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The Effect of Different Temperatures on Fatty-Acid Synthesis and Polyunsaturation in Cell Suspension Cultures

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Abstract. Cell suspension cultures of *Catharanthus roseus* G. Don, *Glycine max* (L.) Merr. and *Nicotiana tabacum* L. were incubated with [^{14}C]acetate, [^{14}C]oleic acid and [^{14}C]linoleic acid at five different temperatures ranging from 15 to 35° C. When the incubation temperature was increased, [^{14}C]acetate was incorporated preferentially into [^{14}C]palmitate, with a concomitant drop in [^{14}C]oleate formation. Between 15 and 20° C, [^{14}C]oleic acid accumulated in *C. roseus* cells. In all cultures, optimum desaturation of [^{14}C]oleic acid to [^{14}C]linoleic acid occurred between 20 and 25° C, and in *G. max* this was also the optimal range for desaturation of [^{14}C]linoleic acid to [^{14}C]linolenic acid. Elongation of [^{14}C]palmitic acid was inhibited when cultures grown at 15° C for 25 h were subsequently incubated with [^{14}C]acetate at 25° C. [^{14}C]oleic acid accumulated in *G. max* and *C. roseus* cultures grown at 35° C for 25 h and subsequently incubated at 25° C. Desaturation of [^{14}C]oleic acid increased up to 25° C, but then decreased or leveled off depending on the cell line and on the temperature prior to incubation.

Key words: *Catharanthus* – Cell suspension cultures – *Glycine* – Fatty acids (synthesis, desaturation) – *Nicotiana* – Linoleic, linolenic, oleic, acids – Temperature and fatty acids.

Introduction

The effect of changing temperatures on fatty-acid synthesis in plants is a well known phenomenon (see review in Hilditch and Williams, 1964, pp. 207–208). Most reports have correlated increased production of unsaturated fatty acids with low environmental temperatures. Hilditch and Williams (1964) have pointed out that plant species capable of survival in different climates normally produced more unsaturated fatty acids in the seeds when grown in cold climates. This effect of temperature had been observed in lower plants (Bedford et al., 1978; Patterson, 1970) and higher plants (Harris and James, 1969; Slack and Roughan, 1978). Increased linolenic-acid content at low temperatures has been implicated in chilling resistance of several higher plant species (Lyons et al., 1964; St. John and Christiansen, 1976; Willemot, 1977). Higher levels of the polyunsaturated fatty acids linoleic and linolenic conferred flexibility on the mitochondrial membranes of chilling-resistant plants grown at chilling temperatures (0–10° C). Membrane inflexibility caused by higher levels of saturated fatty acids was probably responsible for chilling injury in chilling sensitive species (Lyons et al., 1964). Furthermore, a temperature-induced phase-change (ca. 10–12° C) was noted in the phospholipids of mitochondrial, glyoxysomal and proplastid membranes in chilling-sensitive castor bean (Wade et al., 1974).

Temperature effects on fatty-acid synthesis have also been studied in some plant tissue cultures. Breidenbach and Waring (1977) observed that tomato seedlings and tomato cell-suspension cultures both responded to chilling temperatures by synthesizing phospholipids similar to each other in acyl-chain composition. Radwan and Mangold (1976) noted that *Brassica napus* callus cultures incubated at 30° C had lower concentrations of polyunsaturated fatty acids as compared to cultures incubated at 5° C. In general, a low ambient temperature had the effect of increasing C_{18} unsaturated fatty acids (particularly linoleic and linolenic) in many plant species (Hilditch and Williams, 1964; Slack and Roughan, 1978).

In order to examine in more detail these temperature effects, a simplification of the experimental material was preferred. Therefore, cell culture systems grown under defined media and conditions were

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selected and it was, for example, found that such cultures had increased concentrations of oleic acid (*Catharanthus roseus* G. Don), linoleic acid (*C. roseus* and *Glycine max* (L.) Merr.) and linolenic acid (*Nicotiana tabacum* L.) when grown at 15° C (MacCarthy and Stumpf, 1980). This report will record the results of a series of experiments undertaken to probe the effect of different temperatures on the ability of three different tissue culture systems to synthesize fatty acids from [^{14}C]acetate and to desaturate [^{14}C]oleic acid and [^{14}C]linoleic acids.

Materials and Methods

Cell Suspension Cultures

The *Glycine max* cell suspension culture was provided by K.G. Lark, University of Utah, Salt Lake City, Ut., USA; the *Nicotiana tabacum* suspension culture was initiated from callus culture provided by T. Murashige, University of California, Riverside, Cal., USA; the *Catharanthus roseus* cell suspension culture was initiated in this laboratory. The *G. max*, *C. roseus* and *N. tabacum* cell-suspension cultures were maintained on Gamborg's (1975) B5C medium, modified Wood and Braun (1961) medium and Murashige and Skoog (1975) medium, respectively. They were grown in 125-ml Erlenmeyer flasks on a G-10 model gyrotory shaker (New Brunswick Scientific Co., New Brunswick, N.J., USA) at a speed of 131 rpm, at 25 ± 0.5° C and in the light (General Electric fluorescent coolwhite lamps; illuminance 455 lumen m^{-2}). The growth conditions for the cell-suspension cultures have already been described in detail (MacCarthy and Stumpf, 1980).

Incubation Procedure: Fatty-Acid Extraction and Analysis

An aliquot of cell culture (0.5 g fresh weight) was added to 1.5 ml sterile medium (pH 7.3) containing 1.0 μCi of [^{14}C]substrate. The substrates were: 18.5 nmol sodium [^{14}C]acetate (New England Nuclear, Boston, Mass., USA, specific acitivity 54 mCi/mmol), 20.8 or 17.9 nmol [^{14}C]oleic acid (Amersham Corp., Arlington Heights, Ill., USA, specific activity 56 mCi/mmol; New England Nuclear, specific activity 48 mCi/mmol), and 19.8 nmol [^{14}C]linoleic acid (New England Nuclear, specific activity 50.6 mCi/mmol). Incubation vials were capped and then incubated on a gyrotory shaker rotating at 131 rpm in the light and at the desired temperature. Following incubation, the cells were removed from the medium by centrifugation and washed 2 times in deionized water. The final pellet was extracted with 20% KOH in methanol (w/v), acidified with 6N HCl, extracted 3 times with petroleum ether (b.p. 35–60° C), and evaporated to dryness. This fatty-acid extract was methylated using ca. 70% diazomethane in diethyl ether prepared according to deBoer and Backer (1963). Fatty-acid methyl esters were separated and identified using gas-liquid chromatography and thin-layer chromatography. The procedures for incubation and fatty-acid analysis have been described in detail in MacCarthy and Stumpf (1980).

Cultures Incubated from 15 to 35° C; Cultures Pretreated with High or Low Temperatures

Six-day old cell suspension cultures were incubated for 5.5 h at 15, 20, 25, 30, and 35° C at 131 rpm and in the light. In other experiments, 6-day-old cultures were taken from 25° C and placed at either 15 or 35° C for 2 or 25 h. Cultures induced for 2 h (and a control culture) were then incubated for 3 h at 15, 25 or 35° C with [^{14}C]acetate or [^{14}C]oleic acid. Cultures induced for 25 h were subsequently incubated for 4 h at 25° C with [^{14}C]acetate.

Fluorescein-Diacetate Viability Test

Cell viability was determined using a 5 mg/ml solution of fluorescein diacetate (FDA; Sigma Chemical Co., St. Louis, Mo., USA) diluted to 2% (v/v) (Widholm, 1972). One volume of FDA solution was reacted with one volume of cell culture on a glass slide. A Large Fluorescence Microscope (Carl Zeiss, Oberkochen/Württ., West Germany) equipped with an HBO 200 W/4 super-pressure mercury vapor lamp was used. Viable cells fluoresce brightly in ultra-violet light. Results are given as percentage viable cells.

Results and Discussion

Cell Viability

Before the cell cultures could be examined for capacity for lipid biosynthesis at various temperatures, it was essential to determine how viable these cells were under the temperatures that were to be employed. Two temperature shifts were examined: (1) cultures grown at 25° C were transferred to 15° C for increasing lengths of time, and (2) cultures grown at 25° C were transferred to 35° C.

An inspection of Table 1 shows that culture cells were reasonably viable for 10 h but after 24 h a loss was observed differing in extent depending on the cell culture employed. Therefore, 5.5 h at a given incubation temperature was selected to minimize any loss in viability. It is clear from the data in Table 1 that extended periods of growth or incubations at temperatures at or above 35° C would yield meaningless results.

[^{14}C] Acetate Incorporation

When each cell line was incubated with [^{14}C]acetate for 5.5 h, there was an accumulation of [^{14}C]palmitic acid as the incubation temperature increased (Fig. 1). These observations are in line with those of Jaworski et al. (1974) who demonstrated that elongation of palmitoyl acyl carrier protein (ACP) to stearyl ACP decreased with exposure of the cell-free extract to increasing temperatures.

As demonstrated in Fig. 1, [^{14}C]palmitate formation increased, and the formation of [^{14}C]C_{18} fatty acids decreased. Figure 2 summarizes the shifts in the labelled C_{18} fatty acids, i.e. [^{14}C]stearic, [^{14}C]oleic and [^{14}C]linoleic, formed after [^{14}C]acetate incubations at different temperatures. What is strikingly noticeable is the marked difference in the synthesis of unsaturated C_{18} fatty acids by the three different cell cultures at given temperatures. While the formation of [^{14}C]linoleate from [^{14}C]acetate at 15° C was enhanced in cultures of *N. tabacum*, its synthesis in *C. roseus* and *G. max* cultures was very low. The

Table 1. The change in viability of 6-day-old cell cultures of *G. max*, *C. roseus* and *N. tabacum* initially grown at 25° C and then transferred to either 15 or 35° C for 25 h

Time (h)	Viable cells (%)					
	G. max	*C. ros.* 25° C	*N. tab.*	*G. max*	*C. ros.* 25° C	*N. tab.*
0	86.1	92.8	77.3	92.1	99.7	87.6
		15° C			35° C	
2	80.4	86.1	77.9	95.5	91.2	80.1
4	79.1	89.7	74.5	90.0	82.8	63.7
6	75.1	89.5	74.8	90.5	83.9	67.7
10	70.9	90.0	69.2	80.2	81.2	59.2
24	71.2	72.2	61.5	60.3	74.0	52.2

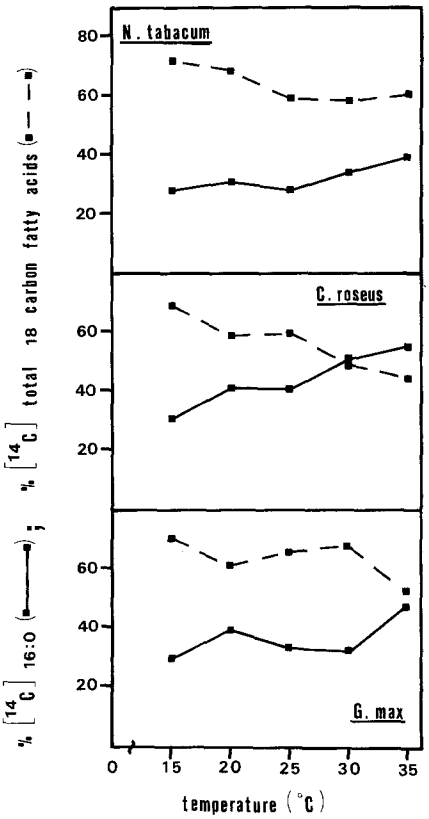

Fig. 1. The incorporation of [^{14}C]acetate into palmitic acid (16:0) and total C_{18}-fatty acids (stearic acid = 18:0, oleic acid = 18:1, linoleic acid = 18:2, linolenic acid = 18:3) as a function of temperature in cell suspension cultures of *N. tabacum*. *C. roseus* and *G. max*. Incubation medium (1.5 ml; pH 7.3) contained 18.5 nmol [^{14}C]acetate. Cell fresh weight was 0.5 g and incubation was for 5.5 h. Less than 5% (in the case of *G. max* and *C. roseus*) and 10% (in the case of *N. tabacum*) of [^{14}C]acetate remained in the medium at the end of incubation. Incorporation of [^{14}C]acetate into total [^{14}C]fatty acids varied from 35 to 15° C as follows: *G. max* between 9.2 and 17.3%; *C. roseus* between 10.8 and 19.5%; *N. tabacum* between 6.5 and 13%. See Table 3 for total nmol [^{14}C]acetate incorporated into [^{14}C]fatty acids over this temperature range, and Materials and Methods for other incubation details

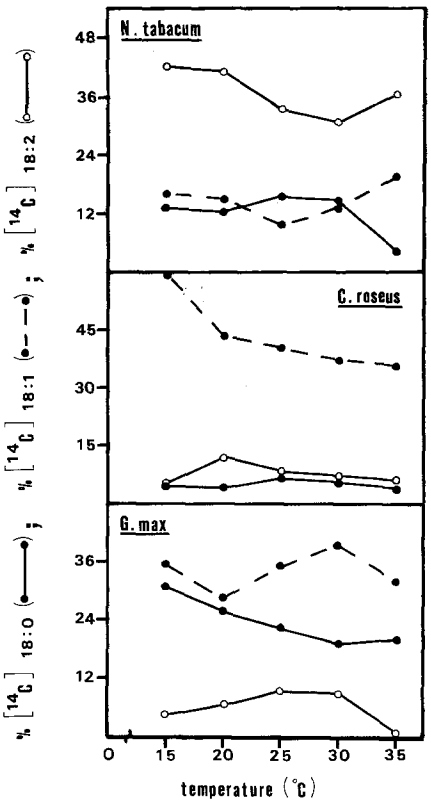

Fig. 2. The incorporation of [^{14}C]acetate into stearic acid (18:0), oleic acid (18:1) and linoleic acid (18:2) as a function of temperature in cell suspension cultures of *N. tabacum*, *C. roseus* and *G. max*. Incubation medium (1.5 ml; pH 7.3) contained 18.5 nmol [^{14}C]acetate. Cell fresh weight, time of incubation, [^{14}C]acetate remaining in the medium at the end of incubation, and incorporation of [^{14}C]acetate into total [^{14}C]fatty acids between 35 and 15° C as in Fig. 1. See Table 3 for total nmol [^{14}C]acetate incorporated into [^{14}C]fatty acids over this temperature range, and Materials and Methods for other incubation details

formation of [^{14}C]oleate was high in both *C. roseus* and *G. max* cells but low in *N. tabacum*. However, [^{14}C]oleate formation declined in *C. roseus* with increasing temperature. At 35° C, [^{14}C]linoleate formation remained high in *N. tabacum*, and very low with the other two cell lines. In general, when the dependence of fatty-acid synthesis on incubation temperatures is examined, no drastic shift is noted within each of the three cell lines. The genotype of the cell line appears to be the determining factor whereas a physical perturbation such as a different incubation temperature had a limited effect.

When the data obtained from the three cell lines are presented (Fig. 3) as ratios of [^{14}C]unsaturated to [^{14}C]saturated fatty acids, definite trends are observed in the *N. tabacum* and *C. roseus* cell cultures. As the incubation temperature was increased, marked reductions in unsaturated fatty acid synthesis occurred in the *C. roseus* cells and a smaller decrease in *N.*

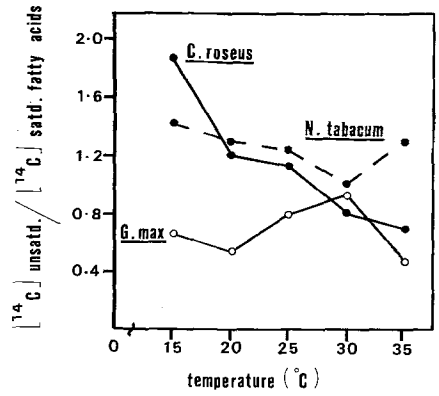

Fig. 3. The ratio of unsaturated fatty acids:saturated fatty acids as a function of temperature in cell suspension cultures of *N. tabacum*, *C. roseus* and *G. max*. Incubation medium (1.5 ml; pH 7.3) contained 18.5 nmol [^{14}C]acetate. Cell fresh weight, incubation time, [^{14}C]acetate remaining in the medium at the end of incubation and incorporation of [^{14}C]acetate into total [^{14}C]fatty acids between 35 and 15° C as in Fig. 1. See Table 3 for total nmol [^{14}C]acetate incorporated into [^{14}C]fatty acids over this temperature range, and Materials and Methods for other incubation details

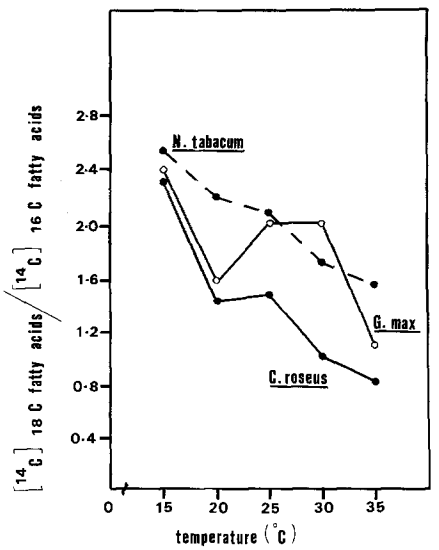

Fig. 4. The ratio of [^{14}C]C_{18}-fatty acids: [^{14}C]C_{16}-fatty acid (palmitic) as a function of temperature in cell suspension cultures of *N. tabacum*, *C. roseus* and *G. max*. Incubation medium (1.5 ml; pH 7.3) contained 18.5 nmol [^{14}C]acetate. Cell fresh weight, incubation time, [^{14}C]acetate remaining in the medium, and incorporation of [^{14}C]acetate into total [^{14}C]fatty acids between 35 and 15° C as in Fig. 1. See Table 3 for total nmol [^{14}C]acetate incorporated into [^{14}C]fatty acids over this temperature range, and Materials and Methods for other incubation details

Table 2. The effect of temperature on formation of [^{14}C]linolenic acid from [^{14}C]oleic acid and [^{14}C]linoleic acid (respectively, 20.8 nmol in 1.5 ml medium and 19.8 nmol in 1.5 ml medium) by *G. max* cell cultures.

Cell fresh weight was 0.5 g and the medium pH was 7.3. Incubation was for 5.5 h. Less than 5% [^{14}C]substrate remained in the medium at the end of each incubation. See Table 3 for total nmol desaturation products, i.e. [^{14}C]linoleate and [^{14}C]linolenate, and Materials and Methods for other details of the incubations

Incubation temp. (°C)	Conversion (%)[a]	
	[^{14}C]18:1 to [^{14}C]18:3	[^{14}C]18:2 to [^{14}C]18:3
15	4.9	14.6
20	12.5	24.8
25	8.9	25.7
30	T[b]	18.9
35	4.6	15.7

[a] 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid
[b] Trace (less than 1%)

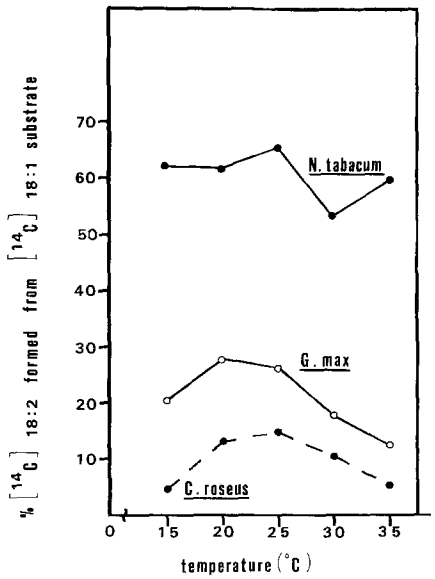

Fig. 5. Desaturation of [^{14}C]oleic acid (18:1) to [^{14}C]linoleic acid (18:2) as a function of temperature in cell suspension cultures of *N. tabacum*, *C. roseus* and *G. max*. Incubation medium (1.5 ml; pH 7.3) contained 20.8 nmol [^{14}C]oleate. Cell fresh weight was 0.5 g and incubation was for 5.5 h. Less than 5% (in the case of *G. max* and *C. roseus*) and 10% (in the case of *N. tabacum*) of [^{14}C]oleate remained in the medium at the end of incubation. See Table 3 for total nmol desaturation products, i.e. [^{14}C]linoleate, and Materials and Methods for other incubation details

tabacum. Essentially no change was observed in *G. max*. However, when the ratio of total [^{14}C]C_{18}-fatty acids to total [^{14}C]C_{16}-fatty acid (palmitic) were plotted (Fig. 4), a noticeable decrease of the ratio was observed as the temperature was increased. These data once more support the results of Jaworski et al. (1974).

Total [^{14}C]acetate incorporated was not affected by changing temperature (Table 3).

Desaturation of [^{14}C]Oleic and [^{14}C]Linoleic Acids

When [^{14}C]acetate is employed as a substrate, biosynthesis of the whole gamut of fatty acids is of course

Table 3. Total nmol of [^{14}C]substrates incorporated or desaturated by cell suspension cultures as a function of temperature. Incubation medium (1.5 ml; pH 7.3) contained 18.5 nmol [^{14}C]acetate, 20.8 nmol [^{14}C]oleate, or 19.8 nmol [^{14}C]linoleate.

Cell fresh weight added was 0.5 g and incubation was for 5.5 h. Less than 5% (in the case of *G. max* and *C. roseus*) or less than 10% (in the case of *N. tabacum*) of each substrate remained in the medium at the end of incubation. See Figs. 1–5 for [^{14}C]fatty acids synthesized, and Materials and Methods for other details of incubation

Incubation temp. (°C)	[^{14}C]acetate incorporated (initial concn. 18.5 nmol)			[^{14}C]oleate desaturated (initial concn. 20.8 nmol)			[^{14}C]linoleate desaturated (initial concn. 19.8 nmol)		
	G. max	*C. ros.*	*N. tab.*	*G. max*	*C. ros.*	*N. tab.*	*G. max*	*C. ros.*	*N. tab.*
15°	3.2	3.6	2.4	5.2	0.62	10.7	1.5	0	0
20°	2.8	2.6	2.2	8.4	1.6	8.2	2.5	0	0
25°	2.8	3.7	2.0	7.3	1.7	13.1	2.7	0	0
30°	2.5	2.4	2.2	3.6	1.4	6.8	2.3	0	0
35°	1.7	2.0	1.2	3.5	0.51	9.2	1.1	0	0

Table 4. The effect of pretreating cell cultures for 25 h at 15 and 35° C on subsequent [^{14}C]fatty-acid synthesis at 25° C. [^{14}C]Acetate (18.5 nmol in 1.5 ml medium; pH 7.3) was the substrate. Cell fresh weight was 0.5 g and incubation at 25° C was 4 h. Less than 3% [^{14}C]acetate remained in the medium at the end of incubation. [^{14}C]Acetate % incorporation into total [^{14}C]fatty acids was as follows: shifting from 15 to 25° C *G. max* incorporated 0.32%, *C. roseus* 9.2%, and *N. tabacum* 6.0%; at 25° C *G. max* incorporated 6.5%, *C. roseus* 19.5%, and *N. tabacum* 23.2%; shifting from 35 to 25° C *G. max* incorporated 3.0%, *C. roseus* 7.6% and *N. tabacum* 8.6%. See Materials and Methods for other details of incubation

Cell culture	[^{14}C]acetate incorporated into fatty acids (total nmol)			[^{14}C]fatty acids[a] synthesized (%)											
				16:0			18:0			18:1			18:2		
	15° → 25°	25°[b]	35° → 25°	15° → 25°	25°[b]	35° → 25°	15° → 25°	25°[b]	35° → 25°	15° → 25°	25°[b]	35° → 25°	15° → 25°	25°[b]	35° → 25°
G. max	0.06	1.2	0.56	50.0	35.1	32.9	10.0	10.8	9.7	20.0	37.8	51.3	15.0	13.5	4.9
C. ros.	1.7	3.6	1.4	45.8	36.8	33.9	9.2	5.3	3.2	36.6	48.7	52.8	5.1	6.6	8.7
N. tab.	1.1	4.3	1.6	45.9	30.7	39.7	8.3	6.9	5.1	12.4	14.9	17.7	30.9	46.5	35.2

[a] 16:0, palmitic acid; 18:0, stearic acid; 18:1 oleic acid; 18:2, linoleic acid

[b] [^{14}C]Fatty acids synthesized by cell cultures grown and incubated at 25° C

observed and interpretation of the results can be difficult. By employing C_{18}-fatty acids as substrates, the number of reactions involved is reduced and a more direct analysis of a specific reaction becomes possible. Thus [^{14}C]oleate and [^{14}C]linoleate were employed as substrates in order to examine the conversion of oleic to linoleic acid and linoleic to linolenic acid.

Figure 5 and Table 3 summarize the data obtained for desaturation of added [^{14}C]oleate as a function of temperature. The *N. tabacum* cells were the most active of the three cell lines in the conversion of oleate to linoleate, and were relatively insensitive to incubation temperatures. The other two cell lines, *C. roseus* and *G. max*, however, showed definite maxima in conversion of oleate to linoleate, namely between 20 and 25° C, with sharp decreases from 20 to 15° C and from 25 to 35° C. A similar result was observed in the conversion of [^{14}C]linoleate to [^{14}C]linolenate by *G. max* cells (Tables 2, 3). A similar effect of temperature on desaturation had been found in *Chlorella sorokiniana* (Patterson, 1970).

Effect of Temperature Pretreatment on [^{14}C]Acetate Incorporation by Cell Cultures Incubated at 25° C

Although viability was reduced when cell suspension cultures were grown at 25° C and then transferred to either 15 or 35° C for 25 h (Table 1), these cultures nevertheless still retained the capacity to absorb [^{14}C]acetate and incorporate the label into [^{14}C]fatty acids (Table 4). In general, there were marked shifts in the patterns of [^{14}C]fatty acids when the cell cultures were exposed to these temperature conditions. Most noticeable in all three cell cultures were the [^{14}C]palmitate shift as well as the [^{14}C]oleate and [^{14}C]linoleate perturbations. [^{14}C]Linolenate was formed in low amounts in all cell cultures and was not further considered. Shifting the temperature from 15 to 25° C increased [^{14}C]palmitate in all cell cultures; when the temperature shift was 35 to 25° C, more [^{14}C]oleate and in *C. roseus* and *N. tabacum* less [^{14}C]linoleate was formed.

Table 5. The effect of pretreatment temperature on the ratio of unsaturated [^{14}C]fatty acids:saturated [^{14}C]fatty acids developed during subsequent incubation at 15, 25 and 35° C.

Pretreatment was for 2 h and incubation was for 3 h. Substrate was [^{14}C]acetate (18.5 nmol in 1.5 ml medium; pH 7.3). Cell fresh weight was 0.5 g. Less than 5% (*G. max*), 7% (*C. roseus*), and 10% (*N. tabacum*) of [^{14}C]acetate remained in the medium at the end of incubation. Incorporation of [^{14}C]acetate into total [^{14}C]fatty acids was as follows: *G. max* between 4.3 and 12.4%; *C. roseus* between 8.6 and 18.4%; *N. tabacum* between 4.3 and 18.9%. See Materials and Methods for other incubation details

Pretreatment of cells[a]	Incubation temp. (°C)	[^{14}C]acetate incorporated into total fatty acids (nmol)			U/S=18:1+18:2+18:3/16:0+18:0		
		G. max	*C. ros.*	*N. tab.*	*G. max*	*C. ros.*	*N. tab.*
Pretreated 2 h at 15°	15	1.1	2.7	0.80	0.65	1.0	0.92
Maintained at 25°	15	1.6	1.9	1.0	2.3	1.8	0.91
Pretreated 2 h at 35°	15	1.7	3.4	3.3	0.69	2.1	1.4
Pretreated 2 h at 15°	25	0.80	2.6	0.85	0.58	0.71	0.81
Maintained at 25°	25	2.3	2.2	1.2	1.4	1.3	0.92
Pretreated 2 h at35°	25	1.5	2.8	3.5	0.33	1.3	0.91
Pretreated 2 h at 15°	35	1.4	2.1	0.80	0.74	0.62	0.65
Maintained at 25°	35	1.2	1.6	1.1	0.92	0.83	0.64
Pretreated 2 h at 35°	35	1.7	2.3	3.3	0.47	1.2	0.74

[a] Temperature in °C

Table 6. The effect of pretreatment temperature on subsequent desaturation of [^{14}C]oleic acid by cell cultures incubated 3.5 h at 15, 25 and 35° C. The substrate was 17.9 nmol [^{14}C]acetate in 1.5 ml medium at pH 7.3. Cell fresh weight was 0.5 g and pretreatment was for 2 h. Less than 5% (*G. max* and *C. roseus*) and 7% (*N. tabacum*) of [^{14}C]oleic acid remained in the medium at the end of incubation. See Materials and Methods for other incubation details

Treatment of cells[a]	Desaturation temp. (°C)	Total [^{14}C]desaturation products (nmol)			[^{14}C]18:2 (%)			[^{14}C]18:3 (%)
		G. max	*C. ros.*	*N. tab.*	*G. max*	*C. ros*[b]	*N. tab.*[c]	*G. max*
Pretreated 2 h at 15°	15	3.1	0.76	6.9	13.7	6.1	48.1	3.7
Maintained at 25°	15	3.0	0.80	6.5	16.5	5.9	44.2	0
Pretreated 2 h at 35°	15	2.3	0.33	7.5	8.2	2.6	49.9	4.7
Pretreated 2 h at 15°	25	5.1	1.2	8.1	18.8	10.2	56.0	9.7
Maintained at 25°	25	5.3	1.4	8.4	25.2	9.7	53.2	4.5
Pretreated 2 h at 35°	25	3.7	1.3	9.1	16.2	10.5	56.2	4.3
Pretreated 2 h at 15°	35	4.8	0.79	9.4	20.8	6.1	62.0	6.1
Maintained at 25°	35	6.3	1.0	8.5	29.2	8.5	55.0	6.2
Pretreated 2 h at 35°	35	3.9	1.3	9.3	18.4	10.2	61.0	3.6

[a] Temperatures in °C
[b] No [^{14}C]18:3 synthesized
[c] Trace levels of [^{14}C]18:3 formed

In another set of experiments, cell cultures grown at 25° C were transferred for 2 h to 15, 25 or 35° C, and were the incubated with [^{14}C]acetate for 4 h at 15, 25, 35° C to determine if a rapid temperature shift could perturb their fatty-acid synthesis patterns. The data in Table 5 are presented in summary form for simplicity. Of interest, the *G. max* cells responded to a brief exposure to different temperatures, i.e. there were shifts in the ratio of unsaturated/saturated products, whereas the *N. tabacum* cells responded only to a slight extent. The *C. roseus* cultures were intermediate between the other two cell cultures. Thus, it would appear that each cell line has mechanisms that respond differently in modulating their lipid (presumably membrane-lipid) synthesis patterns when exposed to different temperatures even for brief periods of time.

In order to limit the number of reactions involved in fatty-acid synthesis and modification, a final set of experiments were carried out with [^{14}C]oleate as the substrate. Table 6 summarizes the results. The response of the three different cell cultures was remarkably different. The *G. max* cell line consistently showed increases in conversion of 18:1 to 18:2 fatty acids when incubated with the substrate at 15, 25 and 35° C, although the capability was modified by

pretreatment at different temperatures. Similar effects were not observed with the *C. roseus* cultures. *N. tabacum* cells showed smaller increases in desaturation as a function of higher temperatures, and pretreatments at different temperatures had essentially no effect.

Concluding Remarks

The data presented here indicate that each of these cell lines is equipped with a genotype that permits maximal synthesis of specific C_{18}-fatty acids from [^{14}C]acetate and that each species has a distinct response in terms of fatty-acid synthesis to changing temperature regimes. For example, at low temperatures the *N. tabacum* cells tend to accumulate linoleic acid whereas the *C. roseus* cells tend to accumulate oleic acid. In all these cultures, the system involved in the elongation of palmitic acid to C_{18}-fatty acids was sensitive to increasing temperature, and this effect was influenced not only by the temperature at which the culture had been maintained prior to incubation, but also by the length of time that the cultures were held at that temperature. Patterson (1970) had noted a similar effect on the average chain length of endogenous fatty acids in *Chlorella sorokiniana*. Moreover, desaturating mechanisms leading to oleic-acid formation in *G. max* and *C. roseus* cells were stimulated by a reduction in temperature, and this response was again related to the initial temperature of the culture and the time spent at that temperature (Table 4, transfer from 35 to 25° C; Table 5, transfer from 25 to 15° C).

Desaturation mechanisms other than those involved in oleic-acid synthesis were also temperature dependent, and production of [^{14}C]linoleic acid increased between 20 and 25° C in *G. max* and in *N. tabacum* cells. The optimum temperature varied with cell line and in *G. max* was associated with stimulation of desaturating mechanisms leading to linolenic-acid formation.

Cell suspension cultures provide thus well-defined, sensitive materials for the study of temperature effects on fatty-acid synthesis. Since the temperature parameters have been established, it should be possible to design growth conditions by which fatty-acid synthesis can be so optimized that cell-free preparations can be successfully obtained for more detailed in-vitro studies.

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