assessing the relative amount of each acid produced is not altogether straightforward, owing to differing rates of absorption. Application of chromatography has led to the identification of smaller quantities of

higher isomers and of formic acid (Gray, Pilgrim, Rodda & Weller, 1951). Subsidiary quantities of volatile fatty acids are also produced in and absorbed from the large intestine.

Although the production of volatile fatty acids in the gut is not confined to ruminants (Phillipson, 1947*a*), the latter present a special problem since it is highly probable that these compounds, previously considered as by-products of digestion, form their chief source of energy. The amount of sugar available

for absorption appears to be very small; it is unlikely that much escapes fermentation in the gut. McClymont & Scarisbrick (unpublished data, quoted by McClymont, 1951) obtained evidence suggesting that at least on a diet of lucerne hay and green kale the amount of reducing sugar available to a sheep from intestinal enzymic digestion is equivalent to only 1–3 g. of glucose per day. The level of volatile fatty acid in the peripheral blood of the sheep is up to about 10 mg./100 ml. (Schambye & Phillipson, 1949; Reid, 1950), which is many times greater than that reported for the dog and man (McClendon, 1944). Conversely, the blood glucose level in adult sheep does not normally exceed 50 mg./100 ml. (Schambye & Phillipson, 1949; Reid, 1950); this is only about one-half that found in most mammals.

Obviously the study of the subsequent fate of the volatile fatty acids is a major problem in ruminant physiology. Few investigations have yet been reported in this field, although numerous studies with the usual laboratory animals have shown that some animal tissues are capable of utilizing these substances, which cannot be stored in the body to an appreciable extent, unlike carbohydrates or the higher fatty acids. Acetic acid is now looked upon as an important metabolic intermediate (Bloch & Rittenberg, 1945) and a precursor of a wide variety of compounds in the body, including higher fatty acids and cholesterol. It can be converted to ketone bodies in the liver. Butyric acid is known to be strongly ketogenic in liver tissue, by which it is readily metabolized. Propionic acid, on the other hand, can give rise to extra carbohydrate; in ruminants, whose supply of carbohydrate is otherwise restricted, this latter fact is probably of considerable importance. Jarrett & Potter (1950) administered acetate and propionate to sheep and found that acetate increased the level of ketone bodies in the blood whilst propionate decreased the ketone bodies and raised the levels of pyruvic and lactic acids. There was little rise in ketone bodies when acetate and propionate were administered together, and they suggest that the oxidation of acetate is linked with that of propionate. Schultz, Smith & Lardy (1949) could detect no increase in blood ketone bodies after administration of acetic acid to goats, although butyric and some higher acids were ketogenic.

The starting point of the work described in the present paper was the observation (Masson & Phillipson, 1951; Kiddle, Marshall & Phillipson, 1951) that the amount of butyric acid in the blood draining the sheep's rumen was less, relative to the level of acetic acid, than the relative amount disappearing from the rumen, a fact which pointed to a utilization of butyrate in the rumen wall. This was probably not simply an esterification, since

McClymont (1951) could find no significant level of esterified lower fatty acids in bovine blood lipids. The *in vitro* metabolism of acetic, propionic and butyric acids by the rumen epithelium has now been investigated in detail and studies have been made with other sheep tissues.

EXPERIMENTAL

Tissues were obtained from sheep killed at the slaughterhouse. They were removed immediately into partly frozen Krebs-Ringer solution (Umbreit, Burris & Stauffer, 1945), and the experiments were normally commenced after approximately 2 hr. Tissue sections were prepared as described below, collected in ice-cold Krebs-Ringer, pooled, gently blotted with filter paper and a weighed amount (usually 2 g.) incubated in 50 ml. conical flasks with 10 ml. of Krebs-Ringer-phosphate, pH 7.2 (Umbreit *et al.* 1945) containing 100 μ moles of the sodium salt of one of the fatty acids. The flasks, after filling with O_2 , were shaken (105 strokes/min.) in a water bath at 39.5° for 3 hr. Any variation in this procedure is stated in the text and tables. Following incubation the tissue was removed and washed twice with distilled water. The dry weight of the tissue was determined by drying at 105° to constant weight. The medium and washings were combined and made up to 25 ml. For determination of the residual volatile fatty acid 1 ml. of 10N- H_2SO_4 and 8 g. of $MgSO_4 \cdot 7H_2O$ were added to 10 ml. of this liquid, followed by steam distillation in a Markham still. A sample of the distillate (100 ml.) was titrated with 0.01N-NaOH under CO_2 -free conditions; each determination was carried out in duplicate. The addition of $MgSO_4$ served not only to increase the rate of distillation of the fatty acid, but also to hold back HCl, which otherwise came over in appreciable quantities. It was found by preliminary tests that approximately 5% of the unused fatty acid was retained by the tissue, even after washing, and all the results obtained were corrected by this amount.

Volatile fatty acid in blood was determined by the procedure of McClendon (1944) with the slight modification due to Phillipson (1947a).

Ketone bodies were determined on the tissue medium and in blood by the method of Greenberg & Lester (1944). The dinitrophenylhydrazine reagent was dissolved in 0.5N-HCl instead of 2N suggested by the authors; this obviated the high blanks which were otherwise sometimes encountered. Also, it was found that rapid shaking for 10 min. was not always adequate for extraction of the colour into the CCl_4 and 15 min. were allowed. A further quantity of ketone bodies, amounting to approximately 10% of the quantity in the medium, could be extracted from the tissue sections by boiling with water under reflux after separation from the medium and washing. All values were corrected for this factor.

In view of the recognized difficulties in the accurate determination of ketone bodies it was felt advisable to compare the method with a different one. The procedure of Weichselbaum & Somogyi (1941) was applied to two filtrates following incubation of rumen epithelial tissue in the butyrate medium and to a sample of arterial blood from a normal sheep. The results were respectively 38.5 and 48.3 μ moles and 21.8 μ moles/100 ml.; the results obtained on the same samples with the Greenberg & Lester method were 47.5 and 44.2 μ moles and 23.3 μ moles/100 ml.

RESULTS

In some preliminary experiments the disappearance of volatile fatty acids when incubated with slices cut from the rumen wall was measured. Table 1 shows the results of experiments with material from three sheep. It is evident that all three acids were metabolized under these conditions and that there

tion of the muscle slices is responsible for this. A further point is that the relative rate of disappearance of butyrate was greater with the epithelium than with the muscle tissue. In subsequent experiments epithelium alone was used. Table 3 shows the results obtained with material from ten sheep; it gives an idea of the extent of the variation in the ability to utilize each fatty acid. In a further

Table 1. *Uptake of volatile fatty acids by sheep rumen slices*

(Ringer-phosphate, pH 7.2, containing 100 μ moles of fatty acid as Na salt. 1 g. (wet wt.) of tissue in sheep no. 1, 2 g. in sheep nos. 2 and 3.)

Sheep no.	Duration (hr.)	Temperature (°)	Fatty acid lost (μ moles)		
			Acetic	Propionic	Butyric
1	3	37.5	5.3 6.1*	4.6 4.1*	18.1 19.0*
2	4	34.0	19.0	15.3	38.3
3	3.5	37.5	18.2	11.1	62.9

* Slices first kept for 6 hr. in ice-cold Ringer.

Table 2. *Uptake of volatile fatty acids by rumen epithelium and rumen muscle*

(Tissue (2 g.) incubated for 3 hr. at 39.5° in Ringer-phosphate containing 100 μ moles of fatty acid as Na salt.)

Sheep no.	Fatty acid lost (μ moles)					
	Epithelium			Muscle		
	Acetic	Propionic	Butyric	Acetic	Propionic	Butyric
1	17.2	10.0	92.5	9.8	8.3	19.2
2	22.7	28.5	79.0	8.3	6.8	11.0

was a marked preference for butyrate; this confirms the conclusion drawn from the absorption studies mentioned above. The stability of the systems responsible, i.e. no measurable loss of activity after several hours in the cold (sheep 1, Table 1), increases confidence that the activity of the rumen wall towards these substances would not be markedly different *in vivo*.

Since the rumen wall comprises predominantly two separate types of tissue, the stratified squamous epithelium and muscle, the activity of each was next studied separately (Table 2). This was easily accomplished by peeling off the epithelium and slicing the muscle in the usual manner. It was unnecessary, and indeed difficult, to slice the epithelium; it was simply cut into pieces about 1 cm. square. Such preparations contained connective tissue in addition to the epithelial cells, but were free from muscle, as shown by histological examination. Rumen epithelium is an ideal tissue for *in vitro* metabolic studies, being thin and tough, with a large surface area owing to its papillae; moreover, large quantities can be prepared in a very short time.

It can be seen from the figures that the epithelium was considerably more active than the muscle, although it is not known to what extent the greater tissue damage necessarily involved in the prepara-

experiment, four parallel runs with butyrate using epithelium from the same animal gave results of 26.2, 27.2, 27.1 and 27.6 μ moles.

Table 3. *Uptake of volatile fatty acids by samples of rumen epithelium from ten sheep*

(Tissue (2 g.) incubated for 3 hr. at 39.5° in Ringer-phosphate containing 100 μ moles of fatty acid as Na salt. The figures in this and subsequent tables (4-8) are calculated to 100 mg. (dry wt.) of tissue.)

	Fatty acid lost (μ moles)			Volatile acid production in control (μ moles)
	Acetic	Propionic	Butyric	
	5.2	7.0	25.1	2.6
	5.6	7.8	25.3	0.7
	4.0	2.2	29.5	2.9
	5.6	10.4	28.9	1.8
	4.9	8.6	30.1	1.1
	8.0	19.0	36.0	0.3
	2.1	1.5	15.3	1.2
	4.5	10.6	26.3	1.5
	4.7	4.6	29.4	1.7
	4.1	3.2	29.3	2.7
Mean	4.9	7.5	27.5	1.6

The figures in the fourth column of Table 3 represent the volatile acid production in control flasks containing no added fatty acid; the figures in

the other columns have been corrected by the addition of the figures in column 4. In this and in subsequent tables the amounts of fatty acid utilized are calculated to 100 mg. of tissue (dry weight) in order to eliminate the effect of any variations in the

Table 4. *Influence of pH of medium upon uptake of volatile fatty acids by rumen epithelium*

(Tissue (2 g.) incubated for 3 hr. at 39.5° in Ringer-phosphate containing 100 μ moles of fatty acid as Na salt.)

pH	Fatty acid lost (μ moles)			Volatile acid production in control (μ moles)
	Acetic	Propionic	Butyric	
7.2	4.9	8.6	30.1	1.1
6.5	5.6	2.1	16.1	1.0
5.8	3.9	0.5	10.3	0.5

wet weight/dry weight ratio. Such variations never exceeded 10% of the ratio in any one experiment. The loss of dry weight during incubation of the tissue amounted to less than 10%, so that the figures are slightly higher than if based on initial dry weight.

It is clear that in every experiment the disappearance of butyrate exceeded that of the other two.

The activity of the tissue appeared to be well maintained during the 3 hr. incubation, since when incubated for only 1 or 2 hr. the amount of acid disappearing was correspondingly less. A longer incubation period was not used owing to the risk of bacterial growth in the flasks.

Some experiments were carried out in which the conditions of incubation were altered. When the concentration of fatty acids was increased eightfold the amounts disappearing were increased 3–5 times. Table 4 shows the effect on the disappearance rates of lowering the pH of the medium by the addition of hydrochloric acid. (The additional chloride thus introduced was very small compared to that already present in the medium and would not, in itself, be expected to influence the results.)

The effect of using bicarbonate buffer of approximately the same pH (Krebs-Ringer-bicarbonate, Umbreit *et al.* 1945) instead of phosphate buffer, in an otherwise identical medium, was also determined. Ciaranfi (1936) found a more rapid utilization of butyric acid by liver slices in a phosphate-buffered than in a bicarbonate-buffered medium. The results of three experiments with different sheep are shown in Table 5.

Table 5. *Influence of buffer and of carbon dioxide upon uptake of volatile fatty acids by rumen epithelium*

(Tissue (2 g.) incubated for 3 hr. at 39.5° in Ringer-phosphate or Ringer-bicarbonate containing 100 μ moles of fatty acid as Na salt.)

Sheep no.	Buffer	Gas phase	Fatty acid lost (μ moles)		
			Acetic	Propionic	Butyric
1	Phosphate	O ₂	5.6	7.8	25.3
	Bicarbonate	O ₂ + 5% CO ₂	5.0	13.7	24.6
2	Phosphate	O ₂	5.6	10.4	28.9
	Bicarbonate	O ₂ + 5% CO ₂	3.6	20.3	29.9
	Phosphate	O ₂ + 5% CO ₂	6.5	18.8	31.4
3	Phosphate	O ₂	4.4	1.2	27.3
	Phosphate	O ₂ + 5% CO ₂	6.0	6.0	27.1

Although the mean of the propionate figures is greater than that of the acetate, this is of little importance owing to the very great variation among the propionate results. (It is of interest that the variance of the latter is 26.98, compared to 2.24 for acetate; a variance ratio of 12 indicates a highly significant difference ($P=0.002$) between the two variances.)

In one of the experiments the rates of distillation of the unused acids were recorded by collecting the distillates in 10 ml. fractions and titrating each fraction. In each case the observed rate corresponded exactly with that of an equivalent quantity of the original fatty acid, thus providing a check that the estimated volatile acid in the experiments represented entirely the unused portion of the original 100 μ moles and not a different volatile acid produced from it by the tissue.

In the first experiment the substitution of bicarbonate for phosphate had a striking effect upon the utilization of propionate, the disappearance rate of which was nearly doubled. This effect was confirmed in the second experiment, where, in addition, the disappearance rates were determined using phosphate buffer but gassing the flasks with oxygen + 5% of carbon dioxide, as used with the bicarbonate buffer. The elevation in the rate of disappearance of propionate was maintained. In the case of the other two fatty acids there were no such marked differences resulting from the presence of carbon dioxide. The relatively small effect upon acetate utilization resulting from the presence of carbon dioxide may not be significant. This will be investigated further.

The important question of the fate of the fatty acids metabolized by the rumen epithelium was

partly answered by testing the media, after incubation, for ketone bodies. Application of Rothera's test invariably gave a strong response following the disappearance of butyrate. A positive response was obtained also on the acetate media but none in the case of propionate, although the controls incubated without fatty acid always gave a little colour. In two subsequent experiments determinations of ketone bodies were carried out (Table 6: results with two sheep).

Table 6. *Production of ketone bodies from volatile fatty acids by rumen epithelium*

(Tissue (2 g.) incubated for 3 hr. at 39.5° in Ringer-phosphate containing 100 μ moles of fatty acid as Na salt. The figures in parentheses are the percentages of the maximum possible production of ketone bodies from the fatty acid utilized.)

Ketone bodies produced (μ moles)
in presence of

Acetic	Propionic	Butyric	Control
1.8 (76)	-2.7	22.7 (77)	4.4
0.4 (19)	-2.7	18.9 (65)	3.3

The figures (calculated as usual to 100 mg. dry weight) are corrected for the ketone production in the controls. The amount of fatty acid utilized was also determined (nos. 9 and 10 in Table 3) and the proportion of this appearing as ketone bodies calculated (assuming that one molecule of ketone is derived from two molecules of acetic acid or one molecule of

butyric acid). It can be seen that more than one-half of the butyric acid disappearing could be accounted for as ketone bodies. Of the total ketone body production less than one-quarter was present as β -hydroxybutyric acid. In the case of acetate the relatively small difference from the controls makes the validity of the calculated percentages doubtful, since it is impossible to say whether the endogenous reactions producing ketone bodies in the controls would continue at the same rate in the presence of added substrate. If not, the corrections are not justified and the figures should consequently be higher. The appearance of ketone bodies in the propionate medium was in each case less than that in the control medium.

In another experiment the initial concentration of butyrate was halved (50 μ moles/flask). The proportion of that lost appearing as ketone bodies was 65 % as compared to 70 % in a parallel run employing the usual concentration of butyrate. In the experiment employing eightfold concentrations of the fatty acids, mentioned above, 59 % of the metabolized butyric acid was converted to ketone bodies.

In further experiments the ability of other tissues of the sheep to utilize the same fatty acids under identical conditions was determined; the ketone body production was measured in each case. The results are summarized in Table 7. Both fatty acid disappearance and ketone production are corrected by the control figures, as in previous tables.

Table 7. *Volatile fatty acid uptake and ketone body production by various sheep tissues*

(Tissue (2 g.) incubated for 3 hr. at 39.5° in Ringer-phosphate containing 100 μ moles of fatty acid as Na salt. Results given as μ moles. The figures in parentheses are the percentages of the maximum possible production of ketone bodies from the fatty acid utilized.)

Tissue	Acetic		Propionic		Butyric		Control	
	Fatty acid used	Ketone bodies produced	Fatty acid used	Ketone bodies produced	Fatty acid used	Ketone bodies produced	Volatile acid produced	Ketone bodies produced
Reticulum	5.3	1.5 (56)	3.9	-0.3	23.8	17.5 (73)	1.3	0.6
epithelium	2.5	0.2 (16)	1.5	-0.2	10.8	5.3 (49)	0.3	0.5
Omasum	4.6	1.9 (83)	1.4	-0.8	30.0	24.2 (81)	0.0	1.7
epithelium	1.6	0.3 (38)	0.7	-0.2	8.6	4.0 (47)	0.8	1.0
Abomasum	18.8	-0.4	14.2	-0.4	18.2	3.3 (18)	0.2	0.9
epithelium	20.2	2.2 (22)	13.7	0.5 (8)	16.9	5.3 (31)	0.0	0.4
Caecum	12.6	0.4 (6)	10.0	0.2 (4)	9.4	1.1 (12)	1.0	0.3
epithelium	10.8	-0.9	6.7	-0.9	10.0	1.0 (10)	1.2	1.8
	13.0	0.3 (5)	8.8	0.0	11.6	0.3 (3)	0.7	1.2
Liver	6.9	0.9 (26)	5.9	0.6 (20)	20.0	14.8 (74)	0.6	0.0
	15.8	1.6 (20)	22.3	-3.8	23.9	16.6 (69)	0.9	1.9
Kidney cortex	42.6	0.0	43.8	0.0	38.5	3.8 (10)	0.0	1.3
	54.5*	0.6 (2)	60.9	0.0	41.2	8.6 (21)	0.0	0.4
Heart muscle	0.0	—	0.0	—	1.4	0.1 (7)	0.4	0.2
	4.2	0.1 (2)	0.4	0.0	1.1	0.2 (18)	0.6	0.0
Brain	0.0	—	0.0	—	0.0	—	1.2	—
	0.0	—	0.0	—	0.0	—	2.1	—

* Only 1 g. of tissue used.

The figures in each line in the table were obtained on tissue from a different sheep. The epithelial tissues were prepared in the same way as the rumen epithelium; although this was rather difficult in the case of epithelium from the abomasum and caecum owing to their greater fragility, satisfactory results were obtained after a little practice. The other tissues were sliced in the standard manner. As a

ketone bodies. On the other hand, kidney cortex, and epithelium from the abomasum (the true stomach of the sheep) and from the caecum, utilized acetate at least as rapidly as butyrate and, moreover, produced a relatively smaller amount of ketone bodies. The results with heart-muscle slices were low and less consistent. Slices of whole brain did not utilize any of the three fatty acids.

Table 8. *Volatile fatty acid uptake and ketone body production by rat tissues*

(Liver tissue (2 g.) or 1 g. of kidney tissue incubated for 3 hr. at 39.5° in Ringer-phosphate containing 100 μ moles of fatty acid as Na salt. Results given as μ moles. The figures in parentheses are the percentages of the maximum possible production of ketone bodies from the fatty acid utilized.

	Acetic		Propionic		Butyric		Control	
	Fatty acid used	Ketone bodies produced	Fatty acid used	Ketone bodies produced	Fatty acid used	Ketone bodies produced	Volatile acid produced	Ketone bodies produced
Liver	27.0	3.1 (23)	23.1	2.1	31.5	19.6 (62)	0.7	7.7
Kidney	92.5	1.2 (3)	56.8	0.9 (3)	58.0	6.0 (10)	0.9	0.0

Table 9. *Changes in blood volatile fatty acids and ketone bodies resulting from the administration of fatty acids into the rumen*

(Results expressed as μ moles/100 ml.)

Liquid in rumen	Rumen blood		Carotid blood	
	Ketone bodies	Fatty acid	Ketone bodies	Fatty acid
Exp. 1				
Water	48	77	58	27
Sodium butyrate, 0.15 M	222	118	199	73
Water	83	46	86	41
Sodium propionate, 0.15 M	83	369	86	100
Water	60	46	58	50
Sodium acetate, 0.15 M	78	611	76	114
Water	19	68	48	45
Sodium butyrate, 0.15 M	219	123	191	64
Exp. 2				
Water	47	50	47	50
Sodium butyrate, 0.15 M	310	375	266	146
Water	110	32	95	27
Sodium propionate, 0.15 M	43	565	43	100
Water	24	27	43	27
Sodium acetate, 0.15 M	100	1088	47	142
Water	33	32	47	36
Sodium butyrate, 0.15 M	195*	320*	134*	96*
	334	246	248	50

* Blood samples taken after 5 min. All other samples taken 30 min. after administration.

check, in each case, rumen epithelium from the same sheep was incubated with butyrate; in every case the amount of butyrate utilized fell within the range of values for butyrate seen in Table 3 and the proportion converted to ketone bodies ranged from 59 to 74%. Inspection of Table 7 shows that epithelium from the reticulum and omasum and also liver tissue behaved in a similar manner to rumen epithelium in that they utilized butyrate most readily and converted a large proportion of it into

For comparison exactly similar experiments were made with liver and kidney tissue from adult male rats. The results (Table 8), which concord with those reported by other workers, show no important differences from those obtained with the corresponding tissues of the sheep (Table 7).

The production of ketone bodies on such a scale by the epithelia of sheep fore-stomachs was rather unexpected, and it was considered desirable to try to confirm this by an *in vivo* experiment. For this

purpose a ewe with a permanent rumen fistula was anaesthetized by intravenous injection of Nembutal and operated upon in the manner described by Masson & Phillipson (1951) (the oesophagus and reticulo-omasal orifice were closed by ligatures and the carotid artery and posterior rumen vein exposed for withdrawal of blood samples). The rumen was emptied and washed clean with warm water. Blood samples were withdrawn for ketone body and volatile acid determination. Portions (3 l.) of each of the liquids indicated in Table 9, warmed to about 40°, were placed in the rumen in the order given in the table. Each was allowed to remain for half an hour, blood samples were then taken and the rumen washed out with warm water before the next insertion. Table 9 records the changes in ketone body and volatile acid levels of the blood in the rumen vein and carotid artery. The experiment was repeated on another sheep; in this case blood samples were taken at 5 min., as well as at 30 min., after the final addition of butyrate.

It may be seen that in each experiment the presence of butyrate in the rumen led to several-fold increases in the level of ketones in the blood. In every case the increase was greater in the blood draining the rumen than in the arterial blood, as would be expected if ketone bodies were produced in the rumen wall. A smaller rise of blood ketone bodies occurred with acetate in the rumen, whilst propionate produced no increase in the first experiment and a pronounced decrease in the second. In the first experiment each of the four solutions, after withdrawal from the rumen, was also analysed for ketone bodies. A total of 44.5 and 22.2 mg. respectively (as β -hydroxybutyric acid) was found in the butyrate solutions and 9.0 mg. in the propionate solution. No ketone bodies appeared in any of the other solutions.

DISCUSSION

The marked ability of the epithelia of sheep stomachs to metabolize the lower fatty acids, shown in these experiments, substantiates the conclusions drawn from previous work that an appreciable portion of the butyric acid absorbed is removed before it can enter the circulation. The results also show that this may apply, though to a lesser extent, to propionic and acetic acids. Although too much reliance cannot be placed on the quantitative significance of such *in vitro* studies, it is perhaps of interest to compute that the mean value for butyrate loss in Table 3 would represent a utilization rate of about 0.3–0.5 g. of butyric acid/hr. by the epithelium of the whole rumen, and correspondingly more than this when the reticulum and omasum are also taken into account. The concentration of butyrate used in these experiments is of the order of that

normally found in rumen liquor. These epithelia are not glandular and hence would not require the energy of oxidation of the fatty acids for secretory purposes. It is possible, however, that such energy may be utilized in connexion with 'active absorption' of substances from the rumen.

The fact that such a large proportion of the butyric acid was converted to ketone bodies by these tissues is also of considerable interest. Ketone body production on an important scale is generally acknowledged to be confined to the liver in the common laboratory animals. In the experiments reported in this paper, the epithelia of the fore-stomachs of the sheep were about as active, weight for weight, as liver tissue in the production of ketone bodies. The total wet weight of these epithelia was determined in one animal and found to be over half the weight of the liver, hence these tissues must be considered as important sources of ketone bodies in the sheep. It is possible that such a supplementary production of ketone bodies, which are known to be utilized by other tissues (Breusch, 1948), assists the animal in dealing with the large fatty acid intake from these organs. However, it remains to be seen whether such speculations are valid under normal conditions where mixtures of fatty acids are absorbed from the rumen.

On comparing the activities of all the tissues studied it is of interest to note that only the tissues which converted a large proportion of the butyrate into ketone bodies showed a marked preference for this fatty acid over the other two. The inability of the sheep-brain tissue to metabolize fatty acids is in accordance with the failure of other workers to observe the oxidation of acetate by brain tissue of other animals and of Quastel & Wheatley (1933) to observe the oxidation of butyrate. It appears that brain tissue may be unique in this respect.

The suggestion of McClymont (1951) that enzymes for metabolizing propionic acid in the sheep are concentrated in the liver is not borne out here. McClymont quotes, in support, the observation of Grafflin (1948) that a cyclophorase preparation from rabbit kidney was incapable of oxidizing propionic acid although acetic acid was readily oxidized; both acids were readily oxidized by the liver preparation. In the present experiments propionate was removed most rapidly by kidney and was utilized by all the tissues with the exception of brain and possibly of cardiac muscle. The fate of the propionate is an important question which is being further studied. As mentioned previously, it seems highly probable, from present knowledge of ruminant digestion, that propionate may be the main source of carbohydrate for these animals. It has been demonstrated (Hitchcock & Phillipson, quoted by Phillipson, 1947*b*) that the administration of propionate to the phlorrhizinized sheep leads to the excretion of an

equivalent amount of glucose in the urine. It will be of interest to see whether the direct conversion of propionate to carbohydrate by sheep tissues can be demonstrated *in vitro* and, if so, whether this process is confined to the liver. The observation that the presence of carbon dioxide enhances the utilization of propionate points to a possible fixation of carbon dioxide in the metabolism of the latter. Carboxylation to succinate followed by carbohydrate synthesis would seem an obvious possibility, although there is no good evidence that propionate can be converted directly to succinate in living tissues.

An important difference between the experimental conditions here used and the conditions *in situ* is that in the latter case the tissues usually have available a mixture of the fatty acids. One possibility then is that propionate could be oxidized simultaneously with the other fatty acids and help to promote complete oxidation of the latter instead of ketone body formation. Quastel & Wheatley (1933) found that propionate inhibited ketone body formation from butyrate by liver slices, although their results could be explained simply by selective utilization of propionate. Jarrett & Potter (1950) in the paper already mentioned suggest that propionate, by conversion to pyruvate, may facilitate the metabolism of acetate. The modern concept of antiketogenesis, namely, the provision of pyruvate, which, by carboxylation to oxaloacetate increases the operation of the Krebs cycle can only be applied to tissues with β -carboxylase activity (Baldwin,

1949). It will be of interest to see whether antiketogenesis can be demonstrated in the rumen epithelium.

SUMMARY

1. The ability of rumen epithelial tissue of the sheep to metabolize butyric acid and, to a lesser extent, acetic and propionic acids, under aerobic conditions has been demonstrated; these compounds are amongst the chief products of fermentation in the rumen.

2. A large proportion of the butyric acid was converted to ketone bodies, mainly acetoacetic acid, by this tissue. Ketone bodies were formed also from acetic acid, but not from propionic acid.

3. The utilization of propionic acid was more rapid under an atmosphere containing 5% of carbon dioxide than under an atmosphere of oxygen.

4. The utilization of the volatile fatty acids by other sheep tissues has also been studied. Ketone bodies were produced from butyric acid by the epithelium of the reticulum and of the omasum as well as by liver tissue; all these tissues metabolized butyric acid more rapidly than acetic or propionic acids. The epithelia of the abomasum and caecum, and kidney tissue, did not show this preference for butyrate and also produced relatively less ketone bodies.

The author wishes to thank Dr A. T. Phillipson for valuable discussion and advice and for performing the operations, Dr J. M. Naftalin for histological examination of the rumen epithelium preparations and Mr R. Green for competent technical assistance.

REFERENCES

- Baldwin, E. (1949). *Dynamic Aspects of Biochemistry*, 1st ed. Cambridge University Press.
- Bloch, K. & Rittenberg, D. (1945). *J. biol. Chem.* **159**, 45.
- Breusch, F. L. (1948). *Advanc. Enzymol.* **8**, 343.
- Ciaranfi, E. (1936). *Biochem. Z.* **285**, 228.
- Elsden, S. R. & Phillipson, A. T. (1948). *Ann. Rev. Biochem.* **17**, 705.
- Graffin, A. L. (1948). *J. biol. Chem.* **176**, 95.
- Gray, F. V., Pilgrim, A. F., Rodda, H. J. & Weller, R. A. (1951). *Nature, Lond.*, **167**, 954.
- Greenberg, L. A. & Lester, D. (1944). *J. biol. Chem.* **154**, 177.
- Jarrett, I. G. & Potter, B. J. (1950). *Nature, Lond.*, **166**, 515.
- Kiddle, P., Marshall, R. A. & Phillipson, A. T. (1951). *J. Physiol.* **113**, 207.
- McClendon, J. F. (1944). *J. biol. Chem.* **154**, 357.
- McClymont, G. L. (1951). *Aust. J. agric. Res.* **2**, 92.
- Masson, M. J. & Phillipson, A. T. (1951). *J. Physiol.* **113**, 189.
- Phillipson, A. T. (1947*a*). *J. exp. Biol.* **23**, 346.
- Phillipson, A. T. (1947*b*). *Nutr. Abstr. Rev.* **17**, 12.
- Quastel, J. H. & Wheatley, A. H. M. (1933). *Biochem. J.* **27**, 1753.
- Reid, R. L. (1950). *Aust. J. agric. Res.* **1**, 182, 338.
- Schambye, P. & Phillipson, A. T. (1949). *Nature, Lond.*, **164**, 1094.
- Schultz, L. H., Smith, V. R. & Lardy, H. A. (1949). *J. Dairy Sci.* **32**, 817.
- Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1945). *Manometric Techniques and Related Methods for the Study of Tissue Metabolism*. Minneapolis: Burgess Publishing Co.
- Weichselbaum, T. E. & Somogyi, M. (1941). *J. biol. Chem.* **140**, 5.