Role of Hexose Monophosphate Pathway in Tomato Catabolism 1, 2

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Materials & Methods

The tomato fruit used in the present experiments were of the Michigan State Forcing variety and were selected on the same basis as described in earlier work (3, 9).

▲ Carbon-14 Labeled Substrates: Gluconate-1-C14 was purchased from the Nuclear-Chicago Corp. Gluconate-2, and -6-C14 were prepared from the correspondingly labeled glucose samples by the method of Moore and Link (16). Ribose-1-C14, glucose-2-C14, and glucose-6-C14 were obtained from the National Bureau of Standards through the kind cooperation of Dr. H. S. Isbell.

The labeled substrates were introduced individually into intact fruit in the form of aqueous solutions (0.2 ml) according to the modified vacuum infiltration method described by Barbour et al. (3). The weights of the tomatoes and the chemical and radiochemical level of each of the substrates used are given in table I. The utilization of the labeled substrates by fruit was followed by means of the radiorespirometric technique using the rate of C14O2 production, particularly from gluconate-1-C14, as the guide. When the production of C14O2 of C-1 of gluconate slowed down to a practically insignificant rate, the fruit were removed from the respiratory chamber for processing.

▲ Isolation Procedures: The fruit employed in the labeled gluconate experiments was individually processed after homogenization in a Waring blender. The homogenate was exhaustively extracted with 80% ethanol. After removal of the solvent, the residue was taken up in water and the solution passed sequentially through a Dowex-50 (H form) column to remove the amino acids and a Dowex-3 (OH form) column to remove the organic acids. The effluent of the second resin column, containing fruit sugars, was concentrated and subjected to paper chromatography for separating individual sugars using butanol-acetic acid-water (4:1:5) as the solvent.

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2 This research was supported by a contract from the Atomic Energy Commission (No. AT(45-1)-573), published with the approval of the Monograph Publication Committee, Research Paper No. 402, School of Science, Oregon State University.
3 A portion of the work described herein is taken from the thesis presented by W. P. Doyle for the degree of Doctor of Philosophy at Oregon State College, 1960. Present address, Texaco Research Laboratories, Beacon, N. Y.
The glucose band on the paper chromatogram was eluted with water and the purity of the isolated glucose was verified again by paper chromatography and radioautography. The organic acids and amino acids were eluted from the respective resin columns with 1 N HCl and assayed for radioactivity.

\[ \text{Degradation of Glucose:} \quad \text{The radioactivity of the glucose samples was determined by a persulfate combustion of glucose to CO}_2 \text{ (6) which was in turn converted to BaCO}_3 \text{ for radioactivity assay. Glucose was degraded microbiologically to CO}_2 \text{, C-1, ethanol (C-2,3), and lactic acid (C-4,5,6) according to the method of Bernstein et al. (5). The ethanol was oxidized to acetate with dichromate and degraded further by the Katz modification of the Phares procedure (13), thus yielding the radioactivity located in C-2 and C-3 of glucose, respectively. The distribution of radioactivity in lactate was determined by the method of Katz (13).} \]

\[ \text{Radioactivity Assay Method:} \quad \text{The radioactivity of various samples was determined by means of a gas flow Geiger-Müller counter equipped with a thin mylar window. All carbonaceous compounds were converted to barium carbonate which was in turn mounted on aluminum planchets by means of the centrifugation technique for radioactivity assay. Countings were carried out to a standard deviation no greater than 1% and the counting data were corrected for self-absorption and background in the conventional manner.} \]

**Results & Discussion**

Previously the catabolism of glucose in tomato fruit has been examined by Barbour, Buhler, and Wang (3) by means of the radiorespirometric method. Estimation of pathway participation, under a set of assumptions, revealed that the administered glucose was catabolized mainly by way of the glycolytic pathway (84%) and to a limited extent (16%) via an alternate route presumably involving phosphogluconate decarboxylation as a key step. The validity of the assumptions set forth by these authors was examined later by Doyle and Wang (9). It was concluded from findings in a series of radiorespirometric experiments employing C14 specifically labeled gluconate substrates, that one of the key assumptions, i.e., "the pentose phosphate formed in the phosphogluconate decarboxylation is not engaged extensively in further catabolic processes", needs further evaluation. Thus it was observed that as much as 10% of C-6 of gluconate was converted to respiratory CO2 in a matter of 33 hours (9). The findings imply that the earlier estimation of catabolic pathways may only represent a limited case of the catabolic mechanisms in tomato fruit.

Moreover, if the pentose phosphate derived from phosphogluconate were utilized in respiratory functions, it should be of great interest to understand the catabolic pathways followed by the pentose phosphate leading to the production of CO2. On the basis of current understanding on pentose metabolism in plants, pentose phosphate may participate in one or more of several possible catabolic sequences. First, pentose phosphate can be incorporated as intact units into plant constituents such as pentosans. Second, pentose phosphate can be converted to fructose-6-phosphate (F-6-P) via the pentose cycle reactions. The fate of the F-6-P so formed is the theme of discussion by several workers. Katz and Wood (12, 18), under the assumption that F-6-P is in perfect isotopic equilibrium with glucose-6-phosphate (G-6-P) have analyzed the recycling of F-6-P via the pentose cycle pathway which is defined as: 1 glucose-6-P \( \rightarrow \) 3 CO2 + glyceraldehyde-3-p. On the other hand, Dawes and Holms (7), in their study of glucose catabolism in Sarcina lutea, estimated the participation of glucose pathways on the basis that the F-6-P formed in the HMP pathway is a component of a pool of hexose monophosphate common to both pathways. The F-6-P is, hence, subject to further degradation by both the EMP and HMP routes in the same proportion as the original glucose. A perfect isotopic equilibrium between F-6-P and G-6-P was neither indicated nor required for the method of pathway estimation devised by these authors. It should be further emphasized that the HMP pathway is defined by Dawes and Holms as: 3 glucose \( \rightarrow \) 3 CO2 + 2 C6 + 2 C5. Neither of these two definitions includes any drainage of intermediates of the HMP pathway for biosynthetic activities, although the definition of Dawes and Holms (7) is more realistic and better suited as a working premise for pathway estimations since the function of the pentose cycle pathway is not restricted solely to respiratory activities.
In the present study, the fate of pentose phosphate, formed in the HMP pathway, in tomato metabolism is traced by the use of gluconate-2-C\textsuperscript{14}, gluconate-6-C\textsuperscript{14}, and ribose-1-C\textsuperscript{14}. Advantage is taken of the fact that neither gluconate nor ribose can be converted directly to glucose (11), therefore, one is permitted to examine exclusively the catabolic mechanism of the HMP pathway particularly with respect to the hexose phosphates reconstructed from the skeleton of either ribose-5-phosphate or 6-phosphoglucose. Under the assumption that the administered substrates behave in the same manner as those formed in situ along the catabolic pathway, the isotope distribution patterns of fruit hexoses derived from the labeled substrate should reveal the extent of recycling of the pentose cycle pathway (12, 18). The findings will then permit one to compare the C\textsuperscript{14}O\textsubscript{2} yields from various carbon atoms of glucose and gluconate so that the complete fate of hexose in fruit respiration can be fully elucidated.

With ribose-1-C\textsuperscript{14}, gluconate-2-C\textsuperscript{14}, and gluconate-6-C\textsuperscript{14} as substrates, the distributions of radioactivity among the fractions isolated from the fruit are given in Table II. An inspection of these data reveals that significant amounts of the labeled substrates are in the soluble carbohydrate fraction. It was found, upon chromatographic analysis of the carbohydrate fraction, that the bulk of the radioactivity from the labeled gluconates was in fruit glucose and fructose with glucose having about one and one-half as much radioactivity as fructose. In the experiment with ribose-1-C\textsuperscript{14}, the radioactivity was distributed mainly in the pulp and respiratory CO\textsubscript{2} and to a less extent in the soluble carbohydrates, including glucose. Since a considerably higher level of substrate was used in the gluconate experiments, as necessitated by the low specific activity of the labeled gluconate available, an effort was not made to compare directly the findings in the ribose and gluconate experiments.

The isotope distribution patterns in glucose samples derived from ribose-1-C\textsuperscript{14}, gluconate-2-C\textsuperscript{14}, or gluconate-6-C\textsuperscript{14} are presented in Table III. These patterns are basically consistent with the transformation of pentose phosphate, derived from the administered ribose or gluconate, to hexose monophosphate by way of the pentose cycle reactions without much recycling, since, theoretically, in the absence of recycling of hexosemonophosphate, C-1 of ribose or C-2 of gluconate should be relocated exclusively in C-1 and C-3 of the reconstructed hexose in the ratio of two to one, respectively (4). However, significant amounts of activity from either C-1 of ribose or C-2 of gluconate also were detected in C-2 of the fruit glucose. This can be accounted for by one of two

### Table II

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Ribose-1-C\textsuperscript{14}</th>
<th>Gluconate-2-C\textsuperscript{14}</th>
<th>Gluconate-6-C\textsuperscript{14}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of total</td>
<td>% of total</td>
<td>% of total</td>
</tr>
<tr>
<td>Substrate administered*</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Pulp</td>
<td>36</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>36</td>
<td>59</td>
<td>66</td>
</tr>
<tr>
<td>Respired CO\textsubscript{2}</td>
<td>24</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Fats</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Acids</td>
<td>5</td>
<td>13</td>
<td>22</td>
</tr>
<tr>
<td>Amino acids</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>15</td>
<td>43</td>
<td>35</td>
</tr>
<tr>
<td>Glucose</td>
<td>9</td>
<td>26</td>
<td>21</td>
</tr>
<tr>
<td>Total recovery</td>
<td>96</td>
<td>82</td>
<td>87</td>
</tr>
</tbody>
</table>

* The radioactivities of the substrates are, respectively: ribose-1-C\textsuperscript{14}, 5.6 \( \mu \)c; gluconate-2-C\textsuperscript{14}, 9.0 \( \mu \)c; gluconate-6-C\textsuperscript{14}, 9.0 \( \mu \)c.

### Table III

<table>
<thead>
<tr>
<th>Carbon atoms of glucose</th>
<th>Ribose-1-C\textsuperscript{14}</th>
<th>Gluconate-2-C\textsuperscript{14}</th>
<th>Gluconate-6-C\textsuperscript{14}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of total</td>
<td>% of total</td>
<td>% of total</td>
</tr>
<tr>
<td>C-1</td>
<td>48</td>
<td>43</td>
<td>20</td>
</tr>
<tr>
<td>C-2</td>
<td>10</td>
<td>14</td>
<td>0.2</td>
</tr>
<tr>
<td>C-3</td>
<td>26</td>
<td>22</td>
<td>0.3</td>
</tr>
<tr>
<td>C-4</td>
<td>4</td>
<td>5</td>
<td>0.2</td>
</tr>
<tr>
<td>C-5</td>
<td>2</td>
<td>3</td>
<td>0.2</td>
</tr>
<tr>
<td>C-6</td>
<td>10</td>
<td>12</td>
<td>77</td>
</tr>
</tbody>
</table>

Specific activity \( \text{cpm} \times 10^{-8} \) per mm glucose

\[
\begin{array}{llll}
\text{Ribose-1-C}\textsuperscript{14} & 37.4 & 150.2 & 124.9 \\
\end{array}
\]
possible mechanisms; namely, A: recycling of hexose phosphates through the pentose cycle pathway, and B: reversed or exchange reactions catalyzed by transketolase or transaldolase (17,18). The first mechanism does not seem to explain in full the observed labeling patterns of glucose since it is impossible to preserve a substantial amount of C\textsuperscript{14} activity at C-1 while a significant amount of C\textsuperscript{14} activity is being transposed from C-3 to C-2 of the reconstructed F-6-P via the pentose cycle pathway. It thus appears that the second mechanism is a more plausible one to account for the observed labeling of C-2 of the reconstructed glucose molecules in fruit. The findings in the ribose experiment are different than that reported by Hiatt (10) with animals. In the latter case, C-1 of ribose was incorporated much heavier into C-3 rather than C-1 of glucose in rat glycogen, and the observation was interpreted as the result of interaction of pentose phosphate with F-6-P via the transketolase and transaldolase reactions.

It is also interesting that as much as 20\% of the radioactivity originally located at C-6 of gluconate was transposed to C-1 of the fruit glucose. The most likely mechanism accounting for this finding is the recombination of triose phosphate to fructose-1,6-diphosphate via the aldolase reaction. Similar findings with strawberries have been reported earlier by Loewus and Jang (14).

Despite the presence of complicating side reactions, the main pathway of gluconate catabolism in tomato fruit appears to be that involving the sequential operation of phosphogluconate decarboxylation followed by the pentose cycle reactions giving rise to the formation of F-6-P. The conclusion, in good agreement with that indicated by Macalchan and Porter in tobacco leaf disks (15), is drawn from a comparison of the observed recovery of gluconate carbon atoms in respiratory CO\textsubscript{2} with that predicted by considering the operation of the foregoing stated sequential reactions. Experimental data used for this comparison are taken from experiments reported earlier (3,9).

The yields of C\textsuperscript{14}O\textsubscript{2} derived from gluconate carbon atoms are calculated under the assumption that the F-6-P, formed biologically, undergoes catabolism in a manner identical to that of glucose. Essentially the calculation is comprised of three steps:

1. The portion of the labeled gluconate that has degraded to pentose phosphate is estimated to be 77\% since 77\% of the radioactivity in gluconate-1-C\textsuperscript{14} was recovered in the respiratory CO\textsubscript{2} presumably yielding the corresponding amount of pentose phosphate.

### Table IV

Conversion of Gluconate Carbon Atoms to Respiratory CO\textsubscript{2}

<table>
<thead>
<tr>
<th>Skeleton of re-formed hexose</th>
<th>Fraction of gluconate carbon atoms* in reformed hexose</th>
<th>Conversion of gluconate carbon atoms to respiratory CO\textsubscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Calculated according to 1-turn pentose cycle mechanism</td>
<td>(b) Calculated according to observed glucose labeling</td>
<td></td>
</tr>
<tr>
<td>Gluconate-2-C\textsuperscript{14}</td>
<td>Gluconate-3,4-C\textsuperscript{14}</td>
<td>Gluconate-6-C\textsuperscript{14}</td>
</tr>
<tr>
<td>1</td>
<td>0.77×0.67=0.52</td>
<td>0.77×0.33=0.25</td>
</tr>
<tr>
<td>2</td>
<td>0.77×0.67=0.52</td>
<td>0.77×0.33=0.25</td>
</tr>
<tr>
<td>3</td>
<td>0.77×0.33=0.25</td>
<td>0.77×0.67=0.52</td>
</tr>
<tr>
<td>4</td>
<td>0.77×0.83=0.64</td>
<td>0.77×0.12=0.09</td>
</tr>
</tbody>
</table>

* Calculation made on the basis that average radiochemical recovery of gluconate-1-C\textsuperscript{14} in respiratory CO\textsubscript{2} is 77\% in three replicate radioisoporic experiments (9). The figure is higher than that observed in the incorporation experiment reported here, since in the latter case, incubation was terminated at 20 hours instead of 33 hours. For the reconstruction of hexose skeleton from the pentose phosphate, see Beevers (4).

** See Barbour, Buhler, and Wang (3), data taken at the end of the experiment.

*** Calculated under the assumptions that C-1 glucuronate is 100\% labeled and that C-1 glucuronate has the same specific activity as C-2 d-glucose.
II. Theoretically, the fate of the carbon atoms of pentose phosphate, with respect to the reconstruction of hexose monophosphate via pentose cycle reactions, can be visualized according to the scheme depicted by Beevers (4) [column (a) of table IV]. For example, C-2 of glucuronate is distributed 67% and 33%, respectively, in C-1 and C-3 of the reconstructed F-6-P. Experimentally, the fate of pentose phosphate is revealed by the observed labeling patterns of fruit glucose, as derived from specifically labeled glucuronates [column (b) of table IV].

III. The yields of CO₂ from individual carbon atoms of the reconstructed hexose monophosphate are taken as those observed in the radiorespirometric experiment on the utilization of specifically labeled glucose substrates (3).

IV. The yield of CO₂ from a given carbon atom of glucuronate is, therefore, calculated according to the following example:

The fraction of glucuronate-2-C₁⁴ participated in catabolism is 77% or 0.77.

The radioactivity of glucuronate-2-C₁⁴ is relocated in the reconstructed F-6-P to the extent of:

\[ 0.77 \times 0.67 = 0.52 \text{ or } 52\% \text{ in C-1 of F-6-P} \]

\[ 0.77 \times 0.33 = 0.25 \text{ or } 25\% \text{ in C-3 of F-6-P} \]

The yield of C₁⁴O₂ from the labeled glucuronate is therefore:

From C-1 of the reconstructed F-6-P:

\[ 0.77 \times 0.67 \times 0.14 = 0.07 \text{ or } 7\% \text{ of the administered glucuronate-2-C₁⁴} \]

From C-3 of the reconstructed hexose:

\[ 0.77 \times 0.33 \times 0.33 = 0.08 \text{ or } 8\% \text{ of the administered glucuronate-2-C₁⁴} \]

The total yield is therefore:

\[ 0.07 + 0.08 = 0.15 \text{ or } 15\% \text{ of the administered glucuronate-2-C₁⁴} \]

The yields of C₁⁴O₂ from individual carbon atoms of the labeled glucuronate, calculated in the stated manner, are given in columns (d) and (e) of table IV along with the respective values observed in the radiorespirometric experiment on glucuronate utilization (9). The proximity of the observed values to the corresponding calculated values, particularly those on the basis of observed isotopic distribution pattern for fruit glucose, thus verifies the stated sequence for the catabolism of F-6-P. It, therefore, appears that in tomato fruit, the HMP pathway is indeed a shunt pathway and the principal function of the HMP pathway is to convert glucose to various carbon skeletons, such as pentoses, heptoses, tetrose, etc., for biosynthetic purposes. The overflow of the HMP pathway, in the nature of F-6-P, is routed mainly to the EMP pathway which, in conjunction with the TCA cyclic pathway, is evidently the principle route for respiratory functions.

The recognition of the complete fate of pentose phosphate in respiratory functions, prompted one to re-examine the equations devised earlier for estimating pathway participations in glucose catabolism (3). The working assumptions set forth for the derivation of the revised equations are essentially the same as that devised and verified in this paper with respect to the one concerned with the catabolism of pentose phosphate. They are summarized as follows:

I. The EMP glycolytic pathway and the HMP pathway account for the primary breakdown of glucose. The operation of other minor pathways, although possible, are not considered to be contributing much to the overall respiratory activities. Thus, preliminary studies in this laboratory indicated that although a small fraction of C-6 of glucuronate can be converted to the respiratory CO₂, the glucose traversing the glucuronate pathway amounts to less than 5% of the total glucose engaged in catabolic functions.

II. The conversion of C-1 of glucose to CO₂ in the phosphogluconate decarboxylation is a rapid process.

III. The triose phosphates formed in the glycolytic process are equivalent to each other with respect to further metabolic reactions.

IV. The pyruvate derived from trioses is decarboxylated oxidatively giving rise to acetate.

V. The pentose phosphate derived from glucose via the HMP pathway is converted via the pentose cycle reactions to F-6-P. The F-6-P, a component of the pool of hexose monophosphate but not necessarily in perfect isotopic equilibrium with glucose-6-phosphate (G-6-P), is in turn catabolized via both the EMP and HMP pathways with pathway distribution identical to that of the original glucose.

VI. The substrate glucuronate used in the radiorespirometric experiment can be readily converted to 6-phosphogluconate which behaves metabolically in a manner identical to that of 6-phosphogluconate formed in the HMP pathway.

On the basis of these assumptions, use can then be made of the radiochemical recoveries in CO₂ from carbon atoms of glucose and glucuronate observed in the radiorespirometric experiments to estimate quantitatively the participation of pathways in glucose catabolism. The estimation actually involves two different aspects, namely: the fraction of the administered glucose actually catabolized and the relative participation of pathways with respect to the catabolized glucose.

Let \( G_1 \), \( G_{1-4} \), and \( G_6 = C^{14}O_2 \) yields from fruit metabolizing equal amounts of glucose substrates labeled with \( C^{14} \) at C-1, C-3 (or 4), or C-6, respectively. Data are taken at the duration of experiments equivalent to 1 relative time unit (RTU). The term, relative time unit, is defined as the duration of experiment at which the production of \( C^{14}O_2 \) from the labeled carbon atoms of substrate, particularly those known to be readily oxidized to \( CO_2 \), have declined to a steady low rate. It is believed that the \( C^{14}O_2 \) yields observed at 1 RTU represent a more concise picture of the fruit catabolism with respect to the administered substrates. The \( C^{14}O_2 \) yields are expressed as fractions of unity.

\( A_1, A_{1-4} \) and \( A_6 = C^{14}O_2 \) yields from fruit metabolizing equal amounts of glucose substrates with \( C^{14} \) at C-1, C-3 (or 4), or C-6, respectively. Data are taken at 1 RTU and are expressed as fractions of
$G_T = \text{Total radioactivity of each labeled substrate administered. The value is always equal to one when it is expressed on the basis of fraction of unity.}$

$G_T' = \text{fraction of the labeled substrate administered that was not catabolized, expressed as a fraction of unity.}$

$G_t = \text{fraction of the administered glucose catabolized, expressed as a fraction of unity,}$

$G_i = G_T - G_T'$

$G_p = \text{fraction of } G_t \text{ catabolized via the HMP pathway, expressed as a fraction of unity.}$

$G_e = \text{fraction of } G_t \text{ catabolized via the glycolytic pathway, expressed as a fraction of unity.}$

In the expression given by Barbour et al. (3),

$G_p = \frac{(G_1 - G_0)}{G_1}$

the term $G_0$ was, in reality, representing the yield of CO$_2$ from C-6 of glucose via exclusively the EMP pathway since it was assumed that there exists no other route for the conversion of this carbon atom to CO$_2$. The realization that C-6 of glucose can also be converted to CO$_2$ via the HMP pathway, thus, necessitates that a correction term be applied to the term $G_p$. The magnitude of the correction term should represent the amount of CO$_2$ originating from C-6 of glucose via the HMP pathway and can be visualized as equal to the product of $A_6$ and $G_p$, i.e., CO$_2$ yield from C-6 of gluconate multiplied by the fraction of glucose substrate engaged in catabolic processes that has been routed through the HMP pathway. It should be emphasized that the relationship is derived from the considerations that gluconate cannot be converted to glucose directly and that the administered gluconate, upon phosphorylation, behaves catabolically in a manner identical to that of the phosphogluconate derived from substrate glucose. Upon applying the correction term to $G_0$ one finds equation I becomes:

$G_p = \frac{[G_1 - (G_i - A_0G_p)]}{G_i}$

II

and

$G_p (G_1 - A_0) = G_1 - G_0$ III

solve for $G_p$, one finds that,

$G_p = \frac{(G_1 - G_0)}{(G_1 - A_0)}$ IV

Since it has been assumed that in tomato fruit, glucose is catabolized by way of the concurrent operation of the EMP and the HMP pathway, it follows that,

$G_e = 1 - G_p$ V

The expression, $G_i$, can be derived in a similar manner by applying the appropriate corrections to the term $G_0$ and $G_2$, thus,

$G_i = G_T - G_T'$

$= [G_1 - (G_0 - A_0G_p)] + [G_{3-4} - A_{3-4}G_p]$

$= G_1 - G_0 + G_{3-4} + G_p (A_0 - A_{3-4})$ VI

By the use of equations IV, V, and VI, it is then possible to estimate the participation of catabolic pathways of glucose if applicable) with radiorespirometric data obtained in experiments using only five labeled substrates, namely: glucose-1-C$^{14}$, 12.5%; glucose-3,4-C$^{14}$, 29%, and glucose-6-C$^{14}$, 7%, and that observed in the glucone experiments (9) at 1 RTU (33 hr) were: glucose-1-C$^{14}$, 77%; gluconate-3,4-C$^{14}$, 23%, and gluconate-6-C$^{14}$, 10%. Employing these data, it was calculated according to equations IV, V, and VI that 27% of the glucose engaged in catabolism was routed through the HMP pathway and 73% via the EMP pathway. The term, HMP pathway, in the present case is applied to that relying on the phosphogluconate decarboxylation as the key step. It is understood that the product of the latter process, pentose phosphate, may engage either in biosynthetic activities or in pentose cycle reactions leading to the reconstruction of F-6-P which is in turn catabolized by both the EMP and HMP pathways.

The participation of the HMP pathway estimated with the revised equations, 27%, is considerably higher than that calculated with the original equation, i.e., 16% (3). It is believed that revised estimation is closer to the true catabolic picture of the fruit. It should be stressed that the estimation of pathway participation is subject to the validity of the assumptions devised for the derivation of equations and, more important, to the correctness of the prevailing understanding in carbohydrate catabolism. Information on pathway participation is very useful in evaluating the function of individual pathways in the overall metabolism of biological systems. However, attachment of absolute quantitative significance to the information of this nature is neither justifiable nor desirable.

**Summary**

The fate of pentose phosphate in tomato catabolism has been examined by the study of the incorporation of ribose-1-C$^{14}$, gluconate-2-C$^{14}$, and gluconate-6-C$^{14}$ into fruit glucose. It appears that pentose phosphate, administered or formed in situ, is catabolized mainly by way of the reactions in the pentose cycle giving rise to the reconstruction of hexose monophosphate. The latter is believed to be further catabolized in a manner similar to that of fruit glucose, i.e., by way of concurrent operation of the Embden-Meyerhof-Parnas (EMP) and hexose monophosphate (HMP) pathways. The HMP pathway functions mainly as the mechanism for the conversion of glucose to various intermediates for biosynthesis. The overflow of the HMP pathway is routed by way of fructose-6-phosphate and fructose-1,6-diphosphate to the glycolytic pathway, the major respiratory mechanism in tomato fruit.

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4 There existed an error in figure 3 of the report by Doyle and Wang (9). The scale of the ordinate for the radiorespirometric pattern of gluconate utilization should read 20, 40, and 60% instead of the 25, 50, and 75% as given.
On the basis of the findings in the present work, the equations given previously for the estimation of pathway participation have been refined to include corrective terms accounting for the production of CO₂ from C-6 and C-3,4 of glucose via the HMP pathway.

Literature Cited