The Elongation of Exogenous Fatty Acids and the Control of Phospholipid Acyl Chain Length in *Micrococcus cryophilus*

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The synthesis of fatty acids *de novo* from acetate and the elongation of exogenous saturated fatty acids (C_{12} – C_{18}) by the psychrophilic bacterium *Micrococcus cryophilus* (A.T.C.C. 15174) grown at 1 or 20°C was investigated. *M. cryophilus* normally contains only C_{16} and C_{18} acyl chains in its phospholipids, and the C_{18}/C_{16} ratio is altered by changes in growth temperature. The bacterium was shown to regulate strictly its phospholipid acyl chain length and to be capable of directly elongating myristate and palmitate, and possibly laurate, to a mixture of C_{16} and C_{18} acyl chains. Retro-conversion of stearate into palmitate also occurred. Fatty acid elongation could be distinguished from fatty acid synthesis *de novo* by the greater sensitivity of fatty acid elongation to inhibition by $NaAsO_2$ under conditions when the supply of ATP and reduced nicotinamide nucleotides was not limiting. It is suggested that phospholipid acyl chain length may be controlled by a membrane-bound elongase enzyme, which interconverts C_{16} and C_{18} fatty acids via a C_{14} intermediate; the activity of the enzyme could be regulated by membrane lipid fluidity.

It is widely accepted that membranes contain lipids with specific acyl constituents to maintain a fluid bilayer, which is required for the correct functioning of the proteins embedded within the membrane (Singer, 1974; Bretscher & Raff, 1975). In micro-organisms a change in environmental temperature usually results in an alteration of membrane lipid acyl constituents (McElhaney, 1976; Esser & Souza, 1976). McElhaney has pointed out that decreasing growth temperature commonly results in an increase in membrane lipid unsaturation; a decrease in average acyl chain length may also occur.

The psychrophilic bacterium *Micrococcus cryophilus* (A.T.C.C. 15174) provides an excellent example of a micro-organism that responds to environmental temperature by modifying its membrane phospholipid acyl chain length: the lipids have a simple acyl composition and the changes are particularly marked (Russell, 1971, 1978). The acyl chains of the membrane phospholipids are composed essentially of palmitoleate and oleate only, and the C_{18}/C_{16} ratio changes from 3.7 to 0.8 when the growth temperature is decreased from 20 to 0°C (Russell, 1971).

Several systems involving different temperature-sensitive mechanisms could account for the growth-temperature-dependent chain length change in *M.*

cryophilus. Fatty acid synthetase may produce a mixture of C_{16} and C_{18} fatty acids whose relative amounts are controlled either directly by temperature affecting synthetase activity, or indirectly by a protein whose induction was regulated by temperature (cf. thioesterase present in mammary gland; Libertini & Smith, 1978). The presence of two independent fatty acid synthetases, such as occur in *Mycobacterium smegmatis* (Odrizola *et al.*, 1977), is considered unlikely in *M. cryophilus*, owing to the narrowness of its phospholipid acyl chain-length range (C_{16} – C_{18}). Alternatively, there might exist a palmitate elongase enzyme, which controlled the proportion of C_{16} and C_{18} fatty acids.

On the basis of the results of the incorporation and modification of exogenous fatty acids, and of inhibitor studies, the present paper provides evidence that *M. cryophilus* possesses a system for the elongation of fatty acids that is separate from fatty acid synthetase. A possible mechanism whereby the proportions of C_{16} and C_{18} acyl chains could be regulated by such an elongase enzyme is discussed.

Experimental

Methods

Sodium [U - ^{14}C]acetate (sp. radioactivity 2.18GBq/mmol) and (1 - ^{14}C)-labelled saturated fatty

acids (C_{12} – C_{18} ; sp. radioactivity 1.18–2.18 GBq/mmol) were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Reagents were of the highest available grade and were obtained from BDH Chemicals, Poole, Dorset, U.K., or from Sigma (London) Chemical Co., Kingston upon Thames, Surrey KT2 7BG, U.K. Fatty acid standards were purchased from Sigma or Nu-Chek Prep., P.O. Box 172, Elysian, MN 56028, U.S.A., and were found to be >98% pure by g.l.c. analysis.

Growth of cultures

The growth and maintenance of cultures of *M. cryophilus* (A.T.C.C. 15174) in a casamino acid/salts medium (NM medium) has been described in detail previously (Russell, 1974). Cultures were grown at 0 or 20°C until they had reached the late logarithmic phase of growth ($A_{600} \approx 0.8 \pm 0.3$ mg dry wt. of cells/ml of culture) at which time portions were withdrawn for incubations with radioactive precursors.

Preparation of bacterial lysate

Bacteria were collected by centrifugation for 20 min at 10000 *g* (r_{av} , 5.35 cm); the bacterial pellet was resuspended in a volume of NM buffer (i.e. NM medium lacking casamino acids) corresponding to one-tenth of the original culture volume. Bacterial cells were disrupted by three passages through a French pressure cell (American Instrument Co Inc., Silver Springs, MD 20910, U.S.A.) at 4.14×10^4 kPa operating pressure. Cell breakage was estimated to be >95% by phase-contrast light microscopy.

Radioisotope-incorporation experiments

Carrier-free sodium [U - ^{14}C]acetate or a (1- ^{14}C)-labelled fatty acid (C_{12} – C_{18}) was added directly to growing bacteria to give a final concentration of radioactivity of 3.7 kBq per ml of culture in an assay volume of 10 ml. Flasks were shaken at 0 or 20°C in a temperature-controlled water bath. The reaction was stopped by the addition of 37.5 ml of chloroform/methanol (1:2, v/v), left to extract overnight and the lipid extract was then washed by the procedure of Garbus *et al.* (1963). The lower chloroform layer was removed and evaporated to dryness. The lipid extract (total lipid) was redissolved in a small volume of chloroform and subjected to t.l.c. on 0.3 mm thick plates of silica gel H (Merck, Darmstadt, Germany) with light petroleum (b.p. 60–80°C)/diethyl ether/acetic acid (85:15:1, by vol.) as the solvent system. The silica gel at the origin (containing phospholipids) was removed and fatty acid methyl esters were prepared by direct transmethylation on the gel with 2M-sodium methoxide in dry methanol (Christie, 1973). Radioactivity in fatty acid methyl esters was determined by counting triplicate portions (1% of

the total) with an Intertechnique PG4000 liquid-scintillation counter; counting efficiency (75–83%) was measured by the external-standard-ratio method. The remainder (97%) of the fatty acid methyl ester sample was analysed by radio-g.l.c. with a Pye–Unicam GCD chromatograph equipped with flame-ionization detectors and connected to a Panax Reigate series Radiogas detector system with a gas-flow-proportional counter. Separations were obtained by using glass columns (1.5 m $\times$ 2 mm internal diam.) packed with 15% (w/w) ethylene glycol succinate silicone (EGSS-X) supported on 80–100 mesh Chromosorb W AW (Supelco, Philadelphia, PA 16823, U.S.A.) operated at 190°C. Fatty acid methyl esters were identified by their retention times compared with authentic standards, and quantified by using the expression (retention time $\times$ peak height) as a measure of peak area, from which percentage composition and relative specific radioactivity ('area' of radioactivity peak/'area' of mass peak) could be calculated.

α -Oxidation procedure

The chemical α -oxidation of reduced phospholipid acyl chains was performed by the procedure of Harris *et al.* (1965). The oxidation products were methylated by using diazomethane and analysed by radio-g.l.c. as described above, except that a temperature gradient of 120–190°C (increasing at 4°C/min) was used.

Assay of succinate dehydrogenase (succinate-acceptor oxidoreductase; EC 1.3.99.1)

The assay used was based on that of Ellis (1959). Incubation mixtures (5 ml) contained 160 μ mol of sodium succinate, 48 μ mol of Tris/HCl, 40 nmol of KCN, 72 μ g of 2,6-dichlorophenol-indophenol and 160 μ g of *N*-methylphenazonium methosulphate. Reactions were started by the addition of portions of bacterial cells or lysates, and the decrease in absorbance at 600 nm was measured at 15 s intervals with a Cecil CE 373 spectrophotometer. There was an initial lag period (0–60 s), after which absorbance decreased linearly for up to 5 min, from which enzymic rates were calculated.

Results and Discussion

Sodium [U - ^{14}C]acetate and (1- ^{14}C)-labelled saturated fatty acids (C_{12} – C_{14}) were incorporated within 15 s into the phospholipids of *M. cryophilus* (Figs. 1 and 2). Table 1 shows the distribution of radioactivity amongst phospholipid acyl chains after a 2 h incubation with each radioactive precursor, when a steady state of incorporation had been obtained.

When acetate was used as a precursor, radioactivity appeared in C_{16} and C_{18} acyl chains only (Fig. 1); no mass or radioactivity was recovered in

Fig. 1. Incorporation of radioactivity from sodium $[U-^{14}C]$ acetate into phospholipid acyl chains in *M. cryophilus*

The fatty acid methyl esters, prepared from phospholipids isolated from cultures incubated with radioactive sodium acetate, were analysed by radio-g.l.c. to determine the percentage distribution of radioactivity in acyl chains, which is presented graphically.

acyl chains longer than C_{18} or shorter than C_{16} , and, moreover, the specific radioactivity (d.p.s./pmol of fatty acid) was the same for all the C_{16} and C_{18} fatty acyl chains at the steady state. The synthesis *de novo* of phospholipid fatty acyl chains from acetate obviously requires fatty acid synthetase, and it appears that the system of controlling fatty acyl chain length is operating normally under these conditions.

The results of incubations in which laurate, palmitate or stearate was used as precursor were similar to those of acetate incubations in that radioactivity appeared in C_{16} and C_{18} acyl chains only (Fig. 2). In contrast, when myristate was used as precursor a small amount (less than 10% of the total incorporated into phospholipids) of radioactivity was present in C_{14} acyl chains, even though C_{14} fatty acyl chain-containing phospholipids do not occur naturally in *M. cryophilus*. The amount of added radioactivity recovered in phospholipid acyl chains was greatest (7–20%) when palmitate or stearate was used as the precursor and was somewhat lower (1–2%) when a precursor such as laurate or myristate (neither of which are normal phospholipid acyl constituents) was used. These differences may reflect the specificity of the fatty acid transport system (if one exists; see e.g. Nunn & Simons, 1978) or the (trans)acylase enzyme(s). It is possible that exogenous fatty acids may suffer a

Table 1. Distribution of radioactivity in phospholipid acyl chains in *M. cryophilus* incubated for 2 h with sodium $[U-^{14}C]$ acetate or $(1-^{14}C)$ -labelled fatty acids

Cultures were incubated with radioisotopic precursor for 2 h and the phospholipid fatty acid methyl esters were prepared and analysed by radio-g.l.c. for determination of the distribution of radioactivity in acyl chains. The results are expressed as means $\pm$ s.d. for the percentages of total areas on chromatographic traces. The numbers of experiments performed with separate cultures is given (*n*). The ratio of radioactivity in C_{18} acyl chains to that in C_{16} acyl chains was calculated directly from areas of peaks on chromatograms.

Radioactive precursor	Bacterial-growth and incubation temperature ($^{\circ}C$)	<i>n</i>	Distribution of radioactivity in phospholipid acyl chains (%)				C_{18}/C_{16} ratio of radioactivity
			$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	
Sodium $[U-^{14}C]$ acetate	20	4	1.9 ± 1.1	13.0 ± 4.7	3.7 ± 2.2	81.4 ± 2.2	5.8 ± 0.5
	0	3	3.3 ± 0.4	48.6 ± 4.2	1.4 ± 0.6	46.7 ± 3.7	0.9 ± 0.1
$(1-^{14}C)$ -labelled $C_{12:0}$ fatty acid	20	2	0.7 ± 0.8	10.8 ± 0.8	2.4 ± 1.5	86.0 ± 3.2	8.2 ± 0.8
	0	3	9.2 ± 1.5	40.9 ± 8.4	5.2 ± 2.0	44.7 ± 8.2	1.0 ± 0.4
$(1-^{14}C)$ -labelled $C_{14:0}$ fatty acid	20*	2	3.2 ± 3.9	12.9 ± 3.7	2.3 ± 0.8	70.9 ± 8.3	5.2 ± 2.9
	0†	3	9.6 ± 1.1	38.7 ± 3.6	5.4 ± 2.4	43.8 ± 2.7	1.0 ± 0.2
$(1-^{14}C)$ -labelled $C_{16:0}$ fatty acid	20	2	2.7 ± 1.8	41.7 ± 21.1	1.9 ± 2.0	58.6 ± 17.1	1.6 ± 1.3
	0	3	16.0 ± 1.6	47.1 ± 9.7	6.9 ± 5.4	30.0 ± 5.9	0.6 ± 0.2
$(1-^{14}C)$ -labelled $C_{18:0}$ fatty acid	20	3	1.7 ± 1.1	7.8 ± 3.1	2.5 ± 1.7	87.3 ± 0.7	10.0 ± 3.0
	0	2	10.6 ± 6.0	21.5 ± 1.5	16.8 ± 3.3	51.0 ± 7.9	2.1 ± 0.4

* $5.5 \pm 5.0\%$ of $C_{14:0}$ fatty acid and $3.0 \pm 2.0\%$ of $C_{14:1}$ fatty acid were also present. C_{16}/C_{14} ratio of radioactivity = 1.9 ± 0.2 .

† $1.5 \pm 0.9\%$ of $C_{14:0}$ fatty acid and $1.6 \pm 0.8\%$ of $C_{14:1}$ fatty acid were also present. C_{16}/C_{14} ratio of radioactivity = 18.7 ± 8.2 .

Fig. 2. Incorporation of radioactivity from (1-¹⁴C)-labelled fatty acids into phospholipid acyl chains in *M. cryophilus*. For details see the legend to Fig. 1.

Table 2. Effect of NaAsO₂ on the ratio of radioactivity in C₁₈/C₁₆ phospholipid acyl chains in *M. cryophilus* incubated for 2 h with sodium [U-¹⁴C]acetate or (1-¹⁴C)-labelled fatty acids

Cultures were incubated with or without 10 mM-NaAsO₂ in the presence of radioisotopic precursor, and the ratios of radioactivity in C₁₈ to C₁₆ acyl chains was determined as described in Table 1. Results are means ± s.d., with the numbers of experiments performed with separate cultures shown in parentheses.

Radioactive precursor	[NaAsO ₂] (mM)	Incubation temperature	Ratio of radioactivity in C ₁₈ /C ₁₆ phospholipid acyl chains	
			20°C P†	0°C P†
Sodium [U- ¹⁴ C]acetate	0		5.8 ± 0.5 (4)	0.9 ± 0.1 (3)
	10		0.6 ± 0.0 (2)	0.6 ± 0.2 (2)
(1- ¹⁴ C)-labelled C _{12:0} fatty acid	0		8.2 ± 0.8 (2)	1.0 ± 0.4 (3)
	10		1.0 ± 0.7 (2)	0.5 ± 0.0 (3)
(1- ¹⁴ C)-labelled C _{14:0} fatty acid*	0		5.2 ± 2.9 (2)	1.0 ± 0.2 (3)
	10		1.1 ± 0.9 (2)	0.4 ± 0.1 (2)
(1- ¹⁴ C)-labelled C _{16:0} fatty acid	0		1.0 ± 1.1 (4)	0.6 ± 0.2 (4)
	10		0.1 ± 0.0 (3)	0.1 ± 0.0 (2)
(1- ¹⁴ C)-labelled C _{18:0} fatty acid	0		10.0 ± 3.0 (3)	2.1 ± 0.4 (2)
	10		9.7 ± 0.6 (2)	5.5 ± 2.7 (2)
			<0.005	<0.10
			<0.10	<0.10
			<0.10	<0.05
			<0.005	<0.005
			n.s.	n.s.

* See Table 3 for C₁₆/C₁₄ ratios of radioactivity.
† P values are obtained by applying the Student's *t* test to the proportions of radioactivity in C₁₈ fatty acid in control and arsenite-treated samples. This is done because the sample proportions have more nearly a normal distribution than do the corresponding ratios, whose distribution is rather skew. n.s., not significant.

different fate inside the cell compared with endogenously generated fatty acids (Cronan, 1978).

The C_{18}/C_{16} ratio of radioactivity in cells grown and incubated at 0°C was markedly lower than that of corresponding cells grown and incubated at 20°C , whether sodium acetate or a fatty acid was used as precursor (Table 1). The smaller amount of elongation of fatty acid precursors at 0°C corresponds to the shorter average acyl chain length in cells grown at this temperature compared with those grown at 20°C .

These experiments demonstrated that *M. cryophilus* was capable of elongating myristate and palmitate, and possibly laurate, but did not distinguish whether an 'elongase' enzyme distinct from fatty acid synthetase was responsible. Some insight into the mechanism of fatty acid elongation was provided by chemical α -oxidation of the phospho-

lipid acyl chains. The α -oxidation pattern of phospholipid acyl chains from incubations with sodium $[\text{U-}^{14}\text{C}]$ acetate showed that radioactivity was distributed uniformly along the acyl chain (Fig. 3a); all α -oxidation fragments were radioactive with a constant specific radioactivity (area of radioactivity peak/area of mass peak). In contrast, when phospholipid acyl chains from incubations containing $(1\text{-}^{14}\text{C})$ -labelled $C_{14:0}$ or $(1\text{-}^{14}\text{C})$ -labelled $C_{16:0}$ fatty acids were subjected to α -oxidation, greater than 98% radioactivity was recovered in α -oxidation fragments corresponding to the chain length of the precursor and longer, whereas less than 5% radioactivity was recovered in α -oxidation fragments shorter than the added radioactive precursor (Figs. 3b and 3c). When $(1\text{-}^{14}\text{C})$ -labelled $C_{18:0}$ fatty acid was used as precursor 97% of the radioactivity was recovered in $C_{16}\text{--}C_{18}$ fragments

Fig. 3. Distribution of radioactivity in α -oxidation products of phospholipid acyl chains in *M. cryophilus*

Cultures were incubated with radioisotopic precursor and phospholipid fatty acids were isolated and reduced using H_2 gas and PtO_2 catalyst. Complete reduction was checked by radio-g.l.c. analysis. The saturated fatty acids were subjected to α -oxidation using KMnO_4 , and the oxidation products methylated by diazomethane before analysing them by radio-g.l.c. An excess of arachidate was added before α -oxidation to facilitate unequivocal identification of radioactive peaks. The mass traces, which are the same for each sample, have been omitted from the Figure to facilitate the comparison of radioactive traces.

(Fig. 3*d*). The small amount of radioactivity in phospholipids isolated from laurate incubations precluded an α -oxidation study.

These results showed that there was direct elongation of myristate and palmitate without prior β -oxidation, which would have randomized the radioactive label along the acyl chain. Support for a direct elongation mechanism was obtained from a study of the effect of NaAsO₂ on the elongation of phospholipid acyl chains in *M. cryophilus*. The rationale for this approach was that NaAsO₂ is known to inhibit palmitate elongation in plants (see e.g. Harwood & Stumpf, 1971).

In most cases NaAsO₂ caused a marked decrease in the proportion of C₁₈ acyl chains in phospholipids of *M. cryophilus* grown at 0 or 20°C (cf. Tables 2 and 3). The mean values of the C₁₈/C₁₆ ratios of radioactivity of arsenite-treated cultures were lower than those of control cultures for all radioactive precursors except stearate. However, in some cases there was considerable variation in the ratios obtained from one experiment to another. Thus the standard deviations of certain of the sample ratios are large in comparison with the corresponding means. Differences involving such ratios, therefore, may well not be statistically significant (see Table 2). Nevertheless, within each individual experiment arsenite lowered the C₁₈/C₁₆ ratio of radioactivity for all radioactive precursors except stearate. A comparison of the effect of NaAsO₂ on the incorporation of acetate and palmitate into phospholipid acyl chains showed that palmitate elongation was more sensitive to inhibition by NaAsO₂ (76% inhibition by 0.1 mM-NaAsO₂) than was palmitate synthesis from acetate (20% inhibition by 0.1 mM-NaAsO₂). The C₁₈/C₁₆ ratios of radioactivity obtained from acetate, laurate and myristate incubations were very similar. From the data in Table 2 average values for this ratio for the three precursors obtained from 20 and 0°C incubations were 7.04 ± 0.64 and 0.94 ± 0.06 respectively; these values were decreased to 0.48 ± 0.10 and 0.54 ± 0.13 by 10 mM-NaAsO₂. However, des-

pite the similar C₁₈/C₁₆ ratios of radioactivity obtained from laurate and myristate incubations there was a major difference in the effect of NaAsO₂ on the incorporation of these two precursors into phospholipid acyl chains. NaAsO₂ (10 mM) caused the accumulation of phospholipids containing C₁₄ acyl chains when myristate was the precursor (Table 3), but no accumulation of phospholipids containing C₁₂ (or C₁₄) acyl chains occurred when laurate was the precursor. The C₁₈/C₁₆ ratios of radioactivity obtained from palmitate incubations were lower than those from laurate and myristate incubations, and this value was decreased still further by NaAsO₂ (Table 2). Conversely, the C₁₈/C₁₆ ratios of radioactivity in stearate incubations were similar to those from laurate and myristate incubations, and NaAsO₂ had no effect on this ratio in bacteria grown and incubated with stearate at 20°C or 0°C (Table 2).

NaAsO₂ inhibits reactions in which vicinal thiol groups play a role, and hence is an inhibitor of enzymes such as pyruvate dehydrogenase [pyruvate-lipoamide oxidoreductase (acceptor-acetylating); EC 1.2.4.1] and α -oxoglutarate dehydrogenase [2-oxoglutarate-lipoamide oxidoreductase (acceptor-succinylating); EC 1.2.4.2] by virtue of their lipic acid content.

It was possible, therefore, that the inhibition of phospholipid acyl chain elongation in *M. cryophilus* was a secondary effect resulting from a decrease in ATP and reduced nicotinamide nucleotide concentrations, which would be a consequence of the inhibition of tricarboxylic acid-cycle activity. *M. cryophilus* grows aerobically and contains enzymes of the tricarboxylic acid cycle (Malcolm, 1968). To investigate this possibility the effect on fatty acid synthesis and elongation of sodium malonate, an inhibitor of succinate dehydrogenase [succinate-(acceptor) oxidoreductase; EC 1.3.99.1] was determined; the activity of succinate dehydrogenase was used as an indicator of tricarboxylic acid-cycle activity. Initial experiments were performed with intact bacteria, in which sodium malonate had little

Table 3. Effect of NaAsO₂ on the distribution of radioactivity in phospholipid acyl chains in *M. cryophilus* incubated for 2 h with [1-¹⁴C]myristate

For details, see the legends to Tables 1 and 2.

[NaAsO ₂] (mM)	Bacterial- growth and incubation temperature (°C)	n	Distribution of radioactivity in phospholipid acyl chains (%)						C ₁₈ /C ₁₆ ratio of radio- activity	C ₁₆ /C ₁₄ ratio of radio- activity
			C _{14:0}	C _{14:1}	C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}		
0	20	2	5.5 ± 5.0	3.0 ± 2.0	3.2 ± 3.9	12.9 ± 3.7	2.3 ± 0.8	70.9 ± 8.3	5.2 ± 2.9	1.9 ± 0.3
10	20	2	42.9 ± 0.8	13.7 ± 14.2	12.8 ± 1.0	9.4 ± 4.5	5.3 ± 3.7	15.9 ± 14.9	1.1 ± 1.0	0.4 ± 0.0
0	0	3	1.5 ± 0.9	1.6 ± 0.8	9.6 ± 1.1	38.7 ± 3.6	5.4 ± 2.4	43.8 ± 2.7	1.0 ± 0.2	18.7 ± 8.2
10	0	2	12.6 ± 5.4	5.3 ± 2.8	44.1 ± 0.6	14.8 ± 0.7	11.9 ± 1.7	11.3 ± 2.1	0.4 ± 0.1	3.3 ± 0.4

Table 4. *Effect of sodium malonate and NaAsO₂ on the incorporation of radioactivity into phospholipid acyl chains in a lysate of M. cryophilus incubated for 2 h with sodium [U-¹⁴C]acetate or [1-¹⁴C]palmitate*

Cultures were grown at 20°C and lysates were prepared by using the French pressure cell as described in the Experimental section. Lysates were incubated at 20°C with or without inhibitor in the presence of radioisotopic precursor, and the ratio of radioactivity in C₁₈ to C₁₆ acyl chains was determined as described in the legends to Tables 1 and 2.

Radioactive precursor	Inhibitor concn.	Ratio of radioactivity in C ₁₈ /C ₁₆ phospholipid acyl chains	P*
Sodium [U- ¹⁴ C]acetate	None	5.84 ± 1.26 (4)	
	Sodium malonate (0.1 M)	4.83 ± 0.85 (4)	n.s.
	Sodium arsenite (10 mM)	2.15 ± 0.37 (6)	<0.005
(1- ¹⁴ C)-labelled C _{16:0} fatty acid	None	0.32 ± 0.02 (3)	
	Sodium malonate (0.1 M)	0.36 ± 0.03 (3)	n.s.
	Sodium arsenite (10 mM)	0.18 ± 0.06 (3)	<0.05

* P values are obtained by applying the Student's *t* test to the proportions of radioactivity in C₁₈ fatty acid in control and malonate- or arsenite-treated samples (see Table 2). n.s., not significant.

effect on elongation. However, the activity of succinate dehydrogenase was inhibited by only 60%. In view of this lack of complete inhibition of succinate dehydrogenase by sodium malonate in intact cells, the experiments were repeated with a lysate prepared from bacteria grown at 20°C, in which 0.1 M-sodium malonate inhibited $99.2 \pm 1.1\%$ (mean $\pm$ S.D., $n = 5$) of the succinate dehydrogenase activity. When acetate or palmitate was used as a precursor in such a lysate, 0.1 M-sodium malonate had no effect on the C₁₈/C₁₆ ratio of radioactivity; in comparison 10 mM-NaAsO₂ decreased this ratio by 63% and 44% for acetate and palmitate respectively (Table 4). Therefore, the inhibition of elongation by arsenite was not due to reduced pyridine nucleotide or energy starvation, although high arsenite concentrations did inhibit both fatty acid synthetase and elongation by up to 17 and 41% respectively in some experiments. Fatty acid synthetase should be more sensitive to a decrease in intracellular NADPH concentrations, because it has a much greater demand for reducing equivalents than does fatty acid elongation.

The suggestion that the elongation system can elongate C₁₄ and C₁₆ acyl chains is supported by the fact that when stearate was used as radioactive precursor up to 32% of the radioactivity incorporated into phospholipid was recovered in C₁₆ acyl chains (Table 1). These could have arisen only by partial β -oxidation ('retroconversion') to C₁₄ acyl chains and subsequent elongation using radioactive fragments, because chemical α -oxidation showed that radioactivity was present in C₁₆–C₁₈ oxidation products only. Radioactive fragments may remain sequestered within an enzyme complex or membrane environment; this would prevent mixing with soluble 'pools' and increase their effective concentration, and thus explain the high amounts of radioactivity in C₁₆ retroconversion products of

stearate incubations. Recent studies have shown that the elongation system is membrane-bound (S. P. Sandercock & N. J. Russell, unpublished work). Retroconversion and elongation might be performed by a single membrane-bound enzyme system, the balance of the two activities being controlled by membrane fluidity, which in turn could be affected by growth temperature. Evidence that acyl-chain-length control by temperature is mediated at the level of fatty acid elongation rather than fatty acid synthesis is provided by the fact that the 7-fold difference between the C₁₈/C₁₆ ratios of radioactivity obtained from 20 or 0°C incubations when acetate was used as the precursor was abolished by NaAsO₂ (Table 2). Perhaps the C₁₈/C₁₆ ratio of radioactivity obtained in the presence of arsenite reflects the chain length ratio of the products of fatty acid synthetase.

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