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The control of fatty acid metabolism in liver cells from fed and starved sheep

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1. Isolated liver cells prepared from starved sheep converted palmitate into ketone bodies at twice the rate seen with cells from fed animals. Carnitine stimulated palmitate oxidation only in liver cells from fed sheep, and completely abolished the difference between fed and starved animals in palmitate oxidation. 2. The rates of palmitate oxidation to CO_2 and of octanoate oxidation to ketone bodies and CO_2 were not affected by starvation or carnitine. 3. Neither starvation nor carnitine altered the ratio of 3-hydroxybutyrate to acetoacetate or the rate of esterification of [1-^{14}C]palmitate. 4. Propionate, lactate, pyruvate and fructose inhibited ketogenesis from palmitate in cells from fed sheep. Starvation or the addition of carnitine decreased the antiketogenic effectiveness of gluconeogenic precursors. Propionate was the most potent inhibitor of ketogenesis, 0.8mM producing 50% inhibition. 5. Propionate, lactate, fructose and glycerol increased palmitate esterification under all conditions examined. 6. Lactate, pyruvate and fructose stimulated oxidation of palmitate and octanoate to CO_2. 7. Starvation and the addition of gluconeogenic precursors stimulated apparent palmitate utilization by cells. 8. Propionate, lactate and pyruvate decreased cellular long-chain acylcarnitine concentrations. Propionate decreased cell contents of CoA and acyl-CoA. 9. It is suggested that propionate may control hepatic ketogenesis by acting at some point in the β-oxidation sequence. 10. The results are discussed in relation to the differences in the regulation of hepatic fatty acid metabolism between sheep and rats.

Studies on the control of hepatic ketogenesis have indicated two major sites of regulation, (i) the partition of long-chain fatty acyl-CoA between esterification and the formation of acylcarnitine esters for transport into the mitochondrion, and (ii) the disposal of intramitochondrial acetyl-CoA between synthesis of ketone bodies and oxidation in the tricarboxylic acid cycle (Mayes & Laker, 1981). In rat liver, the rates of fatty acid oxidation and ketogenesis are thought to be controlled by the activity of carnitine acyltransferase I (EC 2.3.1.21; McGarry & Foster, 1980*a*). This enzyme is inhibited by malonyl-CoA (a key intermediate in the synthesis of fatty acids *de novo*); McGarry & Foster (1980*a*) have proposed that this compound plays a central role in the regulation of hepatic fat and carbohydrate metabolism by ensuring an inverse relationship between the rates of fatty acid synthesis and oxidation. Thus the increased rate of hepatic fatty acid oxidation, observed during either starvation or glucagon treatment, is accompanied by a decrease in the tissue content of malonyl-CoA, caused by an inhibition of both glycolysis and acetyl-CoA carboxylase (EC 6.4.1.2; McGarry & Foster, 1979).

In ruminant liver the role of malonyl-CoA in the regulation of hepatic fatty acid metabolism has not been investigated. Intrahepatic regulatory mechanisms must, however, exist in these species, because the stimulation of ketogenesis by starvation in sheep is accompanied by an increase in the proportion of fatty acid oxidized rather than esterified (Katz & Bergman, 1969). Baird (1981) has proposed that hyperketonaemia in cows may be associated with signs of hepatic carbohydrate insufficiency, as characterized by decreases in the concentrations of glycogen and gluconeogenic intermediates. An imbalance between the rates of gluconeogenesis and precursor supply might result in lowered oxaloacetate availability, thereby causing a fall in citrate synthesis and hence a diversion of acetyl-CoA to ketone-body synthesis. These data are consistent

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with observations that the most severe ketotic states occur during periods of increased gluconeogenesis, and that the administration of glucose and propionate has a marked antiketogenic action (Baird, 1981). However, the precise details of the control of ruminant ketogenesis remain undefined.

The present study examines the effects of feeding and starvation and of gluconeogenic precursors on the disposition of fatty acid in sheep liver. The importance of control at loci before β-oxidation proper is assessed by comparison of the rates of oxidation of palmitate and octanoate; the latter is not esterified and is oxidized by reactions independent of carnitine acyltransferase I (Fritz, 1961).

Materials and methods

Animals

Sheep were 1 year old castrated males and were either pasture fed (during the summer period) or fed on a hay diet with barley concentrate (during winter).

Chemicals and enzymes

Enzymes and biochemicals were purchased from Boehringer Corp. (London), Lewes, Sussex, U.K., with the following exceptions. Glucose oxidase (type II), sodium palmitate and sodium octanoate were from Sigma, and peroxidase (RZ 0.6) was from International Enzymes, Windsor, Berks., U.K. 2-Oxoglutarate dehydrogenase (EC 1.2.4.2) was a gift from Dr. R. M. Denton, University of Bristol; L-carnitine was a gift from Dr. G. D. Baird, Institute for Research into Animal Diseases, Compton, Berks., U.K. [1-^{14}C]Palmitate, [1-^{14}C]octanoate and L-[^{3}H]carnitine were purchased from Amersham International, Amersham, Bucks., U.K. Bovine serum albumin (fraction V: Miles Laboratories, Slough, Berks., U.K.) was freed of fatty acids and other ligands by the method of Chen (1967).

All other chemicals were of the purest grade available from standard suppliers.

Preparation and incubation of cells

Isolated liver cells were prepared from sheep by perfusion of the caudate lobe with buffer (Krebs & Henseleit, 1932) containing 2.3mM-$CaCl_2$ and 0.04% (w/v) collagenase after an initial 5min perfusion with Ca^{2+}-free buffer containing 0.5mM-EGTA (Donaldson *et al.*, 1979). Liver cells were dispersed and washed in buffer containing Ca^{2+} and 2% (w/v) albumin. Metabolic integrity was assessed by measurement of cellular ATP content (Donaldson *et al.*, 1979).

Cells (5–6mg dry wt./vial), in a final volume of 2ml of buffer containing 2% (w/v) albumin, were incubated for 60 or 90min with shaking (100cycles/min) at 37°C in stoppered silicone-treated glass scintillation vials as previously described (Smith *et al.*, 1978). All incubations were performed in triplicate; reactions were stopped by addition of 0.2ml of 2M-$HClO_4$. They were then centrifuged (2000*g* for 5min), to remove denatured protein, and the supernatants were neutralized with 2M-KOH containing 0.5M-triethanolamine hydrochloride.

In experiments with [1-^{14}C]palmitate, the substrate was bound to albumin (Garland & Randle, 1964) and was present throughout the incubation period. Oxidation of 1-^{14}C-labelled fatty acids to $^{14}CO_2$ was measured by using 2ml incubations in 10ml conical flasks (Kean & Pogson, 1979). Parallel incubations were performed to measure the rate of incorporation of [1-^{14}C]palmitate into esterified lipid. Incubations were stopped by separating cells from medium (Whitelaw & Williamson, 1977); lipid was extracted from the cells as described by Folch *et al.* (1957). A portion of the lower phase was dried down and the radioactivity measured. The radioactivity incorporated at 0min of incubation was subtracted from that at other time points to allow for [1-^{14}C]palmitate present in cells or in medium carried down with the cell pellet. The radioactivity at 0min was routinely approx. 35% of the total incorporated into the Folch lower phase after 60min.

The oxidation of 1-^{14}C-labelled fatty acids to ^{14}C-labelled ketone bodies was measured by decarboxylation of acetoacetate with aniline citrate (Krebs & Eggleston, 1945) and collection of $^{14}CO_2$ in 2-phenethylamine/methanol (1:1, v/v). The acetoacetate carbon radioactivity was multiplied by 2 to obtain total acetoacetate radioactivity because carboxyl and carbonyl carbon atoms are equally labelled under these conditions (Weinhouse *et al.*, 1950; Chaikoff *et al.*, 1951). Assuming that acetoacetate and D(−)-3-hydroxybutyrate are in isotopic equilibrium, the radioactivity of ketone bodies was calculated from the radioactivity in acetoacetate and the ratio of the concentrations of acetoacetate and hydroxybutyrate in the medium. This assumption is justified because the rate of incorporation of [1-^{14}C]palmitate into acid-soluble products was similar to that of incorporation into total ketone bodies as calculated above (results not shown). The rate of metabolism of 1-^{14}C-labelled fatty acid to ^{14}C-labelled oxidation products was calculated by the addition of the rates of formation of $^{14}CO_2$ and ^{14}C-labelled ketone bodies.

Ketone bodies were determined enzymically (Mellanby & Williamson, 1974; Williamson & Mellanby, 1974). Intracellular metabolites were determined in incubations in 25ml conical flasks (15–20mg dry wt. of cells, in final volume of 4.0ml). Cells were separated from medium (Hems *et al.*, 1975) after 60min. Metabolic rates were linear with time up to at least this point; it was therefore considered that intracellular metabolites would be at steady state at

this point. The cell-derived supernatants were neutralized and 2-mercaptoethanol was added to a final concentration of 1 mM. CoA, acetyl-CoA and short-chain acyl-CoA esters were assayed fluorimetrically (Kean & Pogson, 1979). Long-chain acyl-CoA was measured as acid-soluble CoA after alkaline hydrolysis (Williamson & Corkey, 1969). Long-chain acylcarnitine was measured as butanol-extractable radioactivity in incubations with unlabelled palmitate (1 mM) and L-[*methyl*-^{3}H]carnitine (1 mM) (Christiansen *et al.*, 1976).

Metabolic activity is expressed as the amount of product accumulated between either 0 and 60 min or 30 and 90 min. The rates of fatty acid metabolism were linear under all conditions except in incubations of palmitate with glycerol or pyruvate, where ketogenesis became non-linear after 60 min.

Results and discussion

There are many points of difference, both qualitative and quantitative, between sheep and rats in respect of hepatic ketogenesis and its regulation. Although starvation leads to increased rates of ketone-body production in both species, these rates are, in absolute terms, much lower in the sheep (Table 1). Thus the value of 88.5 ± 3.9 nmol/h per mg for ketogenesis from 1 mM-palmitate alone in cells from starved sheep compares with values of 300–350 nmol/h per mg for rat cells under similar circumstances (Whitelaw & Williamson, 1977; Kean & Pogson, 1979). These lower rates in the sheep are in accord with reports from studies *in vivo* (Katz & Bergman, 1969).

Carnitine is lost from rat liver cells during isolation procedures (Christiansen & Bremer, 1976); a similar loss may be expected in sheep cells. Such loss might be significant because it has been shown that fatty acid oxidation by sheep liver mitochondria is especially sensitive to the absence of carnitine (Koundakjian & Snoswell, 1970). With sheep cells, addition of carnitine stimulated ketogenesis in fed but not in starved sheep (Table 1). The lack of response in the starved state is perhaps attributable to the high hepatic carnitine content in starved sheep (Snoswell & Henderson, 1970); losses during cell preparation may leave ample carnitine to ensure maximal rates.

The similarity in ketogenic rates of cells, from both fed and starved sheep, supplemented with carnitine, was striking (Table 1), and contrasts with observations in the rat; in this latter species, ketogenesis by cells from fed animals never reaches the rate found with cells from starved individuals,

Table 1. *Effects of starvation and of gluconeogenic precursors on the net release of ketone bodies and the ratio of 3-hydroxybutyrate to acetoacetate in isolated sheep liver cells*

Methods and materials were as given in the text. Sheep were starved for 4 days. Results are means ± S.E.M.; the numbers of independent observations are given in parentheses. Significances between means (presence of gluconeogenic precursor versus control) were assessed by Student's *t*-test: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Substrate (all final concn. 1 mM)	Additions (final concn. 10 mM)	Net release of ketone bodies (nmol/h per mg dry wt.)		[Hydroxybutyrate]/[acetoacetate]	
		Fed	Starved	Fed	Starved
Palmitate	None	37.9 ± 4.5 (14)	88.5 ± 3.9 (7)	0.35 ± 0.05 (14)	0.27 ± 0.07 (7)
	Propionate	7.0 ± 2.1*** (7)	29.1 ± 5.0*** (4)	0.74 ± 0.16* (7)	1.56 ± 0.23** (4)
	Lactate	12.4 ± 4.4** (4)	76.2 ± 5.5 (3)	1.40 ± 0.31** (4)	1.44 ± 0.14* (3)
	Glycerol	24.8 ± 3.0 (4)	87.6 ± 7.1 (3)	0.51 ± 0.15 (4)	0.58 ± 0.20 (3)
	Fructose	14.6 ± 3.2** (5)	86.9 ± 6.1 (3)	0.93 ± 0.25* (5)	0.61 ± 0.07* (3)
	Pyruvate	14.6 ± 3.4** (6)	93.0 ± 16.6 (4)	0.73 ± 0.14** (6)	0.87 ± 0.06*** (4)
Palmitate plus carnitine	None	81.4 ± 7.4 (14)	96.6 ± 6.1 (7)	0.37 ± 0.05 (4)	0.27 ± 0.07 (7)
	Propionate	43.7 ± 8.9** (6)	73.8 ± 10.4 (3)	1.73 ± 0.21*** (6)	1.20 ± 0.30*** (3)
	Lactate	46.2 ± 6.6* (4)	81.1 ± 11.2 (3)	2.02 ± 0.44** (4)	1.26 ± 0.15*** (3)
	Glycerol	57.6 ± 8.0 (4)	89.8 ± 5.1 (3)	0.44 ± 0.10 (4)	0.43 ± 0.10 (3)
	Fructose	45.3 ± 5.2** (5)	88.0 ± 8.3 (3)	0.80 ± 0.20 (5)	0.58 ± 0.10* (3)
	Pyruvate	21.7 ± 8.1*** (5)	111.8 ± 18.0 (3)	1.17 ± 0.09** (5)	1.05 ± 0.14*** (3)
Octanoate	None	73.1 ± 9.1 (5)	86.2 ± 4.5 (4)	0.59 ± 0.07 (5)	0.41 ± 0.04 (4)
	Propionate	15.3 ± 4.5** (4)	28.0 ± 9.6** (3)	1.05 ± 0.22* (4)	1.86 ± 0.32 (3)
	Lactate	74.3 ± 2.7 (3)	64.5 ± 7.4* (3)	1.76 ± 0.10** (3)	1.53 ± 0.32 (3)
	Glycerol	56.8 ± 6.8 (4)	59.2 ± 8.7 (3)	0.67 ± 0.09 (4)	0.66 ± 0.02 (3)
	Fructose	83.1 ± 26.6 (3)	82.5 ± 6.8 (3)	0.55 ± 0.08 (3)	0.55 ± 0.15 (3)
	Pyruvate	27.2 ± 12.3* (3)	47.7 ± 20.8 (4)	1.16 ± 0.21* (3)	1.27 ± 0.21 (4)
Octanoate plus carnitine	None	75.3 ± 13.9 (4)	95.2 ± 3.9 (3)	0.65 ± 0.04 (4)	0.79 ± 0.25 (3)

whatever the carnitine availability (Christiansen, 1977). This difference between fed and starved states has been interpreted in terms of the control of carnitine acyltransferase I by malonyl-CoA (McGarry *et al.*, 1975*b*; McGarry & Foster, 1980*a*; Sugden & Williamson, 1981).

Ketogenesis from octanoate is similar in cells from both fed and starved sheep. Because this fatty acid is oxidized by carnitine-independent reactions (a view supported by the failure of carnitine to enhance octanoate oxidation; Table 1), it would appear that the regulation of fatty acid oxidation during the fed–starved transition is likely to be mediated by effects surrounding the carnitine-dependent uptake of fatty acids across the mitochondrial inner membrane, rather than by effects on β-oxidation itself. Certainly the observation by Varnam *et al.* (1978) that starvation increases the capacity of sheep liver mitochondria to form acetoacetyl-CoA from acetyl-CoA does not seem significant in the context of the whole cell. The known changes in carnitine in the liver during this transition (Snoswell & Koundakjian, 1972) suggest that this compound is a possible regulator. The present study is consistent with such a hypothesis, although the problems associated with loss of carnitine during cell isolation make interpretation difficult.

Glucagon stimulates ketogenesis in rat liver *in vivo* (McGarry *et al.*, 1975*b*), *in vitro* (McGarry *et al.*, 1975*a*) and in isolated cell incubations (Christiansen, 1977). This stimulation has been attributed to the decrease in the cellular content of malonyl-CoA (McGarry & Foster, 1980*a*; Sugden & Williamson, 1981) brought about by the cyclic AMP-dependent phosphorylation, leading to inactivation, of acetyl-CoA carboxylase (Witters *et al.*, 1979; Geelen & Beynen, 1981), the first enzyme of fatty acid synthesis proper.

Addition of glucagon to incubations of cells from fed sheep with palmitate alone, or with palmitate plus carnitine, did not alter the rates of ketogenesis, $^{14}CO_2$ production or esterification (Pogson *et al.*, 1983). This does not arise from any inability of these cells to respond to glucagon, because the hormone elicits increases in intracellular cyclic AMP concentrations (Donaldson *et al.*, 1979). In addition, dibutyryl cyclic AMP, at concentrations effective with rat cells, has as little effect as glucagon on fatty acid metabolism (Lomax *et al.*, 1983; Pogson *et al.*, 1983).

The results of the present study generate questions about the role of malonyl-CoA as a regulatory molecule in sheep liver. In the rat, the concentration of malonyl-CoA is proportional to the rate of fatty acid synthesis (McGarry & Foster, 1980*b*). If the same relationship holds for the sheep, where fatty acid synthesis is poorly expressed (Ballard *et al.*, 1969), then malonyl-CoA is unlikely to be of great significance. Nevertheless, although measurements of malonyl-CoA have not been made in sheep liver, carnitine acyltransferase I from this source exhibits sensitivity to this compound (V. A. Zammit, personal communication).

The ratio of hydroxybutyrate to acetoacetate in incubations is clearly lower for sheep cells than for rat cells (Table 1; Ontko, 1972; Kean & Pogson, 1979). This ratio is increased in the presence of lactate, propionate and pyruvate, but is not affected by starvation, carnitine or the substitution of octanoate for palmitate. The extent to which these ratios should be interpreted in terms of parallel changes in intramitochondrial redox state remains unresolved. The activity of hydroxybutyrate dehydrogenase in ruminant liver is apparently much lower than that in rat liver (Baird *et al.*, 1968; Koundakjian & Snoswell, 1970), so that thermodynamic equilibrium between the ketone bodies may not be achieved in sheep cells, although ratios of more than 1.0 suggest a not insignificant role for the enzyme. Interpretation is further complicated by involvement of the differential ketone-body transport systems in the mitochondrial inner membrane (Siess *et al.*, 1982) and the possible presence of cytosolic (Nielsen & Fleischer, 1969; Koundakjian & Snoswell, 1970) and/or particulate dehydrogenase activities (B. Leighton & C. I. Pogson, unpublished work).

Table 2 demonstrates that starvation is without effect on the production of $^{14}CO_2$ from labelled palmitate and octanoate; carnitine is similarly ineffective. Esterification rates are equally unaffected by starvation and availability of carnitine (Table 3). This contrasts with the situation in rat liver, where the transition from feeding to starvation is accompanied by a switch from esterification to β-oxidation (Mayes & Felts, 1967; Ontko, 1972), and where carnitine decreases absolute rates of esterification (Christiansen *et al.*, 1976). The maintenance of esterification rates in starved sheep is observed *in vivo*, where appreciable accumulation of triacylglycerol occurs (Patterson, 1966).

When the incorporations of label from [1-^{14}C]-palmitate into ketone bodies, CO_2 and esterified products are summed (Table 3), it is apparent that palmitate utilization is increased in starvation. In the fed state, carnitine increases palmitate uptake to the values for starved animals, entirely by stimulating ketogenesis. Propionate, lactate, glycerol and fructose induce similar increases in uptake, but this is in these cases associated with greater esterification (Table 3). In the rat the transition from the fed to the starved state does not alter fatty acid utilization, but redirects flux from esterification to oxidation. This contrast suggests that different regulatory mechanisms may operate in sheep liver.

Table 2. *Effects of starvation and of gluconeogenic precursors on the metabolism of 1-^{14}C-labelled fatty acids to $^{14}CO_2$ and total oxidation products*

Procedures were as given in the text. Sheep were starved for 4 days. Results are means ± S.E.M.; the numbers of independent observations are given in parentheses. Significances between means (presence of gluconeogenic precursors versus control) were assessed by Student's *t*-test: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Substrate (all final concn. 1 mM)	Additions (final concn. 10 mM)	1-^{14}C-labelled fatty acid metabolized to $^{14}CO_2$ (nmol/h per mg dry wt.)		1-^{14}C-labelled fatty acid metabolized to total ^{14}C-labelled oxidation products (nmol/h per mg dry wt.)	
		Fed	Starved	Fed	Starved
Palmitate	None	3.6 ± 0.3 (12)	3.2 ± 0.7 (7)	16.0 ± 1.4 (8)	28.6 ± 7.1 (5)
	Propionate	3.8 ± 0.5 (7)	4.5 ± 0.5 (4)	4.4 ± 0.5*** (6)	10.6 ± 1.9* (4)
	Lactate	7.5 ± 1.4** (5)	6.8 ± 1.3** (4)		
	Glycerol	4.2 ± 0.9 (5)	4.3 ± 0.7 (4)		
	Fructose	8.4 ± 0.6*** (6)	7.3 ± 1.0** (4)		
	Pyruvate	7.2 ± 0.9*** (7)	10.0 ± 1.9** (5)		
Palmitate plus carnitine	None	4.3 ± 0.5 (12)	3.0 ± 0.5 (8)	38.9 ± 4.4 (8)	31.8 ± 6.1 (5)
	Propionate	8.5 ± 1.8** (6)	4.4 ± 0.4 (3)	18.5 ± 2.1** (6)	16.8 ± 2.3* (4)
	Lactate	8.0 ± 0.4*** (5)	7.2 ± 1.1** (5)		
	Glycerol	4.6 ± 0.7 (5)	3.9 ± 0.6 (5)		
	Fructose	8.4 ± 0.7*** (6)	6.7 ± 0.4*** (5)		
	Pyruvate	11.6 ± 0.9*** (7)	7.0 ± 0.1*** (5)		
Octanoate	None	6.8 ± 0.7 (5)	5.4 ± 0.8 (4)		
	Propionate	10.7 ± 0.7** (5)	10.9 ± 2.2 (4)		
	Lactate	9.9 ± 0.5** (4)	11.8 ± 1.2 (4)		
	Glycerol	7.1 ± 0.9 (5)	5.8 ± 1.3 (4)		
	Fructose	15.1 ± 2.8** (4)	13.3 ± 1.9** (4)		
	Pyruvate	19.0 ± 3.5** (3)	22.8 ± 2.8*** (4)		

Table 3. *Effects of starvation and of gluconeogenic precursors on palmitate esterification and utilization by isolated sheep liver cells*

Methods and materials were as given in the text. Sheep were starved for 4 days. Values for palmitate utilization are the sums of the incorporation into ketone bodies and CO_2 (Table 2) and esterification products. Other results are means ± S.E.M.; the numbers of independent observations are given in parentheses. Significances between means (presence of gluconeogenic precursors versus control) were assessed by Student's *t*-test: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Substrate (final concn. 1 mM)	Additions (final concn. 10 mM)	Rate of esterification (nmol/h per mg dry wt.)		Palmitate utilization (nmol/h per mg dry wt.)	
		Fed	Starved	Fed	Starved
Palmitate	None	20.6 ± 2.2 (7)	23.8 ± 3.1 (6)	36.6	52.4
	Propionate	46.3 ± 1.3*** (4)	53.8 ± 9.9** (3)	50.7	64.4
	Lactate	49.9 ± 5.9*** (4)	70.1 ± 8.5*** (3)	60.0	87.4
	Glycerol	42.2 ± 6.9** (4)	60.6 ± 8.7** (3)	57.2	85.9
	Fructose	30.4 ± 3.6* (4)	47.6 ± 4.5** (3)	42.2	80.5
	Pyruvate	19.7 ± 2.5 (4)	35.7 ± 8.3 (3)	28.7	50.0
Palmitate plus carnitine	None	17.6 ± 2.4 (7)	22.2 ± 2.6 (7)	56.5	54.0
	Propionate	49.4 ± 4.3*** (3)	51.0 ± 10.8** (3)	67.9	67.8
	Lactate	47.2 ± 5.8*** (4)	67.0 ± 4.7*** (4)	91.0	91.0
	Glycerol	35.4 ± 7.9* (4)	56.0 ± 6.1*** (4)	78.8	85.5
	Fructose	31.7 ± 3.0** (3)	48.7 ± 3.8*** (4)	70.5	92.1
	Pyruvate	32.5 ± 11.7 (3)	29.3 ± 1.9* (3)	59.4	51.6

In sheep cell incubations, the most effective agent in determining fatty acid disposition was propionate (Tables 1–3). Propionate decreases ketogenesis very markedly, particularly in cells from fed animals and in the absence of carnitine (Table 1), but does not affect oxidation of [1-^{14}C]palmitate to $^{14}CO_2$ (except in cells from fed sheep supplemented with carnitine). The rates of oxidation of palmitate to ketone bodies plus CO_2 were decreased by propionate in both fed and starved sheep (Table 2). The antiketogenic

action of propionate cannot therefore be explained solely on the basis of a redistribution of acetyl-CoA.

Propionate more than doubled esterification of palmitate and promoted a relative redistribution of fatty acid between the oxidation and esterification pathways. In the absence of both propionate and carnitine, therefore, approx. 56% of palmitate in cells from fed sheep was esterified (and approx. 44% was oxidized). With propionate only added, approx. 91% was esterified. With carnitine added, the percentage esterification was approx. 32% in the absence, and approx. 73% in the presence, of propionate. The conversion of octanoate into ketone bodies is also inhibited by propionate (Table 1). Because octanoate is not esterified, changes in the rate of esterification itself cannot account for the antiketogenic effects of propionate. Further, lactate, fructose and glycerol also stimulated esterification of palmitate (Table 3), and there was no clear relationship between this stimulation and the effects of these compounds on ketone-body synthesis. Again, pyruvate, which is antiketogenic, does not affect esterification.

The mechanisms whereby gluconeogenic precursors affect fatty acid disposition have not been investigated. One possibility is that their ability to act as precursors of L-glycerol 3-phosphate is important. One might expect that pyruvate, by biasing the equilibrium of the dehydrogenase towards dihydroxyacetone phosphate, would not stimulate esterification in this way.

Lactate, fructose and pyruvate (all gluconeogenic precursors in sheep liver cells; Ash & Pogson, 1977) all decreased ketogenesis in cells from fed, but not from starved, sheep; lactate and fructose were ineffective when octanoate replaced palmitate. The antiketogenic action of these compounds was attributable to increased oxidation to CO_2 (Table 2) rather than to decreased entry into the β-oxidation sequence. These experiments provide little evidence for a mechanism, but are not inconsistent with the view that mitochondrial oxaloacetate plays a limiting role (Nosadini *et al.*, 1980; Baird, 1981; Siess *et al.*, 1982).

McGarry & Foster (1979) and Christiansen (1979) have demonstrated a positive correlation between the tissue content of long-chain acylcarnitine and the rate of fatty acid oxidation. This correlation was interpreted as supporting the role of carnitine acyltransferase I as the key regulatory point in the control of liver fatty acid oxidation. Table 4 shows the changes in long-chain acylcarnitine in sheep cells incubated with palmitate, carnitine and separate gluconeogenic precursors. Propionate, lactate and pyruvate all produce significant falls in cellular long-chain acylcarnitine, but these changes do not clearly correlate with the corresponding rates of fatty acid oxidation or esterification. The changes in long-chain acylcarnitine may be occasioned rather through the appreciable activities of the carnitine acyltransferase in ruminant liver (Koundakjian & Snoswell, 1970).

Table 4. *Effect of gluconeogenic precursors on long-chain acylcarnitine concentrations in isolated liver cells from fed sheep*

Methods and materials were as given in the text. Measurements were made after 60 min incubation. Results are means ± S.E.M. from three independent observations. Significances between means (presence of gluconeogenic precursor versus control) were assessed by Student's *t*-test: $*P < 0.05$; $**P < 0.01$.

Substrate (final concn. 1 mM)	Additions (final concn. 10 mM)	Long-chain acylcarnitine content (nmol/mg dry wt.)
Palmitate	None	0.29 ± 0.03
Palmitate plus carnitine	None	0.42 ± 0.04
	Propionate	0.13 ± 0.01**
	Lactate	0.24 ± 0.02*
	Glycerol	0.36 ± 0.04
	Fructose	0.30 ± 0.03
	Pyruvate	0.18 ± 0.02*

Of the agents tested, propionate is known to be the most effective gluconeogenic precursor (Lindsay, 1978), and, as an end product of ruminal fermentation, to occupy a key role in ruminant metabolism (Bergman, 1973; Baird, 1981). Because 10 mM is considerably above physiological concentrations, we investigated the effects of lower concentrations of propionate; the results are shown in Fig. 1. Ketogenesis from palmitate was inhibited by 50% at 0.8 mM-propionate in cells from both fed and starved sheep. The concentration of propionate in portal blood is in the range 0.2–0.4 mM in normal fed ruminants (Thompson *et al.*, 1975); it is therefore not unreasonable to suggest that the antiketogenic actions noted above are of physiological significance.

Propionate is the sole precursor which affects both the disposition of acetyl-CoA and the rate of β-oxidation itself. The inhibition of β-oxidation also occurs with octanoate as substrate, suggesting that the locus of control by propionate may be at an intramitochondrial reaction in the β-oxidation sequence [although not, as suggested by Bush & Milligan (1971), at the reaction catalysed by 3-hydroxy-3-methylglutaryl-CoA synthase (EC 4.1.3.5)]. Such a hypothesis is in accord with the location of the enzymes, and presumably the intermediates, of propionate metabolism in the mitochondrial matrix. One possibility is that propionate competes effectively with other fatty acids for intramitochondrial CoA. Table 5 shows the changes in total cellular CoA and acyl-CoA under various conditions. Propionate is associated with

Table 5. *Effects of fatty acid, L-carnitine and propionate on CoA and acyl-CoA contents in isolated liver cells from fed sheep*

Methods and materials were as given in the text. Measurements of intracellular metabolite contents were made after 60min incubation. Results are means ± S.E.M.; the numbers of independent observations are given in parentheses. Significances between means (addition of propionate or carnitine versus corresponding control) were assessed by Student's *t*-test: $*P < 0.05$.

Substrate (final concn. 1 mM)	Addition (final concn. 10 mM)	Cell content (nmol/mg dry wt.) CoA	Acyl-CoA	Short-chain acyl-CoA	Acid-soluble acyl-CoA	Acid-insoluble acyl-CoA
None	None	0.41 ± 0.12 (5)	0.38 ± 0.10 (5)	0.04 ± 0.04 (4)	0.84 ± 0.08 (4)	0.25 ± 0.03 (4)
	Propionate	0.27 ± 0.08 (5)	0.08 ± 0.03* (5)	0.25 ± 0.10 (4)	0.58 ± 0.10 (4)	0.17 ± 0.04 (4)
Palmitate	None	0.29 ± 0.04 (4)	0.60 ± 0.12 (4)	0.01 ± 0.01 (3)	0.93 ± 0.15 (3)	0.44 ± 0.07 (4)
	Propionate	0.16 ± 0.03* (4)	0.13 ± 0.02* (4)	0.10 ± 0.06 (3)	0.41 ± 0.06* (3)	0.41 ± 0.07 (4)
Palmitate plus carnitine	None	0.13 ± 0.02* (4)	0.51 ± 0.05 (4)	0.01 ± 0.01 (3)	0.66 ± 0.08 (8)	0.44 ± 0.06 (4)
	Propionate	0.12 ± 0.03 (4)	0.21 ± 0.05* (4)	0.15 ± 0.08 (3)	0.46 ± 0.05 (3)	0.32 ± 0.04 (4)
Octanoate	None	0.24 ± 0.04 (4)	0.58 ± 0.09 (4)	0.15 ± 0.01 (3)	1.03 ± 0.03 (3)	0.36 ± 0.15 (3)
	Propionate	0.18 ± 0.07 (4)	0.18 ± 0.04* (4)	0.33 ± 0.08 (3)	0.73 ± 0.06* (3)	0.24 ± 0.09 (3)

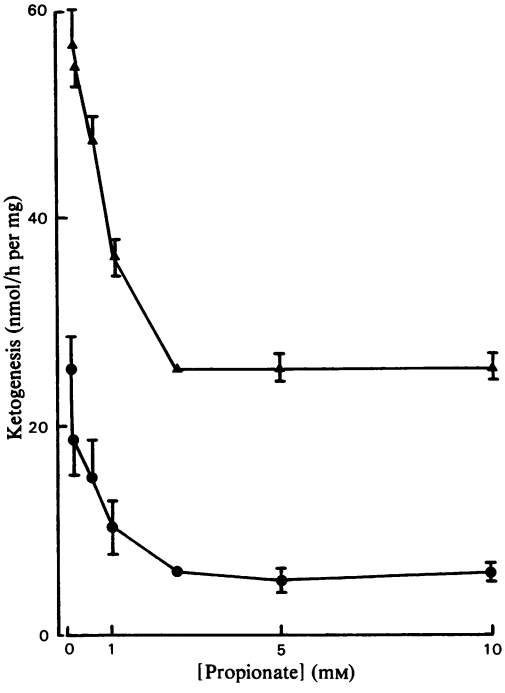

Fig. 1. *Effect of propionate concentration on ketogenesis in sheep liver cells*

The conditions for incubation and assay were as given in the text. Results are means ± S.E.M. for three independent observations: ●, fed sheep; ▲, 4-day-starved sheep.

falls in both free CoA and acetyl-CoA. Carnitine itself, however, decreases free CoA as much in cell incubations without decreasing β-oxidation. Again, propionate does not significantly decrease CoA concentrations below those found with octanoate alone. Although a 'CoA-limitation' hypothesis might still be sustained by arguments based on differential effects on the separate coenzyme pools in the cytosol and the mitochondria, it is not perhaps the most attractive explanation.

In view of the greater significance of propionate in the ruminant, a propionate metabolite may be a more plausible candidate as a regulator (Fishlock *et al.*, 1982). One such is methylmalonyl-CoA (Wahle *et al.*, 1983*a*,*b*). Accumulation of this intermediate is consistent with the changes in CoA distribution in the presence of propionate (Table 5).

The stimulation of ketogenesis by starvation in the sheep *in vivo* (Katz & Bergman, 1969) may be understood in terms of decreased availability of the antiketogenic propionate (which is derived exclusively from ruminal fermentation), although the very large increases in sheep hepatic carnitine content under ketogenic conditions suggest that carnitine may be a further predisposing factor (Snoswell & Henderson, 1970).

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