Aust. J. Biol. Sci., 1976, **29**, 33–42

Production of Endogenous Acetate by the Liver in Lactating Ewes

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Abstract

The production of endogenous acetate by the liver has been investigated in lactating ewes using animals with indwelling arterial, and portal and hepatic venous cannulae. The capacity of the liver to produce acetate from acetyl-CoA *in vitro* has also been examined using homogenates prepared from liver biopsy samples.

Mean arterial, portal and hepatic venous blood acetate concentrations in four ewes at 4 weeks lactation were 0·40, 1·00 and 1·46 mM respectively. The mean exogenous and endogenous acetate production rates were 56 and 54 mmol/h respectively, giving a total of 110 mmol/h. The mean portal–hepatic venous difference in free fatty acid concentration was 81 μM. Converting this uptake of free fatty acids by the liver (based on palmitate as a standard) to 2-carbon equivalents, the acetate produced accounted for 70% of the fatty acids taken up. The correlation coefficient (r^2) between uptake of free fatty acids and production of acetate by the liver was 0·83 ($P < 0·01$).

Calculation of the net acetate production *in vivo* gave a mean value for the production of acetate of 0·75 mmol/min. Calculation of the *in vitro* enzymic capacity of the liver to produce acetate from acetyl-CoA gave a mean of 0·94 mmol/min. These results indicate that enzymic production of acetate from acetyl-CoA, via carnitine acetyltransferase and acetylcarnitine hydrolase (see Costa and Snoswell 1975*a*), can adequately account for the substantial production of acetate by the liver in lactating ewes.

Introduction

Acetate is the major product of fermentation in the rumen and caecum of sheep and other ruminants, and this volatile fatty acid is an important source of energy in these animals (Annison and Armstrong 1970). In addition to this acetate produced in the alimentary tract, endogenous acetate contributes some 25% of total acetate turnover in normal sheep (Annison and White 1962; Bergman and Wolf 1971) and in fed steers and mature cows (Lee and Williams 1962). In fasted sheep this value may rise to 50–75% (Annison and White 1962; Bergman and Wolf 1971).

Although the importance of endogenous acetate in ruminant metabolism has been recognized for more than a decade, we still know little of the control of its release from tissues (see Annison 1973). The carbon source for this endogenous acetate is unclear. All metabolic processes proceeding via acetyl-CoA are potential sources of endogenous acetate; this acetyl-CoA would be formed in the mitochondrial matrix, and the inner mitochondrial membrane is impermeable to acetyl-CoA (Tubbs and Garland 1968). Thus a mechanism for effective release of acetyl-CoA from the mitochondria must exist, or acetyl-CoA must be first hydrolysed to acetate.

A number of authors have suggested that endogenous acetate is probably derived from acetyl-CoA via the acetyl-CoA hydrolase (EC 3.1.2.1) reaction (Annison *et al.*

1967; Ballard 1972; Knowles *et al.* 1974). However, our recent work (Costa and Snoswell 1975*b*) has shown that acetyl-CoA hydrolase is an artifact and that the activity is due to the combined action of carnitine acetyltransferase (EC 2.3.1.7), located in the inner mitochondrial membrane, and acetylcarnitine hydrolase (Costa and Snoswell 1975*a*), located in the outer mitochondrial membrane. The combined action of these two enzymes could account for the production of endogenous acetate.

Baird *et al.* (1974) have recently reported a very substantial production of acetate by the liver of a lactating cow *in vivo* as measured by hepatic–portal differences in blood acetate concentration. Thus, we decided to examine endogenous acetate production in the lactating ewe drawing on our previous experience with the surgical preparation of sheep with indwelling portal and hepatic venous and arterial cannulae (Snoswell and McIntosh 1974). We attempted to associate any production of acetate by the liver, as measured by hepatic–portal differences, with the enzymic capacity of the liver to produce acetate from acetyl-CoA.

The present work has established that there is a substantial net production of endogenous acetate by the liver of lactating ewes. Free fatty acids simultaneously taken up by the liver could account for the acetate produced by the combined carnitine acetyltransferase–acetylcarnitine hydrolase system, which has adequate capacity to account for this endogenous acetate production.

Materials and Methods

Animals

The sheep used were 5-year-old Merino ewes weighing approximately 50 kg. One ewe was a Dorset × Merino. The animals were chosen so that, at the time of intensive sampling, they were at the height of lactation (i.e. 4 weeks into the lactation cycle). The sampling was repeated on one ewe after 8 weeks of lactation. The lambs were left with the ewes to maintain active lactation. Under these conditions milk yields after 8 weeks fall to 1·0 l/day compared with 2·9 l/day after 4 weeks lactation (Snoswell and Linzell 1975). The animals were maintained in pens and fed chaffed lucerne hay *ad libitum*.

Surgical Cannulation of Blood Vessels

Indwelling cannulae were inserted into the portal and hepatic veins under general anaesthesia as described previously (Snoswell and McIntosh 1974). A cannula was also inserted into the femoral artery under local anaesthesia on the morning of sampling by the technique described previously (Snoswell and McIntosh 1974). A further cannula (polyvinyl tube 1·00 mm i.d., 1·5 mm o.d.) was also inserted through a 13-gauge needle into the superficial mammary vein, which was clearly visible on the abdomen anterior to the udder. The cannula was fixed in place with metal clips. All cannulae were kept patent by flushing with heparin–saline mixture (100 i.u. heparin/ml).

Measurement of Hepatic Blood Flow

Total hepatic venous blood flow through the liver was assessed using the bromosulphophthalein (BSP) clearance technique as described by Shoemaker (1964) and Katz and Bergman (1969). A priming dose of 100 mg BSP was administered intravenously followed by a constant infusion of 10 mg/min into the jugular vein over a period of 2·5 h. Thirty min were allowed for equilibration of the dye with the blood, after which simultaneous portal and hepatic venous samples (5 ml) were withdrawn into heparinized syringes every 20 min for the following 2 h. BSP concentrations were determined on plasmas which were prepared on the same day. Packed cell volumes were determined on the blood samples and a sample of hepatic venous blood was drawn prior to commencing the infusion, as an analytical blank. Plasma BSP concentrations were determined by the method of Varley (1963).

Blood flow determinations were made midway through the experimental blood sampling periods, on either one or two occasions, depending on the length of the sampling period. In all blood flow

determinations (with one exception), blood levels of BSP were constant over the period of analysis. There was no evidence of changing packed cell volumes, as might be expected if excessive removal of blood was occurring. Arterial samples drawn together with portal venous samples were shown to contain equivalent BSP concentrations.

Tissue Preparations

The sheep were killed by severing the necks, and samples of liver were immediately freeze-clamped with aluminium-faced tongs previously cooled in liquid N_2 (Wollenberger *et al.* 1960). The $HClO_4$ extracts of frozen tissue were prepared as described by Snoswell and Henderson (1970). Fresh liver samples were collected into 0·25M sucrose–23 mM potassium phosphate (pH 7·4).

Blood Samples

Blood samples for metabolite assays were drawn from all four cannulae (mammary, arterial, and portal and hepatic venous) simultaneously at hourly intervals for 12 h. Blood fractions of 2·0 ml were immediately added to 2·0 ml of 15% perchloric acid for acetate assays and 1·0-ml fractions added to a mixture of 20 ml chloroform plus 6·625 ml of 0·1M sodium phosphate buffer (pH 6·2) for the determination of free fatty acids.

Metabolite Assays

The perchloric acid extracts of the blood samples were adjusted to pH 6·5 with 3M KOH and centrifuged at 5°C to remove potassium perchlorate.

Acetate was measured in duplicate on 30–60-μl aliquots of the neutralized extract using a specific enzyme assay described by Knowles *et al.* (1974). Acetate kinase used in the assay may utilize propionate at 2% of the rate of acetate (Bergmeyer and Möllering 1974) but the propionate concentration even in portal blood is only one-seventh of the acetate concentration (Bergman and Wolf 1971). Thus any interference in the assay of acetate by propionate would be negligible.

Free fatty acids were estimated in the chloroform extracts of the blood samples in the Auto Analyzer (Technicon Instruments Corporation, Ardsley, N.Y.) according to the method described by Dalton and Kowalski (1967).

Alanine was measured using alanine dehydrogenase. The assay system contained 20 mM sodium carbonate (pH 10·0), 5 mM NAD, 25 μg enzyme protein and neutralized extract in a final volume of 1 ml. Reduction of NAD was monitored at 340 nm.

Acetylcarnitine was measured in the neutralized extracts of blood and freeze-clamped livers by the method of Pearson and Tubbs (1964).

Free carnitine was measured in the neutralized extracts of freeze-clamped livers by the method of Marquis and Fritz (1964).

Carnitine Acetyltransferase

This enzyme was assayed in the supernatant fractions obtained by centrifuging (8000 *g* for 3 min) frozen and thawed homogenates (20 % w/v) of liver prepared in hypotonic 0·025M sucrose containing 2·3 mM potassium phosphate (pH 7·4) and 0·1% Triton X-100. The reaction mixture consisted of 400 mM tris–HCl (pH 8·0), 100 μM acetyl-CoA, 330 μM L-carnitine, 100 μM 5,5′-dithio-bis(2-nitrobenzoic acid) and tissue enzyme (2 mg wet weight of liver) in a total volume of 1·0 ml. The reaction was monitored spectrophotometrically by the increase in absorbance at 412 nm due to the yellow 5-thio-2-nitrobenzoic anion at 37°C. The mixture was preincubated at 37°C for 10 min and the reaction started by the addition of L-carnitine.

Carnitine Palmitoyltransferase (EC 2.3.1.21)

The activity of this enzyme was measured in homogenates of liver prepared as described for the carnitine acetyltransferase assay. The reaction mixture consisted of 200 mM tris–HCl (pH 8·2), 100 μM palmitoyl-CoA, 330 μM L-carnitine, 100 μM 5,5′-dithio-bis(2-nitrobenzoic acid) and tissue homogenate (2 mg wet weight liver) in a total volume of 1·0 ml. The reaction was monitored spectrophotometrically by the increase in absorbance at 412 nm and 37°C. The reaction was started by addition of L-carnitine. The increase in absorbance in the absence of L-carnitine was due to palmitoyl-CoA hydrolase (EC 3.1.2.2) activity. The difference in rates of absorbance at 412 nm and 37°C was the true carnitine palmitoyltransferase activity.

Instrumentation for these assays was described previously in Costa and Snoswell (1975*a*).

Results

The results in Table 1 show that, although there is a considerable variation in the concentration of acetate in the portal and hepatic venous blood and arterial blood of the four ewes, there is a substantial and significant ($P < 0.01$) production of endogenous acetate by the liver of each animal as judged by hepatic–portal differences in blood acetate. The mean hepatic–portal difference for the four ewes at 4 weeks lactation is 0·46 μmol/ml and the average hepatic venous blood flow is 1·74 l/min (Table 4), giving a mean net endogenous acetate production of 48 mmol/h. This value is increased to 54 mmol/h if a correction is made for the input into liver via the hepatic artery in addition to the main input via the portal vein (see Bergman and Wolf 1971). This production of endogenous acetate by the liver is almost equal to the production of exogenous acetate in the portal-drained viscera, viz. 56 mmol/h calculated from the mean portal–arterial difference in blood acetate concentration of 0·60 μmol/ml (Table 1) and a calculated portal blood flow rate of 1·56 l/min.

Table 1. Acetate concentrations in arterial, and in portal and hepatic venous blood of lactating ewes
Acetate was estimated in duplicate as described in the text. The values shown are means ± S.E.M. with the number of blood samples assayed given in parentheses. The significance of the differences between hepatic–portal and portal–arterial samples, as determined by paired *t* test, is also indicated

Ewe	Blood acetate concentration (μmol/ml)				
	Hepatic (H)	Portal (P)	Arterial (A)	H−P	P−A
1	2·70±0·18 (6)	1·82±0·19 (6)	0·95±0·17 (6)	0·88±0·24 (6) $P < 0.01$	0·88±0·17 (6) $P < 0.01$
2	1·08±0·08 (6)	0·66±0·11 (6)	0·20±0·04 (6)	0·42±0·06 (6) $P < 0.01$	0·46±0·05 (6) $P < 0.05$
3	1·31±0·13 (11)	1·05±0·13 (11)	0·21±0·05 (11)	0·26±0·06 (11) $P < 0.01$	0·82±0·16 (11) $P < 0.001$
4a[A]	0·76±0·10 (12)	0·46±0·05 (12)	0·22±0·02 (12)	0·29±0·06 (12) $P < 0.01$	0·23±0·05 (12) $P < 0.001$
Mean	1·46	1·00	0·40	0·46	0·60
4b	0·46±0·02 (12)	0·40±0·03 (12)	0·24±0·02 (12)	0·07±0·02 (12) $P < 0.001$	0·16±0·03 (12) $P < 0.001$

[A] Ewe 4a sampled after 4 weeks lactation, 4b is the same ewe sampled after 8 weeks lactation.

Thus the mean total (endogenous plus exogenous) acetate production rate was 110 mmol/h for these lactating ewes. Simultaneously with the production of acetate by the liver, there was a significant ($P < 0.01$) uptake of acetate across the udder, as determined by the arterial–mammary venous difference in blood acetate concentration, with an approximate 50% extraction after 4 weeks of lactation.

The portal blood concentration of free fatty acids exceeded that in the hepatic blood in each ewe (Table 2). In three of the five cases, the portal–hepatic difference was significant. The mean portal–hepatic difference for the four ewes was 81 nmol/ml. The portal and hepatic venous blood concentrations of free fatty acids were significantly ($P < 0.01$ and $P < 0.05$ respectively) less for ewe No. 4 at 8 weeks lactation than those determined at 4 weeks lactation (Table 2). The free fatty acid concentrations are based on the use of palmitic acid as a standard, so that the uptake of fatty acids from the blood across the liver is equivalent to 8×81 or 648 nmol/ml potential 2-carbon

units. Thus acetate produced by the liver, viz. 0·46 μmol/ml (Table 1), represents 70% of the 2-carbon potential of the free fatty acids extracted by the liver.

The portal–hepatic difference in the concentration of free fatty acids in the blood varied considerably over the total sampling periods in each case. Indeed it was found essential to take a considerable number of blood samples over an extended period to

Table 2. Free fatty acid concentrations in hepatic and portal venous blood of lactating ewes
Free fatty acids were determined as described in the text. The values shown are means ± S.E.M. with the number of blood samples given in parentheses. The significance of the differences between portal and hepatic concentrations were determined by paired *t* test. n.s., No significant difference

Ewe	Free fatty acid concentration in blood (nmol/ml)			
	Hepatic (H)	Portal (P)	P−H	Significance
1	251 ± 51 (4)	314 ± 67 (4)	63 ± 33 (4)	n.s.
2	251 ± 29 (9)	365 ± 66 (9)	114 ± 41 (9)	$P < 0{\cdot}05$
3	346 ± 53 (10)	403 ± 73 (10)	57 ± 24 (10)	n.s.
4a[A]	448 ± 16 (10)	535 ± 25 (10)	87 ± 26 (10)	$P < 0{\cdot}01$
Mean	324	404	81	
4b	365 ± 24 (11)	394 ± 20 (11)	29 ± 8 (11)	$P < 0{\cdot}01$

[A] Ewe 4a sampled after 4 weeks lactation, 4b is the same ewe sampled after 8 weeks lactation.

obtain statistically significant results. The results shown in Fig. 1 indicate a significant correlation ($P < 0{\cdot}01$) between the portal–hepatic difference for free fatty acids and the hepatic–portal difference in blood acetate concentrations, with a correlation coefficient (r^2) of 0·83. After 4 weeks of lactation the portal–hepatic difference in free fatty acid concentration for ewe No. 4 was 87 nmol/ml while the hepatic–portal

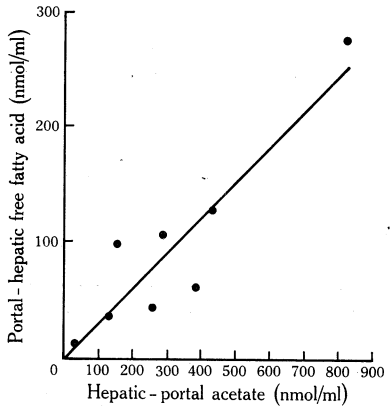

Fig. 1. Portal–hepatic differences in blood free fatty acid *versus* hepatic–portal differences in blood acetate for hourly blood samples. Simultaneous hepatic and portal blood samples were taken from a ewe (No. 4) after 4 weeks lactation. Blood acetate and free fatty acid concentrations were determined as in Tables 1 and 2.

difference in acetate concentration was 0·29 μmol/ml, but after 8 weeks of lactation these values had fallen significantly ($P < 0{\cdot}01$) to 29 nmol/ml and 0·07 μmol/ml respectively. At this later stage of lactation there was no correlation between free fatty acid uptake and acetate production. The portal–hepatic difference in concentration of L-alanine in ewe No. 1 was 0·016 mM. There was no detectable acetylcarnitine in portal or hepatic venous blood.

The activity of carnitine acetyltransferase in the liver of these lactating ewes was 2·2 μmol min^{-1} (g tissue)$^{-1}$ (see Table 3), which is similar to that in normal wethers, as reported previously (Snoswell and Koundakjian 1972). The activity of carnitine

Table 3. The activity of carnitine acetyltransferase, carnitine palmitoyltransferase and enzymic conversion of acetyl-CoA to acetate in liver biopsies from lactating ewes

Liver biopsy samples were removed at the time of surgical insertion of the cannulae and again at autopsy. The activities shown are the means of assays on these two samples. Activities were measured as described in the text. The mean values ±S.E.M. are those for all assays on four ewes. The number of assays is shown in parentheses

Ewe	Liver enzyme activities [μmol min^{-1} (g wet tissue)$^{-1}$]		
	Carnitine acetyltransferase	Carnitine palmitoyltransferase	Acetate production from acetyl-CoA
1	2·1	4·3	0·7
2	3·0	4·2	1·2
3	2·4	5·3	1·5
4a[A]	1·5	2·2	1·3
Mean	2·2±0·23 (10)	4·1±0·33 (8)	1·1±0·14 (10)
4b	2·4	4·2	1·1

[A] Ewe 4a sampled after 4 weeks lactation, 4b is the same ewe sampled after 8 weeks lactation.

palmitoyltransferase, relative to carnitine acetyltransferase activity, of 4·1 μmol min^{-1} (g tissue)$^{-1}$ (see Table 3) is very much greater than that reported previously for wethers (Snoswell and Henderson 1970). The average enzymic activity for converting acetyl-CoA to acetate, via the carnitine acetyltransferase and acetylcarnitine hydrolase reactions (see Costa and Snoswell 1975*b*) in homogenates of liver, was 1·1 μmol min^{-1} (g wet weight)$^{-1}$ (see Table 3).

Table 4. Comparison of the actual *in vivo* production rate of acetate by the liver with the *in vitro* enzymic capacity of the liver to convert acetyl-CoA to acetate as determined on biopsy samples from lactating ewes

Blood and liver samples were collected and assayed as in Tables 1 and 3. Hepatic venous blood flows are the means of two determinations measured by BSP extraction (see Methods)

Ewe	Blood acetate hepatic–portal concentration (μmol/ml)	Hepatic venous blood flow (l/min)	Hepatic acetate production (mmol/min)	Enzymic acetate production rate (μmol min^{-1}g^{-1})	Total liver wet weight (g)	Total enzymic acetate production capacity (mmol/min)
1	0·88	1·35	1·19	0·7	914	0·64
2	0·42	1·74	0·73	1·2	840	1·01
3	0·26	2·06	0·54	1·5	818	1·28
4a[A]	0·29	1·81	0·52	1·3	620[B]	0·81
Mean	0·46	1·74	0·75	1·2	798	0·94
4b	0·07	1·73	0·12	1·1	620	0·68

[A] Ewe 4a sampled after 4 weeks lactation, 4b is the same ewe sampled after 8 weeks lactation.
[B] Liver weight after 8 weeks lactation used, at 4 weeks the weight was probably greater.

The concentrations of free carnitine and acetylcarnitine in the livers of lactating ewes were 117±25 and 90±31 nmol/g respectively (mean±S.E.M. for six samples). This concentration of acetylcarnitine was much greater than that reported by Snoswell

and Koundakjian (1972) for normal wethers fed on a similar diet. The mean total enzymic capacity of the liver to produce acetate from acetyl-CoA for the four ewes after 4 weeks lactation, calculated by multiplying the enzymic capacity determined *in vitro* by the total liver weight, was 0·94 mmol/min (Table 4). The actual mean *in vivo* acetate production by the livers, determined by multiplying the mean hepatic–portal difference in blood acetate concentration by the mean hepatic venous blood flow rate (1·74 l/min), was 0·75 mmol/min (Table 4). The ewe sampled at 8 weeks after lactation showed an *in vitro* enzymic capacity to produce acetate of 0·68 mmol/min but an actual acetate production of only 0·12 mmol/min (Table 4).

Discussion

The results presented here clearly indicate a substantial net production of endogenous acetate by the liver in lactating ewes. The net endogenous acetate production was almost equivalent to the rate of exogenous acetate production. These results differ from those obtained by Bergman and Wolf (1971) working with fed and fasted sheep. These workers observed only a small net production of endogenous acetate by the liver, both in fed and fasted sheep, but in the latter case the total acetate turnover was considerably reduced. However, the total acetate production (i.e. exogenous and endogenous) in lactating ewes was 110 mmol/h, which is considerably higher than the 75 mmol/h production reported by Bergman and Wolf in non-lactating sheep. The net production of endogenous acetate by the liver does appear to be related to the lactation state. The ewe sampled after 8 weeks of lactation showed an *in vivo* acetate production rate of only 0·12 mmol/min compared with 0·52 mmol/min after 4 weeks lactation. Snoswell and Linzell (1975) report that milk production decreases substantially after 8 weeks of lactation and acetate thiokinase activity in the mammary glands is only about 15% of that after 4 weeks lactation (Snoswell and Linzell, unpublished data). Finally, Baird *et al.* (1974) have shown very substantial hepatic–portal differences in blood acetate concentration in the lactating cow, indicating net endogenous acetate production by the liver during lactation in the cow.

In the experiments described here with lactating ewes there was a significant uptake by the liver of free fatty acids from the blood as well as a significant production of endogenous acetate across the liver. On the basis of 2-carbon equivalents, acetate produced accounted for 70% of the free fatty acids taken up. This of course does not prove that free fatty acids are the source of the acetate (this could only be determined by infusing labelled fatty acids), but it does establish that there is sufficient potential carbon in the fatty acids taken up to account for the acetate produced. Further, when the uptake of free fatty acids and the output of acetate by the liver for each successive blood sample were plotted (see Fig. 1), there was a significant correlation ($r^2 = 0{\cdot}83$) between the free fatty acids taken up and the acetate produced. With the reservation that correlation does not imply causation, it would appear likely that free fatty acids could supply the major portion of the acetate produced. Both Palmquist (1972) and West and Annison (1964) have shown that significant incorporation of label from [^{14}C]palmitate into acetate occurs in the sheep.

The production of endogenous acetate from other sources is possible, however. In ewe No. 1 the portal–hepatic difference in alanine concentration was 0·016 μmol/ml compared with a hepatic–portal difference in acetate of 0·88 μmol/ml. Thus alanine

could possibly have contributed up to 18% of the acetate formed. Other amino acids might also have been utilized to a minor extent. If ethanol was utilized, it could be converted to acetate without being metabolized via acetyl-CoA. However, this would appear most unlikely from the work of Baird *et al.* (1974) with the lactating cow, where the small uptake of ethanol from the liver contributed only to a very minor extent to the large amount of acetate produced.

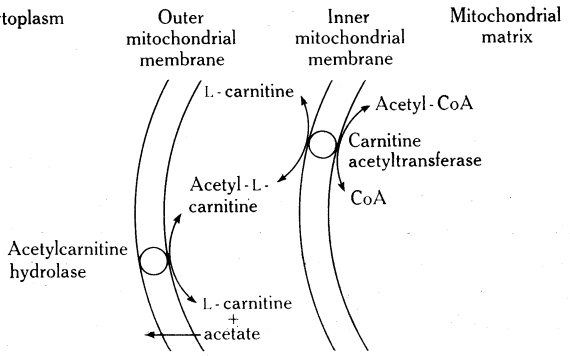

Fig. 2. Proposed mode of action of carnitine acetyltransferase and acetylcarnitine hydrolase in releasing acetate from mitochondrial acetyl-CoA.

The mean enzymic capacity of the liver to produce acetate from acetyl-CoA, as determined *in vitro* using homogenates of biopsy samples, was 0·94 mmol/min, compared with an actual mean net acetate production rate of 0·75 mmol/min. The net production rate may be an underestimate of the actual production rate since some acetate could be used by the liver at the same time as acetate is being produced. This could be demonstrated by infusion experiments with labelled acetate. In spite of this reservation, it would appear that the enzymic capacity of the liver to produce acetate, as measured *in vitro*, could account for the actual endogenous production of acetate observed in the whole animal. The enzymic measurement of the conversion of acetyl-CoA to acetate used here involves a combination of two enzymes, carnitine acetyltransferase associated with the inner mitochondrial membrane (see also Edwards *et al.* 1974) and acetylcarnitine hydrolase associated with the outer mitochondrial membrane (see Costa and Snoswell 1975*a*, 1975*b*).

This enzyme system then establishes a flow, as shown in Fig. 2, which adequately accounts for the breakdown of mitochondrial acetyl-CoA leading to the release of acetate.

The reason why sheep liver should produce considerable amounts of acetate during lactation is of interest. We suggest here that acetate is produced to relieve an intramitochondrial build up of acetyl-CoA derived from fatty acid oxidation. This would appear to be an energetically wasteful process, yet it is no more wasteful than the production of ketone bodies and should be regarded as an alternative process to ketone body production in producing an important metabolite for use in the mammary glands. Sheep liver mitochondria oxidize fatty acids at a relatively slow rate (Koundakjian and Snoswell 1970) and this appears to be due to a lesser ability to oxidize Krebs cycle intermediates. The high activity of carnitine palmitoyltransferase in the livers of lactating ewes reported here (Table 3), together with the significant uptake of fatty acids and acetate production by the livers *in vivo*, suggests that the

limitation on total fatty acid oxidation is not due to a limitation on the conversion of fatty acids to acetyl-CoA but rather on the subsequent oxidation of acetyl-CoA. Baird and Heitzman (1970) found significantly reduced levels of a number of Krebs cycle intermediates in the liver of lactating cows compared with non-lactating cows, suggesting a decreased turnover of the Krebs cycle in bovine liver during lactation. Thus, if Krebs cycle activity in sheep liver is depressed during lactation, the fate of excess acetyl-CoA would largely be determined by the relative activities of carnitine acetyltransferase and of the ketone body-producing enzymes and their affinities for acetyl-CoA. In this respect the activity of carnitine acetyltransferase in liver of lactating sheep is considerable [2·22 μmol min^{-1} (g wet tissue)$^{-1}$; see Table 3]. The question of mechanisms controlling acetate production obviously requires further investigation.

Acknowledgments

The authors are indebted to Mr D. Pethick and Mr G. D. Henderson for assistance in the collection of the blood samples and to Mr R. Fishlock for skilled technical assistance. The authors are also grateful to Mr O. H. Filsell for help in the analyses of the free fatty acids in blood. Grants from the Australian Meat Research Committee and the Australian Research Grants Committee to A.M.S. are gratefully acknowledged. N.D.C. is supported by a grant from the Australian Wool Corporation.

References

Annison, E. F. (1973). In 'Production Disease in Farm Animals'. (Eds J. M. Payne, K. G. Hibbitt and B. F. Sansom.) p. 140. (Bailliere Tindall: London.)

Annison, E. F., and Armstrong, D. G. (1970). In 'Physiology of Digestion and Metabolism in the Ruminant'. (Ed A. T. Phillipson.) pp. 422–37. (Oriel Press: Newcastle.)

Annison, E. F., Brown, R. E., Leng, R. A., Lindsay, D. B., and West, C. E. (1967). *Biochem. J.* **104**, 135–47.

Annison, E. F., and White, R. R. (1962). *Biochem. J.* **84**, 546–52.

Baird, G. D., and Heitzman, R. J. (1970). *Biochem. J.* **116**, 865–74.

Baird, G. D., Symonds, H. W., and Ash, R. (1974). *Proc. Nutr. Soc.* **33**, 70a.

Ballard, F. J. (1972). *Am. J. Clin. Nutr.* **25**, 773–9.

Bergman, E. N., and Wolf, J. E. (1971). *Am. J. Physiol.* **221**, 586–92.

Bergmeyer, H. U., and Möllering, H. (1974). In 'Methods of Enzymatic Analysis'. Vol. 3, 2nd Edn. (Ed. H. U. Bergmeyer.) pp. 1520–8. (Academic Press: New York.)

Costa, N. D., and Snoswell, A. M. (1975*a*). *Biochem. J.* **152**, 161–6.

Costa, N. D., and Snoswell, A. M. (1975*b*). *Biochem. J.* **152**, 167–72.

Dalton, C., and Kowalski, C. (1967). *Clin. Chem.* **13**, 744–51.

Edwards, Y. H., Chase, J. F. A., Edwards, M. R., and Tubbs, P. K. (1974). *Eur. J. Biochem.* **46**, 209–15.

Katz, M. L., and Bergman, E. N. (1969). *Am. J. Physiol.* **216**, 946–52.

Knowles, S. E., Jarrett, I. G., Filsell, O. H., and Ballard, F. J. (1974). *Biochem. J.* **142**, 401–11.

Koundakjian, P. P., and Snoswell, A. M. (1970). *Biochem. J.* **119**, 49–57.

Lee, S. D., and Williams, W. F. (1962). *J. Dairy Sci.* **45**, 893–6.

Marquis, N. R., and Fritz, I. B. (1964). *J. Lipid Res.* **5**, 184–7.

Palmquist, D. L. (1972). *J. Nutr.* **102**, 1401–6.

Pearson, D. J., and Tubbs, P. K. (1964). *Nature (London)* **202**, 91.

Shoemaker, W. C. (1964). In 'The Liver'. (Ed C. Roullier.) pp. 243–66. (Academic Press: New York.)

Snoswell, A. M., and Henderson, G. D. (1970). *Biochem. J.* **119**, 59–65.

Snoswell, A. M., and Koundakjian, P. P. (1972). *Biochem. J.* **127**, 133–41.
Snoswell, A. M., and Linzell, J. L. (1975). *J. Dairy Res.* **42**, 371–80.
Snoswell, A. M., and McIntosh, G. H. (1974). *Aust. J. Biol. Sci.* **27**, 645–50.
Tubbs, P. K., and Garland, P. B. (1968). *Brit. Med. Bull.* **24**, 158–64.
Varley, H. (1963). In 'Practical Clinical Biochemistry'. 3rd Edn. pp. 313–14. (Heinemann: London.)
West, C. E., and Annison, E. F. (1964). *Biochem. J.* **92**, 573–8.
Wollenberger, A., Ristan, O., and Schoffa, G. (1960). *Pflügers Arch. Gesamte Physiol. Menschen Tiere* **270**, 399.

Manuscript received 24 September 1975