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THE PATH OF CARBON IN PHOTOSYNTHESIS. XVI.
KINETIC RELATIONSHIPS OF THE INTERMEDIATES IN STEADY STATE PHOTOSYNTHESIS

A. A. Benson, S. Kawaguchi, P. Hayes and M. Calvin

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THE PATH OF CARBON IN PHOTOSYNTHESIS. XVI. KINETIC RELATIONSHIPS OF THE INTERMEDIATES IN STEADY STATE PHOTOSYNTHESIS

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ABSTRACT

A kinetic study of the accumulation of C^{14} in the intermediates of steady state photosynthesis in $C^{14}O_2$ provides information regarding the sequence of reactions involved. The work described applied the radiochromatographic technique for analysis of the labeled early products. The simultaneous carboxylation reaction resulting in malic acid as well as phosphoglycerate is demonstrated in experiments at high light intensity. A comparison of radioactivities in a number of phosphorylated sugars as a function of time reveals concurrent synthesis of fructose and sedoheptulose phosphates followed by that of ribulose phosphates and later by that of glucose phosphates. The possibility that the cleavage of C_4 compounds to C_2 carbon dioxide acceptors may involve C_7 and C_5 sugars and evidence for this mechanism is presented.

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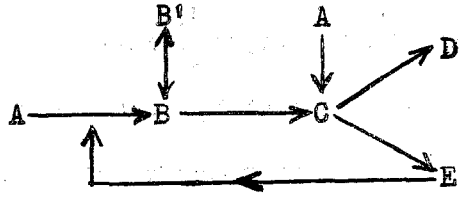
(1) The work described in this paper is sponsored by the U. S. Atomic Energy Commission.

The path of carbon in photosynthesis begins with a small number of carboxylation reactions. A direct approach to the nature and sequence of subsequent reactions requires an observation of the rates of transfer of labeled carbon atoms from carbon dioxide to the successive intermediates of phytosynthesis. The complexity of the inter-relationships among the various intermediate compounds increases considerably as one examines successive steps. Some of the intermediates can undergo transformation into two or more alternative products and thus provide points of branching in the synthetic sequence. Since some of these products may in their turn be involved in the initial synthetic reactions, cycles will appear, and the steady state becomes a rather delicately balanced network of interrelated and interdependent reactions, a change in any one of which must result in some change in most of the others.

This paper constitutes a report of an extension of an experimental quantitative kinetic analysis of this complex system. Although ultimately it should be possible to describe such a system more or less rigorously in terms of the rate of passage of carbon into and through the multitude of compounds (and particular parts or atom positions in compounds) of which it is part, it seems somewhat premature to try and do so in precise algebraic terms now when we are just beginning to discover the nature of what it is we wish to describe, nor is this necessary in order to recognize a number of rather straightforward kinetic relationships which one is likely to encounter.

Some of the more common of these elements which are to be expected may be described schematically in the following way in which at least four essential kinetic elements are represented:



(1) The simple consecutive sequence having a precursor-product relationship would be represented by the transformations A ⟶ B ⟶ C.

(2) The branching sequence would be represented by the transformations B ⟶ C and C ⟶ D and/or E.

(3) The cyclic system in which a product also plays a part in the production of its precursor may be represented in the sequence B ⟶ C ⟶ E and part or all of E serving, in turn, as a contributory source for B.

(4) In a highly organized chemical system, such as the intact cell, there is very likely to be more than one reservoir of a compound serving more than one purpose. This is represented in the diagram by the two reservoirs for the compound B, the second being represented by B'. B' may very well not be in the direct sequence of intermediates preceeding C, and the rate of equilibration between B and B' may have any relation to the rates of the other reactions.

The technique of our kinetic experiment presumably corresponds to a steady state system in which the mass concentration in each of the compounds remains constant. Just how closely we have approached this idealized situation in the present experimental arrangement remains yet to be determined in view of the rather large fluctuations in CO_2 concentration just prior to and during the run. (See experimental part.) A number of simple kinetic consequences between compounds related as above may be immediately set down.[2]

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(2) These considerations are strictly applicable only to individual carbon atoms within each compound. A number of attempts at expressing turnover rates more generally have been made; D. B. Zilversmit, C. Entenman and M. C. Fishler, J. Gen. Physiol., 26, 325 (1943); H. Branson, Bull. Math. Biophys., 8, 159 (1946), 9, 93 (1947).

Into the first member of this system (in this case A corresponds to carbon dioxide) at a time t = 0 is inserted the labeled atom. The measurements then consist in the determination of the rate of appearance of the labeled atom in all successive compounds and atomic positions. From the nature of these appearance curves and the various compounds concerned it should be possible to determine their place in the complex synthetic system which must exist in the cell.

It is worth mentioning at this point that the method of determining precursor-product relation which depends upon the relationship between the specific activity of the two suspected materials is not unequivocally applicable in the highly organized system of the intact cell, although it has been quite successful in its application for simpler reactions which might be studied in isolated enzymatic systems.[3] The reason for this is the very likely occurrence of such reservoirs as represented by B'. The specific activity as usually determined (counts/min./mg. of compound) is not necessarily significant in establishing a precursor-product relationship when the compound is isolated from a complete organism as it is in the present experiments. There may be a number of different sources for any particular compound and these sources may be more or less isolated kinetically, not in equilibrium with each other, so that although the specific activity of a particular precursor in a certain isolated region of the organism might be very high, it would not appear that way when that compound is isolated from the whole organism and thus diluted by the inert (non-radioactive) reservoirs from other sources.

Perhaps the simplest possible consequence, which is easily recognized, is the fact that the appearance curves should have finite initial slopes only for those compounds which have no appreciable stable reservoirs

(3) H. G. Wood, "Advances in Enzymology," Vol. XII, Interscience Publishers, Inc., New York, New York (1951). p. 135; Federation Proc., 9, 553 (1950).

lying between them and the initially labeled material. All others[4] must, of necessity, commence with a zero slope since the reservoirs lying between them and the initially labeled compound must first become labeled.

This leads to two other consequences. First, it is to be expected that the reservoir of a precursor will become saturated before that of its product. Secondly (this is simply another mode of expression of the initial slope idea previously described), if the percentage distribution of label among a particularly selected group of compounds is plotted, that one which precedes the others in time will be the one having a negative initial slope whereas all the others will have positive slopes and approach zero at t = 0. The case of a product which, in turn, serves as source for at least part of its precursor can be very much more complex. However, in its simplest aspect, the rate of incorporation of label into it would consist of the sum of two rates, namely, that in which the label is first incorporated and that in which the label is incorporated into its precursor. Particular examples of these cases will appear in the discussion.

The results of previous investigations of this laboratory[4b] and recently those of other laboratories[5] demonstrated that a predominant carboxylation reaction in photosynthesis results in the formation of C_3 compounds with carboxyl-labeled phosphoglycerate probably being the first product. Analysis of the later products in separate experiments demonstrated the probability

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(4)(a) A. A. Benson, et al., J. Am. Chem. Soc., 72, 1710 (1950).
(b) M. Calvin, et al., Symposia for the Society of Experimental Biology, (Great Britain), 5, 284 (1951).
(c) H. Gaffron, E. W. Fager and L. W. Rosenberg, Symposia for the Society of Experimental Biology (Great Britain), 5, 262 (1951).

(5) H. Gaffron and E. W. Fager, Ann. Rev. Plant Physiol., 2, 87 (1951).

of a sequence of reactions similar, in the reverse order, to those known in glycolysis. A major problem has been the source of the C_2 carbon dioxide acceptor required for phosphoglycerate synthesis. Several years ago the results of work in this laboratory[6] suggested that the source of the carbon dioxide lay in the C_4 compounds related to malic acid. While a C_4 intermediate is not essential to such a cycle[7] the simultaneous and rapid formation of malic acid in steady state photosynthesis suggests that its C_4 precursors may be involved in the process of C_2 synthesis. The results herein reported seem to implicate C_7 and C_5 compounds as well in this process.

Experimental Part

Algae. - Scenedesmus D_3 grown continuously in a shaking apparatus[8] were harvested daily whereby a crop of 2-2.5 cc. of packed cells was obtained from one liter of medium. There is a correlation between the age of the cells and the size of the ribulose diphosphate reservoir. Two-day cells had only 10-50% as much as one-day old cells in each of the six experiments analyzed.[9]

The algae were centrifuged quickly, washed once with deionized water and resuspended at a concentration of 2 cc. of cells per 200 ml. of water in a water jacketed circular illumination vessel with plane sides 1 cm. apart.

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(6) M. Calvin and A. A. Benson, Science, 107, 476 (1948); Science, 105, 648 (1947).

(7) E. W. Fager, J. L. Rosenberg and H. Gaffron, Federation Proc., 9, 535 (1950).

(8) A. A. Benson, et al., "Photosynthesis in Plants," Chapter 19, Iowa State College Press, Ames, Iowa (1949), pp. 381-401.

(9) A. A. Benson, et al., J. Biol. Chem., 196, 703 (1952).

This was illuminated from both sides with 300 w. reflector spot lights with most of the infrared absorbed by water-cooled absorbent glass plates. After 20-30 minutes photosynthesis with 4% carbon dioxide-in-air, the gas bubbled through the suspension was changed to air for five minutes. One mg. of potassium dihydrogen phosphate was added two minutes before t = 0 as buffer for the $NaHC^{14}O_3$ to be added at t = 0.

The temperature measured in the algal suspension was controlled by that of the circulating water in the water jacket.

Time series experiments. - Immediately before adding 100 μc. of $NaHC^{14}O_3$ (9% C^{14}) the gas circulating system was closed whereby air was recirculated from the top of the vessel to the bottom using a rubber tubing pump[10] of relatively small volume. At t = 0 radiobicarbonate in 0.5 ml. was injected into the solution and vigorous stirring accomplished by the rapid gas circulation. During the first five seconds a small U-tube containing 200 μc. of $C^{14}O_2$ previously closed off by an appropriate stopcock was opened to the circulating system. Circulation of this gas was continued throughout the experiment.

Samples of the algae were withdrawn at five-second intervals through an 8 mm. stopcock at the bottom of the illumination vessel into Erlenmeyer flasks containing approximately four volumes of boiling ethanol.

Determination of total fixation curves. - The 80% ethanol suspensions of algae were filtered with Celite and the solids re-extracted with hot 10% ethanol. Aliquot portions of the extracts were taken immediately for colorimetric chlorophyll determination. From a standard curve prepared for extracts of similar batches of algae, the amount of algae taken in each sample was

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J. W. Weigl and D. Stallings, Rev. Sci. Instruments, 21, 395 (1950).

determined. The curves in Figures 1 and 2 were obtained from these values and the activity measured by direct counting of the initial ethanol suspensions and of the final extracts.

Chromatography of algal extracts. - Each extract was concentrated to 1-2 ml. and recounted using micro pipettes to redetermine the amount of extract equivalent to that from 10 $mm.^3$ of fresh cells. The appropriate volume of extract was placed at the origin of oxalic acid-washed Whatman No. 1 paper and developed in the longer direction first with freshly distilled phenol solutions containing 29% deionized water. The second solvent was butanol-propionic acid-water.[4a] Radiograms of these chromatograms required 7-30 days exposure to Eastman "No-Screen" X-ray film.

Enzymatic hydrolysis and rechromatography of radioactive hexose phosphate areas. - In most instances extracts of Scenedesmus give two well-separated radioactive areas, each of which contained hexose monophosphates.[9] The sum total of both areas is hereafter in this paper designated the HMP area (hexose monophosphate area). The faster moving region in both solvents has been called the FMP area (fructose monophosphate area) simply because upon hydrolysis the dominant hexose found here is fructose, while the slower moving region has been called the GMP area (glucose monophosphate area) for a similar reason. In all cases at least one other labeled sugar is found in the hydrolysates of each of these areas.[11]

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(11) In the GMP area the additional radioactive sugar is predominantly sedoheptulose, while that in the FMP area seems to be predominantly mannose. In sugar beet, soy bean and barley there is very little mannose. These two sugars are not well separated either as free sugars or as phosphates by the paper chromatographic methods here used. They are most readily distinguished by the ease with which the heptose is converted to the heptosanhydride in dilute acid, a treatment which leaves the mannose unchanged.

After counting the individual radioactive areas, they were eluted and hydrolyzed with 100 γ of Polidase (Schwarz) for three days.[9] The hydrolysates were rechromatographed on Whatman No.1 paper and developed as far as possible in both solvents. Radioactivity in each of the three sugars was determined by direct counting of the areas defined by darkened areas on the radiogram viewed under the paper on a standard X-ray illuminator. The radioactivities observed on the chromatograms of the hydrolysates approached but did not equal those of the original monophosphate areas due to mechanical and adsorption losses. Hence, the percentage of each sugar in the hydrolysate was determined and the original amount in each phosphate was determined using this fraction and the total activity in the phosphate area. Hydrolysates of the 20° experiment were prepared from the separated fructose and glucose monophosphate areas. Those of the 15° experiment were of the total of both areas. The formation of sedoheptulose at very short times was investigated at two points, 10 seconds and 20 seconds, in the 2° experiment. These times appear to correspond to times less than 5 seconds of photosynthesis at room temperature.

Radioactivity determination in chromatographically separated compounds. - The radioactive areas defined by the radiogram aligned with radioactive ink spots on corners of the paper were counted directly with 5.5-6 cm. diameter, 1.2-1.4 mg./cm. mica window Geiger Mueller tubes.[4a] Large areas were counted in small sections while small sharply defined spots were counted similarly so that geometric or coincidence errors would be relatively constant.[12] The latter

(12) Separate experiments have demonstrated that this method can give reproducible values to within 5% on different papers and independent amounts of carrier added.

type of errors were avoided by restricting the particular amount of activity counted at one time to values giving negligible coincidence error. Radioactivities recorded in the curves for individual compounds are those measured on paper and have been standardized for a constant counter efficiency. Since counting of C^{14} on such chromatograms corresponds to about one-third the values obtained by direct plating, the actual radioactivities fixed in each compound are three times greater than recorded.

The points used for the curves for photosynthesis at 15° represent a weighted average of the radioactivities measured on each of three sets of chromatograms. Where undue diffusion or incomplete separation of a particular area was involved, the value for its radioactivity was given less weight.

Results

The rates of fixation of C^{14} at 2° and 15° are shown in Figures 1 and 2. The products insoluble in both 10% and 80% ethanol diminish to zero at short times. The curve in Figure 2 shows the effect of decrease in carbon dioxide pressure upon the rate of its assimilation. The apparent linearity of the curve after 4 minutes[13] in this particular experiment as well as in a duplicate suggests that a saturation value of a carboxylation reaction may have been reached whereafter the other reactions continue at 40% of the initial rate until the carbon dioxide is practically consumed. The effect of this decrease in assimilation rate is a general decrease in the amount of radiocarbon in most of the soluble intermediates involved.

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(13) The apparent difference between this observation and the usually plotted photosynthetic rate dependence upon CO_2 pressure may be largely a matter of scale.

The algal extracts were chromatographed two-dimensionally[4a], whereupon the phosphorylated products were separated into several major areas, phosphoglycerate, phosphopyruvate, ribulose diphosphate and an as yet unidentified phosphorylated compound with a very labile glucose linkage. The products previously[4a] reported as hexose monophosphates were separated into two major monophosphate areas, one containing most of the glucose monophosphate and a sedoheptulose monophosphate, the other containing most of the fructose monophosphate and a mannose monophosphate. In very short times the glucose monophosphate area from barley extracts contained almost pure sedoheptulose activity and the fructose monophosphate area had only fructose activity.[14] The curves of Figure 3 show the trend observed in Scenedesmus. The rate of appearance of radioactivity in ribulose diphosphate in the same experiments is included for comparison.

The very small amounts of glucose and fructose diphosphates in the ribulose diphosphate area were not determined. The approximate rates of their appearance was the same as that of their monophosphates. The amounts of ribulose monophosphate in the Scenedesmus used under the present condition was small compared to that of its diphosphate.

The results are reported as curves for radioactivity in individual compounds. The C_3 compounds (Figure 4), the sugar phosphates and sucrose (Figure 5) and the C_4 acids with glutamic added for comparison (Figure 6) include the major soluble products of the total fixation (Figure 2). The ordinates in several cases were adjusted to allow direct comparison of

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(14) In an attempt to avoid any change in steady state conditions at the time of the feeding of the $C^{14}O_2$, the entering stream of CO_2 was not interrupted in a 10 second soy bean experiment. In this case the sedoheptulose contained more radioactivity than any other single compound on the paper.

groups of curves. Figure 7 includes all the major products on the same scale. In all such curves the ordinates represent radioactivity emerging from the paper, which is one-third of the amount measured by direct plating, and corresponds in each figure to some arbitrary amount of cells (usually ~10 $mm.^3$).

Discussion

The analyses described by the curves allow certain conclusions to be drawn regarding the sequence of intermediates. It is clear that the curves for products of carboxylation will have finite slopes at zero time while all others have zero slopes. It is apparent, therefore, that there exists no appreciable stable reservoir between phosphoglyceric acid and carbon dioxide. It would also appear that the possibility exists of phosphopyruvate being a primary product. Although its initial slope (Figure 4) appears to be finite, it is saturated with C^{14} after the phosphoglycerate. Radiograms from 5-second photosynthesis showed only phosphoglycerate and malic acid activity. The possibility exists that phosphopyruvate has a zero initial slope followed by very rapid equilibration with phosphoglycerate.

Malic acid (Figure 6) apparently has a finite slope at zero time. The linear fixation observed in the initial 40 seconds intersects the abcissa at about 3 seconds while phosphoglycerate intersects at 2 seconds. It must be pointed out that only infinitely fast and complete mixing of the added $C^{14}O_2$ could yield curves intersecting at about zero time. Following the initial linearity, the malic acid curve increases in slope abruptly by a factor of about four. This greater rate of malic acid labeling is experimentally exactly equal to that of phosphoglycerate. The first slope, then, would correspond to a simple carboxylation rate, while the greater later slope represents carboxylation of labeled C_3 compounds related to and arising from phosphoglycerate. This situation would correspond to the cycling

feedback described in our earlier diagram by the relation B $\longrightarrow$ C $\longrightarrow$ E $\longrightarrow$ B, in which B would correspond to phosphoglycerate. C is something closely related to malate and E is on the path to the C_2 carbon dioxide acceptor.

Approximate equality of the rates of malic acid and phosphoglyceric acid labeling provide strong evidence that a C_4 compound is a primary product in steady state photosynthesis. The slow rate of equilibration of $C^{14}O_2$ with malic acid in the dark precludes the possibility of using this reaction to account for the rapid labeling of malic acid in the light.

The experiments on malonate inhibition[15] indicated that malic acid is probably not directly involved as an intermediate in the regeneration of the C_2 carbon dioxide acceptor but they do permit the participation of a very closely related C_4 acid which normally may be in rapid equilibrium with malic acid and which is present in extremely low concentration.

The relationship of the total fixation curve in Figure 2 and those for phosphoglycerate and malate in Figure 7 for the same experiment should be pointed out. The rate of accumulation of C^{14} in malic acid is relatively undiminished after four minutes compared to that of the C_3 compounds, particularly phosphoglycerate. If the linearity beyond four minutes in the total fixation curve (Figure 2) is at all significant, and if one attempts to relate this to the phosphoglycerate and malate behavior, one comes to the conclusion that there are two independent carboxylation reactions having different dependencies on carbon dioxide partial pressure. The $C_2 \longrightarrow C_3$ (phosphoglycerate) reaction saturates at a higher partial pressure than does the $C_3 \longrightarrow C_4$ (malate) reaction. The first linear component of the total

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(15) J. A. Bassham, et al., J. Biol. Chem., 185, 781 (1950).

fixation, then, would include the most rapid entry via the $C_2 \longrightarrow C_3$ reaction independent of carbon dioxide pressure above the saturation point. The second linear portion could correspond to the slower $C_3 \longrightarrow C_4$ reaction which has become the only one independent of CO_2 partial pressure, the $C_2 \longrightarrow C_3$ reaction having become dependent on CO_2 partial pressure and proceeding more slowly. This might be considered in terms of two adsorption isotherms controlling each of the independent carbon dioxide fixation reactions and in which the points of saturation for these two independent reactions are fairly close together, with that for the $C_2 \longrightarrow C_3$ reaction at slightly higher pressures of CO_2. Thus one finds two regions in which the rate of carbon dioxide fixation is independent of the carbon dioxide pressure and the transition from the one region to the other is rather sharp.

It is of interest to examine the relationship between the sugar phosphates which appear in the hexose monophosphate area. If one plots the percentage distribution of radioactivity among the sugars (glucose, fructose, mannose and sedoheptulose) which occur in this area, in order to determine the priority of their labeling in time, one arrives at the rather clear-cut result that fructose and sedoheptulose appear to be labeled very nearly simultaneously, while glucose and mannose lag behind (Figure 8). This simultaneous appearance would correspond to the branching point $C \longrightarrow D$, $C \longrightarrow E$ in the earlier diagrams.

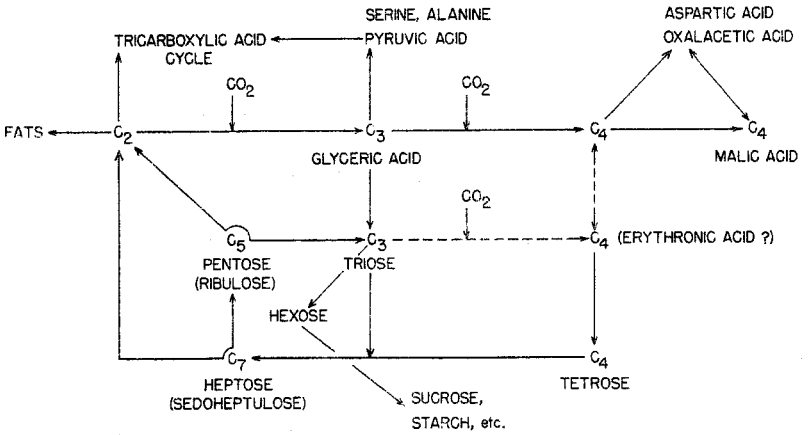
The two or more competing reactions which determine the change of radioactivity in a given compound include its synthesis from its precursor and the exchange or equilibration with, or conversion to, subsequent non-labeled reservoirs. When the flow rate of radiocarbon into the metabolic sequence diminishes as described by the total fixation curve, Figure 2,

equilibration with unlabeled reservoirs of closely related compounds may overtake it and the reservoirs of intermediates along the synthetic sequence most closely related to these unlabeled reservoirs would be observed to diminish most rapidly in radioactivity. The results for the longer periods of photosynthesis are included for the purpose of discussing such effects. It can be seen that the curve for glucose monophosphates, Figures 5 and 7, decreases very rapidly after four minutes when the total fixation rate decreased, Figure 2. Fructose monophosphates do not decrease so rapidly in radioactivity and phosphoglycerate appears to be the last compound suffering dilution. All three compounds are diluted presumably by the inactive polysaccharide reservoirs of the plant. Distribution of radiocarbon within the C_6 and C_3 molecules is not affected by this type of dilution since the cycle for regeneration of carbon dioxide acceptors does not seem to involve hexose synthesis.

While complete degradation data are not yet available for the pentose and heptose or even for some of the simpler compounds, and while the relationships between the pentoses and heptoses are not by any means completely established, it seems worthwhile to proceed on the likely possibility that the heptose precedes the pentose and may be a precursor to it. We are thus led to the following modification of our original proposal for the source of the C_2 carbon dioxide acceptor.

The dotted arrow leading from triose directly to erythronic acid would correspond to the reductive carboxylation of dihydroxyacetone

$$HOCH_2\text{-}CO\text{-}CH_2OH + CO_2 \xrightarrow{2\ [H]} HOCH_2\text{-}CHOH\text{-}CHOH\text{-}CO_2H$$


SERINE, ALANINE
PYRUVIC ACID
TRICARBOXYLIC ACID CYCLE
ASPARTIC ACID
OXALACETIC ACID
CO_2
CO_2
FATS
C_2
C_3
C_4
C_4
GLYCERIC ACID
MALIC ACID
CO_2
C_5
C_3
C_4 (ERYTHRONIC ACID ?)
PENTOSE (RIBULOSE)
TRIOSE
HEXOSE
C_7
C_4
HEPTOSE (SEDOHEPTULOSE)
TETROSE
SUCROSE, STARCH, etc.

which is exactly analogous to the reductive β-carboxylation of pyruvic acid leading to malic acid. The existence of enzymes capable of performing the latter reaction has been demonstrated. The other dotted arrow leading directly to erythronic acid provides a possible alternative route.[16] This is, of course, a diagrammatic chart and is intended to represent primarily the carbon skeletons involved rather than the precise reacting chemical species. Furthermore the carbon skeletons here designated undoubtedly undergo preparative rearrangements of the H and O atoms attached to them between the carbon changes.

It is thus clear that both fructose and sedoheptulose can become labeled simultaneously, or either one slightly ahead of the other, depending upon conditions. The splitting of the four-carbon fragment into two C_2 carbon dioxide acceptors is still essentially here, but in the present proposal it is done while attached to a cyclically migrating three-carbon piece. While further speculation is possible,[17] it seems wiser to await the results of more precise kinetic measurements on more rigorously controlled steady states as well as degradation studies. The data presented in this paper and the available information on the distribution of radiocarbon in the products of short photosynthesis are consistent with the present proposal.

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(16) W. Vishniac and S. Ochoa, Nature, 167, 768 (1951).

(17) M. Calvin, The Harvey Lectures, Vol. 45 (1951), in press.

Captions to Figures

Figure 1 - CO_2 fixation by Scenedesmus during photosynthesis at 2° C. The curve represents the total fixed and there was no appreciable insoluble reaction product at the time interval shown.

Figure 2 - CO_2 fixation by Scenedesmus during photosynthesis at 15° C.

Figure 3 - Radioactivity incorporated in the heptose phosphate areas and the ribulose diphosphate area during photosynthesis by Scenedesmus at 20° C. (10 cu.mm.cells). The sedoheptulose is contaminated with some mannose and the mannose is contaminated with some sedoheptulose as they are given here.

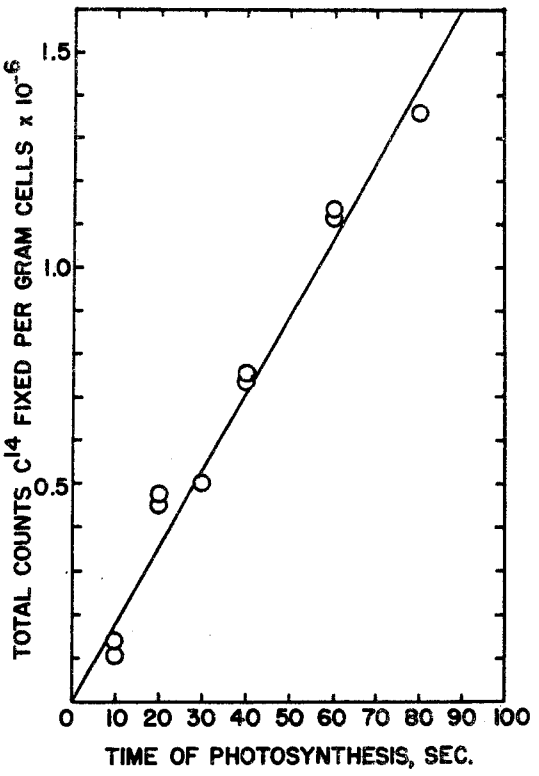
Figure 4 - CO_2 fixation in the two- and three-carbon compounds during photosynthesis by Scenedesmus at 15° C. (10 cu.mm. cells).

Figure 5 - CO_2 fixation in the sugar phosphates and sucrose during photosynthesis by Scenedesmus at 15° C. (10 cu.mm. cells).

Figure 6 - CO_2 fixation in the four-carbon acids and glutamic acid during photosynthesis by Scenedesmus at 15° C. (10 cu.mm. cells).

Figure 7 - CO_2 fixation in the major soluble compounds in the same scale during photosynthesis by Scenedesmus at 15° C. (10 cu.mm. cells).

Figure 8 - Percentage distribution of fixed carbon among the sugars, glucose, fructose, mannose and sedoheptulose during photosynthesis by Scenedesmus at 15° C. The distribution of total count in the heptose area between mannose and sedoheptulose shown above was made from the ratio of these two substances as determined in Figure 3.



Rate of C^{14} fixation by Scenedesmus at 2° C.

Fig. 1

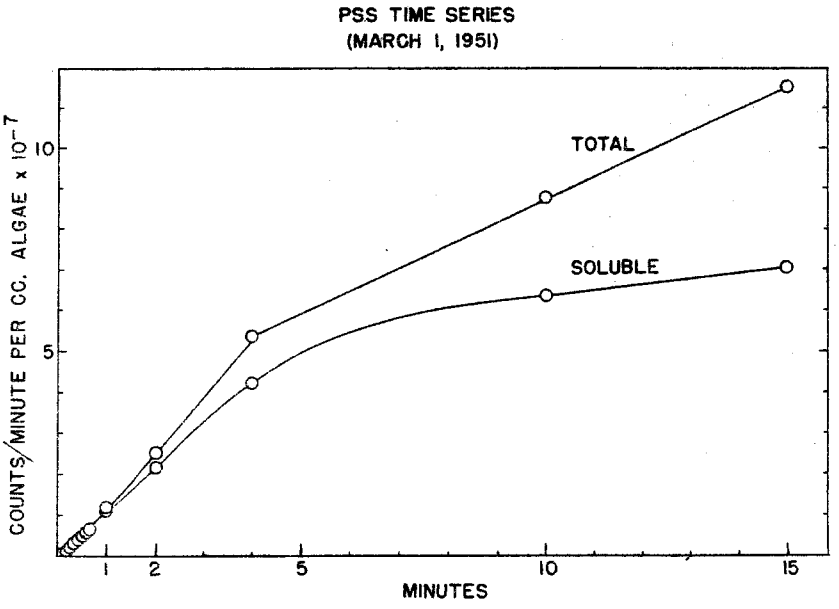
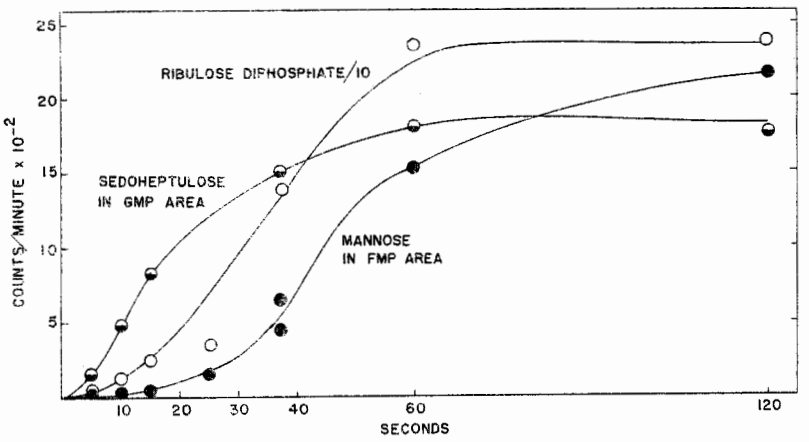


Fig. 2


RIBULOSE DIPHOSPHATE/10
SEDOHEPTULOSE
IN GMP AREA
MANNOSE
IN FMP AREA
COUNTS/MINUTE x 10⁻²
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25
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60
120

Fig. 3

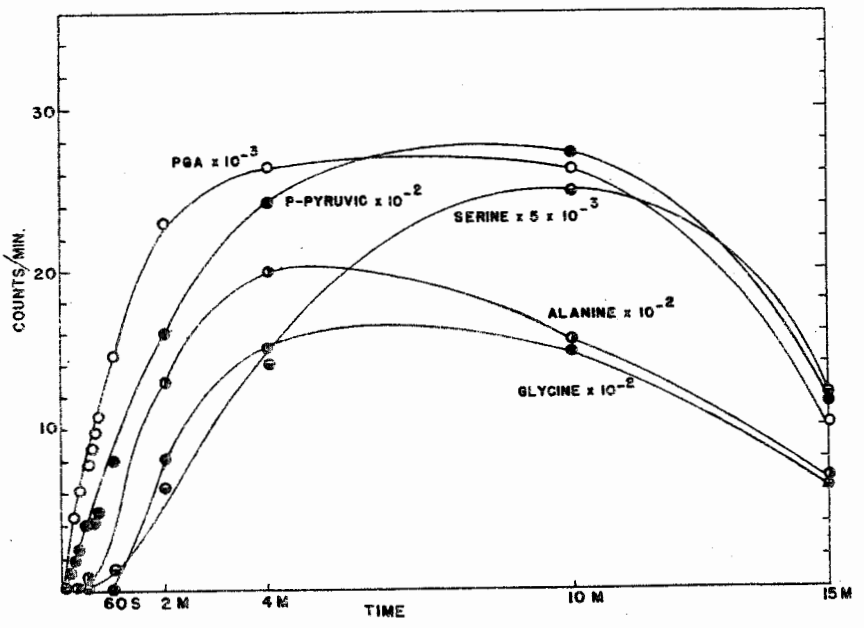
PGA x 10⁻³
P-PYRUVIC x 10⁻²
SERINE x 5 x 10⁻³
ALANINE x 10⁻²
GLYCINE x 10⁻²
COUNTS/MIN.
TIME
30
20
10
60S
2M
4M
10 M
15M

Fig. 4

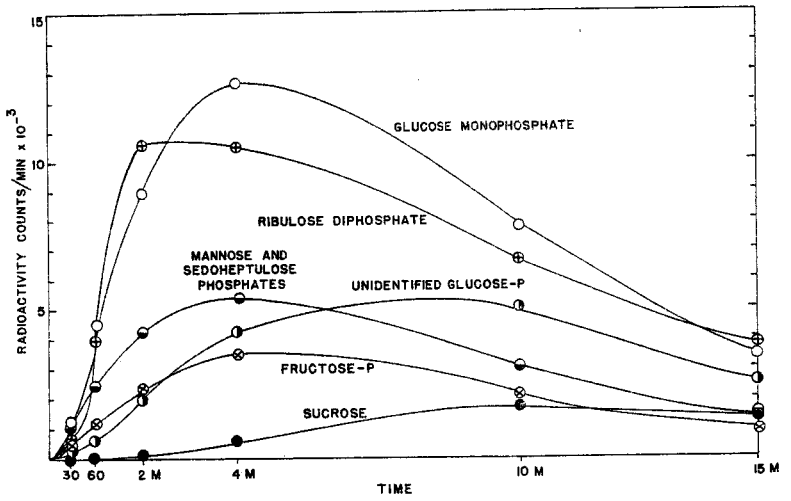
RADIOACTIVITY COUNTS/MIN x 10⁻³
15
10
5
GLUCOSE MONOPHOSPHATE
RIBULOSE DIPHOSPHATE
MANNOSE AND SEDOHEPTULOSE PHOSPHATES
UNIDENTIFIED GLUCOSE-P
FRUCTOSE-P
SUCROSE
30 60
2 M
4 M
10 M
15 M
TIME

Fig. 5

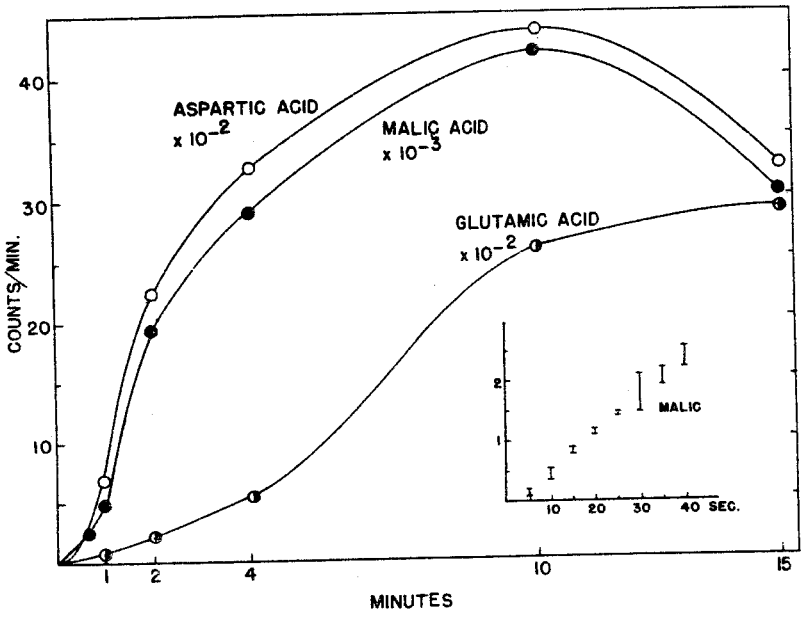
COUNTS/MIN.
40
30
20
10
ASPARTIC ACID
x 10⁻²
MALIC ACID
x 10⁻³
GLUTAMIC ACID
x 10⁻²
MALIC
2
1
10
20
30
40 SEC.
1
2
4
10
15
MINUTES

Fig. 6

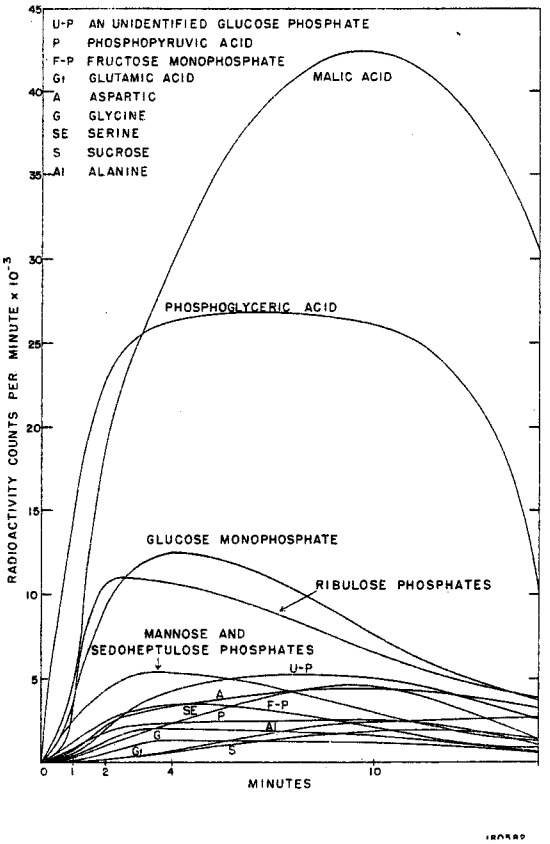
U-P AN UNIDENTIFIED GLUCOSE PHOSPHATE
P PHOSPHOPYRUVIC ACID
F-P FRUCTOSE MONOPHOSPHATE
Gt GLUTAMIC ACID
A ASPARTIC
G GLYCINE
SE SERINE
S SUCROSE
Al ALANINE
MALIC ACID
PHOSPHOGLYCERIC ACID
GLUCOSE MONOPHOSPHATE
RIBULOSE PHOSPHATES
MANNOSE AND
SEDOHEPTULOSE PHOSPHATES
RADIOACTIVITY COUNTS PER MINUTE x 10^-3
MINUTES

Fig. 7

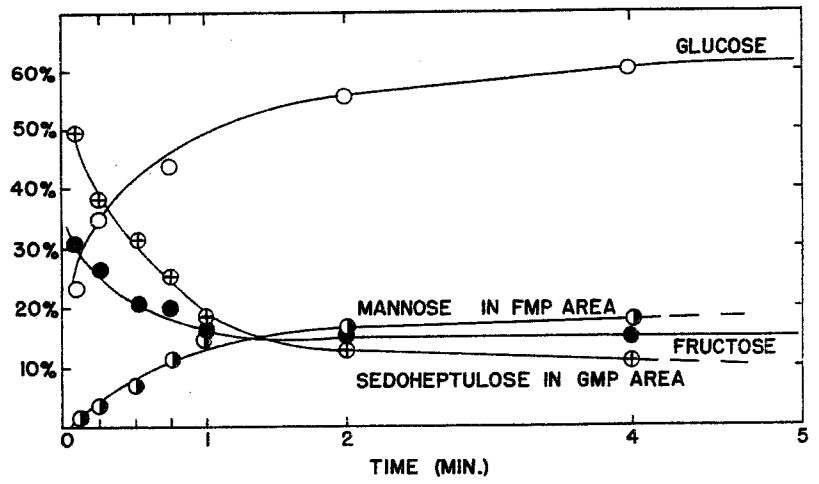
GLUCOSE
MANNOSE IN FMP AREA
FRUCTOSE
SEDOHEPTULOSE IN GMP AREA
TIME (MIN.)

Fig. 8