ON THE BIOSYNTHETIC RELATIONSHIP BETWEEN Lycopene AND COLORLESS POLYENES IN TOMATOES*

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The suggestion of Zechmeister (1) that phytofluene might be a precursor of lycopene has been expanded by Porter and Lincoln (2), who proposed on the basis of experiments with tomatoes that a successive dehydrogenation of more saturated polyenes leads to the biosynthesis of lycopene and other carotenoids. This sequence would involve the intermediary formation of tetrahydrophytoene, phytoene, phytofluene, \( \beta \)-carotene, neurosporene, and lycopene. Although this hypothesis has received wide attention, objections based on genetic and other evidence have been raised to the validity of this concept (3).

Goodwin has determined the concentration of some of these postulated intermediates at intervals during the ripening of tomatoes at various temperatures and has concluded that the Porter-Lincoln hypothesis could not account for the results obtained (4). An alternative scheme which was therefore proposed by Goodwin and Jamikorn (4) and Goodwin (5) and by MacKinney (6) and Jenkins and MacKinney (7) suggests instead that the different polyenes might originate from one single large precursor by parallel pathways. A third possibility concerning the biosynthetic relationship between the various polyenes is one in which lycopene is the source of the more saturated polyenes and is converted to these compounds by successive reduction (4).

Although changes in concentration of substances during the course of metabolic transformations often yield useful information concerning pathways, the interpretation of such data may occasionally be subject to error because an intermediate may be present in small quantity, may not change in concentration, and yet may have a high rate of turnover. This objection can often be overcome by the use of isotopes. In the study reported in this paper, 2-C\(^{14}\)-acetate has been added to ripening tomatoes previously

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1 In this paper the term polyene refers to all forty carbon compounds discussed, and the term carotenoid is used when reference is made to the colored members of the series.
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removed from the vine, and polyenes have been isolated at different periods of time after administration of the isotope. Comparison of the specific activity of lycopene to that of some of the colorless polyenes indicates that neither the Porter-Lincoln hypothesis nor the reverse of this scheme is operative in tomatoes.

EXPERIMENTAL

Administration of Substrate—The administration of 2-C\textsuperscript{14}-acetate was carried out as previously described (8). 1 mg. of sodium acetate, specific activity 0.5 mc. per mmole, was added for each 100 gm. of green ripening tomato. The tomatoes were then allowed to ripen at room temperature (24° ± 2°) for the indicated periods of time.

Isolation Procedures—Three experiments were carried out. In the first, a group of tomatoes weighing 9 kilos was injected with methyl-labeled acetate and allowed to ripen completely for 12 days. At the end of this time, lycopene was isolated and the purity determined as previously described (8). The colorless polyene fraction was obtained from the benzene-methanol mother liquor and was purified by the procedures of Wallace and Porter (9). The colorless, highly fluorescent oil phytofluene, which was obtained from the ignited magnesium oxide-Super-Cel column, was analyzed spectroscopically and, with use of the data of Wallace and Porter (9), was found to be at least 94 per cent phytofluene. The specific activity of this compound remained essentially unchanged after an additional chromatographic treatment on another ignited magnesium oxide-Super-Cel column.

The band which is eluted just before phytofluene consists of the difficultly separable mixture of phytoene or a phytoene-like material (5) and tetrahydrophytoene (2). The quantities of these materials available were insufficient to allow separation. Since spectroscopic analysis of this fraction did not reveal the presence of any other compounds, the specific activity of the mixture was compared to that of phytofluene and lycopene in this experiment.

The second experiment was carried out with 6 kilos of tomatoes. After injection of tracer, the tomatoes were divided into five equal groups, and ripening was allowed to proceed for 1, 3, 6, 9, and 12 days. Lycopene was isolated in each case as before. In order to avoid the use of prohibitively large quantities of materials, phytofluene was not separated from the phytoene-tetrahydrophytoene mixture but was isolated in a single fraction containing the three materials. The complete mixture was subjected to repeated chromatographic purifications, and its specific activity was compared to that of lycopene.

The third experiment was the same as the second, except that groups
of tomatoes were allowed to ripen over a shorter time interval, at 6, 12, 24, and 48 hours after administration of methyl-labeled acetate.

All the samples were converted to carbon dioxide and assayed as barium carbonate. Corrections for self-absorption were carried out by standard methods. Specific activity is defined here as counts per minute per mg. of carbon.

RESULTS AND DISCUSSION

The results of Experiment I, in which ripening was allowed to proceed to completion after administration of 2-C^14-acetate, are shown in Table I. It can be seen that the specific activity in lycopene was more than three times higher than that of phytofluene, and more than five times higher than that of the phytoene-tetrahydrophytoene mixture.

The variations in specific activity during the course of the ripening period were investigated in Experiment II. At each of the time intervals examined (Fig. 1), the specific activity of lycopene was found to be approximately three times higher than that of the mixture of colorless polyenes.

It can be noted that the specific activity in all polyenes reached its highest level during the early part of the ripening period. In order to determine the variations in specific activity immediately after administration of isotope, Experiment III was carried out over a much shorter time interval. This experiment differed from the others also in that the tomatoes used were at a stage of ripening approximately equivalent to the 6 day period of Experiment II. Since the concentrations of the colorless polyenes and of lycopene are very low during the first half of the ripening period and rise rapidly during the last half of this period (4), tomatoes at a later stage of ripening were used in order to insure a sufficient recovery of material for isolation and isotope assay.

The results obtained (Fig. 2) show that the colorless polyenes were slightly higher in specific activity than lycopene at the 6 and 12 hour intervals. At the 24 and 48 hour intervals, however, the specific activity of lycopene became higher than that of the colorless polyenes and exceeded the highest level of isotope found in the colorless polyenes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>C.p.m. per mg. C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lycopene</td>
<td>2500</td>
</tr>
<tr>
<td>Phytofluene</td>
<td>700</td>
</tr>
<tr>
<td>Phytoene-tetrahydrophytoene</td>
<td>450</td>
</tr>
</tbody>
</table>
The characteristics of specific activity-time relationships of precursors and their end products have been noted previously (10). It is necessary that a precursor must have a higher specific activity than its end product during a period when the level of tracer in both substances is rising. The data obtained in this study indicate clearly that the colorless polyenes cannot, therefore, be precursors of lycopene. The data also indicate that lycopene is not a source of carbon for the colorless polyenes. The specific activity of the colorless polyenes does not rise when the isotope concentration in lycopene begins to decrease (Fig. 1), as would be expected if lycopene were a precursor of the more saturated polyenes.

The relationships between specific activities of precursors and end products stated above may not hold when metabolically "active" and "inactive" pools are present. In the experiments reported here, it is hardly likely that this situation exists because the concentration of all polyenes is low at the time of administration of tracer (4). Newly formed radioactive polyenes are diluted very little by pools of inactive polyenes and, therefore, the specific activity relationships of the compounds isolated cannot be affected.

The conclusions drawn here have been based on the specific activities found in mixtures of colorless polyenes. It is, of course, possible that this mixture contains small amounts of substances with either higher or lower specific activities. Small quantities of more highly active materials as
contaminants would mean that the isotope concentrations in the colorless polyenes were really lower than those observed. This would further strengthen the conclusion that these substances are not precursors of lycopene. Contaminants with less activity than the colorless polyenes would also make no difference in the interpretation of the results. A 10 per cent contamination with inactive carbon would raise the true specific activity of the colorless polyenes by 10 per cent, and, in Experiments I and II, lycopene was 300 per cent or more higher in specific activity than the more saturated polyenes. Furthermore, when phytofluene was isolated as a single component in Experiment I, the relative specific activity of this compound compared to that of lycopene was of the same order of magnitude as that observed for the mixture of colorless polyenes compared to that of lycopene in Experiment II.

When the Porter-Lincoln hypothesis was first proposed, phytoene was thought to be a hexadecahydrolycopene. Recent work indicates that the correct structure is probably 7, 8, 11, 12, 12', 11', 8', 7'-octahydrolycopene, a compound with nine instead of five double bonds (11). It has been reported that phytofluene (3, 9) and ζ-carotene (12) are also isomers of phytoene, each differing only in the position in the molecule of the nine double bonds. If these three compounds are simply positional isomers, the Porter-Lincoln hypothesis as first formulated is obviously incorrect, a conclusion supported also by the results of this study. The data presented

![Graph](https://example.com/graph.png)

**Fig. 2.** Specific activity-time curve of Experiment III
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here favor a mechanism of biosynthesis of the various polyenes, similar to that proposed by Goodwin and Jamikorn (4) and Goodwin (5) and MacKinney (6), and Jenkins and MacKinney (7), that these compounds originate from one or, possibly, more than one large precursor by parallel pathways.

SUMMARY

Methyl-labeled acetate was administered to green, ripening tomatoes, and the colorless polyenes and lycopene were isolated from groups of tomatoes at various time intervals during the course of the ripening period. The specific activity-time curves obtained indicate that lycopene is neither a product nor a precursor of the more saturated polyenes.

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