Regulation of *de novo* Fatty Acid Biosynthesis in the *n*-Alkane-utilizing Yeast, *Candida* 107

By C. O. GILL AND C. RATLEDGE

Department of Biochemistry, The University of Hull,
Kingston upon Hull, HU6 7RX

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SUMMARY

Growth of *Candida* 107 on *n*-alkanes (C₁₀, C₁₆ or a mixture) completely repressed formation of acetyl-CoA carboxylase and partially repressed the fatty acid synthetase complex. As all fatty acids must then be derived directly from the substrate no matter what its chain length, the yeast must be able to elongate even-chain acids (C₁₀ to C₁₄) and modify, by unknown reactions, odd-chain acids to give even-chain acids. Short-term control of fatty acid biosynthesis appears to be by long-chain (C₁₆ or C₁₈) fatty acyl-CoA esters feedback-inhibiting the activities of both acetyl-CoA carboxylase and fatty acid synthetase. *n*-Alkanes, *n*-alcohol, free fatty acids or C₁₂ and C₁₄ acyl-CoA esters, had little or no effects on these enzymes. Extracts from *n*-alkane-grown yeast inhibited the carboxylase in extracts from glucose-grown yeast, the pattern of inhibition being similar to that observed with hexadecyl-CoA.

INTRODUCTION

De novo fatty acid biosynthesis in yeast is catalysed by two soluble enzyme systems: acetyl-CoA carboxylase (EC. 6.4.1.2), equation 1, and the multienzyme complex known as the fatty acid synthetase (Sumper & Lynen, 1972), equation 2

Acetyl-CoA carboxylase is subject to allosteric regulation; it is activated by citrate and Mg²⁺ and inhibited by long-chain acyl-CoA thioesters in liver or adipose tissues (Vagelos, 1971; Guynn, Veloso & Veech, 1972) and in crude extracts of *Saccharomyces cerevisiae* (White & Klein, 1965, 1966; Rasmussen & Klein, 1968), though Matsushashi (1969) has reported that the yeast enzyme is not so affected. This enzyme is repressed in *Lactobacillus plantarum* when grown in the presence of unsaturated fatty acids (Henderson & McNeil, 1966, 1967; Birnbaum, 1970; Weeks & Wakil, 1970) and partially repressed in starved rats (Majerus & Kilburn, 1969) when lipolysis is presumably stimulated, but there have been no reports of its repression in yeasts (Hunter & Rose, 1971).

The fatty acid synthetase complex in animals is stimulated by certain hexose phosphates (Wakil, Goldman, Williamson & Toomey, 1966) and is inhibited by hexadecyl-CoA (Tubbs & Garland, 1964; Dorsey & Porter, 1968) which also inhibits the yeast enzyme (Lust & Lynen, 1968). Partial repression of the enzyme complex occurs in starved animals (Butterworth *et al.* 1966; Dahlen, Kennan & Porter, 1968; Burton, Collins, Kennan & Porter, 1969). Changes in the level of the related enzyme system have been reported in *Lactobacillus plantarum* (Birnbaum, 1970; Weeks & Wakil, 1970) but not in yeasts.

Addition of fatty acids, as such, to the medium can inhibit yeast growth (Bell, 1971, 1973; Gill & Ratledge, 1972) although small amounts can be used by *Saccharomyces cerevisiae* (Suomalainen & Keränen, 1968). A more suitable system for the study of control of *de novo* synthesis of fatty acids could therefore be to use alkane-utilizing micro-organisms. In these organisms, fatty acids are directly derived from alkanes (Davis, 1964; Mizuno *et al.* 1966; see also Ratledge, 1970) without inhibition of growth. These fatty acids, in yeasts, may then be metabolized in one or more of three ways depending upon their chain length: (i) from C₁₃ to C₁₈ direct incorporation into lipids can occur; (ii) from C₉ to C₁₅ (and maybe higher) elongation of the chain is indicated to occur; and (iii) degradation to acetate occurs to some extent with all alkanes (Trust & Millis, 1970; Chenouda & Jwanny, 1972; Hornei, Köhler & Weide, 1972; Thorpe & Ratledge, 1972; Hug & Fiechter, 1973). In *Candida* 107, which can oxidize *n*-alkanes to fatty acids even when growing on glucose (Ratledge, 1968; Gill & Ratledge, 1973*a*), one might therefore expect the system for *de novo* fatty acid synthesis to be regulated by repression of enzyme synthesis or feedback inhibition or both. We have now investigated such regulation; a preliminary communication of part of this work has already been made (Gill & Ratledge, 1973*b*).

METHODS

Growth of the organism. *Candida* 107 was grown in shake-culture on a glucose+yeast extract+salts medium (Gill & Ratledge, 1972); after 18 h all glucose had been consumed. To starve the yeast, incubation was continued for a further 24 h. To adapt the yeast to complete alkane utilization, 20 ml of the appropriate sterile alkane was added to each litre of culture after glucose exhaustion and then incubation continued for a further 24 h. Alternatively, the yeast was grown in continuous culture at a dilution rate of 0.1 h⁻¹ on 5% (v/v) *n*-alkanes (C₁₃ to C₁₆ fraction) in a 10-l fermenter (3-l working vol.). The basal salts and vitamins of the medium were as described by Ratledge (1968) except that (NH₄)₂SO₄ was at 2.5 g/l and Span 81 was added to 0.1% (v/v).

Preparation of cell-free extracts and assay of enzymes. *Candida* 107 was harvested by centrifuging for 5 min at 6000 g, washed twice with 0.1 M-phosphate buffer, pH 7.0, and then, as a slurry in fresh buffer, disrupted by passage through a French press. The material was centrifuged for 15 min at 10000 g to remove whole yeast and debris and the supernatant solution re-centrifuged at 15000 g for 30 min to remove mitochondria. The final supernatant was filtered with suction through Whatman fibre-glass paper (grade A) to remove solidified fat. The protein concentration, determined by the method of Lowry, Rosebrough, Farr & Randall (1951), was adjusted for all cell-free extracts to 10 mg/ml with 0.1 M-phosphate buffer. As the effect of some inhibitors, principally long-chain acyl-CoA thioesters, can be reversed by their binding to protein (Numa, Bortz & Lynen, 1965; Marquis, Francesconi & Villee, 1968), a constant protein concentration was maintained to validate comparisons of inhibitory effects between different enzyme preparations.

Acetyl-CoA carboxylase (acetyl-CoA:carbon dioxide ligase (ADP) EC. 6.4.1.2) was assayed using a method based on that described by Matsushashi (1969) but omitting EDTA and including 2 mM-Mn²⁺. The final volume was 1.0 ml, the reaction being started by adding 0.1 ml of cell-free extract. The enzyme preparation and the assay mixture were held at 30 °C for 10 min before mixing. [2-¹⁴C]Acetic acid (2 μmol; 1 μCi) with CoA were sometimes replaced in the assay by [2-¹⁴C]acetyl-CoA (1 μmol; 1 μCi). Effectors of the enzyme were added to the assay mixture before adding the enzyme. The reaction was stopped after 15 min at 30 °C by adding 0.5 ml 5 M-KOH, containing 1 μmol hexadecanoic acid to act as carrier. Hexadecanoic acid was recovered by solvent extraction and, after appropriate washing, its

radioactivity was counted by liquid scintillation. The activity of the enzyme in all preparations used was linear with respect to time of incubation for up to 20 min, and with respect to protein concentration was linear up to 1.5 mg protein/ml of assay mixture in the presence of 75 mM-citrate.

Fatty acid synthetase was assayed according to Lynen (1969), except that bovine serum albumin was omitted. Fatty acids formed from [2-¹⁴C]malonyl-CoA were assayed by the same method used in determination of acetyl-CoA carboxylase activity.

Preparation of CoA-esters. [2-¹⁴C]Acetyl-CoA was prepared from the labelled anhydride according to Simon & Shemin (1953); [2-¹⁴C]malonyl-CoA was prepared from the free acid according to Trams & Brady (1960) and long-chain acyl-CoA esters were prepared from the corresponding acyl chlorides by the method of Seubert (1960).

Chemicals used. All materials used in enzyme assays were obtained from Sigma Chemical Co. Ltd, London. Radiochemicals were obtained from the Radiochemical Centre, Amersham, Buckinghamshire. *n*-Decane, *n*-hexadecane and *n*-decanol, each at 99% purity, were from Koch-Light Laboratories Ltd, Colnbrook, Buckinghamshire. The fraction of *n*-alkanes used for cultivation of the yeast contained 2.3% C₁₃, 55.7% C₁₄, 35.3% C₁₅ and 6.6% C₁₆ *n*-alkanes and was a gift from Texaco Ltd, London.

RESULTS

Glucose-grown Candida 107

Activation and inhibition of fatty acid synthetase complex. The fatty acid synthetase complex, as isolated from pigeon liver, is activated maximally by fructose 1,6-diphosphate but only poorly by inorganic phosphate (Wakil *et al.* 1966). The enzyme from *Candida* 107, however, showed only poor stimulation of activity by fructose 1,6-diphosphate alone and appeared to have an absolute requirement for inorganic phosphate for maximum stimulation (Fig. 1). Subsequent assays of the fatty acid synthetase complex were therefore always performed in 100 mM-phosphate buffer, without fructose 1,6-diphosphate. Fructose 1,6-diphosphate may have exerted its slight activation in tris buffer through hydrolysis by an appropriate phosphatase, to yield some inorganic phosphate in these assays.

In *Saccharomyces cerevisiae*, the fatty acid synthetase complex is inhibited by long-chain acyl-CoA esters (Lust & Lynen, 1968). In *Candida* 107, however, low concentrations of hexadecyl-CoA appeared to stimulate activity of fatty acid synthetase complex, perhaps by helping to disperse the protein of the extract more thoroughly, and only when the ester exceeded 100 μ M was inhibition observed (Fig. 2).

Activation and inhibition of acetyl-CoA carboxylase. The assay of acetyl-CoA carboxylase relies upon excess activity of the fatty acid synthetase complex within the crude extract; this was always present when assaying acetyl-CoA carboxylase in 100 mM-phosphate buffer (see Table 3) and therefore these assays were valid. When studying hexadecyl-CoA as an effector on acetyl-CoA carboxylase activity (see below), care was again taken to ensure that the activity of the fatty acid synthetase, though inhibited, was still above that of the carboxylase.

Citrate was found markedly to stimulate activity of acetyl-CoA carboxylase (Fig. 3). The response to stimulation by citrate, however, depended upon the state of *Candida* 107 from which the extract was obtained. If exponentially growing *Candida* 107 was used, a plot of enzyme activity against citrate concentration was sigmoidal (Fig. 3), but if the extract was prepared from yeast which had been starved for 24 h, the curve became hyperbolic though total activity was now decreased by about 65%. One explanation for this is that during the starvation period enzyme inhibitors, as well as the enzyme itself, were degraded within the

Fig. 1

Fig. 2

Fig. 1. Activation of the fatty acid synthetase complex in extracts from glucose-grown *Candida* 107, starved for 24 h before harvesting, by fructose 1,6-diphosphate in the presence of either 0.1 M-phosphate buffer (pH 7.0) (○), or 0.1 M-tris buffer (●).

Fig. 2. Effect of palmityl-CoA on the fatty acid synthetase complex in extracts from glucose-grown *Candida* 107 starved for 24 h before harvesting.

yeast. Alternative explanations are however possible, as crude extract preparations were used in this work.

The effect of *n*-alkanes and their oxidation products (alcohol, free acid and acyl-CoA) on acetyl-CoA carboxylase activity was subsequently examined both in the presence and absence of citrate (Table 1). *n*-Alkanes and hexadecanoic acid, up to 300 μM, had no effect. *n*-Decanol, however, stimulated activity both in the presence and absence of citrate, possibly by a similar mechanism to that previously noted for the stimulation of fatty acid synthetase activity by low concentrations of hexadecyl-CoA (see Fig. 2). Although 300 μM-hexadecyl-CoA was always strongly inhibitory, hexadecyl-CoA at 100 and 200 μM, and decanoic acid at 300 μM, strongly inhibited acetyl-CoA carboxylase activity only when assayed in the presence of citrate. The nature of the inhibitions brought about by these latter substances was therefore examined further.

Hexadecyl-CoA, at 100 and 200 μM, did not inhibit the basal activity of the enzyme but prevented the activation of the enzyme by citrate. There was a slow, linear increase in enzyme activity with increasing citrate concentration until, with the lower concentration of hexadecyl-CoA, a critical point was reached after which the increase in activity with increasing citrate concentration became extremely rapid (Fig. 4*a*). Presumably a similar pattern would have been observed with the higher concentration of hexadecyl-CoA if the concentration of citrate had been taken beyond 100 mM.

Decanoic acid produced a different pattern of inhibition to that brought about by hexadecyl-CoA, in that low concentrations of citrate continued to stimulate enzyme activity without any effect by added decanoic acid (Fig. 4*b*). Inhibition was only seen when activation by citrate had doubled the initial activity of the enzyme. Full activation was still attainable nevertheless by continuing to increase the citrate concentration. Dodecanoic acid had no effect on the enzyme activity at any concentration of citrate and is therefore, at 100 μM, equivalent in this respect to 300 μM-hexadecanoic acid.

Fig. 3. Citrate activation of acetyl-CoA carboxylase in extracts from glucose-grown *Candida* 107, (○) harvested at the end of exponential growth, or (■) starved for 24 h after the end of growth.

Table 1. Action of possible effectors on activity of acetyl-CoA carboxylase from glucose-grown *Candida* 107 harvested at the end of exponential growth

Effector*	Concn. (μ M)	Enzymatic activity (nmol acetate converted/h/mg protein)			
		Without citrate	Relative activity (%)	With citrate (75 mM)	Relative activity (%)
None	—	5.2	100	31.6	100
Hexadecyl-CoA	100	5.6	108	31.6	100
	200	5.6	108	9.2	29
	300	0	0	1.8	6
Hexadecanoic acid	300	5.8	112	30.4	96
Decanoic acid	300	6.2	119	17.0	54
Decanol	500	7.2	138	54.6	171
<i>n</i> -Alkanes†	300	4.5	87	32.0	101

* Water-insoluble materials were added from a 5% (v/v) emulsion in water.

† A fraction of *n*-alkanes (C_{13} to C_{16}) used.

Inhibition of enzyme activity by long-chain acyl-CoA esters was not confined to hexadecyl-CoA but was dependent upon the chain length of the acyl moiety (Table 2). This is similar to previous observations made with acetyl-CoA carboxylase isolated from rat liver (Bortz & Lynen, 1963). Dodecyl- and tetradecyl-derivatives slightly stimulated enzyme activity at concentrations that were inhibitory when the hexadecyl- and octadecyl-esters were used (Table 2).

Alkane-grown *Candida* 107

Repression of fatty acid biosynthesis. Extracts of *Candida* 107 grown on *n*-alkanes, or adapted to alkane utilization after growth on glucose, had activities of the fatty acid synthetase complex which were repressed to about half the activity observed in glucose-grown *Candida* 107. Even more striking was that the activity of the acetyl-CoA carboxylase had become almost completely repressed by exposure to alkanes (Table 3). The failure to detect

Fig. 4. Effect of long-chain acyl compounds on the activation by citrate of acetyl-CoA carboxylase in extracts from glucose-grown *Candida* 107. (a) Effect of hexadecyl-CoA at 100 μ M (●), and 200 μ M (□); control (no addition), (○). (Yeast starved before harvesting.) (b) Effect of dodecanoic acid at 100 μ M (●) and decanoic acid at 100 μ M (□); control (no addition), (○). (Yeast harvested at end of exponential growth.)

Table 2. Effect of long-chain acyl-CoA esters on activity of acetyl-CoA carboxylase from glucose-grown *Candida* 107 harvested at the end of exponential growth

Acyl group of acyl-CoA	Concn. (μ M)	Enzymatic activity (nmol acetate converted/h/mg protein)	Relative activity (%)
None	—	28.4	100
Dodecyl-	100	34.4	121
	200	31.8	112
Tetradecyl-	100	30.2	107
	200	28.2	99
Hexadecyl-	100	28.6	101
	200	9.6	34
Octadecyl-	100	16.0	56
	200	7.2	25

* 50 mM-citrate included in all assays.

significant activity of this latter enzyme was not due to inability of these extracts to synthesize acetyl-CoA, for when acetyl-CoA was used in the assay system, no increase in the activity of the carboxylase was recorded (Table 3). The slight residual activity was independent of the presence of citrate (up to 200 mM was used) and unaffected by the addition of decanol or the absence of bicarbonate, though it did still show some slight decrease in activity when phosphate was omitted from the assay. These observations indicate that the apparent residual activity of this enzyme as measured is probably spurious, and due mainly to side-reactions involving formation of ether-soluble material, other than fatty acids, from acetate in the assay system. The actual repressed level of acetyl-CoA carboxylase, therefore, is probably much lower than those recorded in Table 3.

Table 3. Activities of acetyl-CoA carboxylase and the fatty acid synthetase complex of *Candida* 107 grown on glucose or alkanes

Growth substrate	Growth conditions	¹⁴ C-substrate for acetyl-CoA carboxylase	Enzyme activity in extracts (nmol substrate converted/h/mg protein)	
			Acetyl-CoA carboxylase*	Fatty acid synthetase complex
Glucose	Batch: harvested after 24 h	Acetate	31.6	70.0
		Acetyl-CoA	21.6	N.D.
Glucose	Batch: starved 24 h after glucose exhaustion	Acetate	10.2	60.5
C ₁₃ -C ₁₆ alkanes	Continuous culture	Acetate	0.7	37.2
		Acetyl-CoA	0.4	N.D.
Glucose	Batch: adapted to hexadecane for 24 h after glucose exhaustion	Acetate	1.0	N.D.
Glucose	Batch: adapted to decane for 24 h after glucose exhaustion	Acetate	0.6	N.D.

* Acetyl-CoA carboxylase activity measured in the presence of 75 mM-citrate. N.D., not determined.

The absence of acetyl-CoA carboxylase activity in *Candida* 107 grown on alkanes could be due to an inhibitor within the extract. This was tested by mixing an extract from alkane-grown *Candida* 107 with an extract prepared from glucose-grown *Candida* 107 (Fig. 5). Although an inhibitor, or inhibitors, were indeed indicated to be present within the extract from alkane-grown *Candida* 107, these were not the cause of the lack of acetyl-CoA carboxylase activity, since in the mixed extract addition of 75 mM-citrate completely restored the activity to the expected value. There was no indication that any activity within this mixture was derived from the extract from alkane-grown *Candida* 107. The relief of inhibition by citrate followed the same pattern as that for the relief of acetyl-CoA carboxylase inhibited with hexadecyl-CoA (see Fig. 4a).

DISCUSSION

De novo fatty acid biosynthesis is absent when *Candida* 107 is grown on *n*-alkanes of any chain length. The enzyme which is principally regulated is acetyl-CoA carboxylase which is apparently totally repressed. Synthesis of this enzyme is clearly not co-ordinate with that of the fatty acid synthetase complex, whose individual genes in yeast are already known to be co-ordinately expressed (Schweizer, Kühn & Castorph, 1971), as only partial repression of the complex was observed even after several days' continuous cultivation of the yeast on *n*-alkanes. The absence of carboxylase activity leads us to conclude that all fatty acids within the *n*-alkane-grown yeast must be derived from the *n*-alkanes themselves. This still applies when a short-chain alkane such as *n*-decane is used as sole carbon source and, with such substrates, elongation of the derived fatty acid must occur. The lipid of *Candida* 107 growing on an alkane with an odd number of carbon atoms, however, still contains a high proportion of even-chain-length acids (Thorpe & Ratledge, 1972) but there is no information regarding the mechanism by which these acids are formed. Some α -oxidation, as well as C₂-unit elongation, may be expected to occur. The absence of *de novo* fatty acid synthesis in alkane-

Fig. 5. Establishment of an inhibitor for acetyl-CoA carboxylase in extracts from alkane-grown *Candida* 107 which can be overcome by citrate. Activity of enzyme from glucose-grown *Candida* 107 starved before harvesting (○) and from alkane-grown *Candida* 107 (□). Activity of 1:1 mixture of both extracts: observed result (●); expected result if no interaction (----). (N.B. Activity of acetyl-CoA carboxylase in alkane-grown *Candida* 107 is still minimal even in the presence of high concentration of citrate; see text.)

grown *Candida* 107 suggests that this yeast may be useful for studying fatty acid modifications, particularly the poorly characterized C_2 -unit elongation mechanism which occurs in many systems.

Acetyl-CoA carboxylase is not only a repressible enzyme but, being sensitive to allosteric effectors, is involved in the short-term, or fine, control of fatty acid biosynthesis. In this respect, the enzyme from *Candida* 107 appears similar to that obtained from other sources which are activated, with aggregation of monomer sub-units (Moss & Lane, 1972), by a number of metabolic intermediates, in particular, citrate (Lane, Moss, Ryder & Stoll, 1971). This activation can be competitively inhibited by long-chain fatty acyl-CoA esters (Numa, Ringlemann & Lynen, 1965; Goodridge, 1972) but it is still not clearly established that these effects constitute a control mechanism *in vivo*. The evidence relating to short-term control of acetyl-CoA carboxylase has been reviewed at length by Vagelos (1971), and doubts regarding the action of citrate and long-chain acyl-CoA esters *in vivo* apply equally to the results we have now obtained with *Candida* 107. More recently, however, Guynn *et al.* (1972), after an examination of possible effectors of fatty acid biosynthesis in freeze-clamped liver, concluded that only the concentration of long-chain fatty acyl-CoA ester varied in a manner which was consistent with that of a controlling metabolite.

The process of alkane oxidation clearly extensively modifies intermediary metabolism within *Candida* 107. Alkanes have already been shown to inhibit simultaneously both glucose transport and its catabolism (Gill & Ratledge, 1973*a*), and these events were conjectured to be instigated by products of alkane oxidation acting as direct feedback-inhibitors of certain

key enzymes. Our results now indicate that of the intermediates of alkane oxidation, only long-chain fatty acyl-CoA esters, produced either directly from a long-chain alkane (C₁₆ to C₁₈ in length) or by elongation of a shorter acyl-CoA ester, could cause the inhibition of fatty acid biosynthesis. Such inhibition would lead to the accumulation of acetyl-CoA, and the consequences of this are likely to be profound as far as further effects on intermediary metabolism are concerned. Direct, or indirect, inhibition of glycolysis, glucose transport and other key processes can be anticipated to occur in keeping with the experimental observations. Since we can produce in *Candida* 107 either complete inhibition of glucose catabolism or the simultaneous utilization of both alkane and glucose, depending upon the state of the yeast and the chain length of the alkane substrate, this organism may be a useful model for studying the inter-relationships between fatty acid and carbohydrate metabolism including the exact nature of control of *de novo* fatty acid biosynthesis.

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REFERENCES

- BELL, G. H. (1971). The action of monocarboxylic acids on *Candida tropicalis* growing on hydrocarbon substrates. *Antonie van Leeuwenhoek* **37**, 385-400.
- BELL, G. H. (1973). The metabolism of long chain fatty acids and alcohols by *Candida tropicalis* and *Saccharomyces carlsbergensis*. *Antonie van Leeuwenhoek* **39**, 137-149.
- BIRNBAUM, J. (1970). Repression of acetyl coenzyme A carboxylase by unsaturated fatty acid: relationship to coenzyme repression. *Journal of Bacteriology* **104**, 171-176.
- BORTZ, W. M. & LYNEN, F. (1963). The inhibition of acetyl coenzyme A carboxylase by long-chain acyl coenzyme A derivatives. *Biochemische Zeitschrift* **337**, 505-509.
- BURTON, D. N., COLLINS, J. M., KENNAN, A. L. & PORTER, J. W. (1969). The effects of nutritional and hormonal factors on the fatty acid synthetase level of rat liver. *Journal of Biological Chemistry* **244**, 4510-4516.
- BUTTERWORTH, P. H. W., GUCHHAIT, R. B., BAUM, H., OLSON, E. B., MARGOLIS, S. A. & PORTER, J. W. (1966). Relationship between nutritional status and fatty acid synthesis by microsomal and soluble enzymes of pigeon liver. *Archives of Biochemistry and Biophysics* **116**, 453-457.
- CHENOUDA, M. S. & JWANNY, E. W. (1972). Utilization of hydrocarbons by micro-organisms; lipids and phospholipids of *Candida lipolytica* grown on hexadecane and on glucose media. *Journal of General and Applied Microbiology* **18**, 181-188.
- DAHLEN, J. V., KENNAN, A. L. & PORTER, J. W. (1968). Effect of alloxan and portacaval shunt on the synthesis of fatty acids and sterols by rat liver. *Archives of Biochemistry and Biophysics* **124**, 51-57.
- DAVIS, J. B. (1964). Microbial incorporation of fatty acids derived from *n*-alkanes into glycerides and waxes. *Applied Microbiology* **12**, 210-214.
- DORSEY, J. A. & PORTER, J. W. (1968). The effect of palmityl coenzyme A on pigeon liver fatty acid synthetase. *Journal of Biological Chemistry* **243**, 3512-3516.
- GILL, C. O. & RATLEDGE, C. (1972). Toxicity of *n*-alkanes, *n*-alk-1-enes, *n*-alkan-1-ols and *n*-alkyl-1-bromides towards yeasts. *Journal of General Microbiology* **72**, 165-172.
- GILL, C. O. & RATLEDGE, C. (1973*a*). Inhibition of glucose assimilation and transport by *n*-decane and other *n*-alkanes in *Candida* 107. *Journal of General Microbiology* **75**, 11-22.
- GILL, C. O. & RATLEDGE, C. (1973*b*). Control of fatty acid biosynthesis in an alkane utilizing yeast. *Journal of General Microbiology* **75**, xvi-xvii.
- GOODRIDGE, A. G. (1972). Regulation of the activity of acetyl coenzyme A carboxylase by palmityl coenzyme A and citrate. *Journal of Biological Chemistry* **247**, 6946-6952.
- GUINN, R. W., VELOSO, D. & VEECH, R. L. (1972). The concentration of malonyl CoA and the control of fatty acid synthesis *in vivo*. *Journal of Biological Chemistry* **247**, 7325-7331.
- HENDERSON, T. O. & MCNEILL, J. J. (1966). The control of fatty acid synthesis in *Lactobacillus plantarum*. *Biochemical and Biophysical Research Communications* **25**, 662-669.
- HENDERSON, T. O. & MCNEILL, J. J. (1967). Further studies on the control of fatty acid synthesis in *Lactobacillus plantarum*. *Bacteriology Proceedings*, 134.

- HORNEI, S., KÖHLER, M. & WEIDE, H. (1972). Das Fettsäurespektrum eines *Candida*-Stammes nach Kultur auf *n*-Alkenen. *Zeitschrift für allgemeine Mikrobiologie* **11**, 19–27.
- HUG, H. & FIECHTER, A. (1973). Assimilation of hydrocarbons by *Candida tropicalis*. II. Fatty acid profiles from cells grown on substrates of different chain length. *Archiv für Mikrobiologie* **88**, 87–96.
- HUNTER, K. & ROSE, A. H. (1971). Yeast lipids and membranes. *The Yeasts*, vol. 2, pp. 211–270. Edited by A. H. Rose and J. S. Harrison. New York and London: Academic Press.
- LANE, M. D., MOSS, J., RYDER, E. & STOLL, E. (1971). Activation of acetyl-CoA carboxylase by tricarboxylic acids. In *Advances in Enzyme Regulation*, vol. 9, pp. 237–251. Edited by G. Weber. Oxford: Pergamon Press.
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**, 265–275.
- LUST, G. & LYNEN, F. (1968). The inhibition of the fatty acid synthetase multienzyme complex of yeast by long-chain acyl-coenzyme A compounds. *European Journal of Biochemistry* **7**, 68–72.
- LYNEN, F. (1969). Yeast fatty acid synthetase. In *Methods in Enzymology*, vol. 14, pp. 17–32. Edited by J. M. Lowenstein. New York and London: Academic Press.
- MAJERUS, P. W. & KILBURN, E. (1969). Acetyl-CoA carboxylase. The roles of synthesis and degradation in regulation of enzyme levels in rat liver. *Journal of Biological Chemistry* **244**, 6254–6262.
- MARQUIS, N. R., FRANCESCONI, R. P. & VILLEE, C. A. (1968). A role for carnitine and long-chain acylcarnitine in the regulation of lipogenesis. In *Advances in Enzyme Regulation*, vol. 6, pp. 31–56. Edited by G. Weber. Oxford: Pergamon Press.
- MATSUHASHI, M. (1969). Acetyl-coenzyme A carboxylase from yeast. In *Methods in Enzymology*, vol. 14, pp. 3–8. Edited by M. J. Lowenstein. New York and London: Academic Press.
- MIZUNO, M., SHIMOJIMA, Y., IGUCHI, T., TAKEDA, I. & SENOH, S. (1966). Fatty acid composition of hydrocarbon-assimilating yeasts. *Agricultural and Biological Chemistry* **30**, 506–510.
- MOSS, J. & LANE, M. D. (1972). Acetyl-coenzyme A carboxylase. III. Further studies on the relation of catalytic activity to polymeric state. *Journal of Biological Chemistry* **247**, 4944–4951.
- NUMA, S., BORTZ, W. M. & LYNEN, F. (1965). Regulation of fatty acid synthesis at the acetyl-coenzyme A carboxylation step. In *Advances in Enzyme Regulation*, vol. 3, pp. 407–423. Edited by G. Weber. Oxford: Pergamon Press.
- NUMA, S., RINGLEMANN, E. & LYNEN, F. (1965). Zur Hemmung der Acetyl Coenzyme A Carboxylase durch Fettsäure-CoA-Verbindungen. *Biochemische Zeitschrift* **343**, 243–257.
- RASMUSSEN, R. K. & KLEIN, H. P. (1968). Activation of fatty acid synthesis in cell-free extracts of *Saccharomyces cerevisiae*. *Journal of Bacteriology* **95**, 157–161.
- RATLEDGE, C. (1968). Production of fatty acids and lipids by *Candida* sp. growing on a fraction of *n*-alkanes predominating in tridecane. *Biotechnology and Bioengineering* **10**, 511–533.
- RATLEDGE, C. (1970). Microbial conversions of *n*-alkanes to obtain economical microbial fats and fatty acids. *Chemistry and Industry*, pp. 843–854.
- SCHWETZER, E., KUHN, L. & CASTORPH, H. (1971). A new gene cluster in yeast: the fatty acid synthetase system. *Hoppe-Seyler's Zeitschrift für physiologische Chemie* **352**, 377–384.
- SEUBERT, W. (1960). S-Palmityl coenzyme A. In *Biochemical Preparations*, vol. 7, pp. 80–83. Edited by H. A. Lardy. New York and London: John Wiley & Sons Inc.
- SIMON, E. J. & SHEMIN, D. (1953). Synthesis of acetyl-CoA. *Journal of the American Chemical Society* **75**, 2520.
- SUMPER, M. & LYNEN, F. (1972). The multienzyme system of fatty acid biosynthesis. *Hoppe-Seyler's Zeitschrift für physiologische Chemie* **353**, 502.
- SUOMALAINEN, H. & KERÄNEN, A. J. A. (1968). The fatty acid composition of baker's and brewer's yeast. *Chemistry and Physics of Lipids* **2**, 296–315.
- THORPE, R. F. & RATLEDGE, C. (1972). Fatty acid distribution in triglycerides of yeasts grown on glucose or *n*-alkanes. *Journal of General Microbiology* **72**, 151–163.
- TRUST, T. J. & MILLIS, N. F. (1970). The isolation and characterization of alkane-oxidizing organisms and the effect of growth substrate on isocitric lyase. *Journal of General Microbiology* **61**, 245–254.
- TRAMS, E. G. & BRADY, R. O. (1960). The synthesis of malonyl-¹⁴C CoA. *Journal of the American Chemical Society* **82**, 2972–2973.
- TUBBS, P. K. & GARLAND, P. B. (1964). Variation in tissue contents of CoA thioesters and possible metabolic implications. *Biochemical Journal* **93**, 550–557.
- VAGELOS, P. R. (1971). Regulation of fatty acid biosynthesis. In *Current Topics in Cellular Regulation*, vol. 4, pp. 119–166. Edited by B. L. Horecker and E. R. Stadtman. New York and London: Academic Press.

- WAKIL, S. J., GOLDMAN, J. K., WILLIAMSON, I. P. & TOOMEY, R. E. (1966). Stimulation of fatty acid biosynthesis by phosphorylated sugars. *Proceedings of the National Academy of Sciences of the United States of America* **55**, 880–887.
- WEEKS, G. & WAKIL, S. J. (1970). Studies on the control of fatty acid metabolism. II. The inhibition of fatty acid synthesis in *Lactobacillus plantarum* by exogenous fatty acid. *Journal of Biological Chemistry* **245**, 1913–1921.
- WHITE, D. & KLEIN, H. P. (1965). Factors affecting fatty acid synthesis in cell-free preparations from *Saccharomyces cerevisiae*. *Biochemical and Biophysical Research Communications* **20**, 78–84.
- WHITE, D. & KLEIN, H. P. (1966). Effects of α -glycerophosphate and of palmityl coenzyme A on lipid synthesis in yeast extracts. *Journal of Bacteriology* **91**, 1218–1223.