- Popjak, G. (1955). *Royal Institute of Chemistry Lectures, Monographs and Reports*, no. 2, p. 17.
- Riniker, B., Arigoni, D. & Jeger, O. (1954). *Helv. chim. acta*, **37**, 546.
- Ruyle, W. V., Chamberlin, E. M., Chemerda, J. M., Sita, G. E., Aliminos, L. M. & Erickson, R. L. (1952). *J. Amer. chem. Soc.* **74**, 5929.
- Schenck, F., Buchholz, K. & Wiese, O. (1936). *Ber. dtsch. chem. Ges.* **69**, 2703.
- Weedon, B. C. L. (1952). *Quart. Rev. chem. Soc., Lond.*, **6**, 380.
- Wieland, H., Rath, F. & Benend, W. (1941). *Liebigs Ann.* **548**, 19.
- Woodward, R. B. & Bloch, K. (1953). *J. Amer. chem. Soc.* **75**, 2023.
- Würsch, J., Huang, R. L. & Bloch, K. (1952). *J. biol. Chem.* **195**, 439.
- Zanetti, C. V. (1891). *Gazz. chim. ital.* **21**, II, 168.

The Metabolism of Short-chain Fatty Acids in the Sheep

5. SOME INTERRELATIONSHIPS IN THE METABOLISM OF FATTY ACIDS AND GLUCOSE BY SHEEP-RUMEN EPITHELIAL TISSUE*

BY R. J. PENNINGTON AND W. H. PFANDER†
Rowett Research Institute, Bucksburn, Aberdeenshire

(Received 19 April 1956)

Acetic, propionic and butyric acids are produced in large quantities by microbial activity in the sheep rumen. Pennington (1952) showed that each of these acids may be metabolized by the rumen epithelial tissue, which produces ketone bodies from butyrate and, to a smaller extent, from acetate. Rumen epithelium also metabolizes glucose (Pennington & Sutherland, 1956*a*), which, although not normally present in appreciable quantity in the rumen, is available to the epithelial tissue from the blood.

It is of obvious physiological interest to study the metabolism of each one of these compounds in the presence of the others. Several workers have studied such interrelationships in other tissues. Quastel & Wheatley (1933) observed that propionic acid, but not glucose, lowered the production of acetoacetic acid from butyric acid by liver slices. Parnes & Wertheimer (1950) found that the utilization of glucose by rat diaphragm was decreased by acetate if the glucose concentration was not higher than 0.05%. Recent studies on the effects of glucose on the oxidation of fatty acids have given rather conflicting results. Allen, Friedmann & Weinhouse (1955) found that glucose had no appreciable effect on the oxidation of butyrate or palmitate by tissue slices and homogenates. However, by the use of a different technique, Lossow & Chaikoff (1955) observed that glucose spared the oxidation of palmitic acid but that the effect decreased with decrease in chain length of the acid.

In the present work mixtures of these compounds were metabolized *in vitro* by rumen epithelium and the uptake of each substrate and the formation of ketone bodies were measured.

METHODS AND RESULTS

The procedures used in the preparation of the tissue sections, incubation of the tissue with the substrates and the method of ketone-body determination were as previously described (Pennington, 1952). The individual fatty acids in the mixtures were determined by gas-liquid chromatography (James & Martin, 1952). Glucose was determined by the method of Somogyi (1945). Tissue from a single sheep was used in each experiment. The sections were pooled before distributing among the flasks. The duplicate figures in Tables 1 and 3 represent the results obtained with different portions of tissue.

Table 1 shows the results obtained in two experiments in which all the possible combinations of acetate, propionate and butyrate were metabolized.

In spite of relatively large variations in the results, there is good evidence for some effects upon the metabolism of the fatty acids. The uptake of propionate was strongly inhibited by butyrate and that of butyrate inhibited by acetate; both effects were significant at the 1% level. The decrease in acetate uptake caused by propionate, the increase in acetate uptake in the presence of butyrate and the increased butyrate uptake when propionate was present were significant only at the 5% level.

The presence of propionate practically abolished ketone-body production from acetate but had only a small and possibly not significant (*P* approx. 0.1) effect on ketogenesis from butyrate.

* Part 4: Pennington & Sutherland (1956*b*).

† Present address: College of Agriculture, Department of Animal Husbandry, University of Missouri, Columbia, U.S.A.

Two unexpected effects were the apparent production of butyrate by the tissue when propionate and acetate were metabolized together, and the appearance of acetate when propionate and butyrate were used. The latter effect was amply confirmed, under various experimental conditions, by another experiment (Table 2).

The results obtained when each of the fatty acids was metabolized together with glucose are given in Table 3.

It is clear that glucose tended to increase the quantities of fatty acid metabolized. The uptake of glucose on the other hand, was, if anything, somewhat lowered in the presence of fatty acids. The

Table 1. *Metabolism of fatty acid mixtures by sheep-rumen epithelium*

The basal medium was Krebs-Ringer-bicarbonate (Cohen, 1945); 400 μ moles of each acid was added. Total vol., 20 ml. Wet wt. of tissue, 2 g. Incubated for 3 hr. at 39.5°. All results are expressed in μ moles. The ketone-body values are given as differences from the amounts produced by the tissue without added substrate (last line). A minus sign before an uptake figure denotes that the acid was actually produced.

Acid(s) added	Uptake of acids			Ketone bodies produced
	Acetic	Propionic	Butyric	
	Expt. 1			
Acetic, propionic, butyric	29, 82	24, -17	238, 185	194, 192
Acetic, propionic	17, 17	167, 143	-23.5, -12.5	1.2, 2.1
Acetic, butyric	93, 41	—	166, 190	200, 208
Acetic	47, 44	—	—	18, 18
Propionic, butyric	0, -36.5	31, 1	270, 307	209, 178
Propionic	—	149, 144	—	0.6, -1.7
Butyric	—	—	288, 248	224, 216
None	—	—	—	4.3, 4.8
	Expt. 2			
Acetic, propionic, butyric	2.7, -8	-58, -8	249, 232	209, 209
Acetic, propionic	-13, 18	144, 91	-23, -23	-2.8, -3.3
Acetic, butyric	92, 84	—	143, 127	235, 213
Acetic	18, 17	—	—	15.7, 12.5
Propionic, butyric	-40, -46	70, 29	214, 240	223, 204
Propionic	—	125, 71	—	-2.8, -3.3
Butyric	—	—	243, 217	235, 213
None	—	—	—	5.2, 6.7

Table 2. *Production of acetate by sheep-rumen epithelium*

Krebs-Ringer-bicarbonate (Cohen, 1945). Total vol., 20 ml. Temp., 39.5°. Quantities of fatty acids are expressed in μ moles.

Propionate added	Butyrate added	Wt. of tissue (g.)	Time (hr.)	Acetate produced
400	400	2	3	47
400	400	2	1½	27
400	400	4	3	72
400	400	2	6	39
400	100	2	3	31
100	400	2	3	46

Table 3. *Metabolism of glucose-fatty acid mixtures by sheep-rumen epithelium*

Krebs-Ringer-bicarbonate. Fatty acid, 100 μ moles; glucose, 111 μ moles. Total vol., 10 ml. Wet wt. of tissue, 2 g. Incubated for 3 hr. at 39.5°. All results are expressed in μ moles. The ketone-body values are given as differences from the amounts produced by the tissues without added substrate (last line).

Additions	Fatty acid uptake		Glucose uptake		Ketone bodies produced	
Acetate	6.2	7.7	—	—	1.3	3.2
Acetate + glucose	10.5	15.5	42.3	41.7	-1.9	-2.6
Propionate	37.7	40.2	—	—	-3.8	-3.1
Propionate + glucose	42.7	48.2	43.4	41.7	—	—
Butyrate	71.2	73.0	—	—	57.8	56.6
Butyrate + glucose	74.2	78.7	35.6	38.3	52.3	48.7
Glucose	—	—	45.0	46.1	-3.7	-2.9
None	—	—	—	—	5.8	5.0

small formation of ketone bodies which accompanied the metabolism of acetate was sharply depressed by glucose, in spite of the increased uptake of acetate. The ketone-body production from butyrate was affected to a relatively much smaller extent by glucose.

DISCUSSION

Although the proportions may vary, all three acids used in this study are known to be present in the rumen, on a wide variety of natural diets. It is evident that a reliable quantitative estimate of the importance of the rumen epithelium in metabolizing the fatty acids produced in the rumen cannot be deduced from experiments with the single acids. In the experiments of Table 1, where equal amounts of the acids were present in the mixtures, the most definite effects were the inhibition of propionate uptake by butyrate and of butyrate uptake by acetate. In the normal rumen, with different proportions of the acids, other interrelationships may become more important.

Insufficient information is available to attempt to interpret the results. However, it does not seem that they can be explained by simple competition of the fatty acids for a common activating enzyme. The apparent interconversion of acetate and butyrate in the presence of propionate is of particular interest and merits further investigation. The effect of propionate and glucose upon ketone-body production from acetate accords with their known antiketogenic activity. However, the results

suggest that, even in the presence of these compounds, butyrate will be an important source of ketone bodies in the sheep.

SUMMARY

1. Butyrate lowered the uptake of propionate by sheep-rumen epithelium, and acetate lowered the uptake of butyrate.
2. Ketone-body production from acetate was suppressed by propionate or glucose.
3. Some acetate was produced when propionate and butyrate were metabolized together.

REFERENCES

- Allen, A., Friedmann, B. & Weinhouse, S. (1955). *J. biol. Chem.* **212**, 921.
- Cohen, P. P. (1945). In *Manometric Techniques and Related Methods for the Study of Tissue Metabolism*. Ed. by Umbreit, W. W., Burris, R. H. & Stauffer, J. F. Minneapolis: Burgess Publ. Co.
- James, A. T. & Martin, A. J. P. (1952). *Biochem. J.* **50**, 679.
- Lossow, W. J. & Chaikoff, I. L. (1955). *Arch. Biochem. Biophys.* **57**, 23.
- Parnes, I. & Wertheimer, E. (1950). *Biochem. J.* **46**, 517.
- Pennington, R. J. (1952). *Biochem. J.* **51**, 251.
- Pennington, R. J. & Sutherland, T. M. (1956a). *Biochem. J.* **63**, 353.
- Pennington, R. J. & Sutherland, T. M. (1956b). *Biochem. J.* **63**, 618.
- Quastel, J. H. & Wheatley, A. H. M. (1933). *Biochem. J.* **27**, 1753.
- Somogyi, M. (1945). *J. biol. Chem.* **160**, 71.

The Influence of Insulin on Free and Fixed Glycogen in the Diaphragm of the Rat

BY ADRIENNE J. M. KITS VAN HEIJNINGEN

Pharmaco-therapeutic Laboratory, University of Amsterdam, Amsterdam, Holland

(Received 28 May 1956)

Because of a difference in solubility in trichloroacetic acid, tissue glycogen can be divided into two forms, a free and a fixed form (Nerking, 1900; Willstätter & Rohdewald, 1934), recently called free and residual glycogen (Bloom, Lewis, Schumpert & Shen, 1951). The free glycogen is soluble in trichloroacetic acid at room temperature whereas the fixed fraction can be extracted only by heating the tissue for 15 min. at 100° in trichloroacetic acid (Kemp & Kits van Heijningen, 1954), or by destruction of the tissue in boiling potassium hydroxide. Previous investigations (Kits van Heijningen & Kemp, 1955) have made it unlikely that the fixation

of glycogen to protein is an artifact, since extraction with an agent which does not precipitate proteins will yield the same values for both fractions, as does extraction with trichloroacetic acid. An investigation into the influence of muscular contraction on these fractions offered no clue as to their respective physiological functions (Kits van Heijningen & Kemp, 1955).

It is well established that insulin promotes the deposition of glycogen in striated muscle (Best, Dale, Hoet & Marks, 1926; cf. Gemmill, 1940). Should this increase in glycogen content be limited to one of these fractions, it might be argued that