The Conversion of Phytoene-14C to Acyclic, Monocyclic, and
Dicyclic Carotenes and the Conversion of Lycopene-15,15'-3H
to Mono- and Dicyclic Carotenes by Soluble
Enzyme Systems Obtained from Plastids
of Tomato Fruits*

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SANTOSH C. KUSHWAHA, GINZABURO SUZUE, C. SUBBARAYAN, AND JOHN W. PORTER

From the Lipid Metabolism Laboratory, Veterans Administration Hospital, and the Department of Physiological Chemistry, University of Wisconsin, Madison, Wisconsin 53706

SUMMARY

Proof is presented in this paper for the formation of acyclic
and cyclic carotenes from phytoene-14C by soluble extracts
of plastids obtained from fruit of different genetic selections
of the tomato. The carotenes biosynthesized from phyto-
ene-14C and the tomato selections used were the following:
(a) cis- and trans-phytofluene, neurosporene, neolycopene,
lycopene, and 7- and 1,3-carotenes, red tomato fruit plastids;
(b) cis- and trans-phytofluene, neurosporene, lycopene, and
7, 8, 8', 9, 9'-carotenes, "Hi-8" tomato fruit plastids;
(c) cis- and trans-phytofluene, neurosporene, lycopene,
7, 8, 8'- and 1,3-carotenes, "Hi-3" tomato fruit plastids, and (d)
cis- and trans-phytofluene, prolycopene, proneurosporene,
lycopene, and 7- and 1,3-carotenes, Golden Jubilee variety
tomato fruit plastids. Experimental evidence for the con-
version of lycopene-15,15'-3H to mono- and dicyclic caro-
tenes by a soluble enzyme system obtained from red tomato
fruit plastids is also presented in this paper.

Proof for the formation of the above carotenes from radio-
active phytoene was obtained by cochromatography with
authentic nonradioactive carotenes on an alumina chromato-
graphic column. A close correspondence between radio-
activity and absorbance for each carotene was observed.
Further proof for the formation of acyclic and cyclic caro-
tenes from radioactive phytoene was obtained by gas-liquid
chromatography of the hydrogenated products. Coincidence
between mass and radioactivity was also observed.

The conversion of phytoene to phytofluene by the tomato
enzyme systems appears to be dependent upon the presence
of NADP, whereas the conversion of the latter compound to
lycopene appears to require FAD and Mn++. The forma-
tion of lycopene is also increased in the presence of Mg++
and dithiothreitol.

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ie.
farnesyl pyrophosphates Shah et al. (16), using the same system, demonstrated the formation of phytoene from geranylgeranyl pyrophosphate-3-14C. Suzue and Porter (17) later reported the incorporation of radioactivity of isopentenyl pyrophosphate-4-14C into phytoene, phytofluene, neurosperene, and lycopene by the above soluble and partially purified tomato enzyme system. Lee and Chichester (18), using a cell-free enzyme system from a P. blakesleeanus R1 mutant, showed the conversion of geranylgeranyl pyrophosphate-4-14C to phytoene, phytofluene, ε-carotene, neurosperene, and lycopene.

Studies on the enzymatic interconversion of carotenes are relatively few. The conversion of phytoene to phytofluene by isolated tomato fruit plastids has been reported by Beeler and Porter (19) and the formation of ε-carotene from phytofluene by chloroplasts of corn leaves has been reported by Costes (20). Decker and Uehleke (21) earlier reported the conversion of lycopene to ε-carotene by spinach chloroplasts and the reverse conversion of ε-carotene to lycopene by tomato slices. Recently, Kushwaha et al. (22) demonstrated the conversion of tritiated lycopene to cyclic carotenes by soluble extracts of spinach chloroplasts and Hi-β and Hi-ε tomato fruit plastids, and Subbarayan et al. (23) demonstrated the enzymatic conversion of isopentenyl pyrophosphophate and phytoene-4-14C to acyclic carotenes by an ammonium sulfate-precipitated spinach enzyme system. From the above results it is generally assumed that the more unsaturated carotenes are formed from phytoene through stepwise dehydrogenation and that lycopene is then converted to mono- and dicyclic carotenes.

Experimental proof for the enzymatic conversion of phytoene to carotenes other than phytofluene has not been reported, except for the data of Subbarayan et al. (23). Hence the present work was undertaken in an effort to show that phytoene-4-14C is converted to the more unsaturated acyclic and cyclic carotenes by a soluble enzyme system obtained from tomato fruit plastids.

**EXPERIMENTAL PROCEDURE**

**Materials**—Red tomato fruits were purchased locally, whereas Hi-β, Hi-ε, and Golden Jubilee tomato fruits were a generous gift from Dr. Mark L. Tomes, Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana. Lycopene-15,15'-3H was a generous gift from Dr. 0. Isler of Hoffmann-La Roche, Inc., Basel, Switzerland. y-Carotene was isolated from fruit of Hi-P, Hi-6, and Golden Jubilee tomato fruits as follows. Plastids were isolated from a strain of Penicillium sclerotiorum, according to the method of Suzue and Porter (24). The acetone powder of these plastids was prepared as reported by Jungalwala and Porter (15). The acetone powder was extracted with phosphate buffer as described previously (16) and then fractionated with ammonium sulfate under nitrogen. The fractions containing radioactivity of isopentenyl pyrophosphate-4-14C into phytoene, phytofluene, neurosperene, and lycopene.

**Preparation of Tomato Enzyme System**—Tomato fruit plastids and an acetone powder of these plastids were prepared as reported by Jungalwala and Porter (15). The acetone powder was extracted with phosphate buffer as described previously (16) and then fractionated with ammonium sulfate under nitrogen. The protein fractions precipitating between 20 and 40% with ammonium sulfate was dissolved in 0.1 M phosphate buffer, pH 7.0, and the protein fraction of the supernatant was dialyzed against 0.001 M DTT and stored as described above.

**Preparation of Substrates**—Radioactive phytoene was prepared according to the method of Suzue and Porter (17). Incubations for the synthesis of radioactive phytoene were carried out as reported by the above authors, but a slightly different procedure was followed for the isolation of the product. Incubations were carried out for 6 hours and then the reaction was terminated by the addition of 4 ml of ethanol to the incubation mixture. Non-saponifiable compounds were extracted five times, each with 5-ml portions of petroleum ether. The combined petroleum ether extract was washed with water and dried over anhydrous sodium sulfate and then concentrated to a volume of 5.0 ml with a gentle stream of nitrogen. The extract was chromatographed on a column, 1.8 x 20 cm, of 1% (v/w) water-desiccated alumina (50 g). Carrier phytoene was not added to the column. The chromatographic column was developed with 0.5% and 1% peroxide-free diethyl ether in petroleum ether. Ten-milliliter eluate fractions were collected and an aliquot (100 μl) was assayed for radioactivity in a toluene-scintillator solution in a Packard Tri-Carb liquid scintillation spectrometer. Usually phytoene was eluted from the column with 1% diethyl ether in petroleum ether. The fractions containing radioactivity were combined, evaporated to dryness under oxygen-free nitrogen, and then redissolved in petroleum ether. The phytoene fraction was rechromatographed once more under the same conditions. An aliquot of rechromatographed material was mixed with authentic nonradioactive phytoene and the mixture was chromatographed on an alumina column. Absorbance at 280 μg and radioactivity were determined on each eluate fraction. Good correspondence was obtained between the plots of radioactivity and absorbance. Aliquots of each of the fractions containing radioactivity were mixed, reduced to a small volume under oxygen-free nitrogen, hydrogenated overnight, and then subjected to gas-liquid chromatography to determine the purity of the product. A single component was found and almost all of the radioactivity was associated with the mass of the hydrogenated phytoene. This chromatographically purified phytoene was then used as a substrate in all incubations.

**Preparation of Tomato Enzyme System**—Tomato fruit plastids and an acetone powder of these plastids were prepared as reported by Jungalwala and Porter (15). The acetone powder was extracted with phosphate buffer as described previously (16) and then fractionated with ammonium sulfate under nitrogen. The protein fractions precipitating between 20 and 40% and between 40 and 60% of saturation were dissolved in 0.1 M phosphate buffer, pH 7.0, containing 0.001 M DTT.1 Each enzyme fraction was found to be stable for at least 4 weeks when stored under nitrogen at -20°. The stored enzyme fractions were dialyzed against 0.05 M phosphate buffer, pH 7.0, containing 0.001 M 2-mercaptoethanol for 3 hours, with a change of buffer at the end of 1.5 hours, just before use.

Soluble enzyme systems were prepared from Hi-β, Hi-ε, and Golden Jubilee tomato fruit plastids as follows. Plastids were added to an approximately equal quantity of 120 mesh glass beads and then ground with a pestle and mortar for 2 min to make a thick slurry. The glass beads and the pestle and mortar were kept at 4° for 24 hours prior to use. The slurry was then extracted twice with 0.1 M phosphate buffer, pH 7.0, and the extract was centrifuged at 105,000 g for 2 hours in a Spinco model L ultracentrifuge. The protein fraction of the supernatant solution precipitating between 0 and 80% of saturation with ammonium sulfate was dissolved in 0.1 M phosphate buffer, pH 7.0, containing 0.001 M DTT and stored as described above. The stored enzyme was dialyzed as described in the previous paragraph just before use.

1 The abbreviation used is: DTT, dithiothreitol.
Enzymatic Synthesis of Carotenes

Incubation System for Synthesis of Acyclic, Monoacyclic, and Dicyclic C40 Carotenes—A typical incubation system for the enzymatic synthesis of carotenes was prepared as follows. A petroleum ether (distilled twice) solution of phytoene-14C was placed in a 20-ml incubation tube and the solvent was removed by a gentle stream of oxygen-free nitrogen. Tween 80, 2 mg, in 200 μl of acetone (purified) was added and the acetone was removed with oxygen-free nitrogen. Borate buffer (pH 7.5, 250 μmoles), DTT (19.4 μmoles), MgCl2 (3 μmoles), NADP (3.66 μmoles), FAD (3.8 μmoles), and enzyme protein (ammonium sulfate precipitate, 20 to 40% fraction, 10 mg; 40 to 60% fraction, 10 mg) were added. The final volume of the incubation was 3 ml. Incubations were carried out for 5 hours at 25° under nitrogen with gentle shaking on a Fisher clinical rotator.

<table>
<thead>
<tr>
<th>Carotene</th>
<th>Total radioactivity (dpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-Phytofluene</td>
<td>9943</td>
</tr>
<tr>
<td>trans-Phytofluene</td>
<td>990</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>2866</td>
</tr>
<tr>
<td>γ-Carotene</td>
<td>1900</td>
</tr>
<tr>
<td>Neurosporene</td>
<td>2350</td>
</tr>
<tr>
<td>Neolycopene</td>
<td>2788</td>
</tr>
<tr>
<td>Lycopene</td>
<td>3883</td>
</tr>
</tbody>
</table>

Hydrogenation of the Separated Carotenes (9)—Radioactive and nonradioactive cis- and trans-phytofluene, proneurosporene, protocarotene, neurosporene, lycopene, α-, β-, γ-, α′-, and β-carotenes, separated by column chromatography as indicated above, were hydrogenated. Hydrogenation was carried out overnight with Adam's catalyst at a pressure of 40 p.s.i. of hydrogen in petroleum ether-isopropyl alcohol in a Parr hydrogenator. After hydrogenation the perhydrocarotenes were extracted with petroleum ether. The extract was washed with water, dried over anhydrous sodium sulfate, and then chromatographed on a column, 1.8 × 20 cm, of 1% (v/w) water-deactivated alumina, 50 g. The upper surface of the alumina column was protected by a small amount of anhydrous sodium sulfate. The chromatogram was developed with increasing amounts of peroxide-free diethyl ether in petroleum ether (see legend to Fig. 1).

Assay of Mass and Radioactivity of Carotenes—A 1-ml aliquot of each eluate fraction, 10 ml, was assayed for radioactivity with a Packard Tri-Carb liquid scintillation spectrometer. The light absorption spectrum of each fraction eluted from the column was then determined with a Beckman DU spectrophotometer. The light absorption maxima and minima for each pigment were also determined with a Beckman DU spectrophotometer. Absorbance for each carotene, at its principal absorption maximum, was measured with a Beckman DU spectrophotometer. Coincidence between the absorbance at the principal absorption maximum for each carotene and radioactivity was obtained.

Gas-Liquid Chromatography of Perhydrocarotenes and Detection of Radioactivity (9)—Perhydrocarotenes were chromatographed in a Packard gas chromatograph. Separation between acyclic, monocyclic, and dicyclic perhydrocarotenes was achieved on a column, 8 feet × 6 mm (internal diameter), of 2% SE-30 on Gas-Chrom-Q at 250° with an Argon flow of 100 ml per min. Fractions emerging from the gas-liquid chromatographic column were collected on glass wool with a Packard fraction collector. Each effluent fraction, trapped on leaving the gas-liquid chromatographic column, was eluted with toluene-scintillator solution and then assayed for radioactivity.

All toluene-soluble samples were assayed for radioactivity in a diphenyloxazole-dimethyl-p-bis-2'-(5'-phenyloxazoyl) benzene-toluene solution in a Packard liquid scintillation spectrometer. Corrections for quenching were made with an automatic external
TABLE III
Enzymatic conversion of phytoene-14C to more unsaturated carotenes by soluble enzyme system obtained from plastids of Hi-β tomato fruits

The incubation mixture contained radioactive phytoene, 1.914 mmoles (200,000 dpm); Tween 80, 2 mg; borate buffer, pH 7.5, 250 pmoles; NADP, 3.66 pmoles; FAD, 3.81 pmoles; MgCl₂, 30 pmoles; MnCl₂, 3 pmoles; DTT, 19.4 pmoles; and enzyme protein (ammonium sulfate precipitate, 0 to 80% saturation, 21 mg) in a final volume of 3 ml. Incubations were carried out for 5 hours at 25° under nitrogen.

<table>
<thead>
<tr>
<th>Carotene</th>
<th>Total radioactivity (dpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-Phytofluene</td>
<td>3400</td>
</tr>
<tr>
<td>trans-Phytofluene</td>
<td>755</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>5006</td>
</tr>
<tr>
<td>γ-Carotene</td>
<td>8803</td>
</tr>
<tr>
<td>Neuporphinene</td>
<td>1100</td>
</tr>
<tr>
<td>Lycopene</td>
<td>1723</td>
</tr>
</tbody>
</table>

TABLE IV
Enzymatic conversion of phytoene-14C to more unsaturated carotenes by enzyme system prepared from plastids of Golden Jubilee tomato fruits (tangerine variety)

The incubation system contained phytoene-14C, 0.957 mmoles (100,000 dpm); Tween 80, 2 mg; borate buffer, pH 7.5, 250 pmoles; NADP, 3.66 pmoles; FAD, 3.81 pmoles; MgCl₂, 30 pmoles; MnCl₂, 3 pmoles; DTT, 19.4 pmoles; and enzyme protein (ammonium sulfate precipitate, 0 to 80% saturation, 17 mg) in a final volume of 2 ml. Incubations were carried out for 5 hours in the dark at 25° under nitrogen.

<table>
<thead>
<tr>
<th>Carotene</th>
<th>Total radioactivity (dpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-Phytofluene</td>
<td>3602</td>
</tr>
<tr>
<td>trans-Phytofluene</td>
<td>906</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>457</td>
</tr>
<tr>
<td>γ-Carotene</td>
<td>1105</td>
</tr>
<tr>
<td>Neuporphinene</td>
<td>3390</td>
</tr>
<tr>
<td>Prolycopene</td>
<td>9112</td>
</tr>
<tr>
<td>Lycopene</td>
<td>1224</td>
</tr>
</tbody>
</table>

Fig. 1. The separation of carotenes synthesized from phytoene-14C by a soluble enzyme system obtained from plastids of Hi-β tomato fruits on a 1% (v/w) water-deactivated alumina column. The chromatogram was developed with petroleum ether containing increasing amounts of peroxide-free diethyl ether. Radioactivity (●) and absorbance (○) were measured for each eluate fraction collected. Absorbance measurements were made at 348, 444, 345, 450, 425, 455, 460, 440, and 470 nm, respectively for cis-phytofluene, α-carotene, trans-phytofluene, β-, γ-, and δ-carotenes, neuporphinene, and lycopene. The agreement between radioactivity and absorbance was nearly the same, as shown when each carotene synthesized from either radioactive phytoene or lycopene-15,15'-3H by a soluble enzyme system obtained from plastids of red, Hi-β, or Golden Jubilee tomato fruits was chromatographed on alumina. The eluate fractions from this chromatogram were each 50 ml (Fractions 1 to 9) or 10 ml (Fractions 10 to 150). The per cent of ether and the fractions collected were the following: 0.5, 1 to 3; 1, 4 to 6; 2, 7 to 9; 3, 10 to 15; 4, 16 to 20; 5, 21 to 25; 6, 26 to 30; 7, 31 to 35; 8, 36 to 40; 9, 41 to 45; 10, 46 to 50; 11, 51 to 55; 12, 56 to 60; 13, 61 to 65; 14, 66 to 70; 15, 71 to 75; 16, 76 to 80; 17, 81 to 85; 18, 86 to 90; 19, 91 to 95; 20, 96 to 100; 21, 101 to 105; 22, 106 to 110; 23, 111 to 120; 24, 121 to 130; 25, 131 to 140; 26, 141 to 150. Unreacted phytoene was recovered in Fractions 1 to 6. The total radioactivity in phytoene was approximately 40% of that added to the incubation mixture. Presumably the major loss of phytoene on incubation resulted from oxidation of this very labile compound. It should be pointed out that each lot of alumina has somewhat different adsorptive characteristics. Hence, each new lot needs to be standardized as to the per cent of diethyl ether and the volume to use to effect the separation shown on this figure. The separation of neuporphinene, prolycopene, and neolycopene is not shown on this figure. These compounds were each separated from only a single genetic selection of tomatoes. Each carotene was identified by its light absorption spectrum and by its position on chromatography as reported previously: neolycopene (25, 26), prolycopene (formerly called protetrahydrolycopene and unidentified I) (1, 27), and prolycopene (1, 25 27).
FIG. 2. The gas-liquid chromatographic separation of acyclic perhydrocarotenes. The carotenes were synthesized from radioactive phytoene by a soluble enzyme system obtained from plastids of Hi-6 tomato fruits. The carotenes were then purified, hydrogenated, and subjected to gas-liquid chromatography as reported under "Experimental Procedure." The effluents leaving the gas chromatograph were trapped on glass wool for 1 min as indicated. The trapped samples were eluted with toluene-scintillator solution and then assayed for radioactivity with a Tri-Carb liquid scintillation spectrometer. The shaded areas indicate radioactivity. Similar results were obtained when carotenes were synthesized by soluble enzyme systems obtained from plastids of Hi-β, red, or Golden Jubilee tomato fruits.
standardization unit and the appropriate calibration curve. All values for radioactivity are reported in disintegrations per min.

The conversion of radioactive phytoene to phytofluene, proto-neurosporene, protolycopene, neurosporene, lycopene, \( \gamma \)-, \( \delta \)-, \( \gamma \)-, \( \alpha \)-, and \( \beta \)-carotenes was shown by the coincidence of radioactivity and mass of perhydrocarotene on gas-liquid chromatography.

RESULTS

Conversion of Phytoene-\(^{14}C\) to Acyclic and Cyclic Carotenes by Soluble Enzyme Systems Prepared from Plastids of Tomato Fruit of Different Genetic Selections

Red Fruit—The formation of cis- and trans-phytofluenes, neurosporene, neolycopene (25, 26), lycopene, and \( \gamma \)- and \( \gamma \)-carotenes from radioactive phytoene by a soluble enzyme system obtained from plastids of red tomato fruits is shown in Table I. The incorporation of radioactivity into cis-phytofluene was greater than that for any other carotene reported in this table. The amount of radioactivity in trans-phytofluene was approximately 10% of that in the cis-isomer.

Hi-\( \delta \) Fruit—The conversion of phytoene-\(^{14}C\) to cis- and trans-phytofluenes, neurosporene, lycopene, and \( \gamma \)-, \( \delta \)-, \( \gamma \)-, \( \alpha \)-, and \( \beta \)-carotenes by a soluble enzyme system (0 to 80% ammonium sulfate precipitate) obtained from plastids of Hi-\( \delta \) tomato fruits is reported in Table II. The quantity of radioactivity found in \( \delta \)-carotene was greater than that found in any of the other carotenes, as expected from the pigment composition of these fruits.

Hi-\( \beta \) Fruit—The conversion of phytoene-\(^{14}C\) to cis- and trans-

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**FIG. 3.** The gas-liquid chromatographic separation of monocyclic perhydrocarotenes. The carotenes were synthesized from radioactive phytoene by a soluble enzyme system obtained from plastids of Hi-\( \delta \) tomato fruits. The carotenes were then purified, hydrogenated, and subjected to gas-liquid chromatography as reported under “Experimental Procedure.” Effluents were trapped on glass wool for 1 min as indicated. The trapped samples were eluted with toluene-scintillator solution and then assayed for radioactivity with a Tri-Carb liquid scintillation spectrometer. The shaded area indicates radioactivity. Similar results were obtained when carotenes were synthesized by soluble enzyme systems prepared from plastids of Hi-\( \delta \), Golden Jubilee, or red tomato fruits. Monocyclic carotenes synthesized from lycopene-\( 15,15'-{^3}H \) by a soluble enzyme system obtained from red tomato fruit plastids behaved similarly when treated as indicated above.

**FIG. 4.** Gas-liquid chromatographic separation of dicyclic perhydrocarotenes. The carotenes were synthesized from radioactive phytoene by a soluble enzyme system prepared from plastids of Hi-\( \delta \) tomato fruits. The carotenes were then purified, hydrogenated, and subjected to gas-liquid chromatography as reported under “Experimental Procedure.” Effluents leaving the gas chromatograph were trapped on glass wool for 1 min as indicated. The trapped samples were eluted with toluene-scintillator solution and then assayed for radioactivity with a Tri-Carb liquid scintillation spectrometer. The shaded area indicates radioactivity. Similar results were obtained when carotenes were synthesized by soluble enzyme systems prepared from plastids of Hi-\( \delta \), Golden Jubilee, or red tomato fruits. Dicyclic carotenes obtained on incubation of tritiated lycopene with a soluble enzyme system obtained from plastids of red tomato fruits behaved similarly when treated as indicated above.
phytoene-14C to phytofluene and lycopene by a soluble enzyme system obtained from plastids of red tomato fruits (22). When 14C-labeled cis- and trans-phytofluene, proneurosporene, prolycopenone, neurosporene, and y-carotene were isolated from incubation mixtures, they were separately hydrogenated and then passed through an alumina column. The conversion of lycopene to cyclic carotenes by soluble extracts of Hi-β and Hi-β tomato fruit plastids and spinach plastids (22), provide proof that tritiated lycopene is converted to α-, β-, δ-, and γ-carotenes by plant enzyme systems obtained from a diversity of sources.

### Recovery of Radioactivity in Mass of Each Perhydrocarotene on Gas Liquid Chromatography

When 14C-labeled cis- and trans-phytofluene, proneurosporene, prolycopenone, neurosporene, and y-carotene were isolated from incubation mixtures, they were separately hydrogenated and then passed through an alumina column. The conversion of lycopene to cyclic carotenes by soluble extracts of Hi-β and Hi-β tomato fruit plastids and spinach plastids (22), provide proof that tritiated lycopene is converted to α-, β-, δ-, and γ-carotenes by plant enzyme systems obtained from a diversity of sources.

### Cofactor Requirements

Data on the effect of various cofactors on the conversion of phytene-14C to phytol and lycopene by a soluble enzyme system obtained from plastids of red tomato fruits are presented in Table VIII. When the incubations were carried out without FAD, phytene was converted only to phytol. No radioactivity was detected in other carotenes. When NADP was omitted the conversion of phytene to phytol was decreased considerably. However, some lycopene was formed. The omission of Mn++ had the same effect as the omission of FAD. Phytol was not converted to lycopene but conversion to phytol was unaffected. These results suggest that NADP is required for the conversion of phytene to phytol and that FAD and Mn++ are absolute requirements for the conversion of phytene to lycopene. Some reduction in the formation of lycopene was also observed when Mg++ and DTT were omitted from the incubation mixture.

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**Table V**

Enzymatic conversion of tritium-labeled lycopene to cyclic carotenes by soluble enzyme system prepared from plastids of red tomato fruits

<table>
<thead>
<tr>
<th>Carotene</th>
<th>Radioactivity (dpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Carotene</td>
<td>5,009</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>4,925</td>
</tr>
<tr>
<td>δ-Carotene</td>
<td>13,510</td>
</tr>
<tr>
<td>γ-Carotene</td>
<td>31,427</td>
</tr>
</tbody>
</table>

α-Carotene was converted to β-carotene in tomato fruit plastids (22).

**Golden Jubilee Fruit**—The conversion of radioactive phytol to cis- and trans-phytofluene, proneurosporene, prolycopenone (1, 25, 27), lycopene, and δ- and β-carotenes by a soluble enzyme system obtained from plastids of fruit of a Golden Jubilee type tomato is reported in Table IV. The incorporation of radioactivity was maximal in prolycopenone, neurosporene, lycopene, and δ-, β-, α-, and γ-carotenes, isolated from incubation mixtures, were separatedly hydrogenated and then passed through an alumina column containing 90 to 95% of radioactivity originally present in the carotene was recovered in the perhydrocarotene fraction. However, when the tritiated cyclic carotenes formed on incubation of tritiated lycopene with the soluble enzyme obtained from plastids of red tomato fruits were hydrogenated, each perhydrocarotene contained only 35 to 40% of the radioactivity originally present in the cyclic carotene. Presumably, the decrease in tritium content of the carotenes during hydrogenation is due to an exchange of tritium with hydrogen gas. Most of the radioactivity (either 14C or tritium) of each perhydrocarotene was recovered with the mass peak of the hydrogenated carotene on passage through the gas-liquid chromatographic column. The recovery of this radioactivity ranged between 78 and 98% as shown in Tables VI and VII.


**TABLE VII**

Recovery of radioactivity of hydrogenated 14C-labeled carotenes on gas-liquid chromatography

<table>
<thead>
<tr>
<th>Hydrogenated carotene</th>
<th>Red tomato</th>
<th>H-4</th>
<th>H-β</th>
<th>Golden Jubilee</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-Phytofluene</td>
<td>7,550</td>
<td>7,380</td>
<td>3,208</td>
<td>2,991</td>
</tr>
<tr>
<td>trans-Phytofluene</td>
<td>2,150</td>
<td>2,000</td>
<td>1,075</td>
<td>840</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>1,891</td>
<td>1,821</td>
<td>3,196</td>
<td>3,039</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>2,650</td>
<td>2,442</td>
<td>2,000</td>
<td>1,918</td>
</tr>
<tr>
<td>γ-Carotene</td>
<td>1,788</td>
<td>1,638</td>
<td>5,011</td>
<td>4,388</td>
</tr>
<tr>
<td>Neophycophane</td>
<td>2,300</td>
<td>2,350</td>
<td>2,333</td>
<td>2,218</td>
</tr>
<tr>
<td>Neolycopene</td>
<td>2,714</td>
<td>2,501</td>
<td>2,411</td>
<td>2,301</td>
</tr>
<tr>
<td>Lycopene</td>
<td>3,635</td>
<td>3,327</td>
<td>2,374</td>
<td>2,190</td>
</tr>
</tbody>
</table>

*The values given are the radioactivities recovered with each perhydrocarotene trapped on emergence from the gas-liquid chromatograph.*

**TABLE VIII**

Effect of various factors on enzymatic conversion of phytoene-14C to more unsaturated carotenes

<table>
<thead>
<tr>
<th>System</th>
<th>Phytofluene</th>
<th>β-Carotene</th>
<th>Lycopene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cis-</td>
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<td></td>
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<tr>
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<tr>
<td>- NADP</td>
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<td>58</td>
<td>1171</td>
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<tr>
<td>- MnCl₂</td>
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<tr>
<td>- MgCl₂</td>
<td>3046</td>
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<td>2865</td>
</tr>
<tr>
<td>- DTT</td>
<td>1906</td>
<td>334</td>
<td>1561</td>
</tr>
</tbody>
</table>

*The complete incubation system contained phytoene-14C, 0.354 mmole (37,050 dpm); Tween 80, 2 mg; borate buffer, pH 7.5, 250 μmole; NADP, 3.66 μmole; FAD, 1.27 m.pmoles; MgCl₂, 30 μmole; MnCl₂, 3 μmole; DTT, 19.4 μmole; and enzyme protein (nucleonium sulfate precipitate, 20 to 40% saturation, 15 mg, and 40 to 60% saturation, 15 mg) in a final volume of 2 ml. Incubations were carried out for 5 hours in the dark at 25° under nitrogen.

DISCUSSION

Except for the demonstrated conversion of phytoene to phytofluene by tomato plastids (19), and the recent report by Subbarayan et al. (23) on the conversion of phytoene to phytofluene and lycopene by a spinach enzyme system, no reports have been made on the conversion of phytoene to more unsaturated carotenes. In the present paper we have demonstrated that phytoene-14C is converted to each of the principal acyclic and cyclic carotenes found in four genetic types of tomato fruits. Furthermore, these conversions were effected by soluble enzyme systems obtained from the plastids of fruits of these selections. Proof for the conversion of phytoene to acyclic and cyclic carotenes was obtained through the demonstration of (a) the coincidence of radioactivity and absorbance for each carotene separated on chromatography on alumina, and (b) the coincidence of mass and radioactivity for each hydrogenated carotene subjected to gas-liquid chromatography.

An examination of the cofactor requirements for the conversion of radioactive phytoene to more unsaturated carotenes has shown that the conversion of phytoene to phytofluene was greatly reduced when NADP was omitted from the incubation mixture. When FAD was omitted from the incubation mixture lycopene was not formed. However, the conversion of phytoene to phytofluene was unaffected. These results suggest that NADP participates in the conversion of phytoene to phytofluene, whereas FAD functions in the conversion of phytofluene to lycopene. The elimination of Mn⁺⁺ from the incubation mixture had the same effect as the removal of FAD on the formation of lycopene. Hence, Mn⁺⁺ appears to be an absolute requirement for the conversion of phytoene to lycopene. The removal of Mg⁺⁺ and DTT from the incubation mixture also reduced the conversion of phytoene to lycopene.

We have also demonstrated in the present work that lycopene-15,15'-14H is converted to β-, γ-, α-, and δ-carotenes by a soluble enzyme system obtained from plastids of red tomato frruits. Previously, we had shown (22) that tritiated lycopene is converted to cyclic carotenes by spinach chloroplasts and by soluble preparations of these chloroplasts. We also showed that the same conversion could be effected with plastids (or soluble extracts thereof) of fruit of several genetic types of tomatoes.

The results reported in this paper on the conversion of tritiated lycopene to more unsaturated carotenes have been obtained when NADP was omitted from the incubation mixture. When FAD was omitted from the incubation mixture lycopene was not formed. However, the conversion of phytoene to phytofluene was unaffected. These results suggest that NADP participates in the conversion of phytoene to phytofluene, whereas FAD functions in the conversion of phytofluene to lycopene. The elimination of Mn⁺⁺ from the incubation mixture had the same effect as the removal of FAD on the formation of lycopene. Hence, Mn⁺⁺ appears to be an absolute requirement for the conversion of phytoene to lycopene. The removal of Mg⁺⁺ and DTT from the incubation mixture also reduced the conversion of phytoene to lycopene.
Enzymatic Synthesis of Carotenes

Fig. 5. A proposed pathway for the conversion of phytoene to more unsaturated acyclic, monocyclic, and dicyclic carotenes by enzymes of plastids of fruits of various genetic selections of tomatoes.

Prior to the present investigation a report appeared in the literature (23, 29) on the conversion of bacterial phytoene (de-hydrosqualene) to \( \delta \)-carotene by enzymes extracted from \( S. \) aureus. However, the identification of \( \delta \)-carotene from this bacteria was dependent only upon its light absorption spectrum (30). Therefore, proof of the incorporation of radioactivity into \( \delta \)-carotene in this system is equivocal. However, the suc-
cessive dehydrogenation of dehydrosqualene (29) with N bromosuccinimide to C₃₀ phytofluene-like and C₄₀ β-carotene-like substances, as identified by light absorption spectra, might suggest the presence of a C₃₀ carotene series in this microorganism. If dehydrosqualene were the precursor of the C₄₀ carotenes, the Porter-Lincoln series of reactions (1) would need some modification. However, it is very doubtful that a pathway for the formation of carotenoids from dehydrosqualene exists in tomatoes, inasmuch as it has been shown that geranylgeranyl pyrophosphate is converted to phytoene by soluble extracts of tomato fruit plastids (16).

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REFERENCES
The Conversion of Phytoene-$^{14}$C to Acyclic, Monocyclic, and Dicyclic Carotenes and the Conversion of Lycopene-15,15'-$^{3}$H to Mono- and Dicyclic Carotenes by Soluble Enzyme Systems Obtained from Plastids of Tomato Fruits

Santosh C. Kushwaha, Ginzaburo Suzue, C. Subbarayan and John W. Porter


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