100/120 3 mm i.d., 2 ft column at 210 °C. The ionizing current was 60 μA and the ionizing voltage 70 eV. There were two GLC peaks, most likely due to the pyro and the isopyro forms of the derivative. The first peak was very much larger than the second. Several spectra were obtained from the front, apex, and rear of the two peaks from the muscle extract and all were identical with that from the authentic 25-OH-D_3. A typical spectrum is shown in Figure 2 which shows molecular ion with m/e at 578 representing the 25-OH-D_3 diheptafluorobutyrate less a molecule of heptafluorobutyric acid. This loss takes place during GLC/mass spectrometry as the parent ion 792 was obtained when the derivative from the pure compound was introduced by the direct probe. Even by direct probe mass spectrometry, the first loss was a molecule of heptafluorobutyric acid, and thereafter the fragments were identical with that by GLC/mass spectrometry. The characteristic ions of 25-OH-D_3 diheptafluorobutyrate are indicated in the spectrum.

Recovery Data. Table I shows the recovery of 25-OH-D_3 from the liver, muscle, and kidney samples, respectively. In these experiments known amounts of 25-OH-D_3 in ethanol were added to the tissue in the blender cup at the extraction step and carried through the entire procedure. An unfortified sample was run along with each set of fortified samples. The concentrations of 25-OH-D_3 in the fortified and unfortified samples were calculated from peak height responses by comparison with the responses from appropriate standards. The fortification levels were in the range of 10–70 ng/g for the liver and kidney and 10–100 ng/g for the muscle. The average values for the unfortified samples were subtracted from the fortified ones in calculating the percent recovery which were 88 ± 4.2, 86 ± 5.4, and 89 ± 8.4% for the liver, muscle, and kidney, respectively.

Endogenous Level of 25-OH-D_3. Since the endogenous level of 25-OH-D_3 in the blood will vary according to the diet and exposure to sunlight, it was expected that the endogenous level in the liver, muscle, and kidney will vary from animal to animal. Replicate analyses were made for each tissue obtained from the same cow raised in Michigan, and these are shown in Table II. No definite conclusions can be made because of the limited number of tissues analyzed. It appears that among the three tissues, the kidney level was the highest and the muscle the lowest. The levels observed were 5 to 10 ppb in the kidney, 3 to 5 ppb in the liver, and 1.5 to 3.4 ppb in the muscle. The data also indicate good reproducibility for replicate analyses for each tissue from the same cow.

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Effect of High Temperature on CPTA-Induced Carotenoid Biosynthesis in Ripening Tomato Fruits

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Detached fruits of the normal red and high-beta tomato genotypes (*Lycopersicon esculentum* cv.) were dipped in an aqueous solution containing CPTA. The treated fruits with corresponding untreated control fruits were ripened at either 21 or 32 °C for 6 and 12 days in order to determine the effect of the higher temperature on CPTA-induced carotenoid biosynthesis in the ripening tomato. The lycopene content of both the normal red and high-beta fruits treated with CPTA increased during ripening at 21 °C and decreased when ripened at 32 °C but the inhibitory effect of high temperature was more pronounced in the high-beta genotype. CPTA treatment did not overcome the temperature-inhibited step in carotenoid biosynthesis in the tomato.

The alteration of the carotenoid biosynthetic pathway induced by CPTA, 2-(4-chlorophenylthio)triethylamine hydrochloride, has been reported in numerous carotenogenic systems (Batra et al., 1973; Coggins et al., 1970; Elahi et al., 1973; Hsu et al., 1972; Poling et al., 1975; Yokoyama et al., 1971, 1972). The inhibition of the biosynthetic pathway at the cyclization step(s) with consequent accumulation of the acyclic intermediates is the most commonly observed effect of this bioregulator. The changes in the biosynthetic pattern are complex and extend beyond simple cyclase inhibition to include increased synthesis of the more saturated polyene precursors (Elahi et al., 1973) as well as other lipids (Hayman et al., 1974).

Lycopene synthesis in the fruit of the normal red tomato genotype (*Lycopersicon esculentum* cv.) is inhibited at a temperature of 32 °C or higher while β-carotene synthesis is unaffected (Goodwin and Jamikorn, 1952; Tomes, 1963). Rabinowitch and Rudich (1972) reported that treatment

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of "Moneymaker" tomato fruits with CPTA with or without "Ethephon" (2-chloroethylphosphonic acid) prior to ripening at 32 °C resulted in the accumulation of lycopene such that the ripe fruit appeared red. Although no qualitative or quantitative data were presented, it was suggested that CPTA induced lycopene synthesis by an alternate pathway which is not affected by high-temperature ripening. Tomes (1963) and Tomes et al. (1956) have previously shown that ripening at 32 °C reduced the amount of β-carotene synthesized by the high-beta fruit. The effect on lycopene synthesis was rather inconclusive because of the small amount of the pigment present in the high-beta fruit (Tomes, 1963; Tomes et al., 1956, 1958).

The objectives of this investigation were to study the effects of CPTA on the carotenoid composition of detached tomato fruits of the high-beta and normal red genotypes (*Lycopersicon esculentum* cv.) ripened at a higher temperature and to determine if CPTA can overcome a temperature-inhibited step in carotenoid biosynthesis.

MATERIALS AND METHODS

High-beta tomato fruits were harvested from field-grown plants at the beaker stage of maturity. Seeds were generously provided by Dr. M. L. Tomes, Purdue University, Lafayette, Ind. Tomato fruits of the normal red line were purchased from a local market at the mature green stage of maturity. Genotypes were cultivars of *Lycopersicon esculentum*.

The fruits were washed in distilled water, dried, and dipped for 1 min in a CPTA solution (4300 μg/mL) containing 1% Tween 80 as a surfactant. Controls were dipped in water containing 1% Tween 80 without CPTA. CPTA was supplied in a water-soluble form by AmChem Products Inc., Ambler, Pa. Fruits were allowed to ripen at 21 and 32 °C in dual environmental growth chambers under constant illumination for 6 and 12 days at each temperature. The fruits were then frozen until analyzed in duplicate. Samples were composite homogenates of five fruits of uniform size.

Solvents and chromatographic adsorbents were prepared as described (Raymundo et al., 1967). The extraction and purification procedure reported previously (Raymundo et al., 1967, 1970) was slightly modified as follows: when ζ-carotene was contaminated with other pigments, it was rechromatographed on neutral Alumina II and eluted with 2% Me_2CO in light petrol. γ-Carotene was rechromatographed on neutral Alumina III and eluted with light petrol. Carotenoids were identified on the basis of their position on the MgO column and their absorption spectra (Davies, 1976).

RESULTS AND DISCUSSION

Treatment of normal red tomato fruit with CPTA generally resulted in an increased synthesis of phytoene, phytofluene, ζ-carotene, lycopene, and γ-carotene with a concomitant decrease in the synthesis of β-carotene when ripened for 6 and 12 days at 21 °C (Table I). At 32 °C the β-carotene content of both treated and control fruits was higher than the corresponding fruits ripened at 21 °C for 6 and 12 days. Treatment with CPTA, however, resulted in the reduction of β-carotene at 21 °C as well as at 32 °C. However, lycopene synthesis in the normal red tomato fruit was not stimulated by CPTA treatment and storage at 32 °C contrary to the preliminary findings of Rabinowitch and Rudich (1972). In fact, the lycopene content of CPTA-treated fruits was consistently lower than that of the control fruits when ripened at 32 °C for 6 and 12 days. Although lycopene synthesis was not completely inhibited by further ripening at 32 °C for 12 days, the concentration of the pigment was still much lower than that found in fruits ripened at 21 °C either with or without CPTA.

The high-beta fruit produces relatively large amounts of β-carotene and small amounts of lycopene (Table II). The synthesis of β-carotene is partially inhibited by ri-

Table I. Effect of CPTA Treatment (4300 μg/mL) on the Carotenoid Content of Detached Normal Red Tomato Fruit[a]

		Concentration, μg/g dry wt			
		6 days		12 days	
Polyene	0 days	21 °C	32 °C	21 °C	32 °C
Phytoene		72.8			
		151.4	Tr[b]	344.2	
Phytofluene		103.0	10.0	157.5	15.1
		133.3	33.4	169.9	34.6
ζ-Carotene		1.5		2.9	0.8
		1.9	2.6	8.8	4.4
Lycopene		477.7	99.7	515.0	235.7
		555.1	59.2	581.4	217.4
β-Zeacarotene					
γ-Carotene		0.6	0.3	2.1	5.0
		2.8	5.8	1.4	2.3
β-Carotene	7.30	52.8	61.1	21.0	31.8
		12.0	38.0	4.1	14.1
Total	7.30	708.4	171.1	698.5	288.4
		856.5	139.0	1109.5	272.8

[a] Top figures are the values for untreated control fruits (μg/g dry wt). Bottom figures are the values for CPTA treated fruits (μg/g dry wt). [b] Tr = trace.

Table II. Effect of CPTA Treatment (4300 μg/mL) on the Carotenoid Content of Detached High-Beta Tomato Fruit[a]

		Concentration, μg/g dry wt			
		6 days		12 days	
Polyene	0 days	21 °C	32 °C	21 °C	32 °C
Phytoene	23.5	104.8	19.5	159.6	24.0
	23.1	148.8	22.3	218.8	26.5
Phytofluene	19.6	47.7	16.3	70.6	17.2
	15.6	62.8	17.1	94.3	19.0
ζ-Carotene	2.4	1.8	1.0	9.0	3.1
	3.1	7.3	1.1	12.2	3.5
Lycopene	0.2	0.9	2.1	120.0	0.2
	0.3	302.1	12.5	495.2	7.1
β-Zeacarotene	1.1	1.4	0.5	2.0	1.1
	0.9	1.3	0.8	1.6	0.7
γ-Carotene	9.3	31.2	11.7	51.1	10.0
	7.5	54.3	22.3	76.9	21.6
β-Carotene	557.2	1708.7	654.7	1665.9	761.5
	462.3	870.1	580.4	1152.7	596.9
Total	613.3	1985.5	705.8	2078.2	817.1
	512.8	1448.9	656.5	2051.7	675.3

[a] Top figures are the values for untreated control fruits (μg/g dry wt). Bottom figures are the values for CPTA-treated fruits (μg/g dry wt).

pening at 32 °C and the total carotenoid content of the control fruits decreased. The reduced lycopene content of controls ripened at 32 °C for 12 days is consistent with earlier reports on the effect of temperature on carotenoid synthesis in ripening tomato (Tomes, 1963; Tomes et al., 1956, 1958).

High-beta fruit treated with CPTA and ripened at 21 °C exhibited a reduction in β-carotene after 6 and 12 days concomitant with an increased synthesis of lycopene and γ-carotene. The results suggest a partial shift from β-carotene to lycopene synthesis in the presence of CPTA. Ripening at 32 °C effectively blocked the CPTA-induced synthesis of lycopene and γ-carotene. The β-carotene content similarly decreased upon ripening at 32 °C. Furthermore, at 32 °C the CPTA-treated high-beta fruits contained greater quantities of lycopene and γ-carotene than the corresponding controls. This increase appears to have occurred at the expense of β-carotene which is slightly lower than the untreated controls. The synthesis of the more saturated acyclic intermediates was not affected by treatment with CPTA when the fruit was ripened at 32 °C.

The high-beta tomato line possesses the dominant gene *B* and the recessive modifier mo_B (Kirk and Tilney-Bassett, 1967). Studies by several workers suggested that in the high-beta genotype the formation of β-carotene from lycopene was enhanced in the presence of the dominant *B* allele (Kohler et al., 1947; Porter and Lincoln, 1950). Tomes (1963) and Tomes et al. (1956) reported that some of the β-carotene in the high-beta tomato could be inhibited by ripening at high temperature while β-carotene synthesis in the normal red fruit was not. The increased biosynthesis of lycopene and γ-carotene at 32 °C in response to CPTA treatment is insufficient to make lycopene the major pigment of the fully ripe high-beta tomato fruit. Thus, the ripe fruit has a yellow-orange color due to the high concentration of β-carotene. Therefore, CPTA fails to overcome a temperature-inhibited step in the conversion of lycopene to β-carotene in the high-beta tomato fruit.

The data presented are in variance with the earlier hypothesis of Rabinowitch and Rudich (1972) and suggest that in both the normal red and high-beta tomato fruits, an inhibition of carotenoid biosynthesis by high temperature cannot be overcome by treatment with CPTA.

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Carotenoid Induction in Orange Endocarp

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The effects of synthetic bioregulators of carotenogenesis on orange endocarp are reported for the first time. Valencia oranges were treated with 2-(4-chlorophenylthio)triethylamine hydrochloride, 2-(*p*-ethylphenoxy)triethylamine hydrochloride, and 4-[β-(diethylamino)ethoxy]benzophenone hydrochloride. There is a large increase in the xanthophyll fraction and a twofold increase in total carotenoids. Bioregulators sprayed on the fruit during preharvest treatment caused no lycopene to accumulate. Postharvest treatment by pressure infiltration caused less lycopene accumulation in the endocarp than usually seen in treated peel.

A large number of bioregulators of carotenoid biosynthesis have been developed (Hsu et al., 1975; Poling et al., 1975; Poling et al., 1976) and shown to have a profound effect on carotenogenesis in a number of organisms (Coggins et al., 1970; Yokoyama et al., 1971; Rabinowitch and Rudich, 1972; Gertman and Fuchs, 1973; Hayman et al., 1974; Elahi et al., 1975; Jahn and Young, 1975; Hayman and Yokoyama, 1976). These compounds cause accumulation of the acyclic carotene, lycopene, and induce increased carotenogenesis. The first effect is caused by an inhibition of the cyclase enzyme(s) which normally functions in most carotenogic systems to produce the cyclic carotenes and xanthophylls from the acyclic precursor

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