Biochem. J. (1980) **186**, 287–294
Printed in Great Britain

Transacylation as a Chain-Termination Mechanism in Fatty Acid Synthesis by Mammalian Fatty Acid Synthetase

SYNTHESIS OF BUTYRATE AND HEXANOATE BY LACTATING COW MAMMARY GLAND FATTY ACID SYNTHETASE

Jan Krogh HANSEN and Jens KNUDSEN
Institute of Biochemistry, Odense University, Campusvej 55, *DK*-5230 *Odense M, Denmark*

(*Received* 18 *June* 1979)

1. Purified cow mammary gland fatty acid synthetase synthesized long-chain unesterified and short-chain esterified fatty acids. 2. A direct relationship was observed between the amount of short-chain products synthesized and the concentration of acetyl-CoA in the incubation medium. 3. The short-chain products were identified as butyryl-CoA and hexanoyl-CoA. 4. Inhibition of the terminating thioester hydrolase of the fatty acid synthetase complex with phenylmethanesulphonyl fluoride did not inhibit the synthesis of short-chain products. 5. It is suggested that the synthesis of short-chain fatty acids involves the reverse of the 'loading' reaction.

The products of fatty acid synthesis by avian and by mammalian fatty acid synthetases are generally accepted to be long-chain ($C_{14:0}$ to $C_{18:0}$) saturated fatty acids. However, fatty acid synthetases from lactating mammary glands of cow, rabbit and guinea pig and from rat liver synthesize butyric acid and hexanoic acid in addition to long-chain fatty acids (Carey & Dils, 1970*a*,*b*; Hansen *et al.*, 1970; Knudsen, 1972; Strong & Dils, 1972).

A proportion of the short-chain acids synthesized by fatty acid synthetase from lactating rabbit mammary gland and from rat liver are released into the incubation medium as esterified fatty acids (Carey & Dils, 1970*b*; Hansen *et al.*, 1970). The authors suggested that these products are acyl-CoA esters.

The terminating thioester hydrolase moiety of lactating rabbit mammary gland synthetase only shows significant activity towards long-chain acyl-CoA esters (Knudsen *et al.*, 1975). The very low activity of this hydrolase towards short-chain acyl-CoA esters together with the synthesis of short-chain esterified fatty acids by these fatty acid synthetases suggest that the mechanism of synthesis of short-chain fatty acids is different from that of long-chain fatty acids.

In the present investigation the composition and nature of the products formed by lactating cow mammary gland fatty acid synthetase have been determined. The role of the terminating thioester hydrolase in the synthesis of short-chain fatty acids has been investigated, and a possible mechanism for chain termination leading to the synthesis of these acids is discussed.

Materials and Methods

Materials

Lactating red Danish dairy cows, 6–7 months *post partum*, were used. Dithiothreitol and NADPH were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A., and CoA was from Boehringer, Mannheim, Germany. [1-^{14}C]Acetic anhydride and [1-^{14}C]butyric acid were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Sephadex G-10 was from Pharmacia Fine Chemicals A.B., Uppsala, Sweden, and cellulose powder MN 300 was from Macherey, Nagel and Co., 516 Düren, Germany.

Methods

Fatty acid synthetase from lactating cow mammary gland was purified as described by Knudsen (1972). Fatty acid synthetase from lactating cow adipose tissue was purified by this method, except that the adipose tissue was homogenized at room temperature in 2 vol. of 100 mM-potassium phosphate buffer, pH 7.0, 250 mM-sucrose, 4.0 mM-EDTA and 1.0 mM-dithiothreitol in a Waring blender at full speed for 30 s. Acetyl-CoA and [1-^{14}C]-acetyl-CoA were prepared from acetic anhydride as described by Stadtman (1957). [1-^{14}C]Butyryl-CoA was synthesized as described by Marshall &

0306-3283/80/010287-08 $1.50/1

Knudsen (1977). Malonyl-CoA was synthesized by the method of Eggerer & Lynen (1962).

Incubations

(*a*) *Incubation conditions for fatty acid synthetase.* Fatty acid synthetase was incubated at 37°C in 200mM-potassium phosphate buffer (pH 6.7)/1mM-EDTA/240μM-NADPH/60μM-malonyl-CoA and [1-^{14}C]acetyl-CoA in the volume indicated. The reaction was stopped by adding 30μl of 18M-H_2SO_4 per ml of incubation medium. Unesterified fatty acids were extracted with 2vol. of diethyl ether. The extraction was repeated three times and the extracts were pooled. The recoveries of $C_{4:0}$ and $C_{16:0}$ fatty acids using this extraction procedure were 94$\pm$4% and 97$\pm$2% (mean$\pm$S.D. for 12 determinations) respectively. The acidified incubation mixture obtained after diethyl ether extraction was made 2.5M with respect to NaOH and hydrolysed for 2h at 80°C. The amount and the composition of the fatty acids in the pooled diethyl ether extracts and in the alkaline aqueous phase were determined as described by Knudsen (1976).

(*b*) *Incubation conditions for fatty acid synthetase inhibited with phenylmethanesulphonyl fluoride.* Fatty acid synthetase (specific activity 443nmol of NADPH oxidized/min per mg of protein) was treated with phenylmethanesulphonyl fluoride as described by Grunnet & Knudsen (1978). Synthetase incubated under similar conditions without phenylmethanesulphonyl fluoride was used as a control. Both treated and control-treated synthetases were incubated as described above or were incubated with rate-limiting concentrations of malonyl-CoA, which were added by the slow infusion of malonyl-CoA (1–3μl/min) throughout the incubation period. A Harvard infusion pump (model 971) fitted with eight 1ml disposable syringes was used. To ensure efficient mixing of the infused malonyl-CoA with the incubation medium, the infusion tubes were attached to the bottom of the incubation vessel which was shaken with a rotatory motion by using an Evapomix (Buckler Instruments, Fort Lee, NJ, U.S.A.) at shaker speed 6. The reaction was stopped by adding on equal volume of 5M-NaOH and the mixture was analysed for total fatty acids (Knudsen, 1976).

Isolation of short-chain esterified fatty acids synthesized by fatty acid synthetase

(*a*) *Separation of the acidified reaction mixture by gel filtration.* The acidified aqueous phase obtained by using incubation conditions (*a*) was centrifuged at 1200$g_{av.}$ for 5min to remove precipitated protein and then applied to a column (2.5cm$\times$60cm) of Sephadex G-10 equilibrated with water. The column was eluted with water (25ml/h) and the eluate was collected in fractions of 4.6ml. The absorption of the eluate at 254nm was measured continuously, and the conductivity and radioactivity in each fraction were determined. Fractions containing short-chain esterified fatty acids were pooled and freeze-dried.

(*b*) *Separation of short-chain acyl-CoA esters by preparative cellulose t.l.c.* The freeze-dried fractions from the Sephadex G-10 column were dissolved in a small volume of water and analysed by preparative cellulose t.l.c. The plates (20cm$\times$20cm) were coated with MN300 cellulose (0.75mm) and developed in n-butanol/water/acetic acid (5:3:2) as described by Pullman (1973). The developed plates were scanned for radioactivity by using a Berthold LB 2722 thin-layer scanner. [1-^{14}C]Acetyl-CoA was identified by running an authentic standard. The band corresponding to [1-^{14}C]acetyl-CoA partly overlapped a faster moving band that contained the short-chain esterified products. The latter band was scraped off and separated again by preparative cellulose t.l.c. as described above. The band containing the short-chain esterified products from the second preparative t.l.c. was scraped off, eluted with water, freeze-dried and dissolved in a small volume of water.

Identification of short-chain esterified fatty acids synthesized by fatty acid synthetase

(*a*) *Analysis by cellulose t.l.c.* The product isolated by preparative cellulose t.l.c. (see above) was then separated by analytical t.l.c. on cellulose plates (DC-Alufolien Cellulose AH 5552; Merck, Darmstadt, Germany). [1-^{14}C]Acetyl-CoA and [1-^{14}C]butyryl-CoA were used as standards. The plates were developed in n-butanol/water/acetic acid (5:3:2) and then scanned for absorption at 254nm and for radioactivity.

(*b*) *Analysis by radio-g.l.c.* The product isolated by preparative cellulose t.l.c. was hydrolysed in 2.5M-NaOH for 2h at 80°C and was then analysed for total fatty acids as described by Knudsen (1976).

Synthesis of triacylglycerols that contain short-chain fatty acids

The short-chain esterified fatty acids formed by fatty acid synthetase and isolated by preparative cellulose t.l.c. were used as substrates for the microsomal 1,2-diacylglycerol acyltransferase (EC 2.3.1.20) of lactating cow mammary gland with the assay described by Marshall & Knudsen (1977). The incubation (0.3ml) contained 0.63mg of microsomal protein, 64.5nM-dipalmitoyl[2-^{3}H]glycerol (sp. radioactivity 0.74nCi/nmol) and either 5μM-[1-^{14}C]butyryl-CoA (sp. radioactivity 3.95μCi/μmol) or ^{14}C-labelled short-chain esterified fatty acids (5μM) produced by fatty acid synthetase. The concentration of the latter was calculated from the specific radioactivity of the [1-^{14}C]acetyl-CoA used in the incubation of fatty acid synthetase.

Results

Synthesis of unesterified and esterified fatty acids by fatty acid synthetase from lactating cow mammary gland

Cow mammary gland synthetase was found to synthesize both long-chain unesterified fatty acids and short-chain esterified fatty acids (Table 1). With the incubation conditions used, almost all of the short-chain fatty acids were esterified and all of the long-chain products were unesterified fatty acids. These proportions were unaffected by the incubation time (Table 1, *a*) or by the wide range of concentrations of acetyl-CoA used (Table 1, *b*).

By contrast, these results indicate that the amount of esterified fatty acids synthesized depended on the time of incubation and on the concentration of [1-^{14}C]acetyl-CoA used. These incubations were done more than three times with essentially similar results. Fig. 1 shows that the amount of short-chain esterified fatty acids synthesized increased with time until a constant amount had been formed, which depended on the acetyl-CoA concentration. There was a direct relationship between the amount of short-chain product synthesized and the concentration of [1-^{14}C]acetyl-CoA in the incubation medium. A 4-fold increase in the latter from 9μM to 36μM produced a similar increase in the amount of short-chain esterified fatty acids synthesized.

Similar results as those described above were obtained with fatty acid synthetase obtained from lactating cow adipose tissue (results not shown).

Control incubations without added malonyl-CoA were used in all these experiments. No fatty acid synthesis was observed in the absence of malonyl-CoA, showing that the synthesis of both long- and short-chain fatty acids is dependent on malonyl-CoA.

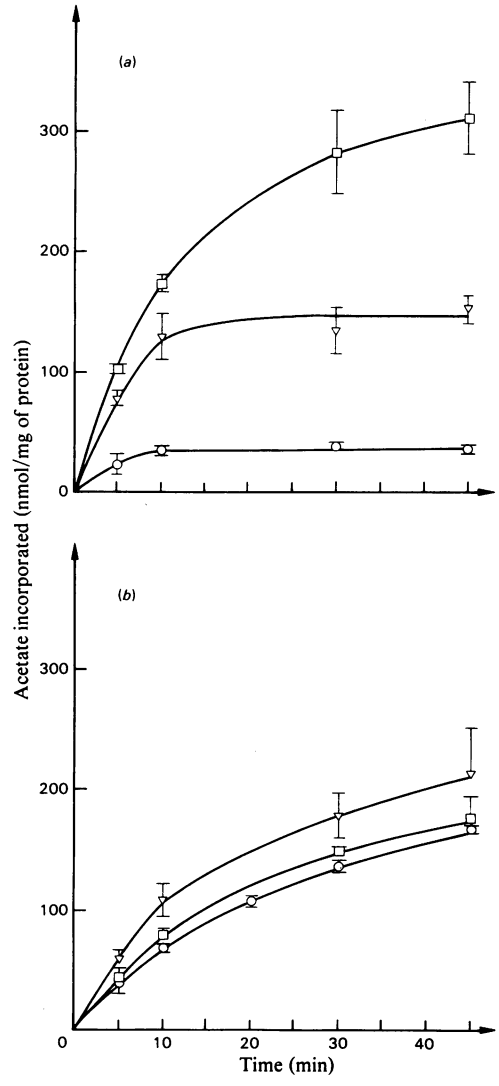

Fig. 1. *Synthesis of esterified (a) and unesterified fatty acids (b) by fatty acid synthetase from cow mammary gland*

Fatty acid synthetase (18.5μg; specific activity 1060nmol of NADPH oxidized/min per mg of protein) was incubated for the times indicated in a total volume of 1.0ml as described in the Materials and Methods section (incubation conditions *a*). The concentrations of [1-^{14}C]acetyl-CoA (sp. radioactivity 2.8μCi/μmol) used were 9.2μM (O), 36.8μM (△) and 73.6μM (□) respectively. All incubations were in duplicate. The values for acetate incorporation are means ± S.D. for two, three and two independent experiments using 9.2, 36.8 and 73.6μM of [1-^{14}C]acetyl-CoA respectively.

Isolation and identification of the esterified product(s) synthesized by fatty acid synthetase from cow mammary gland

Isolation. The results of a typical analysis by gel filtration of the products formed by fatty acid synthetase from cow mammary gland are shown in Fig. 2 (the incubation conditions used are described in the legend to Fig. 2). There were two radioactive peaks: one coincided with the peak absorbing at 254nm and the other was shown to contain unesterified fatty acids. Preparative cellulose t.l.c. analysis of the freeze-dried pooled fractions that absorbed at 254nm showed that the majority of the radioactivity was due to [1-^{14}C]acetyl-CoA. The faster-moving band, which contained the esterified product of the synthetase, was almost completely free from [1-^{14}C]acetyl-CoA after the second separation by preparative cellulose t.l.c.

In a similar incubation system the recovery of radioactive esterified fatty acids after the second preparative cellulose t.l.c. was 50–60% of that

Table 1. *Effect of incubation time (a) and of acetyl-CoA concentration (b) on the synthesis of esterified and unesterified fatty acids by lactating cow mammary gland fatty acid synthetase*

Incubations that were carried out as described in the Materials and Methods section contained fatty acid synthetase (18.5μg; specific activity 1060nmol of NADPH oxidized/min per mg of protein) and [1-^{14}C]acetyl-CoA (sp. radioactivity 3.8μCi/μmol) as indicated in a total volume of 1.0ml. In (*a*) the incubation time varied as indicated. In (*b*) the incubation time was 30min. Values for the percentage distribution of radioactivity in the fatty acids are mean values for duplicate incubations. The values for the total nmol of acetate incorporated are means ± half the difference between duplicate incubations.

	Acetyl-CoA concn. (μM)	Incubation time (min)	Product	Percentage distribution of radioactivity in fatty acids (mol/100mol) $C_{4:0}$	$C_{6:0}$	$C_{8:0}$	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	Total acetate incorporated from [1-^{14}C]acetyl-CoA (nmol)
(*a*)	36.0	5	Esterified	100								1.30 ± 0.10
			Unesterified							100		1.20 ± 0.09
	36.0	10	Esterified	98						2		2.03 ± 0.10
			Unesterified	5					8	82	5	2.17 ± 0.01
	36.0	30	Esterified	92	8							2.54 ± 0.09
			Unesterified	3					10	82	5	3.62 ± 0.06
	36.0	45	Esterified	93	7							2.68 ± 0.10
			Unesterified	3					15	78	4	4.40 ± 0.02
(*b*)	9.2	30	Esterified	88	12							0.67 ± 0.01
			Unesterified						11	89		2.46 ± 0.01
	36.8	30	Esterified	92	8							2.54 ± 0.09
			Unesterified	2					10	83	5	3.06 ± 0.06
	73.6	30	Esterified	85	13	2						4.71 ± 0.01
			Unesterified						13	87		2.73 ± 0.07

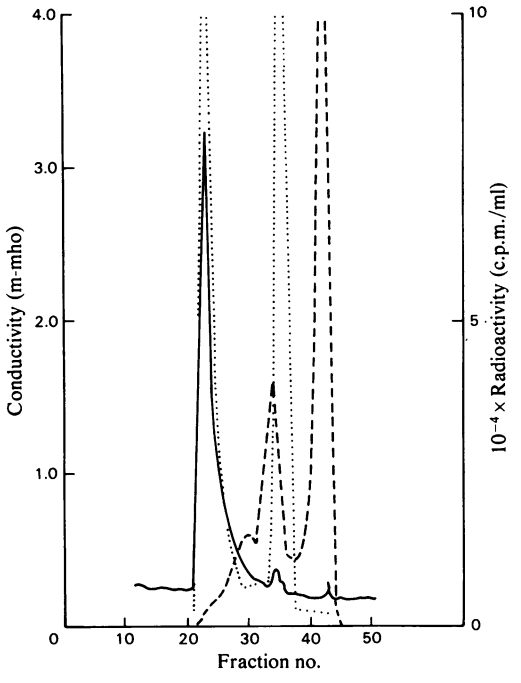

Fig. 2. *Sephadex G-10 gel filtration of the products of fatty acid synthesis*

Fatty acid synthetase (92μg; specific activity 1060nmol of NADPH oxidized/min per mg of protein) in a total volume of 4.0ml was incubated at 37°C for 30min as described in the Materials and Methods section (incubation conditions *a*), except that the reaction was stopped by adding 20μl of 11.7M-$HClO_4$/ml instead of 30μl of 18M-H_2SO_4/ml. ——, A_{254}; ····, radioactivity; ----, conductivity.

recovered in the material that was not extractable by diethyl ether.

Identification. The isolated esterified product from cow mammary gland fatty acid synthetase co-chromatographed with butyryl-CoA on analytical cellulose t.l.c. In addition, radio-g.l.c. analysis of the isolated esterified product showed that the acyl chain corresponded to butyric acid plus a small proportion of hexanoic acid. The isolated product also contained traces of radioactivity corresponding to acetate.

Synthesis of triacylglycerols

The isolated esterified fatty acid product(s) of fatty acid synthetase was also found to act as a substrate for cow mammary gland microsomal diacylglycerol acyltransferase. The rate of incorporation was 0.033 ± 0.0012nmol/min per mg of microsomal protein (mean ± S.D. for three independent experiments), compared with a value of 0.153 ± 0.0 (mean ± S.D. for two independent experiments) when butyryl-CoA was used. In both cases, the product was identified as triacylglycerol that contained short-chain fatty acids.

The results presented indicate strongly that the esterified short-chain products synthesized by cow mammary gland fatty acid synthetase are butyryl-CoA and hexanoyl-CoA. However, the possibility that part of the products could be other C_4-CoA derivatives like crotonyl-CoA, β-hydroxybutyryl-CoA and acetoacetyl-CoA cannot be excluded.

Fatty acid synthesis by fatty acid synthetase inhibited by phenylmethanesulphonyl fluoride

Phenylmethanesulphonyl fluoride specifically inhibits the terminating acyl-thioester hydrolase moiety of the fatty acid synthetase complex (Kumar, 1975). When cow mammary gland fatty acid synthetase was assayed spectrophotometrically with optimum substrate concentrations, phenylmethanesulphonyl fluoride completely inhibited the enzyme. A similar result was obtained under identical conditions when the fatty acid synthetase activity was measured by the incorporation of [1-^{14}C]acetyl-CoA into fatty acids (Table 2).

By contrast, the addition to the inhibited synthetase of rate-limiting concentrations of malonyl-CoA by slow infusion resulted in the synthesis of butyrate. The control-treated synthetase under these conditions synthesized both short- and long-chain fatty acids (Table 2). When malonyl-CoA was omitted from the incubation, neither the inhibited nor the control-treated synthetase synthesized fatty acids (Table 2). These results further indicate that the synthesis of both short- and long-chain fatty acids are dependent on malonyl-CoA and that the mechanism involved in the termination of fatty acid synthesis leading to the formation of short-chain acids is different from that involved in the synthesis of long-chain fatty acids.

K_m values of cow mammary gland fatty acid synthetase for acetyl-CoA, butyryl-CoA and hexanoyl-CoA

Acetyl-CoA, butyryl-CoA and hexanoyl-CoA were all found to be excellent substrates for fatty acid synthetase from cow mammary gland. Butyryl-CoA was the preferred substrate [$K_m = 6.4 \pm 0.12\mu$M (mean ± S.D.)], since the K_m values for acetyl-CoA and for hexanoyl-CoA were 11.9 ± 0.17 and $10.8 \pm 0.24\mu$M respectively (means ± S.D. for duplicate determinations). The K_m values for acetyl-CoA and for butyryl-CoA are similar to those published by Maitra & Kumar (1974) i.e. 12.5 and 6.65μM respectively.

Table 2. *Effect of phenylmethanesulphonyl fluoride on the composition of fatty acids synthesized by fatty acid synthetase from cow mammary gland in the absence and presence of different concentrations of malonyl-CoA*

In experiments (*a*)–(*c*) and (*e*)–(*g*) 275 μg of control-treated synthetase or phenylmethanesulphonyl fluoride-treated synthetase were incubated at 37°C for 15 min as described in the Materials and Methods section (incubation system *b*) in a total volume of 0.5 ml. The incubation contained 42 μM-[1-^{14}C]acetyl-CoA (sp. radioactivity 3.8 μCi/μmol) and malonyl-CoA was infused as indicated. In experiments (*d*) and (*h*), 15 μg of control-treated synthetase or phenylmethanesulphonyl fluoride-treated synthetase were incubated as described above except that 60 μM-malonyl-CoA was added at the start of the incubation. Incubations were stopped by adding an equal volume of 5 M-NaOH. Total fatty acid synthesis and the radioactivity in individual fatty acids were measured as described in the Materials and Methods section. Values for the total incorporation of acetate are means ± half the difference between duplicate incubations. Values for the percentage distribution of radioactivity are means for duplicate incubations. The results presented have been reproduced in two independent experiments.

	Expt.	Mode of addition of malonyl-CoA	Percentage distribution of radioactivity in fatty acids (mol/100 mol)								Total acetate incorporated from [1-^{14}C]acetyl-CoA (nmol)
			$C_{4:0}$	$C_{6:0}$	$C_{8:0}$	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	
Control-treated fatty acid synthetase	(*a*)	0									0
	(*b*)	Infused at 0.5 nmol/min	40	17	7	8	12	16			1.43 ± 0.09
	(*c*)	Infused at 2.5 nmol/min	14	3	2	0	2	55	24		2.19 ± 0.09
	(*d*)	60 μM-Malonyl-CoA added at start of incubation	60	3					37		3.64 ± 0.21
Fatty acid synthetase treated with phenylmethanesulphonyl fluoride	(*e*)	0									0
	(*f*)	Infused at 0.5 nmol/min	100								0.76 ± 0
	(*g*)	Infused at 2.5 nmol/min	100								0.67 ± 0.10
	(*h*)	60 μM-Malonyl-CoA added at start of incubation									0

Discussion

Butyrate and hexanoate synthesized by cow mammary gland fatty acid synthetase are released almost entirely as esterified acids, whereas long-chain fatty acids are released as the unesterified acids (Table 1). The behaviour of the purified esterified products on analytical cellulose t.l.c. and the results of radio-g.l.c. analysis indicate that the products are butyryl-CoA and hexanoyl-CoA. This is further supported by our findings that the esterified products could be used as substrates for microsomal diacylglycerol acyltransferase. These results are different from those published for lactating rabbit mammary gland and rat liver fatty acid synthetases, where various amounts of both short- and long-chain products were reported to be released as esterified fatty acids (Carey & Dils, 1970*b*; Hansen *et al.*, 1970).

There is a direct relationship between the maximum synthesis of short-chain fatty acids and the concentration of acetyl-CoA used. This can be explained by the fact that acetyl-CoA, butyryl-CoA and hexanoyl-CoA are all excellent substrates for fatty acid synthetase. Hence butyryl-CoA and hexanoyl-CoA only accumulate to a limited concentration in the incubation medium, since they effectively compete with acetyl-CoA for the 'loading' transacylase of the synthetase.

There was a complete lack of fatty acid synthesis in the absence of malonyl-CoA. This shows that cow mammary gland and adipose tissue synthetases cannot synthesize fatty acids from acetyl-CoA alone. These results agree with those reported for lactating goat mammary gland by Grunnet & Knudsen (1979*a*,*b*). The present results also agree with those of DeKay *et al.* (1976) who found that more than 95% of fatty acid synthesis in cow mammary gland cytosol was malonyl-CoA-dependent. The synthesis by fatty acid synthetase of butyrate from acetyl-CoA alone reported by Carey & Dils (1970*a*,*b*), Hansen *et al.* (1970) and Knudsen (1972) is most probably due to inability of the radio-g.l.c. method available to these authors to separate completely [^{14}C]acetate from other radioactive short-chain fatty acids. The method used in the present experiments quantitatively separates acetate from butyrate and from hexanoate.

The terminating hydrolase of cow mammary gland synthetase shows a very low hydrolytic activity towards butyryl- and hexanoyl-CoA compared with palmitoyl-CoA (Grunnet & Knudsen, 1979*b*). Furthermore, when this synthetase was treated with phenylmethanesulphonyl fluoride it was still able to synthesize butyrate even though the terminating thioester hydrolase of the synthetase was inhibited. It is therefore very unlikely that the terminating hydrolase is involved in the synthesis of short-chain fatty acids. Fatty acid synthetase that had been inhibited by phenylmethanesulphonyl fluoride was unable to synthesize fatty acids at high malonyl-CoA concentrations. This is most probably due to saturation of the synthetase by long-chain acyl groups, which cannot be released from the synthetase.

The function of the transacylase in terminating short-chain fatty acid synthesis cannot be identified from these experiments. However, we suggest that the reaction involves the reverse of the 'loading' reaction. The low K_m value (6.4 μM) for butyryl-CoA shows that the 'loading' transacylase has a high affinity for butyrate. If this reaction is reversible it would involve the transfer of butyrate from the 4-phosphopantetheine moiety of synthetase to CoA after one round of the chain-elongation reactions of the synthetase complex. This reaction is also very likely to occur from an energetic point of view, since in both compounds butyrate is esterified to 4-phosphopantetheine. The high affinity of the 'loading' enzyme for butyrate compared with hexanoate (K_m 10.8 μM) probably explains why butyrate is the predominant product of this chain-terminating pathway. However, the chain-length specificity of the transacylase function of the synthetase, which transfers the growing acyl chain from the central acyl-carrier protein thiol group to the peripheral cysteine thiol group may also be involved.

We thank Professor R. Dils, University of Reading, U.K., for critical reading and correction of the manuscript and Miss Annelise V. Andersen for excellent technical assistance. We also thank Dr. M. O. Marshall for carrying out the experiments with lipid synthesis from esterified fatty acid synthetase products and the Wellcome Trust of Great Britain for a travel grant to Jens Knudsen.

References

Carey, E. M. & Dils, R. (1970*a*) *Biochim. Biophys. Acta* **210**, 371–387

Carey, E. M. & Dils, R. (1970*b*) *Biochim. Biophys. Acta* **210**, 388–399

DeKay, D. E., Bauman, D. E. & Davis, C. L. (1976) *J. Dairy Sci.* **59**, 1513–1517

Eggerer, H. & Lynen, F. (1962) *Biochem. Z.* **335**, 540–547

Grunnet, I. & Knudsen, J. (1978) *Biochem. Biophys. Res. Commun.* **80**, 745–749

Grunnet, I. & Knudsen, J. (1979*a*) *Eur. J. Biochem.*, in the press

Grunnet, I. & Knudsen, J. (1979*b*) *Eur. J. Biochem.*, in the press

Hansen, H. J. M., Carey, E. M. & Dils, R. (1970) *Biochim. Biophys. Acta* **210**, 400–410

Knudsen, J. (1972) *Biochim. Biophys. Acta* **280**, 408–414

Knudsen, J. (1976) *Comp. Biochem. Physiol.* **53B**, 3–7
Knudsen, J., Clark, S. & Dils, R. (1975) *Biochem. Biophys. Res. Commun.* **65**, 921–926
Kumar, S. (1975) *J. Biol. Chem.* **250**, 5150–5158
Maitra, S. K. & Kumar, S. (1974) *J. Biol. Chem.* **249**, 111–117
Marshall, M. O. & Knudsen, J. (1977) *Eur. J. Biochem.* **81**, 259–266
Pullman, M. E. (1973) *Anal. Biochem.* **54**, 188–198
Stadtman, E. R. (1957) *Methods Enzymol.* **3**, 931–941
Strong, C. R. & Dils, R. (1972) *Int. J. Biochem.* **3**, 369–377