Title
PHOTOSYNTHESIS: FIRST REACTIONS

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May 20, 1954

Berkeley, California

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The reactions of phytosynthesis include all metabolic reactions in the plant which result in the synthesis of organic compounds. Of primary importance among these are the reactions of photosynthesis which store the energy of light as chemical energy by converting carbon dioxide and water through a series of intermediate compounds to carbohydrate and oxygen. Thus, photosynthesis supplies both the materials and the energy for all other reactions of phytosynthesis.

The studies of photosynthetic $C^{14}O_2$ fixation in this laboratory have now led to an understanding of the first steps of carbohydrate synthesis. From these few compounds are derived the multitude of compounds in the plant.

The fundamental process of photosynthesis is the conversion of electromagnetic energy of the light quantum to chemical energy of the reducing agent(s) required for carbon dioxide reduction. A plausible proposal for the mechanism of this puzzling process has been advanced from our laboratory[1] and is supported by a growing collection of circumstantial, if not positive, evidence.

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

This paper is a review of recent work in this laboratory, most of which is described in more detail elsewhere, J. A. Bassham, A. A. Benson, L. D. Kay, A. Z. Harris, A. T. Wilson and M. Calvin, J. Am. Chem. Soc., 76, 1760 (1954).

(1) Melvin Calvin, Chem. and Engin. News, 31, 1622, 1735 (1953).

It is the function of this photochemically produced reducing power in photosynthesis to which this survey is addressed.

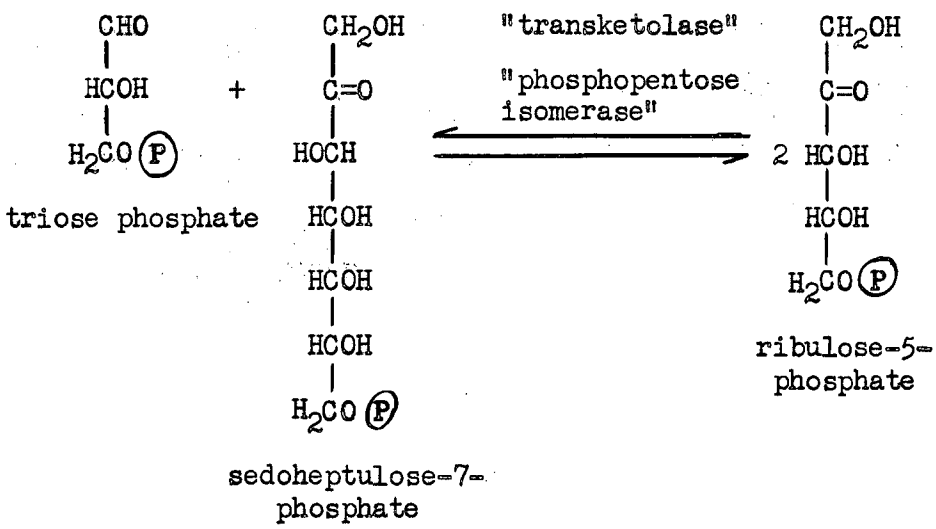
The major first product of $C^{14}O_2$ fixation by all plants is carboxyl-labeled phosphoglycerate. Phosphoglyceric acid (PGA) becomes the major radioactive product. The first hexoses formed are 3,4-labeled. This indicates that two C_3 molecules condense, head to head, to give a C_6. As time progresses, the α and β carbons of PGA acquire C^{14} and the 1,2- and 5,6-carbon atoms of the hexoses become labeled. The typical distribution of label is given in Figure 1. Such data are obtained by chemical degradation of pure radioactive compounds separated from each other by two-dimensional paper chromatography.

It is now clear that the plant has made sucrose by a sequence of reactions similar if not identical to the process of glycolytic breakdown of sugars in animal tissue and yeast. In fact, each of the intermediates has been identified and observed to acquire radioactivity in the sequence shown in Figure 2.

As radiocarbon saturates successive reservoirs of these intermediates it would be expected that specific radioactivity (concentration of C^{14}) will be greater for fructose phosphates than for glucose phosphates. This is verified experimentally by an examination of the earliest labeled sucrose. Upon acid hydrolysis and chromatography of the two hexoses, the fructose moiety contained the greater part of the C^{14} (Figure 3). The exact mechanism of the enzymatic synthesis of sucrose is being studied in several laboratories and soon may be well understood.

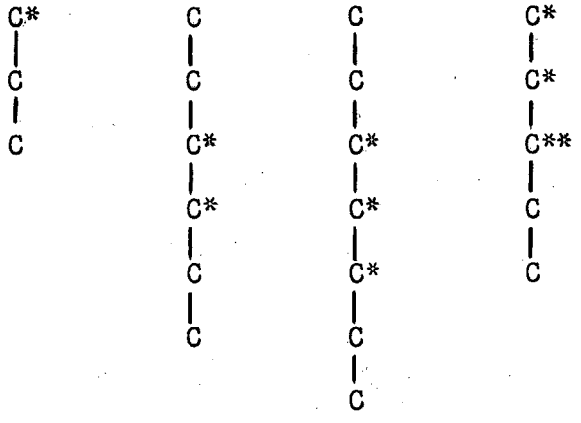
The carboxylation reaction by which phosphoglycerate is formed from CO_2 and some constantly generated acceptor molecule has occupied our attention for some time. The biochemistry of microbiological or animal metabolism offered few clues on the nature of the cyclic process for making the carboxylation substrate. Such clues appeared in radiograms of short photosynthesis in $C^{14}O_2$.

The intermediates of sucrose synthesis were not the only phosphorylated sugars to acquire label in the first seconds of photosynthesis in $C^{14}O_2$. Sedoheptulose, previously found only in succulent plants, sedums, and ribulose, known only as an uncrystallized synthetic sugar, became labeled in all photosynthetic organisms. They had not been observable before the advent of the tracer method with its great sensitivity for detecting their low concentrations (10^{-4} M) and the paper chromatographic method for separating efficiently the numerous sugars and their phosphate esters. The stereostructures of sedoheptulose and ribulose bear little relationship to that of the hexoses. By splitting a glycolaldehyde fragment (C_2) from sedoheptulose, however, one obtains a ribose, the epimer of ribulose. Further, ribulose might lose a C_2 to give triose which we see is the reduction product of PGA. These relationships led to the discovery of an ubiquitous enzyme system, transketolase, in laboratories of B. L. Horecker[2] and E. Racker. Their discovery of the transketolase enzyme supported and clarified our proposal of its function in photosynthesis. With transketolase, the acyloin-type cleavage of ketoses allows a transfer of glycolyl groups from one phosphorylated aldose to another.



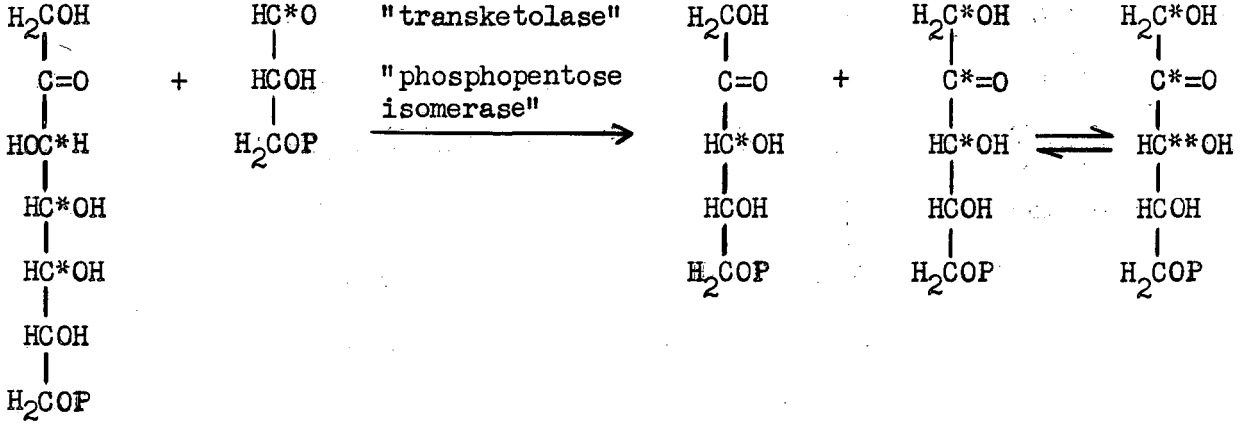
(2) B. L. Horecker, P. Z. Smyrniotis and H. Klenow., J. Biol. Chem., 205, 661 (1953).

These phosphorylated sugars have been isolated from the products of brief $C^{14}O_2$ fixation experiments and chemically degraded to determine the C^{14} distribution within each molecule. The results for products of the first few seconds of photosynthesis are schematically summarized:

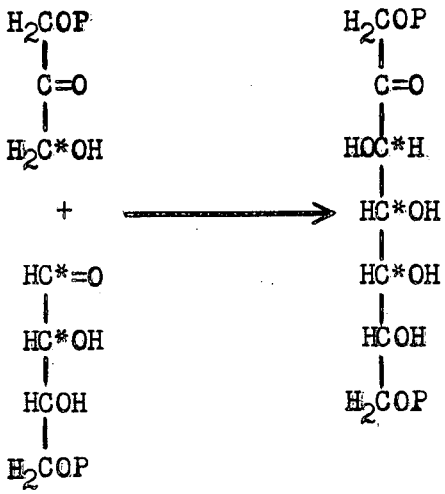


triose hexose sedoheptulose ribulose

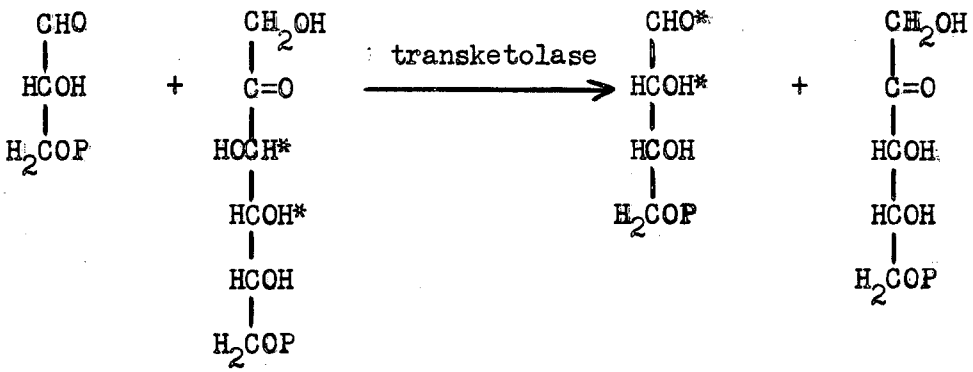
In time, of course, they all become uniformly C^{14}-labeled. The observed labeling of the C_5 molecule does not appear to result from C_2 cleavage from C_7. Transketolase, however, catalyzes C_2 transfer from C_7 to a C_3 aldehyde to give:



This accounts for the pentose labeling but we must have a mechanism for the heptose labeling. Its trans configuration of carbons 3 and 4 suggested that it was formed, like fructose, by aldol condensation of erythrose-4-phosphate (C_4) with glyceraldehyde-3-phosphate (C_3):



The obvious source of such a 1,2-labeled erythrose phosphate among the first products of phytosynthesis is fructose-6-phosphate.



This hypothesis was elegantly proved by E. Racker, et al.[3] with crystalline transketolase and pure fructose-6-phosphate. As soon as the plant has produced some labeled fructose-6-phosphate, then, there is a widespread equilibration of label among the ketoses and it becomes impossible to define the path of carbon in such an arbitrary system.

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(3) E. Racker, G. de la Haba and I. G. Leder, Arch. Biochem. and Biophys., 48, 238 (1954).

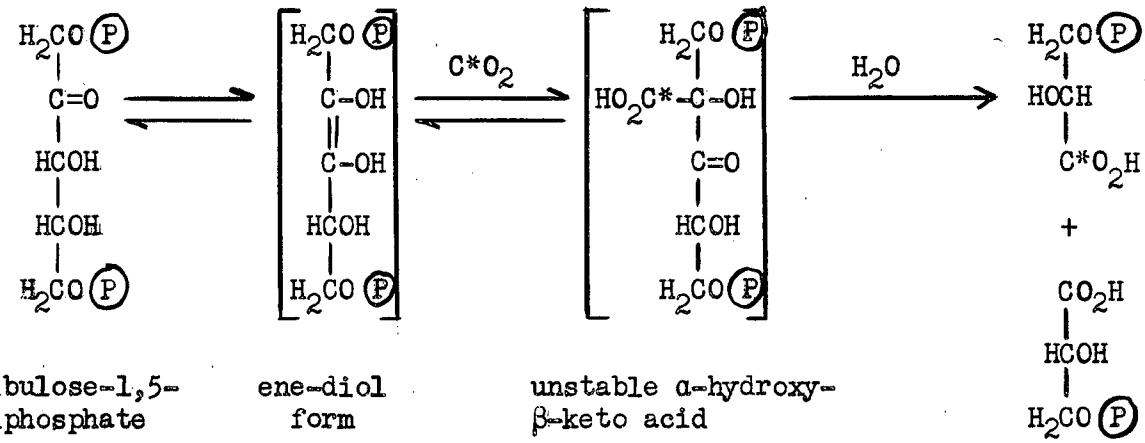
The general trend of this synthetic system is towards the production of ribulose phosphate. Apparently this is the precursor of the CO_2 acceptor and must be produced constantly. The glycolyl enzyme complex produced by each transketolase operation condenses with triose phosphate, one of the most available free aldehydes.

We are suggesting that such a pentose is required as the CO_2 acceptor of photosynthesis. Such a hypothesis is verified experimentally in the following manner. Green algae (Scenedesmus) were given $C^{14}O_2$ for twenty minutes, whereupon the reservoirs of the early photosynthetic intermediates became saturated with radiocarbon and the radioactivity in each compound became a measure of its concentration. In experiments by Mr. A. T. Wilson of this laboratory, the CO_2 pressure was suddenly dropped from 1% to 0.003%. Small samples of algae were withdrawn from the illumination vessel every five seconds and their components separated on paper chromatograms. The radioactive areas, defined by radiograms, were counted with a large-windowed Geiger tube. The concentration of phosphoglycerate and ribulose diphosphate showed profound changes. The concentration of phosphoglycerate, the carboxylation product, went down as would be expected when the carboxylation step, Figure 4, is blocked. Ribulose diphosphate concentration, on the other hand, rose rapidly. When the CO_2 pressure is kept constant and the light is turned off the cycle is blocked at the reduction step and phosphoglycerate accumulates. Ribulose diphosphate and triose phosphate concentrations drop markedly. In fact, several oscillations in these concentrations were observed as might be characteristic of any electrical or hydraulic closed circuit where an external condition is suddenly changed. Ribulose diphosphate is the largest C_5 reservoir of most plants and it seems

to represent the outlet for ribulose-5-phosphate formed in the transketolase system. The phosphorylating power somehow developed during photosynthesis drives this synthesis

$$\text{Ribulose-5-P} + \text{ATP} \longrightarrow \text{Ribulose-1,5-P} + \text{ADP}$$

In considering ribulose-1,5-diphosphate as a possible CO_2 acceptor we notice that it cannot assume a ketal form as does fructose diphosphate but must have a true carbonyl group. This might well enolize and add carbon dioxide to form an unstable α-hydroxy-β-keto acid



ribulose-1,5-diphosphate | ene-diol form | unstable α-hydroxy-β-keto acid

Hydration of the C_2-C_3 bond in such a keto acid should be possible and would lead to one molecule of labeled phosphoglycerate and one unlabeled one. As time of photosynthesis with $C^{14}O_2$ progresses the acceptor becomes labeled and both phosphoglycerates contain C^{14}. The change in free energy of this reaction has been estimated to be -5±5 Kcal. and is extremely favorable compared to many other possible carboxylation reactions. This possibility of low energy carboxylation arises because of oxidation of C_3 from a -CHOH to a -COOH group. The cyclic system, then, is currently considered to be represented by Figure 5.

While the existence of such a mechanism for the primary CO_2 fixation step of photosynthesis is not yet certain, it would seem that when biochemical

transformations were being selected it should have merited the consideration of the plant world. It is particularly interesting that this reaction, through which almost all of the earth's reduced carbon passes, is not yet clearly defined.

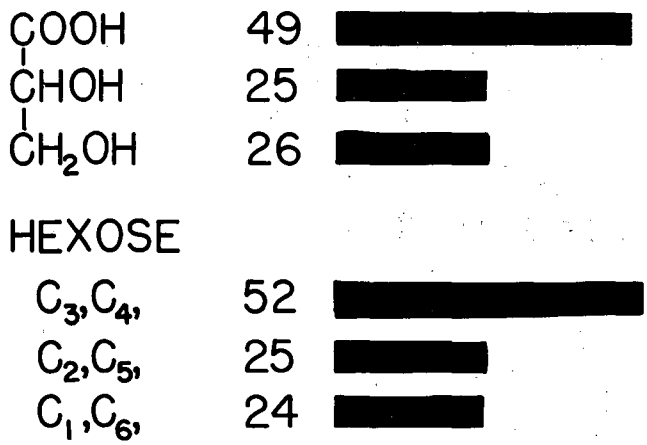


Fig. 1. C^{14}-distribution in phosphoglycerate and hexoses formed during 15 seconds photosynthesis by barley seedling leaves.

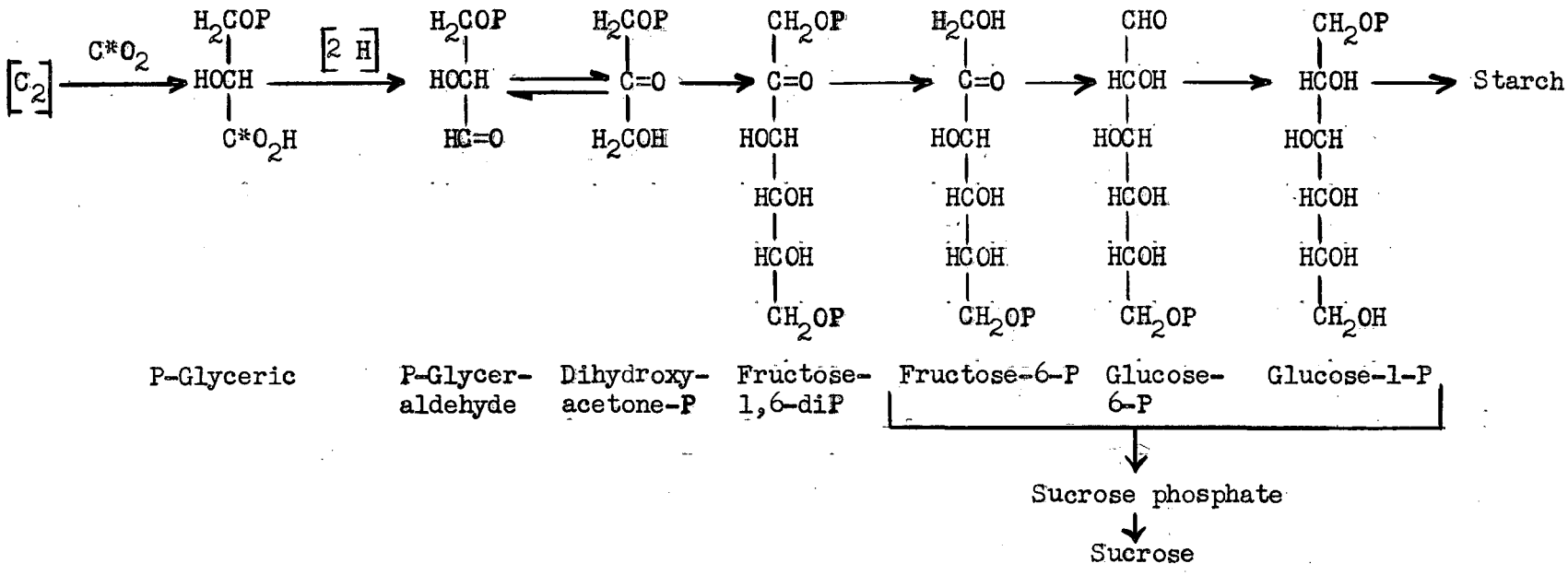


Figure 2

Mechanism of Carbohydrate Phytosynthesis

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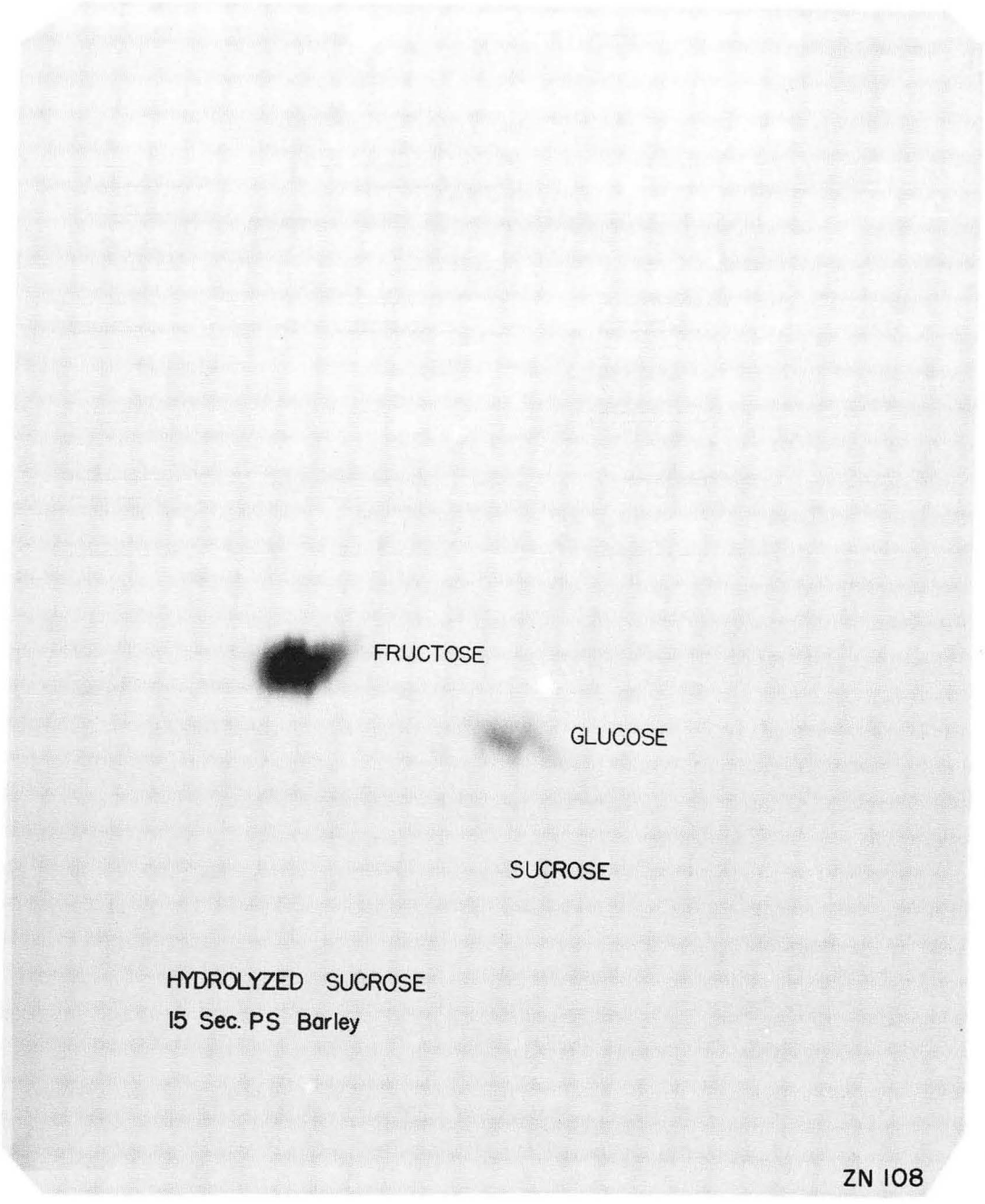
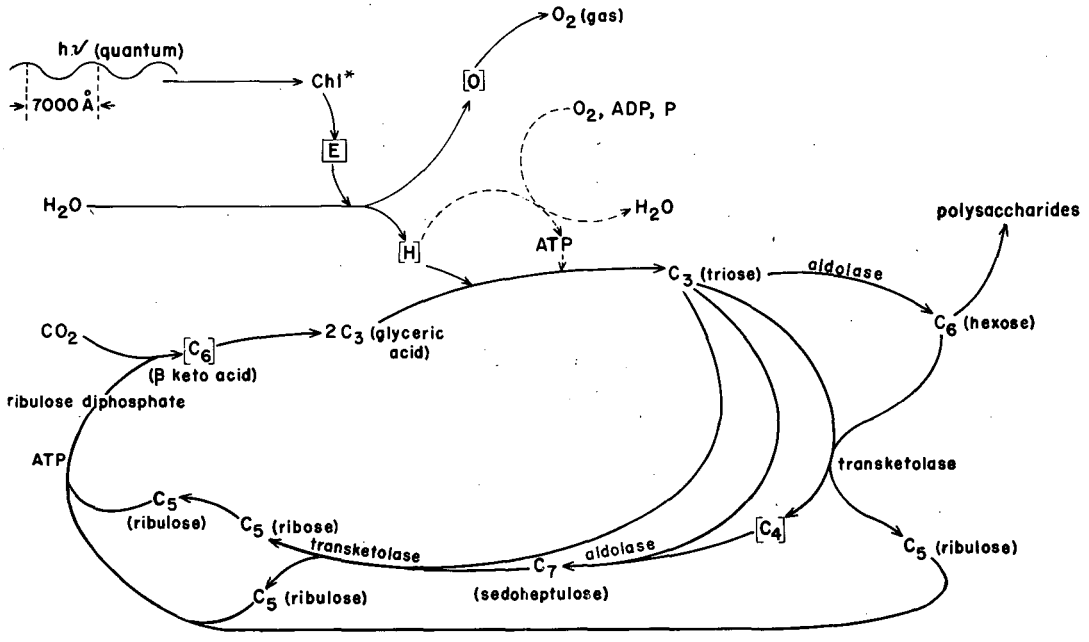


Fig. 3. Hydrolysate of sucrose formed during 15 seconds photosynthesis in $C^{14}O_2$ by barley seedling leaves.



PROPOSED CYCLE FOR CARBON REDUCTION
IN PHOTOSYNTHESIS

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Fig. 4. Cyclic pathway for regeneration of carbon dioxide acceptor of photosynthesis.

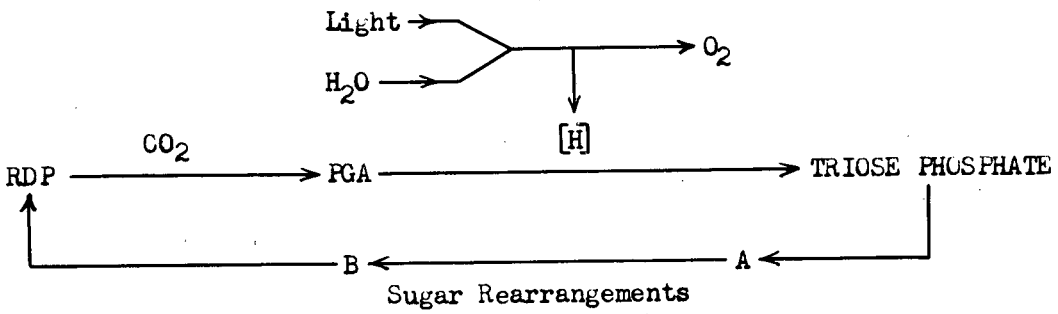
Light
H_2O
O_2
[H]
CO_2
RDP
PGA
TRIOSE PHOSPHATE
B
A
Sugar Rearrangements

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Fig. 5.