Transport and Metabolism of Fatty Acids by Isolated Rumen Epithelium

By F. J. R. HIRD, R. B. JACKSON* AND M. J. WEIDEMANN†

The Russell Grimwade School of Biochemistry and School of Agriculture, University of Melbourne, Parkville, N.2, Victoria, and Fodder Conservation Section, C.S.I.R.O., Highett, Victoria, Australia

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1. The metabolism of even-numbered saturated (acetic acid to stearic acid) and unsaturated (oleic acid and linolenic acid) fatty acids by diaphragms of isolated rumen epithelium has been investigated. 2. When fatty acids are presented to the papillae surface, ketone bodies are released from the opposite (muscle) side of the tissue. 3. When the concentration of octanoate or decanoate is increased to a critical value, which varies inversely with the chain length of the fatty acid, the respiration of the tissue is inhibited and ketone body synthesis is diminished. Under these conditions unmetabolized fatty acid crosses the tissue down a concentration gradient. 4. The inhibitions by octanoate and decanoate are more marked when the fatty acid is presented to both surfaces of the rumen epithelium. 5. During the oxidation of octanoate and decanoate at non-inhibitory concentrations, small quantities of shorter chain fatty acids, including acetate, are produced.

The rumen epithelium of the sheep is in contact with short- and long-chain fatty acids. The former are the products of microbial fermentation (see review by Warner, 1964) and the latter are derived in much smaller amount mainly from the galactosyl-glycerides of fodder plants (Weenink, 1959; Garton, 1960; van der Veen & Olcott, 1964). These are hydrolysed and the free fatty acids substantially hydrogenated by the rumen flora (Shorland, Weenink & Johns, 1955; Garton, Lough & Vioque, 1961). Acetate, propionate and butyrate are metabolized, the last-named to ketone bodies, by the rumen epithelium both *in vivo* and *in vitro* (Pennington, 1952). When presented to the papillae surface of such tissue *in vitro*, butyrate gives rise to a preferential release of ketone bodies at the opposite (muscle) surface of the tissue (Hird & Weidemann, 1964a). Hexanoate (Plaut & Smith, 1951), octanoate and decanoate (Schultz, Smith & Lardy, 1949) have been shown to give rise to increased blood ketone bodies when administered orally to goats. In experiments with isolated rumen epithelium of the sheep, Jackson, Taylor, Hatcher & Carter (1964) have shown that palmitic acid, stearic acid, oleic acid and linoleic acid are also precursors of ketone bodies.

In the present paper the metabolism and trans-

port of fatty acids by isolated sheets of rumen epithelium of the sheep have been investigated.

EXPERIMENTAL

Materials

Sodium butyrate, hexanoate, octanoate and decanoate were prepared from their redistilled acids (British Drug Houses Ltd., Poole, Dorset). Lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid and linoleic acid were purchased from the Hormel Institute, Austin, Minn., U.S.A., and were converted into the sodium salts before use. Crystalline bovine serum albumin was obtained from the Commonwealth Serum Laboratories, Melbourne, Australia. The other reagents used were of analytical grade.

Methods

Experimental procedure. Preparation of the rumen epithelium tissue (from which the accompanying muscle layers had been removed) and its incubation at 38° as a diaphragm supported by two Perspex plates was carried out as described by Hird & Weidemann (1964a), except that both compartments of the apparatus were of the same size (diam. 6.8 cm., depth 0.7 cm.) with a capacity of approx. 25 ml. Oxygen was passed through channels drilled into the Perspex plates and the stream of gas bubbles served to oxygenate and to mix the contents. The use of four units allowed four separate comparisons to be made on tissue from the same animal.

The effect of concentration of octanoate and decanoate on the consumption of O₂ of rumen epithelium was followed in a Warburg apparatus at 38° over a period of 2 hr. Rumen

* Present address: Division of Soils, C.S.I.R.O., Glen Osmond, South Australia.

† Present address: Department of Biochemistry, University of Oxford.

epithelium bearing small papillae was obtained from the dorsal sac and the preparation of the tissue and its incubation in the presence of substrates were performed as described by Hird & Symons (1959). Results are expressed as μ moles/50 mg. dry wt./2 hr.

Estimation of fatty acids. Estimations were made in duplicate. Butyrate and acetate were estimated by steam-distillation and titration as described by Pennington (1952).

With hexanoate, octanoate and decanoate the estimations were performed by gas-liquid chromatography. The method used was as follows.

Samples of the incubation mixtures (2.0 or 5.0 ml.) were adjusted approximately to pH 11 or greater by addition of NaOH (2M) and a known amount of the sodium salt of a suitable fatty acid (Table 1) was added to each sample to provide an internal standard. The solution was then evaporated to dryness at 95–100° in a stream of N₂. The residue was acidified with saturated NaHSO₄ and diethyl ether (4 ml.) was added, followed by anhydrous Na₂SO₄ (1.5 g.). The mixture was ground and mixed and the ethereal solution transferred to a 15 ml. tapered centrifuge tube. A second extraction of the Na₂SO₄ was made with diethyl ether (2 ml.) and the combined extracts were concentrated to about 0.3 ml. in a water bath at 45–50°.

The free acids were analysed in a gas chromatograph (model 300; F & M Scientific Corp., Acton, London, W. 3). The detector was operated with a filament current of 165 mA and at a temperature of 185°. The stainless-steel column (122 cm. $\times$ 4 mm.) was packed with diethylene glycol-

succinate (5%, w/w) and orthophosphoric acid (0.7%, w/w) adsorbed on a fluorethylene polymer (Haloport F; F & M Scientific Corp.). Samples (up to 0.1 ml.) of the ethereal solutions were applied directly to a plug of glass wool at the top of the column to avoid adsorption of fatty acids in the injection port. After starting the carrier gas, the temperature was maintained at 70° for 5 min. to remove ether and any residual water from the sample. The temperature was then raised rapidly (about 80°/min.) to an operating temperature within the experimental range 125–145° (Table 1). The flow-rate and operating temperature were selected to give a retention time of 20–30 min. for the slowest component.

Estimation of ketone bodies. This was done by the method described by Hird & Symons (1959) as modified by Hird & Weidemann (1964b).

Calculations. The results in Tables 2 and 3 give the distribution of ketone bodies on both sides of the tissue after 2-hr. incubations. The total number of μ moles of fatty acid appearing on the opposite side of the tissue is shown as fatty acid 'transported'. The loss or gain of fatty acid is given for comparison; this figure represents the difference between the number of μ moles of fatty acid added initially and the recovery of fatty acid on both sides of the tissue at the end of the experiment. It is assumed that loss of fatty acid in this context includes some retention of un-metabolized fatty acid in the tissue, as well as loss by metabolism to ketone bodies, CO₂ and water. The extent of retention in the tissue has not been estimated.

RESULTS

Table 1. *Conditions for gas chromatography of fatty acids*

Other experimental details are given in the text.

Substrate	Internal standard	Carrier gas-flow rate (ml./min.)	Operating temp.
Hexanoate	Valerate	25	125°
Octanoate	Nonanoate	50	135
Decanoate	Nonanoate	50	145

Transport experiments with 10 mM solutions. Table 2 shows the results of experiments where fatty acids were applied to the papillae surface of isolated rumen epithelium. In each case ketone bodies appeared on the muscle side of the tissue and very little appeared on the papillae side. With acetate as substrate, total ketone-body production was only slightly higher than with no added substrate and there was no net disappearance of acetate from the system. A small amount of acetate

Table 2. *Transport and metabolism of fatty acids by isolated rumen epithelium*

Substrates were added only to the papillae surface of isolated rumen epithelium (bearing small papillae). Incubation at 38° for 2 hr. was as given in the text. Results are calculated as μ moles/diaphragm/2 hr. Standard deviations are given in parentheses.

Additions (10 mM) to the papillae side	No. of expts.	Total ketone bodies (μ moles)		Fatty acid (μ moles)	
		Muscle side	Papillae side	Transported to muscle side	Loss or gain
None	2	3 ($\pm$ 1)	0.4 ($\pm$ 0.1)	—	+3 ($\pm$ 2)
Acetate	3	4 ($\pm$ 0.5)	0.4 ($\pm$ 0.3)	11 ($\pm$ 2)	+1 ($\pm$ 3)
Butyrate	2	24 ($\pm$ 3)	4 ($\pm$ 0)	8 ($\pm$ 2)	-40 ($\pm$ 4)
Hexanoate	4	33 ($\pm$ 16)*	4 ($\pm$ 2)	9 ($\pm$ 4)	-38 ($\pm$ 12)
Octanoate	3	17 ($\pm$ 3)	3 ($\pm$ 2)	25 ($\pm$ 3)	-18 ($\pm$ 5)
Decanoate	4	3 ($\pm$ 1)	0.6 ($\pm$ 0.4)	30 ($\pm$ 1)	-15 ($\pm$ 4)

* The large variation is due to one low figure (9 μ moles).

Table 3. *Transport and metabolism of fatty acids by isolated rumen epithelium*

Substrates were added only to the muscle surface of isolated rumen epithelium; other conditions were as described for Table 2.

Additions (10mM) to the muscle side	No. of expts.	Total ketone bodies (μ moles)		Fatty acid (μ moles)	
		Muscle side	Papillae side	Transported to papillae side	Loss or gain
None	2	3 ($\pm$ 1)	0.4 ($\pm$ 0.1)	—	+3 ($\pm$ 2)
Acetate	4	5 ($\pm$ 0)	0.7 ($\pm$ 0.4)	12 ($\pm$ 6)	-23 ($\pm$ 6)
Butyrate	3	52 ($\pm$ 9)	3 ($\pm$ 2)	5 ($\pm$ 1)	-63 ($\pm$ 8)
Hexanoate	4	44 ($\pm$ 18)*	4 ($\pm$ 2)	21 ($\pm$ 6)	-51 ($\pm$ 12)
Octanoate	1	18	2	37	-17
Decanoate	2	3 ($\pm$ 2)	0.8 ($\pm$ 0.2)	37 ($\pm$ 1)	-20 ($\pm$ 1)

* The large variation is due to one low figure (14 μ moles).

was transported to the muscle side under these conditions.

The distribution of ketone bodies across the diaphragm and the amount of intact fatty acid appearing on the muscle side when hexanoate was the substrate were similar to results previously obtained with butyrate (Hird & Weidemann, 1964a). However, as the chain length of the fatty acid increased to octanoate and decanoate, the amount of unmetabolized fatty acid transported was considerable and was greater than the total number of moles of ketone bodies formed. With decanoate at this concentration (10mM), ketone-body production was very small, but with hexanoate and octanoate, the number of moles of fatty acid disappearing from the system was similar to the number of moles of ketone bodies synthesized. The results suggest that with butyrate, hexanoate and octanoate substantial metabolism of acetyl groups occurs to products other than ketone bodies. With decanoate there was a much greater loss of fatty acid not accounted for by ketone-body production. It is assumed from the later results, which show a low rate of respiration at this concentration of decanoate, that the fatty acid substantially remained in the tissue at the end of the experiment.

The results of related experiments, where the fatty acids were applied to the muscle surface of the tissue, are given in Table 3. It is apparent that the marked distribution of ketone bodies in favour of the muscle side was similar with each fatty acid and that once again decanoate was metabolized to only a small extent. The results also indicate that fatty acid moved through the tissue to the papillae side. This was again greatest with octanoate and decanoate and on a mole to mole basis exceeded total ketone-body production. As before, there was a substantial disappearance of fatty acid from the system, which was mostly related to ketone-body formation (on a molar basis) except with acetate

Fig. 1. Effect of octanoate concentration on the rate of oxygen consumption by isolated rumen epithelium. Incubations were carried out in Warburg flasks at 38° for 2 hr. as described in the text. Results are expressed in μ moles/50 mg. dry wt. of tissue. ■, No added substrate; ▲, butyrate (10mM). Concentrations of octanoate: ○, 1mM; △, 2mM; □, 10mM; ○, 20mM.

and decanoate. The greater metabolism of acetate by the muscle surface is probably a reflection of increased access of acetate to metabolic sites through greater permeability of this surface. With butyrate and hexanoate, but not with octanoate and decanoate, application to the muscle surface also led to a greater conversion of the fatty acid carbon atoms into ketone bodies. The large disappearance of decanoate in relation to the small

total ketone-body formation is probably due again to retention of unmetabolized fatty acid in the tissue.

Effect of concentration of octanoate and decanoate on fatty acid transport and metabolism by rumen epithelium

Oxygen consumption. The results given in Tables 2 and 3 for decanoate (10mm) may indicate an

Fig. 2. Effect of decanoate concentration on the rate of oxygen consumption by isolated rumen epithelium. Experimental details were as for Fig. 1. ■, No added substrate; ▲, butyrate (10mm). Concentrations of decanoate: ○, 1 mm; △, 2 mm; □, 5 mm; ○, 10 mm.

inhibition of tissue respiration at this concentration of the fatty acid. A similar inhibition has been shown for adipose tissue in the presence of fatty acid by Hagen & Ball (1961). Accordingly, the influence of decanoate and octanoate on tissue respiration has been investigated over a range of concentrations.

Fig. 1 shows the oxygen consumption by rumen epithelium in relation to the concentration of octanoate. Inhibition of respiration occurred above 2mm, and the loss of activity was progressive. Butyrate (10mm) was also included in the experiment for comparison. At 20mm-octanoate the total oxygen consumed was less than half that observed with only endogenous substrates. Fig. 2 shows a similar experiment in the presence of increasing concentration of decanoate. In this case there was a progressive decline in total oxygen consumption at concentrations above 1 mm, and respiration was inhibited to below the endogenous rate at less than 5mm concentration. Table 4 shows that the production of ketone bodies in the presence of increasing concentration of both fatty acids paralleled oxygen consumption. Thus, for the longer chain fatty acids, as shown previously for butyrate (Hird & Weidemann, 1964b), the oxygen consumption of the tissue in the presence of the fatty acid is related to the metabolism of fatty acids to ketone bodies. In these experiments the fatty acids were in contact with both surfaces of the tissue. Accordingly it was necessary to study the system in which the normal surface (papillae) was presented to the fatty acid.

Transport experiments. Tables 5 and 6 give the relationship between ketone-body production and the concentration of octanoate and decanoate when these acids were presented to the papillae surface

Table 4. Effect of fatty acid concentration on oxygen consumption and ketone-body production by isolated rumen epithelium

Tissue was incubated in Warburg flasks at 38° for 2 hr. as described in the text. Results are expressed as μmoles/50 mg. dry wt. of tissue/2 hr. for duplicate experiments.

Concn. of substrate (mm)	Octanoate		Decanoate	
	Oxygen consumption (μmoles)	Total ketone bodies (μmoles)	Oxygen consumption (μmoles)	Total ketone bodies (μmoles)
0	10.0	0.6	10.5	0.6
	11.3	0.7	10.8	0.6
	15.5	5.4	14.1	3.4
1	18.6	5.9	13.1	2.7
	16.4	6.8	12.9	3.2
	21.5	8.8	11.1	2.2
5	—	—	6.5	0.4
	—	—	6.3	0.2
10	10.4	2.4	4.0	0.1
	9.7	1.7	5.2	0.01
20	4.0	0.6	—	—
	2.7	0.3	—	—

of rumen epithelium in diaphragm-type experiments. It can be seen that concentrations above 2mm-octanoate and 1mm-decanoate gave a progressive decrease in the total ketone bodies appearing on the opposite (muscle) side of the tissue. This effect was more marked with decanoate. When octanoate was applied on the papillae side

the inhibition was less than when it was in contact with both surfaces (Table 4). This is consistent with the view that the oxidizing system is closer, or more accessible, to the surface normally attached to the muscle (Hird & Weidemann, 1964a). The oxidizing enzymes are therefore likely to be in contact with a higher concentration of the inhi-

Table 5. *Metabolic products formed during the metabolism and transport of octanoate by isolated rumen epithelium*

Conditions were as described for Table 2. Results, calculated as $\mu\text{moles/diaphragm/2hr.}$, are given for two separate experiments. See Table 6 for endogenous values. Substrates were added to the papillae surface.

Additions to the papillae side	Gain on muscle side (μmoles)				Gain or loss on papillae side (μmoles)			
	Ketone bodies	Fatty acid			Ketone bodies	Fatty acid		
		Acetate	Hexanoate	Octanoate		Acetate	Hexanoate	Octanoate*
Octanoate (1mm)	23	2.6	0.1	0.27	4	2.1	0.1	-19
	22	2.9	0.1	0.30	2	0.1	0.1	-17
Octanoate (2mm)	30	5.6	0.4	3.1	7	2.2	0.8	-28
	32	3.7	0.6	1.2	4	0.2	0.7	-27
Octanoate (5mm)	21	2.1	0.5	15	6	1.9	1.1	-38
Octanoate (10mm)	18	3.2	0.5	28	5	2.6	1.1	-53
	20	2.1	0.5	21	2	0.1	0.2	-38
Octanoate (20mm)	13	2.0	0.4	34	1	0.0	1.0	-63

* The loss of octanoate includes fatty acid metabolized plus the amount remaining in the tissue at the end of the experiment.

Table 6. *Metabolic products formed during the metabolism and transport of decanoate by isolated rumen epithelium*

Conditions and calculations were as described for Table 2. The decanoate added contained 5% of octanoate; it was added to the papillae surface.

Additions to the papillae side	Gain on muscle side (μmoles)				Gain or loss on papillae side (μmoles)			
	Ketone bodies	Fatty acid			Ketone bodies	Fatty acid		
		Hexanoate	Octanoate	Decanoate		Hexanoate	Octanoate	Decanoate*
None	3	0.02	0.00	0.03	0.0	0.03	0.02	-0
	4	0.05	0.04	0.00	0.2	0.04	0.01	-0
Decanoate (1mm)	14	0.6	0.7	1.9	1	0.5	1.0	-15
	11	0.5	0.7	2.1	1	0.4	1.2	-14
	22	0.4	0.4	1.5	1	0.6	1.1	-18
	20	0.5	0.8	1.9	0.3	0.2	1.8	-16
Decanoate (2mm)	3	0.3	1.0	8	1	0.4	0.8	-16
	6	0.4	1.2	6	1	0.4	1.7	-17
Decanoate (5mm)	2	0.3	1.3	19	0.4	0.4	-0.1	-30
	4	0.3	1.2	17	0.1	0.3	-0.1	-26
Decanoate (10mm)	2	0.0	1.4	28	0.2	0.0	-0.8	-56
	2	0.2	1.6	30	0.2	0.3	-1.7	-43

* The loss of decanoate includes fatty acid metabolized plus the amount remaining in the tissue at the end of the experiment.

bitory fatty acid when this is in contact with the muscle surface.

Analysis of the medium on both sides of the tissue by gas chromatography (Table 5) indicated that small amounts of acetate and hexanoate were also produced during the oxidation of octanoate. These fatty acids of lower molecular weight usually appeared on both sides of the tissue, although more acetate was found on the muscle side than the papillae side. The results suggest the presence of a deacylase(s) acting on intermediates produced during the oxidation of octanoate. The possibility of leakage of acyl-CoA intermediates from the tissue and their subsequent deacylation during the estimation procedure would not account for more than 10% of the lower fatty acids obtained, if amounts of CoA did not exceed those found in sheep liver (Jarrett & Filsell, 1964). Table 5 also indicates that, as the ketone-body production fell off at higher concentrations of the fatty acid, the proportion of unmetabolized fatty acid moving across the tissue increased as the concentration increased.

The results of a similar experiment with decanoate are given in Table 6. In this case also, lower fatty acids were produced by the tissue; the gas chromatograms again showed the formation of acetate, but as this acid was not included in the calibration mixture used for these analyses, it is not possible to give a quantitative figure for its appearance. With decanoate the passage of larger amounts of fatty acid to the muscle side at the higher concentrations coincided with a marked fall in ketone-body production and oxygen consumption. This indicates a simultaneous loss of selective permeability and oxidative processes. The rate of passage of fatty acid across the tissue in the absence of marked ketone-body production (2mm-decanoate and above) appears to be proportional to the concentration of the fatty acid (Table 6).

Production of ketone bodies from long-chain fatty acids

Table 7 shows the effect of adding long-chain fatty acids (1mm) to the papillae side of rumen epithelium. Bovine serum albumin (0.5%) was added to the incubating medium in each case to increase the solubility of the fatty acid. It can be seen that serum albumin alone produced a rise in total ketone-body production above the endogenous level. In the presence of the fatty acids, ketone-body production was consistently higher than that found with serum albumin alone, and the distribution of ketone bodies at the end of the experiment was always in favour of the muscle side. It is possible that the lower activity associated with palmitate and the higher fatty acids was due to decreasing

Table 7. *Ketone-body production accompanying the metabolism of long-chain fatty acids added to the papillae surface of isolated rumen epithelium*

The fatty acids, added to give a concentration of 1mm, were applied to the papillae surface of the epithelium (bearing small papillae). Bovine serum albumin (0.5%) was present in the fatty acid-containing solution. Other conditions are given in the text. Results are the mean of duplicate estimations.

Additions to the papillae side	Appearance of total ketone bodies (μ moles)	
	Muscle side	Papillae side
None	2.2	0.2
	3.8	0.5
Bovine serum albumin (0.5%)	5.7	2.1
	4.7	1.9
	6.1	0.3
	6.2	0.2
	5.4	0.5
Laurate (1 mm)	5.2	0.9
	12.6	0.5
	14.7	0.2
Myristate (1 mm)	14.6	0.2
	17.3	0.6
Palmitate (1 mm)	7.9	0.3
	10.5	1.0
Stearate (1 mm)	6.6	2.7
	7.0	2.1
Oleate (1 mm)	8.1	3.5
	8.9	3.7
Linolenate (1 mm)	8.0	3.8
	6.2	3.5

solubility, as solution of fatty acids higher than myristate was incomplete. It is apparent from Table 7 that long-chain fatty acids normally found in the rumen (palmitate, stearate, oleate and linolenate) are, like the shorter-chain fatty acids tested, precursors of ketone bodies during their transport through the tissue.

DISCUSSION

The application of even-numbered fatty acids to the papillae surface of rumen epithelium has been shown, in each case, to lead to the preferential release of ketone bodies on the muscle side of the tissue. Apart from acetate, which is not extensively converted into ketone bodies (Pennington, 1952; Hird & Symons, 1961), the longer-chain fatty acids are transported and metabolized by the tissue in a similar fashion to butyrate (Hird & Weidemann, 1964a). In the series acetate to decanoate, there is little transport of the unmetabolized fatty acid unless the concentration of fatty acid in the medium

is raised to a value at which respiration is inhibited and selective permeability is lost. At concentrations of the fatty acids which are not inhibitory, the production of ketone bodies and their selective release on the side of the tissue normally in contact with the blood supply would therefore appear to be a part of the mechanism of absorption of these fatty acids. The slow rate of fatty acid transport (as such) may be related to the pH of the incubating medium (7.4), under which conditions absorption *in vivo* is slow (Danielli, Hitchcock, Marshall & Phillipson, 1946; Gray, 1948).

Experiments with octanoate and decanoate suggest that 'deacylase' activity leads to the production of shorter-chain fatty acids from intermediates formed during oxidation. The existence of deacylase(s) in rumen epithelium is consistent with the observation of Duncombe & Glascock (1954) that labelled volatile fatty acid appears in blood after the feeding of tritiated stearic acid to goats. A similar pathway in the liver of the ruminant is probably partly responsible for the release of radioactive acetate into the blood when labelled palmitate is infused (Connolly, Head & Williams, 1964; West & Annison, 1964).

The known effects of fatty acids on tissue respiration (Hagen & Ball, 1961; Enser, 1964) and oxidative phosphorylation (Björntorp, Ells & Bradford, 1964; Hird & Weidemann, 1966) suggests that the metabolism of these compounds to ketone bodies achieves several useful results. The tissue is able to use the fatty acids as a substrate for its energy requirements (Hird & Weidemann, 1964b) and the excess of carbon atoms are available for distribution through the body as ketone bodies etc. In this way deleteriously high concentrations of fatty acids are avoided.

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