Evidence for separate elongation enzymes for very-long-chain-fatty-acid synthesis in potato (*Solanum tuberosum*)

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Aging potato (*Solanum tuberosum*) tuber discs in a Ca^{2+} -containing medium resulted in increased rates of fatty acid labelling from $[1-^{14}\text{C}]$ acetate with time. Maximal labelling rates were seen after 6–8 h aging in a number of varieties. Saturated very-long-chain fatty acids (C_{20} and particularly C_{22} and C_{24}) were very poorly labelled in freshly cut tissue. They were synthesized in increasing amounts and in a homologous sequence with progressive aging times. Use of increasing induction times and cycloheximide or puromycin as protein-synthesis inhibitors indicated that the sequence of fatty acid elongation was dependent on protein synthesis *de novo* and was controlled by three separate specific elongase enzymes.

INTRODUCTION

Very-long-chain fatty acids (VLCFA) are significant constituents of the cuticular surface layer of leaves and stems. They are also important intermediates in the biosynthesis of many of the individual components of the surface layers (cutin, suberin and waxes) of plant tissues (Harwood, 1975; Kolattukudy, 1975, 1980).

The biosynthesis of VLCFA has been studied in several tissues which are capable of high rates of fatty acid elongation (cf. Harwood, 1979). One such system is the use of discs from aged potato (*Solanum tuberosum*) tubers (e.g. Willemot & Stumpf, 1967a). When a dormant potato tuber is sliced, at room temperatures, there is an increase in respiration and the synthesis of protein, RNA and fatty acids increases (Laties, 1962, 1964; Willemot & Stumpf, 1967a,b). There is also an increase in the rate of membrane and mitochondrial formation. These phenomena are reported to be dependent on the production of protein and RNA, and to involve de-repression of enzyme synthesis (Click & Hackett, 1963; Tuan & Bonner, 1964). Thus, in contrast with other tissues such as pea (*Pisum sativum*) (Bolton & Harwood, 1977) and leek (*Allium porrum*) (Cassagne & Lessire, 1978), the formation of significant quantities of VLCFA in potato is highly dependent on the synthesis of new protein and, possibly, RNA.

The synthesis of saturated fatty acids by aged potato slices involves, like that in pea, at least three enzyme systems (Bolton & Harwood, 1976a). The first, a 'de novo' fatty acid synthase system, has been partially purified from potato by Huang & Stumpf (1971). They demonstrated that the chain length of the fatty acid products could be altered by varying the incubation conditions, yet the maximum chain length was always C_{16} . About 50–70% of the newly synthesized products occurred as non-esterified fatty acids, but the palmitate content of the esterified fraction was present as the acyl carrier protein (ACP) thioester. An enzyme system for elongation of palmitate to stearate has been found in a

variety of plant tissues (Hawke & Stumpf, 1965; Harwood & Stumpf, 1971; Jaworski *et al.*, 1974; Weaire & Kekwick, 1975). This system, shown to utilize palmitoyl-ACP as substrate, was inhibited by arsenite in germinating peas (Harwood & Stumpf, 1971). It seems probable that palmitate-elongation systems differ from fatty acid synthase only in their use of a C_{16} -specific condensing enzyme (β -oxo acyl-ACP synthase II) (Shimikata & Stumpf, 1982) and that the other enzymes involved are synonymous with those used in fatty acid synthesis *de novo* (cf. Slabas *et al.*, 1984).

An additional elongation system in germinating pea converts stearate into VLCFA, is temperature-sensitive and is inhibited by fluoride and thiocarbamate herbicides (Harwood & Stumpf, 1971). Thiocarbamate herbicides also had an inhibitory effect on VLCFA synthesis in aged potato discs, indicating the presence of a similar stearate-elongating system (Bolton & Harwood, 1976b). In jojoba (*Simmondsia chinensis*) seeds (Pollard *et al.*, 1979) and leek epidermal-cell fractions (Cassagne & Lessire, 1978) stearoyl elongase demonstrated high activity with stearoyl-CoA as substrate. However, in the microsomal fraction from germinating pea, neither exogenous stearoyl-CoA nor stearoyl-ACP was utilized for VLCFA formation (Sanchez & Harwood, 1981).

It is not known how many elongation enzymes are required for the chain lengthening of stearate to the various VLCFA. Indirect evidence based on differential effects of ACP (or CoA) and ATP on the leek system have been interpreted as indicating more than one system (Cassagne & Lessire, 1978). Recent studies with Triton X-100-solubilized preparations from leek provided evidence that one of these systems was specific for the elongation of stearoyl-CoA to arachidoyl-CoA (Agrawal & Stumpf, 1985). Studies with barley (*Hordeum vulgare*) mutants also suggested several elongation systems (von Wettstein-Knowles, 1979).

The question of the substrate specificity for individual elongation systems could be answered if purified proteins were available. However, these membrane-bound enzymes

Abbreviations used: VLCFA, very-long-chain fatty acids; ACP, acyl carrier protein.

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have proved difficult to purify (cf. Agrawal & Stumpf, 1985). The availability of the aged-potato-disc system, where elongation activity could be induced, did, however, afford us the opportunity to tackle the question as to whether a single elongation system could produce VLCFA up to C_{24} or whether separate elongation systems could be demonstrated. The results, which are now reported, provide evidence for three elongation systems, namely those responsible for the formation of arachidic, behenic and lignoceric acids respectively.

MATERIALS AND METHODS

Potatoes (*Solanum tuberosum* var. King Edward, Romano, Wilja and Cambridge) were obtained from the local market and stored for up to a week at 4 °C in the dark. Sodium [$1-^{14}C$]acetate (sp. radioactivity 2.146 GBq/mmol) was purchased from Amersham International, Amersham, Bucks., U.K. Other chemicals were of the best available grades and were obtained from Sigma Chemical Co. or BDH (both of Poole, Dorset, U.K.)

Tissue preparation

Cores of peeled potato tissue were taken with a 5 mm-diameter cork borer and then sliced into 1 mm-thick discs. Aging solution [$100 \mu M$ -CaSO₄/chloramphenicol ($50 \mu g/ml$)] was used to rinse the freshly cut discs. The discs were then aged as described by Willemot & Stumpf (1967a) at 25 °C for up to 24 h. In some experiments the aging solution also included cycloheximide at a concentration of $10 \mu g/ml$ or puromycin at a concentration of $100 \mu M$. After various periods, discs were removed and quickly rinsed with distilled water.

Assay of fatty acid synthesis

Assay incubations were carried out in 1 ml of 0.1 M-potassium phosphate buffer, pH 5.5, containing $50 \mu g$ of chloramphenicol and $1 \mu Ci$ of sodium [$1-^{14}C$]acetate at 25 °C for 2, 4 or 6 h. All assays were carried out in triplicate with ten discs per sample. The assays were stopped by the addition of 0.2 ml of 20% (v/v) H₂SO₄. After the mixture had been left for 2 min, the acid was removed and the tissue rinsed three times with distilled water. The discs were then heated in sealed tubes at 70 °C for 30 mins with 2.5 ml of methanol. After the addition of 5 ml of chloroform, extraction was continued overnight (~15 h) at room temperature. The discs were then re-extracted for 2 h with 2.5 ml of chloroform/methanol (2:1, v/v). The combined extracts were then evaporated to dryness under N₂. Fatty acid methyl esters were prepared by transmethylation with 1 ml of 2.5% (v/v) H₂SO₄ in anhydrous methanol at 70 °C for 2 h. Samples of the light-petroleum (b.p. 60–80 °C) extract were then taken for radioactivity measurement and g.l.c. analysis. These procedures had previously been found to be quantitative. The methyl esters were routinely separated in 3% (w/v) SE-30 on 80/100 mesh Supelcoport columns using a Pye 104 gas chromatogram coupled to a Panax gas flow proportional counter. A temperature program was used that consisted of a 17 min isothermal period at 175 °C, a rise to 230 °C in 3 min, followed by maintenance at the latter temperature for a further 20 min. Fatty acids were also identified and separated as previously reported (Bolton & Harwood, 1976b). All radioactive samples were counted in a scintillant consisting of PCS (Amersham/Searle; The Radiochem-

Fig. 1. Effect of aging time on fatty-acid-synthetic activity in three varieties of potato

Discs of potato tuber were aged for periods up to 24 h and then assayed for fatty-acid-synthesis activity for 4 h as described in the Materials and methods section. Results are means $\pm$ s.d. for an experiment in triplicate [Cambridge (○) and King Edward (●)] or quadruplicate (Romano, □).

ical Centre, Amersham, Bucks., U.K.) / xylene (2:1, v/v). The efficiency of counting was corrected for each sample by the external-standards-ratio method.

RESULTS AND DISCUSSION

It has long been known that sliced potato tissue shows a rapid increase in fatty-acid-synthetic activity in the presence of CaSO₄ solution (Willemot & Stumpf, 1967a). Moreover, the results in Fig. 1 demonstrate that, for a number of varieties of potato, a peak in fatty-acid-synthetic activity occurs between 6 and 8 h of aging. As would be expected with plant tissue of such variable supply, overall levels of activity varied considerably between experiments. However, a peak in activity was always found between 6 and 8 h. This indicates that total fatty-acid-synthetic activity is under some common control mechanism in response to wounding. Although various natural plant hormones have been suggested as possible regulators (cf. Smith & Rappaport, 1961), no conclusive results for their control in fatty-acid-synthetic activity have yet been found.

Even after 24 h there was often about 50% more fatty-acid-synthetic activity present than in the initial stages. Kolattukudy & Dean (1974) found that the major part of suberin deposition in sliced potato tuber occurred between 5 and 7 days after excision. Thus the major rise in fatty-acid-synthetic activity occurs very quickly compared with the actual wound healing and probably reflects an immediate requirement for membrane replacement. However, Willemot & Stumpf (1967b) have speculated that slicing causes de-repression of short-lived RNA species that can then direct synthesis of proteins, including fatty-acid-producing enzymes.

The distribution of radioactivity in the fatty acids synthesized as a function of aging time is shown for the potato variety Romano in Table 1. Fatty acid methyl esters were usually separated on the basis of carbon chain length. In this particular experiment it was also shown that radioactivity was present in palmitate, palmitoleate

Table 1. Effect of aging on products of fatty acid synthesis from [1-¹⁴C]acetate

Discs of Romano potato tuber were aged as described in the Materials and methods section. Results represent means $\pm$ S.D. ($n = 3$) Abbreviations: tr, $< 0.5\%$; n.d., not detected. Fatty acid synthesis assay was for 4 h.

Aging time (h)	Chain length . . .	Distribution of radioactivity (% of total [¹⁴ C]fatty acids)					
		$< C_{16}$	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}
0		1 $\pm$ tr	44 $\pm$ 1	51 $\pm$ 1	4 $\pm$ 1	n.d.	n.d.
2		3 $\pm$ tr	50 $\pm$ 3	42 $\pm$ 4	5 $\pm$ 1	1 $\pm$ tr	n.d.
4		2 $\pm$ 1	56 $\pm$ 2	35 $\pm$ 2	6 $\pm$ 1	2 $\pm$ 1	n.d.
8		1 $\pm$ tr	50 $\pm$ 2	41 $\pm$ 2	5 $\pm$ 1	3 $\pm$ 1	1 $\pm$ tr
12		1 $\pm$ tr	47 $\pm$ 3	45 $\pm$ 3	5 $\pm$ 1	3 $\pm$ 1	1 $\pm$ tr
16		2 $\pm$ tr	47 $\pm$ 4	39 $\pm$ 2	5 $\pm$ 1	4 $\pm$ 1	2 $\pm$ tr
20		1 $\pm$ 1	44 $\pm$ 1	39 $\pm$ 1	8 $\pm$ tr	5 $\pm$ tr	4 $\pm$ 1
24		1 $\pm$ tr	46 $\pm$ 1	37 $\pm$ 5	5 $\pm$ tr	6 $\pm$ 3	5 $\pm$ 2

(traces), stearate, oleate and linoleate, and the VLCFA were arachidic, behenic and lignoceric acids. The maximum chain-length fatty acid produced during these aging experiments was lignoceric acid, in agreement with other workers (Kolattukudy, 1975; Willemot & Stumpf, 1967a). Relative proportions of radioactivity present in C_{16} and C_{18} acids varied between varieties (cf. Table 3 below). However, all varieties demonstrated a decrease in the relative percentage of radiolabelled C_{18} acids during the initial increase in the percentage of VLCFA produced with aging time. This would be expected if some of the newly synthesized C_{18} fatty acids were to act as substrates for elongation to the VLCFA. Bolton & Harwood (1976a) had previously shown that VLCFA represent an increasing percentage of the radioactive fatty acids produced in King Edward potato discs up to 18 h aging. Similarly, in the variety Romano the relative production increased for up to 20 h of aging, after which time it appeared to be at a maximum relative level. Careful examination of the first appearance of arachidic, behenic and lignoceric acids revealed a chronological appearance of these acids (Table 1). Similar results were also found for King Edward, Wilja and Cambridge varieties (cf. Table 3 below and results not shown). Thus, in all the potato varieties we examined, a very similar appearance of VLCFA of increasing chain length was seen. Since freshly cut potato discs were not capable of synthesizing behenic and lignoceric acids (Table 1), this would suggest that the production of these acids, at least, is under separate control from the production of fatty acids up to a chain length of C_{18} . Thus the aging of potato discs is responsible for the synthesis of new fatty acids not normally produced in detectable quantities in the dormant tuber. This situation differs from the germinating pea (Bolton & Harwood, 1977) and leek epidermal-cell systems (Cassagne & Lessire, 1978), where the VLCFA are produced in ratios depending upon the substrate applied to the assay *in vitro*.

In order to check that the appearance of increasing-chain-length fatty acids was not simply a function of substrate availability, discs of King Edward potato were aged for various times and the duration of the assay varied. The results in Table 2 show that the period of the assay had much less effect on the distribution of radioactivity in the fatty acid products than the aging period. The general chronological appearance of radio-

Table 2. Effect of varying assay time on products of fatty acid synthesis from [1-¹⁴C]acetate

Discs of King Edward potato tuber were aged as described in the Materials and methods section, then assayed for fatty-acid-synthesis activity for 2, 4 or 6 h. Results are means $\pm$ S.D. ($n = 3$) Abbreviations are as for Table 1.

Aging time (h)	Assay time (h)	Chain length . . .	Distribution of radioactivity (% of total [¹⁴ C]fatty acids)			
			$C_{16} + C_{18}$	C_{20}	C_{22}	C_{24}
0	2		88 $\pm$ 2	6 $\pm$ 3	n.d.	n.d.
	4		89 $\pm$ 2	6 $\pm$ 1	3 $\pm$ 1	n.d.
	6		88 $\pm$ 1	6 $\pm$ 1	4 $\pm$ 1	n.d.
2	2		92 $\pm$ 3	3 $\pm$ 1	2 $\pm$ 1	n.d.
	4		86 $\pm$ 1	6 $\pm$ 1	4 $\pm$ 1	n.d.
	6		85 $\pm$ 1	7 $\pm$ 1	7 $\pm$ 1	n.d.
4	2		88 $\pm$ 3	4 $\pm$ 1	7 $\pm$ 2	n.d.
	4		87 $\pm$ 2	5 $\pm$ tr	7 $\pm$ 1	n.d.
	6		84 $\pm$ 1	7 $\pm$ tr	7 $\pm$ tr	1 $\pm$ 1
6	2		85 $\pm$ 3	6 $\pm$ 3	7 $\pm$ 1	n.d.
	4		82 $\pm$ 2	7 $\pm$ 1	7 $\pm$ 2	2 $\pm$ tr
	6		79 $\pm$ 3	7 $\pm$ 1	8 $\pm$ 1	3 $\pm$ 1
8	2		73 $\pm$ 3	9 $\pm$ 1	10 $\pm$ 1	5 $\pm$ 1
	4		72 $\pm$ 2	10 $\pm$ 1	10 $\pm$ 2	5 $\pm$ 2
	6		71 $\pm$ 3	11 $\pm$ 1	12 $\pm$ 1	5 $\pm$ 1

activity in a homologous series of fatty acids was still observed. Thus, after 4 h aging, lignoceric acid becomes labelled only when provided with acetate for 6 h. Although the assay medium did not contain any CaSO_4 , residual Ca^{2+} in the tissues would catalyse slower rates of aging, and one would therefore expect to find longer-chain-length fatty acids becoming labelled at longer incubation times. Thus, although the period of incubation has a small influence on the maximum-chain-length fatty acid produced, the initial appearance of a given chain length is still linked primarily to the period of tissue aging.

Cycloheximide inhibits DNA and protein synthesis and prevents translation by blocking the formation of the

Table 3. Effect of cycloheximide on products of fatty acid synthesis from [1-¹⁴C]acetate

Discs of King Edward potato tuber were aged for various times in the presence or absence of cycloheximide (10 µg/ml), then assayed for fatty acid synthesis for 6 h as described in the Materials and methods section. Results are means ± S.D. (*n* = 3). Abbreviations are as for Table 1.

Treatment	10 ⁻³ × Total radioactivity (c.p.m.)	Aging time (h)	Chain length . . .	Distribution of radioactivity (% of total [¹⁴ C]fatty acids)					
				< C ₁₆	C ₁₆	C ₁₈	C ₂₀	C ₂₂	C ₂₄
Control	211.6 ± 10.2	0		2 ± 1	25 ± 2	68 ± 3	5 ± 2	n.d.	n.d.
	289.6 ± 16.5	2		2 ± 1	26 ± tr	59 ± 3	6 ± 1	7 ± 2	2 ± tr
	292.3 ± 13.6	4		2 ± 1	30 ± tr	53 ± 2	6 ± 1	7 ± tr	3 ± 1
	351.5 ± 6.3	6		3 ± 2	24 ± 1	52 ± 2	9 ± 2	8 ± tr	4 ± 2
	263.2 ± 14.3	8		6 ± 4	26 ± 3	46 ± 3	9 ± 1	10 ± 1	4 ± 2
+ Cycloheximide	250.7 ± 10.7	2		2 ± 1	21 ± 4	75 ± 4	2 ± 1	n.d.	n.d.
	270.0 ± 14.3	4		4 ± 2	25 ± 2	68 ± 3	3 ± 1	n.d.	n.d.
	316.1 ± 21.3	6		3 ± 2	25 ± tr	70 ± 1	3 ± 2	n.d.	n.d.
	169.4 ± 10.2	8		4 ± 2	30 ± 2	63 ± 5	3 ± 2	n.d.	n.d.

Table 4. Effects of cycloheximide additions on the assay of fatty acid synthesis

Discs of King Edward potato tuber were aged for various times in the presence or absence of cycloheximide (10 µg/ml) and then assayed for fatty acid synthesis for 4 h in the presence or absence of cycloheximide (10 µg/ml) as described in the Materials and methods section. Results are means ± S.D. (*n* = 3). Abbreviations are as for Table 1.

Aging time (h)	Cycloheximide addition		10 ⁻³ × Total radioactivity (c.p.m.)	Chain length . . .	Distribution of radioactivity (% of total [¹⁴ C]fatty acids)				
	Aging incubation	Assay incubation			C ₁₆	C ₁₈	C ₂₀	C ₂₂	C ₂₄
0	—	—	57.4 ± 9.1		27 ± 4	71 ± 4	2 ± 1	n.d.	n.d.
	—	+	68.8 ± 22.8		26 ± 1	72 ± 3	1 ± tr	n.d.	n.d.
8	—	—	185.2 ± 25.5		33 ± 2	56 ± 2	4 ± 1	4 ± 2	2 ± 1
	—	+	202.2 ± 31.9		29 ± 3	56 ± 8	4 ± 1	5 ± 1	2 ± 2
8	+	—	89.7 ± 3.4		19 ± 4	79 ± 5	1 ± tr	n.d.	n.d.
	+	+	92.2 ± 25.2		20 ± 6	80 ± 6	n.d.	n.d.	n.d.

peptide bond at the level of the ribosome (de Kloet, 1966). Willemot & Stumpf (1967*b*) found that cycloheximide at concentrations of 2 µg/ml would almost completely inhibit the rise in fatty acid synthesis normally observed during aging of discs of the potato varieties White Rose or Russet. This was also shown to inhibit protein and RNA synthesis, thus demonstrating that the rise in fatty acid synthesis upon slicing was dependent on increased protein and RNA synthesis. However, no results for the effect of cycloheximide on the distribution of radioactivity in fatty acids were presented.

A cycloheximide concentration of 10 µg/ml did not fully inhibit the rise in fatty acid synthase activity in discs of King Edward potato. However, the maximum levels of activity after 8 h was only about 60% of the discs aged without cycloheximide (cf. Table 3). The results in Table 3 show the effect of cycloheximide at 10 µg/ml in the aging solution with time upon the products of fatty acid synthesis. In this particular experiment the assay time was 6 h to achieve maximal possible incorporation into the longest-chain-length fatty acids, and cycloheximide was not included in the assay medium. 2 h of aging was required before radioactivity was detected in behenic and

lignoceric acids in the control samples. Whereas the relative proportion of label in palmitate remained comparatively constant at all aging times, that of stearate declined in proportion to the increase in VLCFA labelling (which increased from 5 to 23%). Considering that the total production rate of fatty acids increases considerably during this period (cf. Fig. 1) it is clear that VLCFA production is increased dramatically during the initial hours of aging. Cycloheximide was found to abolish completely the induction of behenic and lignoceric acid production and also to inhibit the increase in the relative proportion of arachidic acid normally observed. Thus cycloheximide, by inhibiting nuclear-coded protein synthesis, is able to prevent both the absolute and relative increases in VLCFA synthesis. The results in Table 4 demonstrate that additions of cycloheximide to the assay incubation cause no significant difference to the pattern of fatty acids produced, although the overall production rate was usually marginally increased. Aging for 8 h in the presence of cycloheximide at 2, 5 or 20 µg/ml and with cycloheximide at 10 µg/ml in the assay medium produced a distribution of labelled fatty acids essentially identical with that obtained with the

Table 5. Effect of sequential treatment of aged King Edward potato discs with cycloheximide

Discs of King Edward potato tuber were aged for up to 8 h; after the periods shown, some discs were rinsed and transferred to aging solution containing 10 µg of cycloheximide/ml. Assays for fatty acid synthesis were for 4 h. Results are means ± s.d. ($n = 3$). Abbreviations are as for Table 1. The asterisk (*) means five discs each of 8 h control + 8 h cycloheximide treatment.

Treatment	Aging time (h)	$10^{-3} \times$ Total radioactivity (c.p.m.)	Chain length . . .	Distribution of radioactivity (% of total [^{14}C]fatty acids)					
				< C ₁₆	C ₁₆	C ₁₈	C ₂₀	C ₂₂	C ₂₄
Control	0	38.0 ± 6.1		9 ± 2	24 ± 5	69 ± 5	tr	n.d.	n.d.
	2	64.3 ± 16.6		3 ± 2	27 ± tr	57 ± 1	7 ± tr	6 ± tr	n.d.
	4	73.9 ± 3.8		2 ± 1	25 ± 3	56 ± 4	9 ± tr	8 ± 1	n.d.
	6	168.3 ± 21.6		1 ± tr	25 ± 1	51 ± 2	10 ± 1	11 ± 1	3 ± 1
	8	235.9 ± 5.2		3 ± 1	23 ± tr	48 ± 4	10 ± 2	11 ± 1	5 ± tr
+ Cycloheximide	8	88.2 ± 7.9		4 ± 3	24 ± 1	70 ± 4	1 ± tr	n.d.	n.d.
Control period + cycloheximide treatment period	1 + 1	85.5 ± 2.7		4 ± 1	19 ± 1	75 ± 2	3 ± tr	n.d.	n.d.
	2 + 2	87.4 ± 11.3		4 ± tr	24 ± 1	71 ± 3	2 ± tr	tr	n.d.
	3 + 3	137.3 ± 11.5		3 ± 3	24 ± 2	67 ± 3	4 ± 2	2 ± 1	n.d.
	4 + 4	166.2 ± 2.4		3 ± 2	28 ± tr	59 ± 1	5 ± tr	5 ± 1	1 ± tr
Hybrid sample*	8	187.5 ± 7.2		3 ± 2	25 ± 3	56 ± 4	7 ± 1	6 ± 1	3 ± tr

aging treatment in Table 4 (10 µg of cycloheximide/ml; results not shown). These results confirm those in Table 2 and indicate that the treatment during the aging incubation is the determining factor in the pattern of fatty acids produced.

Since we had found, firstly, that there was a sequential production of VLCFA of increasing chain length (Table 1) and, secondly, that cycloheximide could inhibit completely the induction of behenic and lignoceric acid synthesis, we tried experiments where cycloheximide was added after different aging periods. The rationale behind these experiments was to attempt to block selectively the induction of the C₂₀- and C₂₂-fatty-acid elongation systems and so provide more evidence that chain-length-specific elongases were present. The results for a typical experiment with King Edward potato are shown in Table 5. All assays were carried out in the absence of cycloheximide. The control showed a typical chronological development in the appearance of radioactivity in C₂₀, C₂₂ and C₂₄ fatty acids. At the same time the relative proportion of radioactivity in C₁₈ fatty acids decreased as that in VLCFA increased. Aging in the presence of cycloheximide for 8 h gave radioactive-fatty-acid pattern very similar to zero-time samples, except for slightly more arachidate. This again confirmed the inhibitory effect of cycloheximide on the formation of behenic and lignoceric acids. In order to check that the effect of cycloheximide was not a direct enzyme inhibition, equal numbers of discs from 8 h aging in the presence and absence of cycloheximide were mixed and assayed. The distribution of radioactivity in fatty acids from these samples (Table 5) is seen to resemble very closely the average distribution for samples obtained from 8 h aging in the presence and absence of cycloheximide. Thus the effect of cycloheximide on VLCFA synthesis was not by direct enzyme(s) inhibition. The results of delayed cycloheximide addition to the aging solution (Table 5) indicated that the enzymes responsible for the production of C₂₀, C₂₂ and C₂₄ fatty acids become active in a chronological sequence related to the aging time and that this activation is due to new protein synthesis. Thus, for instance, although discs in the transfer treatment of 3 h aging in the absence + 3 h

aging in the presence of cycloheximide had been aged for a total of 6 h, there was no radioactivity in lignoceric acid. The control sample for 6 h aging had 3% of total radioactivity in lignoceric acid; moreover, the total radioactivity in the two treatments was not significantly different. Similarly, aging for 4 h in the absence then 4 h in the presence of cycloheximide resulted in the production of only about 10% of the total products as VLCFA. Lignoceric acid was only synthesized in very small amounts, whereas in the control (8 h aging), 5% of the radiolabelled product was lignoceric acid. Thus in the presence of cycloheximide the induction of VLCFA synthesis appears to be completely blocked.

The control samples seemed to produce slightly higher percentages of VLCFA for a given aging time period when compared with the test samples aged for the same time in the absence and then aged further in the presence of cycloheximide (compare 2 h control with 2 + 2 h test, or 4 h control with 4 + 4 h test; Table 5). We consider this is probably due to continued aging during the assay as discussed above in connection with Tables 2 and 4. Indeed, experiments on Ca²⁺ uptake during aging and subsequent loss during incubations confirmed that high levels of Ca²⁺ were retained within potato discs and could, presumably, lead to further enzyme induction in control tissues. Such induction was prevented during the assay of test samples, where it was shown (by monitoring [^{14}C]leucine incorporation into protein) that sufficient residual cycloheximide was present to prevent protein synthesis (K. A. Walker & J. L. Harwood, unpublished work).

It is well known that some lipid-metabolizing enzymes (e.g. lipolytic acyl hydrolase and lipoxygenase activities) display very different activities in different potato varieties (cf. Galliard & Matthew, 1973; Moreau, 1985). Therefore, in order to check that the results obtained for King Edward were applicable generally, we repeated the cycloheximide experiments of Table 5 with another potato variety (Wilja). The original percentages of C₁₆ and C₁₈ products were different from King Edward, with higher percentages of C₁₆ products in the 4 h assay period. However, a chronological sequence in the appearance of

behenic and lignoceric acids was seen again upon aging (results not shown). Aging in the absence, and then the presence, of cycloheximide resulted in a pattern of fatty acid synthesis which was again typical of the period of aging in the absence of cycloheximide. It is clear, therefore, that in this potato variety also, the induction of enzymes required for VLCFA synthesis is blocked by cycloheximide. Furthermore, the ability of this inhibitor to prevent the sequential appearance of increasing-chain-length products provides further evidence for separate elongation systems.

The effect of puromycin, another protein-synthesis inhibitor (cf. Colombo *et al.*, 1966) was of interest because of the different mode of inhibition. Puromycin causes premature release of partially formed protein chains from ribosomes. Used at a concentration of 100 μ M, in experiments similar to that in Table 3, puromycin had essentially the same effects as cycloheximide upon the products of fatty acid synthase in aged King Edward potato discs (results not shown).

The experiments with protein-synthesis inhibitors in aged potato tuber discs therefore indicate a sequential induction of three elongation systems, each responsible for the synthesis of a different-chain-length fatty acid. These results give additional evidence for separate elongases, one of which was also suggested by experiments with leek tissue (Agrawal & Stumpf, 1985; Lessire *et al.*, 1985). The increase in relative and absolute quantities of VLCFA during the initial hours of aging of the potato discs suggests that the elongase enzymes responsible are themselves synthesized *de novo* in a homologous sequence in response to the slicing stimulus. Willemot & Stumpf (1967*b*) speculated that slicing causes de-repression of short-lived RNA species that can then direct synthesis of proteins including the enzymes for fatty acid synthesis. With respect to the control of the sequential production of C₂₀, C₂₂ and C₂₄ fatty acids, this possibility requires further investigation.

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