Lawrence Berkeley National Laboratory
Recent Work

Title
RECENT KINETIC STUDIES ON THE CARBON REDUCTION CYCLE

Permalink
https://escholarship.org/uc/item/1d46n6m2

Author
Bassham, J.A.

Publication Date
1963-10-01

eScholarship.org Powered by the California Digital Library
University of California

UCRL-11064

University of California

Ernest O. Lawrence Radiation Laboratory

TWO-WEEK LOAN COPY

This is a Library Circulating Copy which may be borrowed for two weeks. For a personal retention copy, call Tech. Info. Division, Ext. 5545

RECENT KINETIC STUDIES ON THE CARBON REDUCTION CYCLE

Berkeley, California

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

Proceedings Symposium on Photosynthesis of Green Plants Oct. 14-18, 1963 Airlie, Va.

UCRL-11064

UNIVERSITY OF CALIFORNIA
Lawrence Radiation Laboratory
Berkeley, California

Contract No. W-7405-eng-48

RECENT KINETIC STUDIES ON THE CARBON REDUCTION CYCLE

J. A. Bassham

October 1963

RECENT KINETIC STUDIES ON THE CARBON REDUCTION CYCLE

J.A. Bassham

The photosynthetic carbon reduction cycle (PSCR cycle) was proposed in essentially its present form about ten years ago [1], following some seven years of study by Calvin and coworkers, who used ^{14}C as a tracer to follow the path of carbon in photosynthesis. Since that time, it has been rather widely, though not universally, accepted as being the correct primary pathway of carbon dioxide reduction during photosynthesis. Studies from this laboratory [2] showed the PSCR cycle, or a cycle utilizing the same intermediate compounds, to be responsible for by far the greater part of the carbon dioxide reduction. Other later studies showed that carbohydrates are not unique as secondary products formed from the intermediates of the carbon cycle. For example, it was shown that the synthesis of carbon skeletons of amino acids could account for 30% or more of the rate of carbon uptake by the carbon reduction cycle during photosynthesis in Chlorella pyrenoidosa [3]. The pursuit of such quantitative studies led to the development of more sophisticated steady-state, steady tracer level techniques[2,3]. Development of these techniques in turn permitted us to restudy some kinetic properties of the PSCR cycle, and to investigate some puzzling facets of the ^{14}C labeling patterns which had been noted earlier in this and other laboratories.

Before presenting these questions and our efforts to find answers to them, I will discuss briefly the PSCR cycle, shown in Fig. 1. In this cycle, the first stable carboxylation product is 3-phosphoglyceric acid (PGA) [4]. All the remaining stable intermediates are sugar phosphates and diphosphates, with carbon skeletons from 3 to 7 carbon atoms in length.

There are four stages in the cycle: 1) Ribulose-1,5-diphosphate (RuDP) is carboxylated and gives two molecules of PGA; 2) PGA is reduced to triose phosphate; 3) A series of reactions convert five triose phosphate molecules to three ribulose monophosphate molecules; 4) The ribulose monophosphate molecules are then phosphorylated with ATP to give RuDP.

Carbon which enters this cycle as CO_2 is later "drained off" in the form of reduced carbon compounds such as PGA or sugar phosphates, by secondary photosynthetic pathways. Such pathways lead to the synthesis of fats, proteins, carbohydrates and other products. Part of these products are then used in the synthesis of new chloroplast structure while the remainder is "exported" to non-green parts of the plant cell, or to other parts of the plant in multicellular organisms.

The asterisks are intended to show the degree of labeling of various carbon atoms in the cycle following a short period (a few seconds) of photosynthesis with $^{14}CO_2$. Newly incorporated $^{14}CO_2$ becomes the carboxyl group of PGA and the unphosphorylated terminal carbon atom of phosphoglyceraldehyde and of phosphodihydroxyacetone. Condensation of these two triose phosphates with each other results in fructose-1,6-diphosphate labeled in carbon atoms 3 and 4. However,

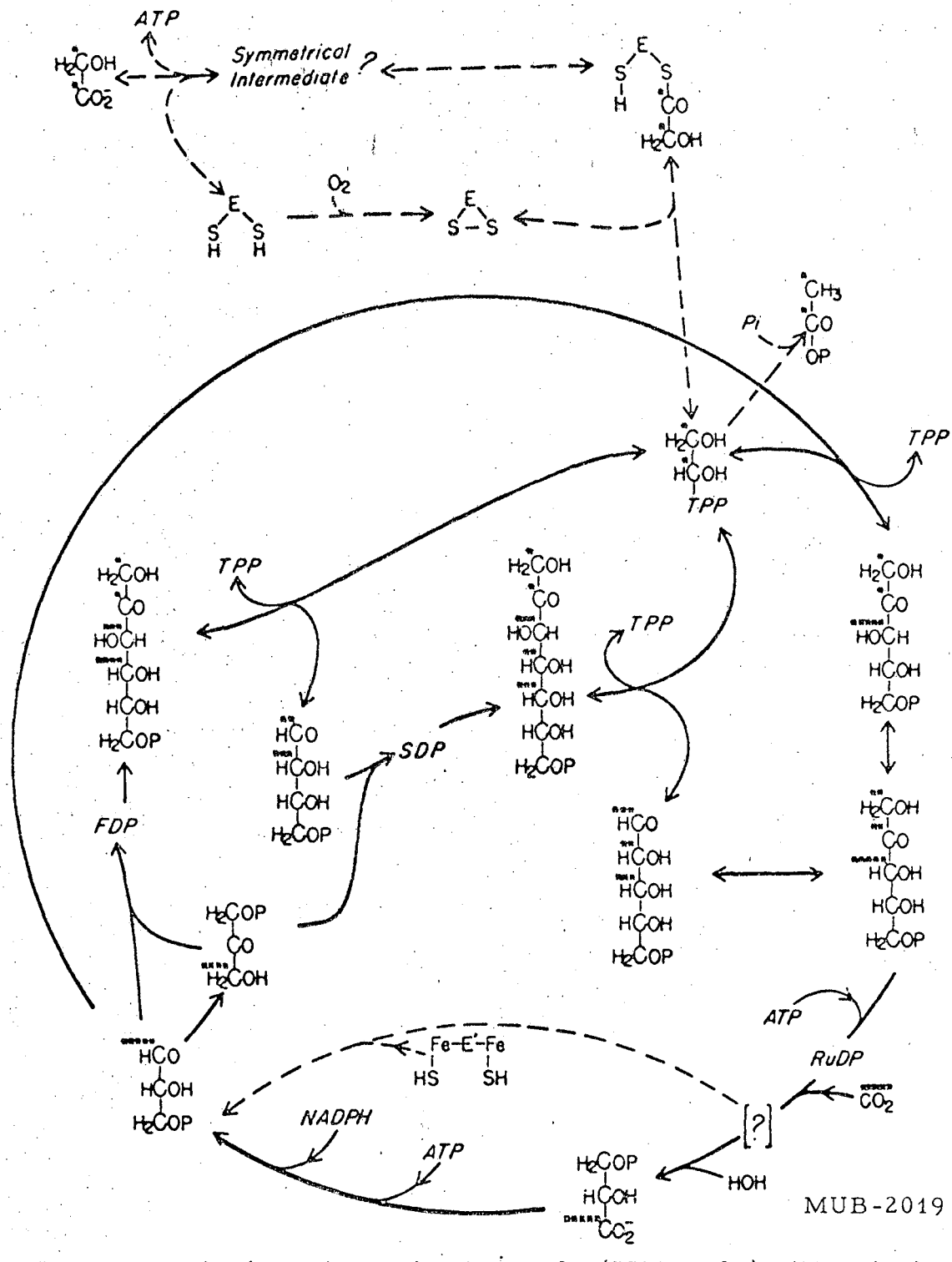


Fig. 1. The photosynthetic carbon reduction cycle (PSCR cycle). Abbreviations include: FDP, fructose-1,6-diphosphate; SDP, sedoheptulose-1,7-diphosphate; RuDP, ribulose-1,5-diphosphate; TPP, thiamine pyrophosphate; E, E', unspecified enzymes or proteins. The E'$(FeSH)_2$ symbolizes a reduced ferredoxin-type electron carrier with a potential of -0.4 v. The asterisks denote the order of labeling (not the accurate magnitude) of various carbon atoms of sugar phosphates, and other intermediates, following a short period (such as 10 sec) of photosynthesis with $^{14}CO_2$. Depending on pool sizes, particularly that of triose phosphates, asymmetry of labeling in hexose and heptose phosphates may be more or less than shown.

there is a higher label in carbon no. 4 than in carbon no. 3 if the equilibration of label between the two triose phosphates has been incomplete so that the specific activity of the glyceraldehyde phosphate is higher than that of phosphodihydroxyacetone.

A transketolase reaction on fructose-6-phosphate, and a subsequent condensation of the resulting tetrose phosphate with dihydroxyacetone phosphate leads to the formation of sedoheptulose diphosphate and of sedoheptulose monophosphate with the label predominantly in carbons 3,4 and 5 with more label in 3 and 5 than in 4. Such a labeling pattern was observed when leaves were exposed to $^{14}CO_2$ during photosynthesis for a second or less (1). This was evidence for the existence of differentially labeled triose phosphate pools and was so recognized at the time (1).

Another transketolase reaction on sedoheptulose-7-phosphate leads to the formation of a ribulose-5-phosphate labeled in carbon atoms 1,2 and 3. The carbon atoms 1 and 2 resulting from the transketolase reactions on fructose-6-phosphate and sedoheptulose-7-phosphate are considered to be in equilibrium with a pool of thiamine pyrophosphate-glycolaldehyde addition compound. Two-carbon moieties from this pool undergo a reversible reaction with glyceraldehyde phosphate to give molecules of xylulose-5-phosphate (labeled in carbon atom no. 3) which in turn is in equilibrium with a ribulose-5-phosphate pool and the ribose-5-phosphate pool mentioned above. Rapid reversible equilibration among these pentose phosphate pools and the thiamine pyrophosphate glycolaldehyde pool results in the feedback of labeling from carbon atoms 1 and 2 of ribose phosphate through ribulose phosphate, xylulose phosphate to glycolaldehyde thiamine pyrophosphate addition compound and thence to the number 1 and 2 carbon atoms of fructose-6-phosphate and sedoheptulose-7-phosphate. This is essentially the explanation which we have given previously (6) for the asymmetric labeling of hexose discovered by Kandler and Gibbs (7).

This rapid reversible equilibration is entirely to be expected, since the free energy changes associated with the transketolase and eperimisation reactions under steady state conditions probably are all in the range $F^S = +1.5$ to -1.5 Kcal. This close to equilibration, and with low activation energies for the reactions involved, the ratio between forward and back reactions is given by $F^S = -RT \ln f/b$ where f is the rate of the forward reaction and b is the rate of the back reaction. (5) At 25°C, $F^S = -1.37 \log f/b$, so that the back reaction is approximately 10% of the rate of the forward reaction.

Other reactions for the glycolaldehyde thiamine addition compound are its conversion by phosphoroclastic splitting to acetyl phosphate, and its oxidation to glycolic acid. Evidence for the acetyl phosphate formation by this path in photosynthesis is lacking. The stimulation of formation of labeled glycolic acid during photosynthesis with $^{14}CO_2$ at high O_2 levels has been demonstrated(8).

The Gibbs effect is often quoted as an argument against the correctness of the PCR cycle (9). This effect, asymmetric labeling of hexose following short periods of photosynthesis with $^{14}CO_2$, consists of two parts: carbon atom no. 4 is more highly labeled than carbon atom no. 3, and carbon atoms 1 and 2 are more highly labeled than carbon atoms 5 and 6.

There is a difficulty in the explanation of the greater labeling of carbon atom 4 as compared with carbon atom 3. The pools of the two kinds of triose phosphate would have to be incompletely equilibrated with respect to carbon labeling. By the argument presented above, one would expect these two types of

triose phosphate to be in rather rapid equilibrium with each other. However, if the relation between phosphoglyceraldehyde and phosphodihydroxyacetone were different from that which exists in glycolysis, it is possible that the labeling of the dihydroxyacetone phosphate pool would not be reflected into the phosphoglyceraldehyde pool. Such a situation might exist if the phosphoglycolaldehyde moiety were bound to an enzyme and if its conversion to dihydroxyacetone phosphate resulted in a larger negative free energy change than is associated with the conversion of free phosphoglyceraldehyde to dihydroxyacetone phosphate.

The possibility that phosphoglyceraldehyde and perhaps erythrose-4-phosphate as well, may exist only in a form bound to the enzyme such as enzyme-S-CO-R is most attractive. It could explain why neither the triose phosphate nor tetrose phosphate are normally seen as labeled intermediates during studies of photosynthesis with $^{14}CO_2$. If the enzyme-bound phosphoglyceraldehyde were unable to react with inorganic phosphate to make phosphoryl-phosphoglycerate, as it does in glycolysis, the oxidation of triose phosphate to phosphoglyceric acid could be blocked. Such a block might be most advantageous to the photosynthetic mechanism, in that it would prevent reoxidation of newly formed sugar phosphates during short periods of darkness. I shall return to this point later.

It is also possible that the existence of such a bound form of phosphoglyceraldehyde, with a block towards its oxidation, could account for the reported lack of aldolase in some photosynthetic organisms (10,11,12). A reversal of the condensation reaction leading to fructose-1,6-diphosphate would give dihydroxyacetone phosphate and bound glyceraldehyde phosphate. Aldolase is known to bind dihydroxyacetone phosphate (13) as a Schiff base. Conceivably some organisms bind both triose phosphates so tightly that they can only be liberated by the condensation reaction leading to fructose-1,6-diphosphate. One might expect the pool of free dihydroxyacetone phosphate, commonly observed in studies of photosynthesis in leaves and in Chlorella with $^{14}CO_2$, to be missing from such organisms.

I would now like to focus attention on our more recent kinetic studies. In most of these experiments we establish a condition of steady state photosynthesis in which all reactions are proceeding at a constant rate and in which the intermediate pool sizes are maintained at constant levels (2,3). We then introduce $^{14}CO_2$ in a step function in such a way that the specific activity of the added tracer comes immediately to its final value and is maintained there during the course of the experiment. The addition of the tracer is accompanied by no other environmental change. Samples may be taken immediately following the addition of the $^{14}CO_2$ at frequent intervals and continuing through the time when intermediates of the carbon reduction cycle have become "saturated" with radiocarbon.

From the initial slopes of the labeling curves upon introduction of $^{14}CO_2$, we can calculate rates of flow of ^{14}C through specific intermediate pools. From the level of radioactivity in specific compounds when they are "saturated" we can determine the concentration of the actively turning over pool, by dividing the total radioactivity by the specific radioactivity of the $^{14}CO_2$ which is maintained constant and is measured. Following "saturation" of intermediates of the carbon cycle, we may vary some environmental factor, such as light or CO_2 pressure, and follow the changes in concentration in the actively turning over pools which are seen as changes in the total radioactivities of these pools (1,2,3,14,15).

In the first of our more recent studies, we investigated the kinetics of the labeling of ribulose diphosphate and of PGA in vivo. We found that from the beginning of the period of steady state synthesis with $^{14}CO_2$ until saturation of the intermediates, the specific radioactivity of PGA was always considerably higher than that of RuDP (2). If the carboxylation of RuDP results in the formation of two molecules of PGA, then the carboxyl group of one of every two PGA molecules will contain the newly incorporated ^{14}C.

To test this model, we subtracted from the total PGA radiocarbon an amount of ^{14}C radioactivity which would correspond to the radioactivity expected in this carboxyl if the model were correct. Since this carboxyl group would rapidly saturate if the model were correct, this involved subtracting 1/2 x 1/3 = 1/6 of the saturation level of radiocarbon in PGA after about 30 seconds. The remaining ^{14}C which would have to be derived from the RuDP carbon atoms was compared with the ^{14}C in RuDP. The remaining, or residual, PGA carbon atoms were found to be labeled to a higher degree of saturation than the average of the five atoms of RuDP, suggesting that this model (2 molecules of PGA per carboxylation) was incorrect.

Using a different model in which only one PGA molecule was formed, and in which it was formed from the newly incorporated $^{14}CO_2$ and carbon atoms 1 and 2 of RuDP, a similar calculation showed that the residual carbon atoms of PGA were not labeled more rapidly than the average of the five RuDP carbon atoms until after 50 seconds. In this case there was no contradiction between model and data even after 50 seconds, since we know that carbon atoms 1 and 2 of RuDP are more quickly labeled than the carbon atoms 3, 4 and 5 (1).

From this data and reasoning, we concluded that the carboxylation of RuDP leads to only one molecule of PGA in equilibrium with the PGA pool. The other three carbon atoms from RuDP appear to have been converted either to a form of bound PGA not in equilibrium with the pool, or to some other molecule. We speculated that if the in vivo reaction were reductive, the other molecule might be triose phosphate.

It was noted earlier that the radioactivity in PGA does not always extrapolate to 100% at zero time (1). Sometimes such extrapolation gives 10-15% ^{14}C in sugar phosphates at zero time. This finding suggests that it is not only the three carbon moiety derived from carbons 3,4 and 5 of RuDP which may not be in equilibrium with the PGA pool. It appears that some of the PGA labeled with the newly incorporated ^{14}C in the carboxyl group is also bound, perhaps to an enzyme, and converted to sugar phosphates without freely equilibrating with the PGA pool.

The experimental evidence suggesting that the newly incorporated ^{14}C does not all pass through the free pool of PGA prompted us to perform further kinetic studies on the labeling of intermediates of the carbon cycle during photosynthesis. Light-dark transient studies were performed (16) under conditions of steady state photosynthesis. As in earlier light-dark transient studies (14,17), the level of PGA rose and the level of ribulose diphosphate fell when the light was turned off. However, under the more nearly steady state conditions used in the more recent study, the concentration of fructose diphosphate was higher than that of ribulose diphosphate and both diphosphates fell to zero in concentration in the dark with equal rapidity. Sedoheptulose diphosphate concentration also dropped, and the sum of the drops of these three diphosphates was not more than equal to the transient rise in PGA concentration. The levels of dihydroxyacetone phosphate and of fructose-6-phosphate

also declined, suggesting that some of the rise in PGA concentration might be due to a momentary reoxidation of sugar phosphates in addition to that resulting from continuing carboxylation of ribulose diphosphate coupled with a cessation of the reduction of PGA to sugar phosphate.

To investigate this point further, we undertook a kinetic study of the carbon labeling of cycle intermediates in preilluminated Chlorella. Following a period of steady state photosynthesis the light was turned off and $^{14}CO_2$ was immediately added without any alteration in the total level of CO_2 which was maintained at about 2%.

Some of the results of this experiment are shown in Fig. 2. There was a rapid

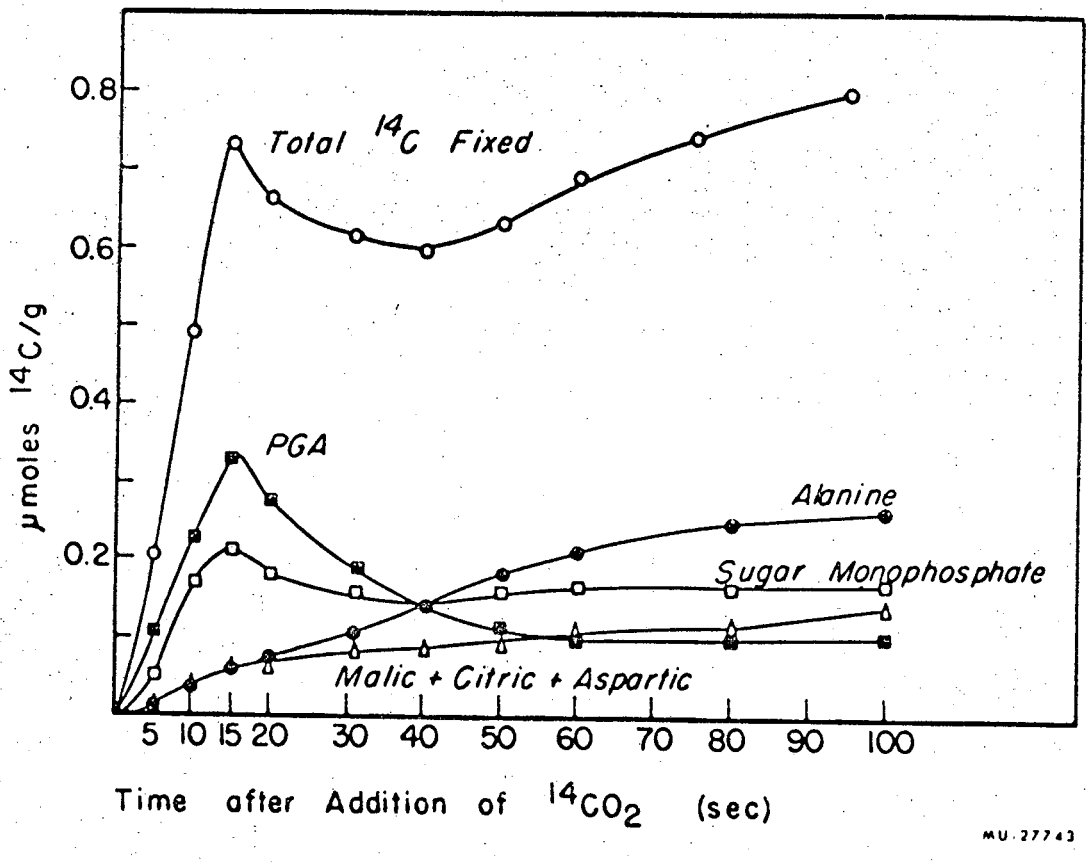


Fig. 2. Fixation of ^{14}C into compounds in Chlorella following preillumination. Sugar monophosphates include monophosphates of fructose, glucose, sedoheptulose, ribose and ribulose. "Total ^{14}C fixed" includes all radioactive compounds, including those on the origin, found on the chromatogram.

initial uptake of $^{14}CO_2$ during the first 15 seconds. This was followed by a temporary decrease in ^{14}C-labeling of the compounds. Presumably some of the newly incorporated ^{14}C was respired. After this drop, a slight loss of newly incorporated ^{14}C in such products as sugar monophosphate occurs during the period from 15-30 seconds. Thereafter, ^{14}C in these compounds remains essentially constant.

This ^{14}C labeling curve of sugar phosphates suggests that there is a mechanism which allows only a small amount of reoxidation of the newly photosynthesized sugar phosphates and then blocks further oxidation of these sugar phosphates by triose phosphate dehydrogenase type reactions. From the usual concept of the PCR cycle in which PGA is reduced by triose phosphate dehydrogenase in the presence of ATP and NADPH, one might have predicted that once these cofactors were exhausted in the dark, the triose phosphate dehydrogenase reaction would be reversed and the newly formed sugar phosphates rapidly oxidized. Thus the result obtained here suggests once again the possibility that phosphoglyceraldehyde in the PCR cycle exists only in an enzyme-bound form which cannot readily be oxidized. This hypothesis would require a corollary - the formation of the bound phosphoglyceraldehyde directly from an intermediate in the carboxylation reaction rather than from reduction of PGA.

Another interesting point seen in the preillumination study is the rapid rate of labeling of sugar monophosphates between 5 and 10 seconds. This rate is equal in magnitude to that of the labeling of PGA despite the fact that during this period the PGA carboxyl group in all probability can be no more than 20 - 30% saturated. (2) This rapid labeling of sugar monophosphates at a time when the PGA pool is only partly labeled appears to be rather direct evidence for the reduction of some bound form of newly incorporated $^{14}CO_2$ which is not in equilibrium with the PGA pool.

I have mentioned several bits of evidence which suggest the possibility that a carboxylation intermediate preceeding PGA might be converted directly to the sugar phosphates without equilibration of label between the intermediate compound and PGA. From the standpoint of chemical possibilities the most likely reaction for the accomplishment of this direct conversion would seem to be a reductive carboxylation reaction. However, the in vitro enzyme system for carboxylation of ribulose diphosphate accomplishes only the non-reductive dismutation to give two molecules of PGA. There is to date no enzymic evidence whatever for a reductive carboxylation. One must suppose that if such a reaction exists, it is mediated by some organized or multifunctional enzyme system which is most difficult to isolate intact from the living system.

In looking for reasons why such a system might be difficult to isolate, one could propose that the system is particulate or an enzyme of high molecular weight, and that the system is easily disrupted into soluble enzymes of smaller molecular weight which lack the necessary organization. A more plausible reason would be that in the in vivo system there is some direct link between the photochemical reactions which produce ATP and electrons, and the carbon reducing system. This link might then be lost when the system is isolated. We know that the green lamellar structures which carry out the photochemical reactions can be rather easily separated from the soluble carbon fixing enzymes (18).

When phosphopyridine nucleotide reductase (PPNR), discovered by San Pietro (19,20), was shown by Tagawa and Arnon (21) to be a non-heme iron protein of the ferredoxin type (22,23) it became a good candidate as the link between the photochemical apparatus and the carbon photosynthetic apparatus of photosynthesis. It has been generally supposed that chloroplast ferredoxin or PPNR mediates the transfer of electrons from the light reaction to NADPH, with the latter cofactor then being used to bring about the reductions of the carbon reduction cycle. However, ferredoxin is a stronger reducing agent than NADPH, being comparable to H_2. If it were used directly in the carbon reducing reactions, only one molecule of ATP per two electrons of reducing agent would be required rather than one and one half molecules of ATP per two electrons as would be the case with NADPH as sole reducing cofactor (5,24). I have proposed

that ferredoxin reduces an enzyme disulfide to disulfhydryl and that the enzyme disulfhydryl in turn functions directly in the reductive carboxylation reaction (24).

It is also possible that ferredoxin could function directly in the carbon reducing reaction. Valentine et al. (25) have shown that in the oxidation of pyruvate in Chlostridium acidi-urici, ferredoxin takes the place occupied by lipoic acid in other systems and mediates the electron transfer between pyruvate and NAD. Since ferredoxin contains three sulfide groups we may suppose that it is functioning here as a disulfide cofactor similar to lipoic acid, and that it is accepting an acetaldehyde moiety from the carboxylation of pyruvate. By analogy with the lipoic acid system, we may suppose that the intermediate is acetyl ferredoxin sulfhydryl which then reacts with inorganic phosphate to produce acetyl phosphate and ferredoxin disulfhydryl.

Possibly ferredoxin contains chains of FeS-FeS-FeS.. arranged so that two of the terminal sulfides have the same orientation as the disulfide of a lipoic acid molecule. At the other ends of these FeS chains, the Fe^{+3} atoms could accept electrons which would then be transported along the chain to the disulfide grouping which would then become disulfhydryl. In this way, the ferredoxin could function as a mediator between one electron and two electron oxidation reduction reactions.

Accepting electrons singly from the photochemical apparatus of the green cell, ferredoxin might transfer two electrons at the potential of hydrogen gas to the enzyme system responsible for the reductive carboxylation reaction. Without attempting to guess the detailed mechanism of this carboxylation reaction, we may nonetheless note that it bears a formal similarity to a reversal of the pyruvate oxidation discussed above.

With these thoughts in mind, we have attempted to investigate the kinetics of the carbon cycle of photosynthesis in the presence of added chemical agents which might interact with disulfide disulfhydryl systems. In one such study (26) we allowed Chlorella to photosynthesize in the presence of $^{14}CO_2$ for about 10 minutes under steady state conditions at pH 5.0. Without disrupting these conditions we introduced an amount of 8-methyl lipoic acid which gave an approximately 0.5 millimolar solution of this lipoic acid analog. Preliminary studies had shown that such an addition caused an immediate complete inhibition of oxygen evolution and CO_2 uptake.

The effect of the addition of this inhibitor upon the levels of various intermediates of the carbon reduction cycle and other photosynthetic products are shown in Figs. 3 and 4. The most dramatic effect is the immediate drop in the level of PGA which falls during the first 15 seconds after addition of the inhibitor to about 1/4 of its steady state value. At the same time, the levels of fructose diphosphate and of sedoheptulose diphosphate rise quite rapidly. Surprisingly, in view of the PGA effect, ribulose diphosphate undergoes only a small positive transient and then a slight decrease to a constant level. It was noted that lipoic acid itself also caused inhibition of photosynthesis, and in more recent but unpublished experiments we find that the effects of 8-methyl lipoic acid are reproduced by the same concentration of lipoic acid.

If the disulfide compound which we have added is accepting electrons from the light reaction and thereby keeping them from being used in carbon reduction, one might expect the resulting transient changes in the intermediates of the carbon cycle to resemble those seen upon turning off the light. It is clear

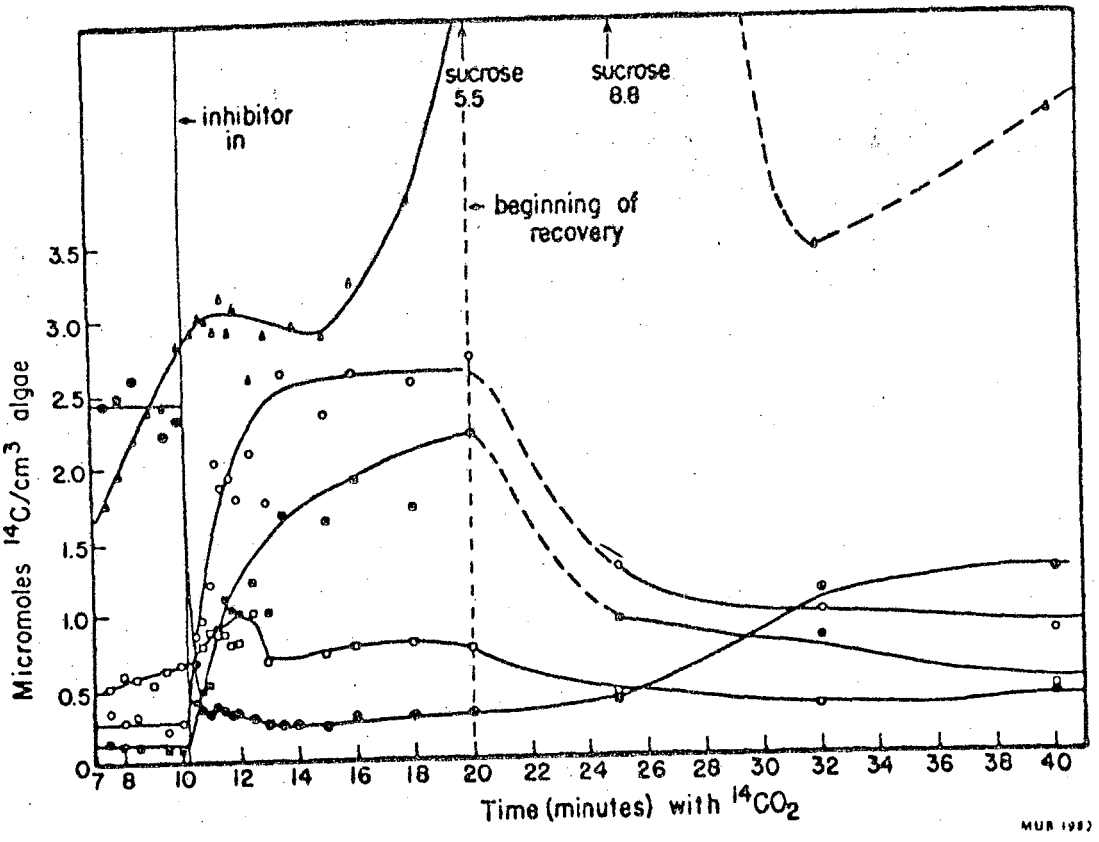


Fig. 3. Effects of 8-methyl lipoic acid on labeling of compounds with ^{14}C during photosynthesis with $^{14}CO_2$. ▲ Sucrose, ○ fructose-1,6-diphosphate, ▣ sedoheptulose-1,7-diphosphate, □ glucose "diphosphate", ● PGA.

that this is by no means the case, since PGA drops instead of increasing and since ribulose diphosphate does not drop immediately to zero concentration. The extremely rapid drop in PGA concentration requires either that the carboxylation reaction leading to the formation of PGA be inhibited or that the reduction of PGA be greatly accelerated. The latter possibility is not borne out by the transient behavior of other carbon fixation products. Although there is a rise in the fructose and sedoheptulose diphosphates, there is a corresponding drop in their monophosphates, which would not be expected from an acceleration of PGA reduction.

Thus we are left with a dramatic inhibition of reactions leading to PGA, but without a concomittant increase in the level of ribulose diphosphate which we would expect if the carboxylation of ribulose diphosphate to give PGA were stopped. Perhaps in addition to the inhibition of the carboxylation of ribulose diphosphate to give PGA, there is a subsequent inhibition of the formation of ATP. This would prevent the level of ribulose diphosphate from rising.

Another possibility is that in the in vivo system phosphate groups from the no. 1 carbon atoms of fructose and sedoheptulose diphosphates are transferred to ribulose-5-phosphate to make ribulose-1,5-diphosphate. This would account for the apparent inhibition of the conversion of the hexose and heptose diphosphates to their monophosphates. Whatever the precise explanation of the various effects of the disulfide compounds may prove to be, it is clear that these substances interfere suddenly and dramatically with the carbon reduction cycle.

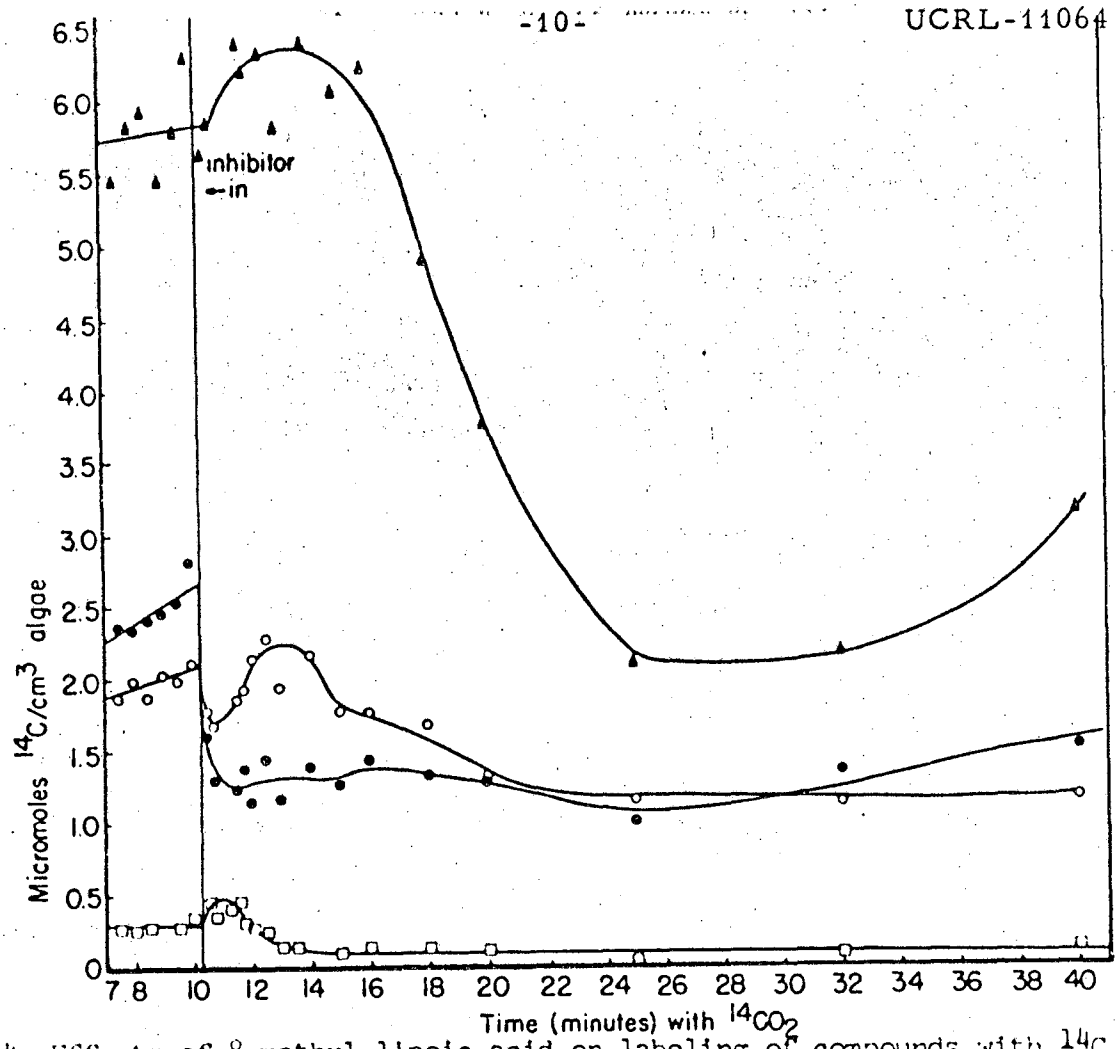
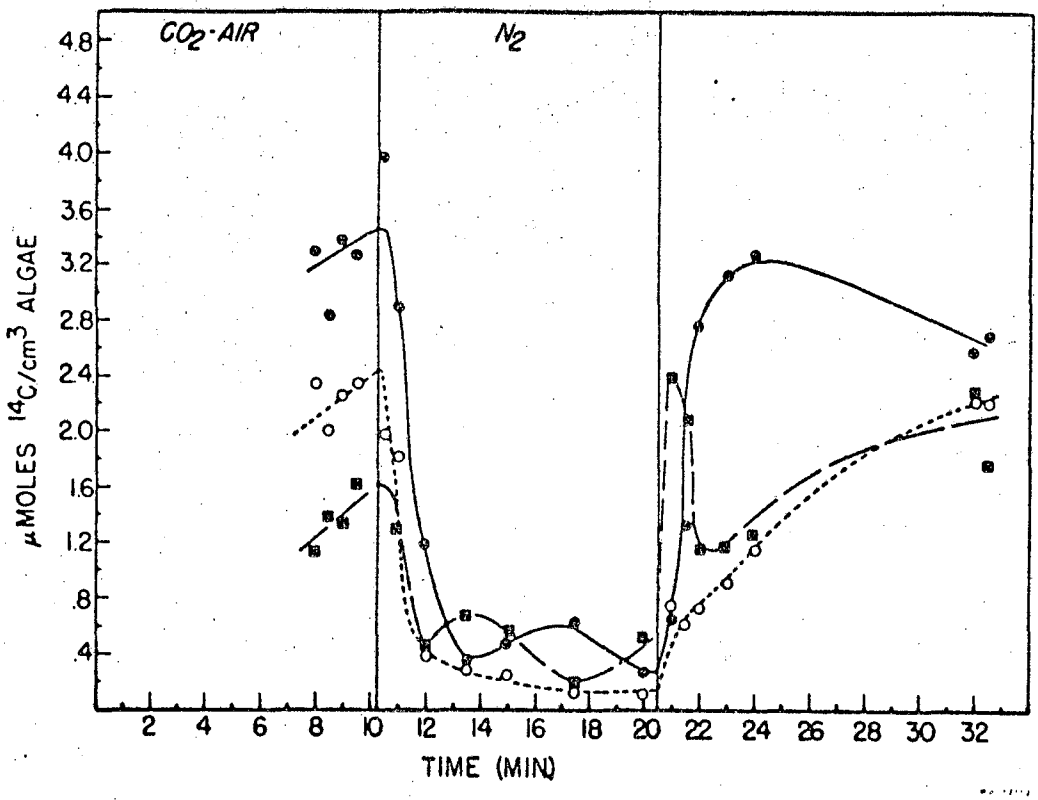


Fig. 4. Effects of 8-methyl lipoic acid on labeling of compounds with ^{14}C during photosynthesis with $^{14}CO_2$. ▲ Glucose-6-phosphate, ● sedoheptulose-7-phosphate, ○ fructose-6-phosphate, □ ribulose-1,5-diphosphate.

The immediacy of their effects makes it seem plausible to suppose that they are interferring in some way with electron transport by compounds with disulfide groups.

We have recently carried out experiments under our steady state conditions of photosynthesis with $^{14}CO_2$ in which CO_2 in air is suddenly replaced by nitrogen. After about 10 minutes the $^{14}CO_2$ in air is again added to the photosynthesizing algae. The results of these experiments are shown in Figs. 5 and 6. The drop in the level of PGA and the rise and fall in the level of ribulose diphosphate are similar to those noted by Wilson and Calvin (15). The behavior of the intermediates on addition of $^{14}CO_2$ again is quite interesting. As would be expected, the level of ribulose diphosphate falls while that of PGA rises as the carboxylation reaction resumes. What is intriguing is the fact that the level of fructose diphosphate rises as rapidly as that of PGA during the first few seconds. This is an additional bit of evidence for the direct conversion of the carboxylation intermediates to sugar phosphate without equilibration through the PGA pool.

Another interesting point is the fact that fructose diphosphate and sedoheptulose phosphate rise and then fall after the readdition of the $^{14}CO_2$. Why do they fall? One could argue that the initial carboxylation reaction is faster than usual because of the high level of ribulose diphosphate, and that once the ribulose diphosphate has been depleted, the carboxylation reaction becomes slower. However, the level of PGA which continues to rise for many minutes belies this argument. Perhaps the explanation is to be found in the previously

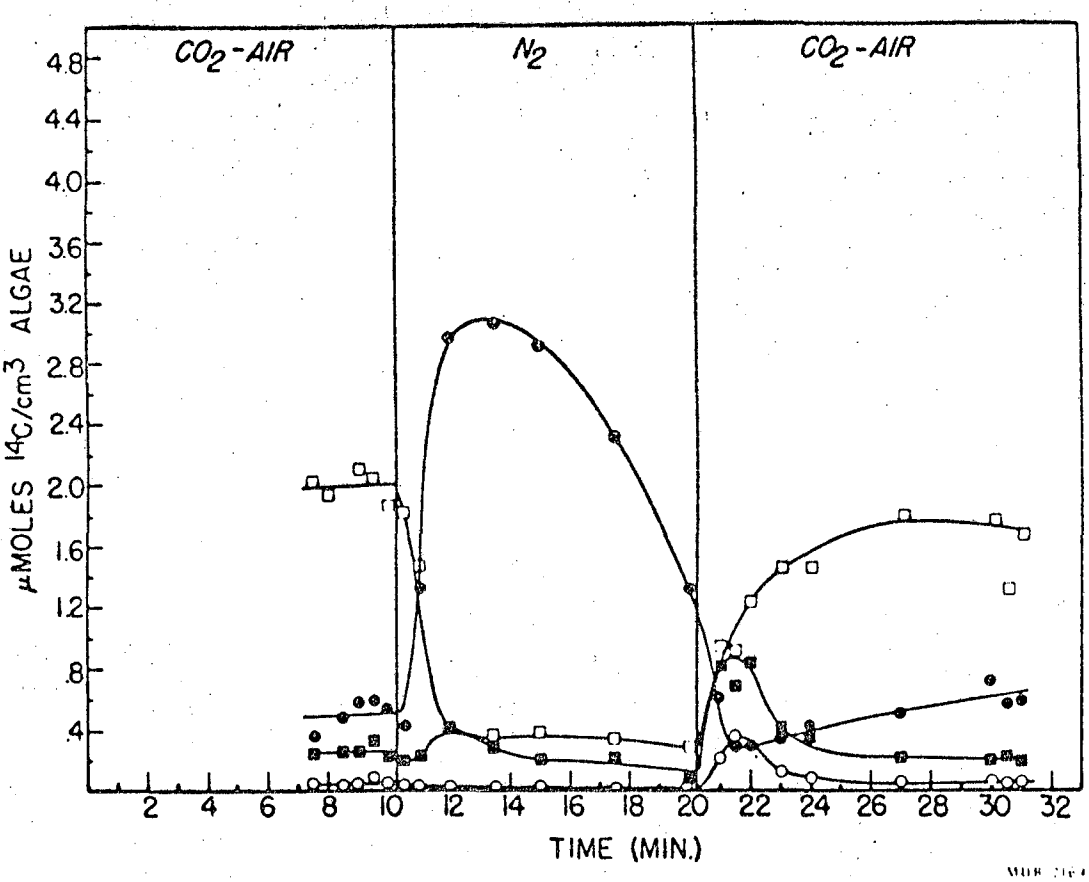


MU-32112

Fig. 5. Effects of sudden removal and readdition of CO_2 on levels of PSCR cycle. Sugar monophosphates. ● Glucose monophosphate, ○ sedoheptulose monophosphate, ■ fructose monophosphate.

suggested transfer of C_1 phosphate groups from fructose diphosphate and sedoheptulose diphosphate to ribulose monophosphate. This transfer which might initially be inhibited by the high level of ribulose diphosphate would accelerate as the level of ribulose diphosphate decreases.

In summary, while recent kinetic studies of the carbon reduction cycle of photosynthesis confirm the cycle in essentially the form in which it was proposed ten years ago, there is a scattering of evidence which suggests that the in vivo cycle might vary somewhat in its mechanistic details from the cycle which was proposed at that time. First there is a variety of kinetic evidence which suggests the direct conversion of the carboxylation intermediate compound to the level of sugar phosphate without complete equilibration with the free pool of PGA. Secondly, there is some indication that when the light is turned off there is only a partial brief reoxidation of sugar phosphate via triose phosphate dehydrogenase and that this period is followed by a block on this reoxidation. Third, some experiments suggest the possibility of direct transfer of phosphate groups from the no. 1 carbon atoms of hexose and heptose diphosphates to ribulose monophosphate. Fourth, the dramatic effects of disulfide compounds on the carbon reduction patterns, and other biochemical considerations argue for a role of disulfide compounds, perhaps including ferredoxin, in mediating a reductive carboxylation of ribulose diphosphate to give sugar phosphates directly. A separate but related possibility is that some of the newly incorporated carboxyl carbon is also reduced to the level of sugar phosphate without an opportunity for equilibration with the free PGA pool. Such a direct



MUB 2.169

Fig. 6. Effects of sudden removal and readdition of CO_2 on levels of PSCR cycle. Sugar diphosphates and PGA. ● Ribulose-1,5-diphosphate, □ 3-phosphoglyceric acid, ■ fructose-1,6-diphosphate, ○ sedoheptulose-1,7-diphosphate.

reduction of newly incorporated carbon to the level of bound triose phosphate would be required if we are to invoke the bound glyceraldehyde phosphate moiety as an explanation for the asymmetry of the hexose phosphate labeling and the slow labeling of carbon atom 4 of sedoheptulose phosphate.

The mediation of the reactions of the cycle may be accomplished by a multifunctional, organized enzyme system. This system should be closely linked to the photochemical apparatus, perhaps by such a compound as PPNR (chloroplast ferredoxin). The system should include enzyme "handles" for holding some of the intermediate compounds in a bound form. Bound forms might include glycolaldehyde, bound to thiazolium groups in transketolase reactions, and aldehyde moieties bound to disulfide/disulfhydryl groups in carboxylation, condensation and epimerisation reactions. Attempts to isolate active enzymes from the cell may result in the loss of the organization and primary enzymic activities. Residual enzymic activities found in the soluble isolated protein could mimic the reactions of the intact system. Such activities might well be variable and in many cases inadequate to accomplish the reactions of the PSCR cycle at anything like the in vivo rate.

The work described in this paper was sponsored by the United States Atomic Energy Commission.

REFERENCES

1. 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).
2. J.A. Bassham, and Martha Kirk, Biochim. et Biophys. Acta 43, 447 (1960).
3. D.C. Smith, J.A. Bassham and Martha Kirk, Biochim. et Biophys. Acta 48, 299 (1961).
4. M. Calvin, J.A. Bassham, A.A. Benson, V. Lynch, C. Ouellet, L. Schou, W. Stepka and N. Tolbert, Symposia of the Society for Experimental Biology, No. V, p. 284 (1951).
5. J.A. Bassham, J. Theor. Biol. 4, 52 (1963).
6. J.A. Bassham and M. Calvin, "The Path of Carbon in Photosynthesis", Prentice Hall, Englewood Cliffs, New Jersey, 1957.
7. O. Kandler and M. Gibbs, Plant Physiol. 31, 411 (1956).
8. J.A. Bassham and Martha Kirk, Biochem. Biophys. Res. Commun. 9, 376 (1962).
9. M. Stiller, Ann. Rev. Plant Physiol. 13, 151 (1962).
10. G. Richter, Naturwissenschaften 21, 604 (1959).
11. C.A. Fewson, M. Al-Hafidh and M. Gibbs, Plant Physiol. 37, 402 (1962).
12. C.A. Fewson, P.K. Kindel and M. Gibbs, Plant Physiol. 36, Suppl. IX (1961).
13. E. Frazi, P.T. Rowley, T. Cheng, O. Tchola and B.L. Horecker, Biochem. Biophys. Res. Commun. 9, 38 (1962).
14. M. Calvin and P. Massini, Experientia VIII, 445 (1952).
15. A.T. Wilson and M. Calvin, J. Am. Chem. Soc. 77, 5948 (1955).
16. J.A. Bassham and Martha Kirk, Microalgae and Photosynthetic Bacteria (Special issue, Plant and Cell Physiol., Japan) 493 (1963).
17. J.A. Bassham, K. Shibata, K. Steenberg, J. Bourdon and M. Calvin, J. Am. Chem. Soc. 78, 4120 (1956).
18. R.B. Park and N.G. Pon, J. Mol. Biol. 3, 1 (1961).
19. A. San Pietro, in "The Photochemical Apparatus, its Structure and Function", R.C. Fuller, Ed., Brookhaven National Laboratory, Upton, New York, 1959, p. 262.
20. A. San Pietro and H.M. Lang, J. Biol. Chem. 231, 211 (1958).
21. K. Tagawa and D.I. Arnon, Nature 195, 537 (1962).
22. L.E. Mortenson, R.C. Valentine, and J.E. Carnahan, Biochem. Biophys. Res. Commun. 7, 448 (1962).
23. R.C. Valentine, R.V. Jackson and R.S. Wolfe, Biochem. Biophys. Res. Commun. 7, 453 (1962).
24. J.A. Bassham, Advances in Enzymol. XXV, 39 (1963). F.F. Nord, Ed., Interscience Publishers, New York.
25. R.C. Valentine, W.J. Brill and R.D. Sagers, Biochem. Biophys. Res. Commun. 12, 315 (1963).
26. J.A. Bassham, H. Egeter, F. Edmonston and Martha Kirk, Biochem. Biophys. Res. Commun. 13, 144 (1963).

This report was prepared as an account of Government sponsored work. Neither the United States, nor the Commission, nor any person acting on behalf of the Commission:

A. Makes any warranty or representation, expressed or implied, with respect to the accuracy, completeness, or usefulness of the information contained in this report, or that the use of any information, apparatus, method, or process disclosed in this report may not infringe privately owned rights; or

B. Assumes any liabilities with respect to the use of, or for damages resulting from the use of any information, apparatus, method, or process disclosed in this report.

As used in the above, "person acting on behalf of the Commission" includes any employee or contractor of the Commission, or employee of such contractor, to the extent that such employee or contractor of the Commission, or employee of such contractor prepares, disseminates, or provides access to, any information pursuant to his employment or contract with the Commission, or his employment with such contractor.