REFERENCES

- Boekenoggen, H. A. (1952). *Angew. Chem.* **64**, 339.
 Boldingh, J. (1950). *Rec. Trav. chim. Pays-Bas*, **69**, 247.
 Greaves, W. S., Linstead, R. P., Shephard, B. R., Thomas, S. L. S. & Weedon, B. C. L. (1950). *J. chem. Soc.* p. 3326.
 Herb, S. F., Witnauer, L. P. & Riemenschneider, R. W. (1951). *J. Amer. Oil Chem. Soc.* **28**, 505.
 Holman, R. T. & Hagdahl, L. (1950). *J. biol. Chem.* **182**, 421.
 Howard, G. A. & Martin, A. J. P. (1950). *Biochem. J.* **46**, 532.
 Joubert, F. J. & Sutton, D. A. (1952). *J. Amer. Oil Chem. Soc.* **29**, 287.
 Kritchevsky, D. & Calvin, M. (1950). *J. Amer. chem. Soc.* **72**, 4330.
 Ramsey, L. L. & Patterson, W. I. (1948). *J. Ass. off. agric. Chem. Wash.* **31**, 441.
 Riemenschneider, R. W., Herb, S. F. & Nichols, P. L. (1949). *J. Amer. Oil Chem. Soc.* **26**, 371.
 Vandenheuvel, F. A. & Hayes, E. R. (1952). *Analyt. Chem.* **24**, 960.
 White, M. F. & Brown, J. B. (1948). *J. Amer. chem. Soc.* **70**, 4269.

The Metabolism of Short-Chain Fatty Acids in the Sheep

2. FURTHER STUDIES WITH RUMEN EPITHELIUM

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Short-chain fatty acids are produced in considerable quantities as a result of microbial activity in the fore-stomachs and large intestine of ruminants and are undoubtedly an important source of energy for these animals. In the first paper in this series (Pennington, 1952) it was shown that acetic, propionic and *n*-butyric acids can be utilized by many tissues of the sheep including the epithelia of the alimentary tract. Interest was focused principally upon the activity of the rumen epithelium, which proved to be a very useful tissue for *in vitro* metabolic studies. Some further experiments on the metabolism of short-chain fatty acids by this tissue are described in the present paper.

Particular attention has been given to propionic acid since little is known with certainty about the pathways of metabolism of this acid in animal tissues. It can be completely oxidized under suitable conditions by rat-liver preparations (Grafflin & Green, 1948; Huennekens, Mahler & Nordmann, 1951). The latter authors could demonstrate its oxidation by kidney preparations only when supplemented with a fraction from liver dispersions. Propionic acid is also known to be a precursor of carbohydrate in the dog (Ringer, 1912) and rat (Deuel, Butts, Hallman & Cutler, 1935-6). In this respect it may be of special importance to the ruminant, whose carbohydrate supply is limited owing to the nature of its diet and means of digestion. There is no generally accepted evidence that either acetic or butyric acid can give rise to a net increase of carbohydrate in animal tissues (Deuel, Johnston, Morehouse, Rollman & Winzler, 1945; Wood, 1946).

EXPERIMENTAL

The tissue was collected, prepared and incubated as described previously (Pennington, 1952) with the modification that the tissue sections, before incubation, were shaken vigorously for 5 min. in ice-cold Ringer solution in order to ensure a more complete removal of rumen micro-organisms possibly adhering to the tissue. In manometric experiments, where smaller amounts of tissue were needed, it was found convenient to use papillae, shaved from the rumen wall. These were pooled and usually 150 mg. (wet wt.) were introduced into each flask; since this quantity represents upwards of a dozen papillae, a uniform distribution of tissue between the flasks was ensured. Such preparations of papillae have a rate of respiration and fatty acid utilization somewhat higher than that of the tissue prepared in the usual manner. Chopping the tissue finely with scissors appreciably diminishes the rate of utilization of fatty acids.

Volatile fatty acids and ketone bodies were determined as before (Pennington, 1952). As previously mentioned, high blank values may be encountered in the determination of ketone bodies by the dinitrophenylhydrazine method of Greenberg & Lester (1944) unless the reagent is dissolved in a lower concentration of HCl (0.5N, instead of 2N recommended by these authors). Recrystallization of the reagent from butanol did not reduce the high blank values obtained when 2N acid was used. Low blanks may also be obtained by dissolving the reagent in 2N-HCl and extracting the solution twice with half its volume of CCl₄. The latter procedure is rather more convenient than the use of 0.5N-HCl, owing to the difficulty in dissolving the reagent in acid of this concentration.

Glucose was determined by the method of Somogyi (1945) on ZnSO₄/Ba(OH)₂ filtrates. Glycogen was determined by the procedure of Good, Kramer & Somogyi (1933). Succinic acid was determined manometrically using a sheep-heart succinoxidase preparation (Umbreit, Burris & Stauffer, 1945).

Table 1. *Utilization of volatile fatty acids by rumen epithelium in media with and without calcium ion*

(Tissue (2 g.) incubated for 3 hr. at 39.5° with 100 μ moles of the Na salt of the fatty acids in the media indicated. The flasks containing the Ringer-phosphate media were gassed with O₂, and the Ringer-phosphate-bicarbonate with 80% O₂/20% CO₂. The figures are calculated to 100 mg. (dry wt.) of tissue.)

Expt. no.	Medium	Calcium	Fatty acid lost (μ moles)		
			Acetic	Propionic	Butyric
1	Ringer-phosphate-bicarbonate	+	6.6	12.5	23.2
		-	6.8	13.3	25.5
2	Ringer-phosphate-bicarbonate	+	.	27.5	.
		-	.	25.6	.
	Ringer-phosphate	+	.	2.8	.
		-	.	3.9	.

Table 2. *Utilization of propionic acid by sheep-rumen epithelium in presence of various concentrations of carbon dioxide*

(Tissue (2 g., wet wt.) incubated for 3 hr. at 39.5° in buffered Ringer containing 100 μ moles of sodium propionate. The values are calculated to 100 mg. (dry wt.) of tissue. The figures in parentheses are the percentages of CO₂ in the gas phase.)

Expt. no.	Propionic acid lost (μ moles)		
1	7.8 (0)	13.7 (5)	.
2	8.5 (0)	17.2 (5)	21.7 (10)
3	2.8 (0)*	6.5 (0)	.
4	12.2 (0)	15.4 (5)	17.6 (10)
5	13.6 (0)	23.4 (20)	22.7 (30)
6	4.5 (0)	29.4 (20)	23.0 (40)

* Flask contained KOH in a centre well to remove any CO₂ produced by endogenous respiration.

Standard manometric techniques were employed for the measurements of gas exchange by the tissue. In the measurement of O₂ uptake in 100% O₂ the conventional Warburg procedure, with 5% KOH in the centre well, was employed. The Dickens & Šimer (1931) method was used for measurements in a 95% O₂/5% CO₂ atmosphere. Anaerobic glycolysis was measured under 95% N₂/5% CO₂, with yellow phosphorus in the centre well of the flasks to remove any traces of O₂. All *Q* values are expressed in μ l. of gas/mg. dry wt./hr., the dry wt. being the final dry wt.

Media. The inorganic medium used for incubating the tissue was Krebs-Ringer solution (Umbreit *et al.* 1945), buffered with phosphate or bicarbonate, or both, as described in the text and legends. Since the calcium ion can exert certain inhibitory effects upon the metabolism of tissue preparations (see, for example, Krebs & Eggleston, 1940), it was first ascertained whether the presence of Ca²⁺ in this medium inhibits the utilization of fatty acids. Table 1 shows that this is not the case, and the complete medium containing Ca²⁺ was used in all the experiments.

All the values given in the tables are the means obtained from two runs with separate lots of tissues; analyses on each run were performed in duplicate. The tissues used in any one experiment were all obtained from the same animal. This was necessary since there was sometimes marked variation in activity between tissues from different sheep; duplicate tissue samples from the same animal usually gave results in close agreement.

RESULTS

Influence of carbon dioxide on fatty-acid metabolism. It was noted previously (Pennington, 1952) that the rate of disappearance of propionic acid

incubated with rumen epithelial tissue of the sheep was greater under an atmosphere containing 5% of carbon dioxide than under oxygen alone, although there was little or no effect upon acetate or butyrate utilization. This was investigated further by measuring the disappearance of propionate with various levels of carbon dioxide in the atmosphere of the flask, the remaining gas being oxygen. The medium was Ringer-phosphate with the addition of the appropriate quantity of 0.154M sodium bicarbonate to produce the same initial pH in each flask. Table 2 shows the results obtained.

The effect of 5% of carbon dioxide in the gas phase upon the rate of disappearance of propionate was confirmed and the rate was further increased when the carbon dioxide level was raised above 5%. Since there was little change of pH during the metabolism of propionate this effect cannot have been due to the increased buffering concomitant with the increase in carbon dioxide tension. The uptake in flasks gassed with oxygen alone may be largely or wholly dependent upon the presence of carbon dioxide produced from endogenous substrates, since in one experiment (expt. 3) in which the centre well in a flask contained potassium hydroxide the uptake was further diminished.

It has not been found possible to demonstrate any effect of carbon dioxide upon the rate of metabolism of propionate by sheep-liver or -kidney slices. Possibly propionate is metabolized by a different

Table 3. *Utilization of acetic and butyric acids by sheep-rumen epithelium in presence and absence of carbon dioxide*

(Tissue (2 g.) incubated for 3 hr. at 39.5° in buffered Ringer containing 100 μ moles of fatty acid as Na salt. The figures are calculated to 100 mg. (dry wt.) of tissue.)

CO ₂ in gas phase %	Fatty acid lost (μ moles)	
	Acetic	Butyric
0	7.1	32.5
20	6.9	33.0

Table 4. *Utilization of propionic acid by rumen epithelium in presence of various concentrations of fumarate*

(Tissue (2 g.) incubated for 3 hr. at 39.5° with 100 μ moles of sodium propionate and various concentrations of sodium fumarate in the media indicated. The flasks with Ringer-phosphate were gassed with O₂ and Ringer-phosphate-bicarbonate with 80% O₂/20% CO₂. The figures are calculated to 100 mg. (dry wt.) of tissue.)

Expt. no.	Medium	Fumarate concn. (M)	Propionic acid lost (μ moles)
1	Ringer-phosphate-bicarbonate	.	21.8
		0.010	21.2
		0.025	22.0
		0.050	20.4
2	Ringer-phosphate	.	1.0
		0.002	1.7
		0.010	2.6
		0.025	4.2
		0.050	4.5
3	Ringer-phosphate-bicarbonate	.	14.7
	Ringer-phosphate	.	1.5
		0.025	4.8
	Ringer-phosphate-bicarbonate	.	9.0
		0.025	8.8

pathway in these tissues, or it may be merely that a higher rate of production of carbon dioxide from endogenous substrates (or a different distribution of enzymes in the cells) may have made unlikely any effect of adding carbon dioxide.

Table 3 shows that the presence of 20% of carbon dioxide in the gas phase had no effect upon the utilization of acetate or butyrate.

In view of existing knowledge of the metabolic functions of carbon dioxide, the observed stimulation of propionate metabolism could be related to the supply of acids which constitute the Krebs's tricarboxylic acid cycle. Accordingly, the influence of fumaric acid upon the rate of disappearance of propionic acid was determined (Table 4). Fumarate, although increasing propionate uptake, was considerably less effective than carbon dioxide.

Effect of malonate. Malonate has been shown to inhibit the oxidation of fatty acids by liver tissue (Jowett & Quastel, 1935; Weinhouse, Millington &

Friedman, 1949). Geyer & Cunningham (1950) reported an increase in the formation of ketone bodies by liver tissue from fatty acids in the presence of malonate; Weinhouse *et al.* (1949), however, found that malonate decreased the formation of ketone bodies. Jowett & Quastel (1935) found that malonate increased the endogenous production of acetoacetic acid. Table 5 shows the effects of malonate upon fatty-acid utilization and production of ketone bodies by rumen epithelium. It may be seen that the utilization of each of the acids was inhibited by malonate. Formation of ketone bodies from butyrate was depressed but there was little difference in the proportion of the butyrate converted into ketone bodies in the presence of the inhibitor. Malonate (0.025M) did not influence the endogenous ketone-body production of the tissue, nor lead to the production of any ketones from propionate.

Table 6 shows the effect of adding fumarate upon the inhibition of propionate utilization by malonate.

Table 5. *Metabolism of volatile fatty acids by rumen epithelium in presence of malonate*

(Tissue (2 g.) incubated for 3 hr. at 39.5° in Ringer-phosphate-bicarbonate containing 100 μ moles of fatty acid as Na salt and the concentration of sodium malonate shown. Gas phase, 80% O₂/20% CO₂. Figures in parentheses are μ moles of ketone bodies produced. The figures are calculated to 100 mg. (dry wt.) of tissue.)

Expt. no.	Malonate concn. (M)	Fatty acid lost (μ moles)		
		Acetic	Propionic	Butyric
1	.	.	18.8	.
	0.008	.	15.5	.
	0.025	.	6.7	.
	0.081	.	0.5	.
2	.	5.4	18.2 (0)	24.5 (17.1)
	0.025	1.5	8.5 (0)	16.9 (12.6)

Table 6. *Utilization of propionic acid by rumen epithelium in presence of various concentrations of malonate and fumarate*

(Tissue (2 g.) incubated for 3 hr. at 39.5° in Ringer-phosphate-bicarbonate containing 100 μ moles of sodium propionate and other substances in the concentrations shown. Gas phase, 80% O₂/20% CO₂. The figures are calculated to 100 mg. (dry wt.) of tissue.)

Expt. no.	Concn. of malonate (M)	Concn. of fumarate (M)	Propionic acid lost (μ moles)
1	.	.	26.5
	0.025	.	17.1
	0.025	0.025	16.2
	0.025	0.050	16.9
2	.	.	22.4
	0.009	.	17.1
	0.018	.	11.7
	0.009	0.050	18.2
	0.018	0.050	16.8

The inhibitory effect of malonate suggested that oxidation of succinate may be an essential step in the metabolism of propionate. It was, therefore, of interest to determine whether, in the presence of malonate to inhibit succinate disappearance, the production of succinate from propionate could be demonstrated. The data given in Table 7 show that an appreciable part of the propionate disappearing under these conditions can be accounted for as succinate. The succinate figures would include any α -oxoglutarate which was present, since the ether extraction of the succinate was preceded by permanganate treatment to destroy malonate.

Effect of the ammonium ion. Edson (1935) showed that production of acetoacetic acid by liver slices is increased in the presence of ammonium chloride. In the course of experiments to determine whether ammonium chloride similarly influenced ketogenesis by rumen epithelium, it was observed that ammonium chloride partially inhibits the uptake of propionate by the tissue (Table 8). No

such effect could be observed with butyrate, although the uptake of acetate, which is small with this tissue, appeared to be depressed. There was no effect on the production of ketone bodies.

The magnitude of the effect with propionate varied greatly from experiment to experiment. In some of the flasks, as indicated, the added ammonium chloride replaced an equivalent amount of sodium chloride in the medium; in the others the ammonium chloride was an addition to the medium; it was found, however, that the addition of equivalent amounts of sodium chloride did not depress the utilization of propionate.

Manometric studies. Some studies have been made of the respiration and glycolysis of the rumen epithelium. Fig. 1 comprises typical curves which were obtained showing the effect of fatty acids and of glucose upon the oxygen uptake of the tissue under 100 % oxygen. Respiration data from several experiments are summarized in Table 9.

A totally unexpected result was the marked inhibition of respiration by propionate. Exactly similar results were obtained using propionate purified by steam distillation or by the use of 'purified propionic acid' (Hopkin and Williams Ltd.).

In view of the fact that carbon dioxide affects the rate at which propionic acid is metabolized (Table 1), the effect of propionate upon the respiration of the tissue was also examined in an atmosphere of 5 % $\text{CO}_2/95\%$ O_2 (Table 10). Under these conditions quite a different result was obtained. Propionate markedly increased the Q_{O_2} of the tissue. It may be noted that the R.Q. of the tissue, which was low, was hardly altered when propionate was metabolized. There was an increase in bicarbonate, suggesting the conversion of propionate into non-acidic products.

Table 7. *Production of succinate by rumen epithelium*

(Tissue (4 g.) incubated for 3 hr. at 39.5° in Ringer-phosphate-bicarbonate containing the additions indicated. The percentage conversion is calculated after subtraction of the succinate production in the corresponding flask without propionate.)

Addition to medium	Propionate lost (μ moles)	Succinate* produced (μ moles)	Conversion (%)
None	.	2.8	.
Propionate (200 μ moles)	118	5.9	2.6
Malonate (0.009 M)	.	4.7	.
Propionate (200 μ moles) + malonate (0.009 M)	98	20.0	15.6

* Includes α -oxoglutarate: see text.

Table 8. *Metabolism of volatile fatty acids by rumen epithelium in presence of various concentrations of ammonium ion*

(Tissue (2 g.) incubated for 3 hr. at 39.5° in Ringer-phosphate-bicarbonate containing 100 μ moles of fatty acid as Na salt and the concentration of NH_4Cl shown. Gas phase, 80% $\text{O}_2/20\%$ CO_2 . The ketone body values are given in parentheses and were derived by subtraction of the corresponding control figures. The figures are calculated to 100 mg. (dry wt.) of tissue.)

Expt. no.	Concn. of NH_4Cl (M)	Fatty acid lost and ketone bodies produced (μ moles)			
		Acetic	Propionic	Butyric	Control
1	—	5.8 (2.0)	27.6 (-1.1)	28.1 (24.9)	(2.2)
	0.015	5.0 (0.9)	22.6 (-1.2)	28.7 (26.4)	(2.3)
	0.030	4.5 (1.5)	18.2 (-1.2)	29.4 (26.9)	(2.4)
2	.	.	23.7	28.8 (24.8)	(2.0)
	0.030*	.	21.9	29.6	.
	0.060*	.	18.5	29.3	.
	0.090*	.	17.1	28.6 (23.8)	(2.0)
3	.	4.3 (1.6)	17.9	.	(2.3)
	0.030	2.8 (1.1)	4.5	.	(2.7)
	0.030*	.	7.2	.	.
	0.090*	.	3.1	.	.

* NH_4^+ replaced an equivalent amount of Na^+ .

Table 9. *Rate of oxygen uptake of rumen epithelium in presence of fatty acids and glucose*

(Medium: 3 ml. of Ringer-phosphate containing 30 μ moles of substrate. Gas phase, 100% O_2 . Temp. 39.5°. Values not marked by an asterisk were obtained using tissue sections as in preceding experiments. Where a pair of values are given, the first represents the initial rate, the second the rate after 3 hr. Where only one value is given the rate was constant for at least 3 hr.) (Q_{O_2} = μ l. O_2 /mg. dry wt./hr.)

Expt. no.	Q_{O_2}					Propionate + glucose
	No substrate	Glucose	Acetate	Butyrate	Propionate	
1	3.24	3.30	.	.	.	.
	2.41	.	.	.	.	.
2	3.28	3.85	3.04	5.67	2.93	.
	1.71	2.97	1.32	.	0.47	.
3	2.78	3.36	2.66	3.59	2.65	3.70
	.	.	.	.	0.66	.
4	3.69	.	.	6.75	3.75	.
	2.33	.	.	4.44	1.14	.
5	2.13	.	1.75	.	1.00	.
	0.97	.	0.60	.	0.25	.
	4.30*	.	.	.	.	.
	1.80*	.	.	.	.	.
6	2.61*	.	.	.	2.22*	.
	1.72*	.	.	.	0.59*	.
7	2.13*	.	.	.	1.18*	.
	0.11*	.	.	.	0.07*	.

* Using papillae only.

Fig. 1. Oxygen uptake of rumen epithelium in presence of fatty acids and glucose. Each flask contained 150 mg. (wet wt.) of tissue, 3 ml. Ringer-phosphate and 30 μ moles of the appropriate substrate. Fatty acids were added as Na salts. Gas phase, O_2 .

Table 10. *Respiration of rumen epithelium in bicarbonate-buffered medium in presence and absence of propionate*

(Medium: 3 ml. of Ringer-bicarbonate containing 30 μ moles of sodium propionate, where added. Gas phase, 95% O_2 /5% CO_2 . Temp. 39.5°. The tissue used in expt. 2 was from the same rumen as that used in expt. 7, Table 9. (Q_{O_2} = μ l. O_2 /mg. dry wt./hr.))

Expt. no.	No substrate		Propionate	
	Q_{O_2}	R.Q.	Q_{O_2}	R.Q.
1	2.55	0.70	3.70	0.68
2	1.88	0.78	4.64	0.65

The rate of glycolysis of the tissue (carbon dioxide evolution with the bicarbonate medium under 95% N_2 /5% CO_2) was found to be low ($Q_{CO_2}^N$ = 0.64). This was increased to 2.24 in the presence of glucose and was not measurably influenced by propionate, either in the presence or absence of glucose. This is in contrast with the results of Villasante & Ballowitz (1948), who reported that propionic acid completely inhibited the anaerobic glycolysis of liver slices.

Table 11. *Metabolism of volatile fatty acids by ox-rumen epithelium*

(Tissue (2 g.) incubated for 3 hr. in Ringer-phosphate containing 100 μ moles of fatty acid as Na salt. Gas phase, 100% O₂. The figures are calculated to 100 mg. (dry wt.) of tissue. The last three ketone body figures have been corrected by subtraction of the control value.)

Substrate	Change in volatile acid (μ moles)	Ketone bodies produced (μ moles)
None	+ 1.6	2.7
Acetate	- 5.8	1.5
Propionate	- 10.3	- 2.3
Butyrate	- 22.3	16.8

As with other tissues, the presence of a relatively high endogenous respiration places a serious limitation on the usefulness of gas-exchange measurements. With some tissues, e.g. mammary-gland slices (Turner, 1951) it is possible to diminish this considerably by preliminary incubation in an inorganic medium. As may be seen from Table 9, the rate of oxygen uptake of rumen epithelium tends to fall off with time, although to greatly varying degrees. However, it was found that the increment due to added substrate (butyrate) was diminished when the substrate was added after a few hours.

Fatty-acid utilization by ox-rumen epithelium. The rumen of cattle is lined with an epithelium similar to that of the sheep. The data of Table 11 show that the uptake of volatile fatty acids by ox-rumen epithelium parallels that previously reported for the sheep.

DISCUSSION

Evidence for the occurrence of another site of carbon dioxide fixation has been obtained recently by Barban & Ajl (1951). They showed that the decarboxylation of succinate to propionate by *Propionibacterium pentosaceum* can be reversed. Although not necessarily a single-step transformation, it does not seem capable of an explanation involving any of the biological carboxylations previously reported, such as the conversion of pyruvate into oxaloacetate. The suggestion that animal tissues may convert propionate into dicarboxylic acids by a pathway not involving pyruvate has been put forward on the basis of isotope experiments (Lorber, Lifson, Sakami & Wood, 1950; Schreeve, 1952). Marshall & Friedberg (1952) have found that following injection of ¹⁴C-labelled bicarbonate into rats the rate of appearance of radioactivity in the dicarboxylic acids of the liver was by far the most rapid in the cases of oxaloacetate and succinate.

The influence of carbon dioxide upon propionate metabolism in rumen epithelium shown above suggests that a carboxylation step is involved. The possibility that propionate is metabolized to

pyruvate (Baldwin, 1952) and that carbon dioxide is acting to produce oxaloacetate from the latter must be considered; the oxaloacetate would be necessary for the oxidation of further pyruvate via Krebs's cycle. If this were so, it would be expected that the dicarboxylic acids of Krebs's cycle would be as effective as carbon dioxide in stimulating propionate metabolism. Similar reasoning was advanced by Ajl & Werkman (1948) to explain the ability of these acids to replace carbon dioxide for growth of *Escherichia coli*. The failure of fumarate to substitute completely for carbon dioxide (Table 4) may be taken as evidence against this possibility. It cannot have been due to an additional, competitive action of fumarate for enzymes or coenzymes involved in the metabolism of both substrates, since fumarate did not depress propionate uptake in the presence of carbon dioxide. Although fumarate did stimulate propionate uptake to some extent, this may have been due to carbon dioxide produced by oxidation of the fumarate. Respiration data (unpublished) indicate that fumarate is readily oxidized by this tissue. We have recently obtained further evidence (Pennington & Sutherland, unpublished results) that propionate is not necessarily metabolized via pyruvate in this tissue.

It is hardly possible as yet to draw any definite conclusion from the inhibition by malonate of the uptake of fatty acids by this tissue (Tables 5 and 6) in view of evidence (Pardee & Potter, 1949; Weinhouse *et al.* 1949) that the action of malonate is not confined to the inhibition of succinic dehydrogenase.

The failure to produce ketone bodies from propionate in the presence of malonate is a further fact which is difficult to reconcile with the conversion of propionate into pyruvate, since, in liver tissue, pyruvate is largely or wholly converted into acetoacetate in the presence of malonate (Lehninger, 1946; Recknagel & Potter, 1951).

The involvement of the tricarboxylic acid cycle in the metabolism of propionic acid could be put forward to explain the inhibitory effect of the ammonium ion upon the metabolism of the latter (Table 8). Recknagel & Potter (1951) investigated further the ketogenic effect of ammonium chloride reported by Edson (1935), and concluded that it was a result of the blocking of the tricarboxylic acid cycle owing to removal of α -oxoglutaric acid by reductive amination to glutamic acid. On the other hand, Crane & Ball (1951) postulated that ammonia also inhibits the fixation of carbon dioxide in animal tissues; this fits in with the above suggestion that propionate or one of its metabolic derivatives may undergo carboxylation. It may be noted that Huennekens *et al.* (1951) have reported an inhibitory effect of ammonia upon propionate metabolism by the liver 'cyclophorase system'. The

effect of ammonia upon the metabolism of the rumen epithelium may possibly be of physiological importance, since the concentration of ammonia in the sheep's rumen was found by McDonald (1948) to reach a level as high as 35 mg. ammonia N/100 ml. (0.025M). This is higher than the lowest level found to exert an inhibitory effect *in vitro* (Table 8). McDonald also showed that ammonia is absorbed into the blood draining the rumen; levels of about 1.5 mg. ammonia N/100 ml. in the blood were found, although it appeared all to be removed by the liver.

The difference between the effect of propionate upon the oxygen uptake of the tissue in the presence and absence of carbon dioxide is very striking (Tables 9 and 10). In this instance the more commonly used Warburg procedure for measuring oxygen uptake gives totally misleading results. A tentative explanation of the difference may be suggested. The initial step in the metabolism of propionate may be combination with coenzyme A to form propionyl coenzyme A, the propionyl moiety being carboxylated in this form and further metabolized. In the absence of carbon dioxide, the coenzyme A may be immobilized as propionyl coenzyme A and thus rendered unavailable for the metabolism of endogenous substrates. The identities of the endogenous substrates responsible for the oxygen uptake are not known. The tissue does not contain measurable amounts of glycogen and the R.Q. is low (Table 10). It is of interest that Grafflin & Green (1948) found that propionate inhibits the oxidation of acetate by the kidney 'cyclophorase system', and Hill (1952) found that propionate inhibition of the growth of *Streptococcus faecalis* can be reversed by acetate.

It must be emphasized that this explanation of the effect of propionate upon respiration is entirely speculative, although Stadtman (1952) has produced evidence that propionate combines with coenzyme A in extracts of *Clostridium kluyveri*.

SUMMARY

1. The rate of metabolism of propionic acid by sheep-rumen epithelium increased with the proportion of carbon dioxide in the atmosphere up to about 20% CO₂/80% O₂. This level of carbon dioxide had no influence upon the rate of metabolism of acetic or butyric acid.

2. Malonate inhibited the uptake of each acid without showing any differential action on the formation of ketone bodies from butyrate. Malonate inhibition of propionate uptake was only partially reversed by fumarate. Succinate was produced when propionate was metabolized in the presence of malonate.

3. Ammonium ion inhibited the metabolism of

propionate and possibly of acetate, but not of butyrate. It had no appreciable effect upon ketogenesis.

4. Propionate depressed the oxygen uptake of rumen epithelial tissue in the absence of carbon dioxide, but stimulated respiration in the presence of the latter. Butyric acid and glucose increased the rate of oxygen uptake.

5. The uptake of acetic, propionic and butyric acids, and production of ketone bodies by ox-rumen epithelium paralleled that of the corresponding tissue of the sheep.

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REFERENCES

- Ajl, S. J. & Werkman, C. H. (1948). *Arch. Biochem.* **19**, 483.
 Baldwin, E. (1952). *Dynamic Aspects of Biochemistry*, 2nd ed. p. 273. Cambridge University Press.
 Barban, S. & Ajl, S. J. (1951). *J. biol. Chem.* **192**, 63.
 Crane, R. K. & Ball, E. G. (1951). *J. biol. Chem.* **188**, 819.
 Deuel, H. J. jun., Butts, J. S., Hallman, L. F. & Cutler, C. H. (1935-6). *J. biol. Chem.* **112**, 15.
 Deuel, H. J. jun., Johnston, C., Morehouse, M. G., Rollman, H. S. & Winzler, R. J. (1945). *J. biol. Chem.* **157**, 135.
 Dickens, F. & Šimer, F. (1931). *Biochem. J.* **25**, 973.
 Edson, N. L. (1935). *Biochem. J.* **29**, 2082.
 Geyer, R. P. & Cunningham, M. (1950). *J. biol. Chem.* **184**, 641.
 Good, C. A., Kramer, H. & Somogyi, M. (1933). *J. biol. Chem.* **100**, 485.
 Grafflin, A. L. & Green, D. E. (1948). *J. biol. Chem.* **176**, 95.
 Greenberg, L. A. & Lester, D. (1944). *J. biol. Chem.* **154**, 177.
 Hill, C. H. (1952). *J. biol. Chem.* **199**, 329.
 Huennekens, F. M., Mahler, H. R. & Nordmann, J. (1951). *Arch. Biochem.* **30**, 66.
 Jowett, M. & Quastel, J. H. (1935). *Biochem. J.* **29**, 2181.
 Krebs, H. A. & Eggleston, L. V. (1940). *Biochem. J.* **34**, 442.
 Lehninger, A. L. (1946). *J. biol. Chem.* **164**, 291.
 Lorber, V., Lifson, N., Sakami, W. & Wood, H. G. (1950). *J. biol. Chem.* **183**, 531.
 McDonald, I. W. (1948). *Biochem. J.* **42**, 584.
 Marshall, L. M. & Friedberg, F. (1952). *J. biol. Chem.* **199**, 783.
 Pardee, A. B. & Potter, V. R. (1949). *J. biol. Chem.* **178**, 241.
 Pennington, R. J. (1952). *Biochem. J.* **51**, 251.
 Recknagel, R. O. & Potter, V. R. (1951). *J. biol. Chem.* **191**, 263.
 Ringer, A. I. (1912). *J. biol. Chem.* **12**, 511.
 Schreeve, W. W. (1952). *J. biol. Chem.* **195**, 1.
 Somogyi, M. (1945). *J. biol. Chem.* **160**, 71.
 Stadtman, E. R. (1952). *Fed. Proc.* **11**, 291.
 Turner, C. (1951). *Biochem. J.* **50**, 145.
 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.
 Villasante, J. G. & Ballowitz, K. (1948). *Z. ges. inn. Med.* **3**, 61.
 Weinhouse, S., Millington, R. H. & Friedman, B. (1949). *J. biol. Chem.* **181**, 489.
 Wood, H. G. (1946). *Physiol. Rev.* **26**, 198.