Lawrence Berkeley National Laboratory
Recent Work

Title
CONTROL OF PHOTOSYNTHETIC CARBON METABOLISM

Permalink
https://escholarship.org/uc/item/1xz4n2h5

Author
Bassham, James A.

Publication Date
1972-08-01

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

Presented at Symposium 27,
Society for Experimental Biology,
Oxford, England, August 27-Sept. 1, 1972

LBL-1034
c.1

RECEIVED
LAWRENCE
RADIATION LABORATORY

NOV 30 1972

LIBRARY AND
DOCUMENTS SECTION

CONTROL OF PHOTOSYNTHETIC CARBON METABOLISM

James A. Bassham

August 1972

AEC Contract No. W-7405-eng-48

For Reference

Not to be taken from this room

LBL-1034
c.1

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.

0 0 0 0 3 8 0 0 9 7 1

CONTROL OF PHOTOSYNTHETIC CARBON METABOLISM

James A. Bassham

Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory,

University of California, Berkeley, California 94720

NECESSITY FOR METABOLIC REGULATION IN PHOTOSYNTHETIC CELLS

The reductive pentose phosphate cycle (RPP cycle, Calvin cycle, Figure 1) is the basic pathway for carbon dioxide fixation, reduction, and formation of carbohydrates during photosynthesis in green plants (Bassham _et al._, 1954). This cycle apparently occurs in all green plants and algae (Norris, Norris & Calvin, 1955), although in some species of higher plants, notably certain tropical grasses such as sugar cane, the RPP cycle is supplemented by a pyruvate-malate cycle (Kortschack, Hartt & Burr, 1965; Hatch & Slack, 1966; Hatch & Slack, 1970). Other metabolic pathways also occur inside and outside the chloroplasts, and these pathways share intermediate compounds and enzymes with the RPP cycle. The interactions between these pathways impose the necessity for rapid metabolic regulation.

The light-driven reactions in the chloroplast membranes supply ATP and reduced cofactors for the synthetic reactions in the chloroplasts. Thus the RPP cycle would cease to function, even without metabolic control, when the light is turned off, or when the light reactions are blocked for some reason. Additional control is required to prevent wasteful reactions from occurring. One example is the carboxylation reaction, required for incorporating carbon dioxide in the light. In the dark, this reaction would only use up carboxylation substrate, ribulose-1,5-diphosphate (RuDP) which must be made at the expense of ATP utilization.

Equally important is the necessity for matching the availability of intermediate compounds of the RPP cycle to the requirements of the green cell as these intermediate compounds are used as starting materials for biosynthetic pathways. It was once thought that photosynthesis produced some unique product such as starch which then served as the sole substrate for subsequent biosynthetic reactions. This biosynthesis was considered to be operating independent of photosynthesis. We now know that several intermediate compounds of the RPP cycle serve as key starting materials for biosynthesis both inside and outside the chloroplasts. These compounds are withdrawn from the cycle at rates which vary with the needs of the cell. The concentrations of these compounds must nevertheless be maintained at levels which will permit the continued efficient operation of the basic cycle. This concentration maintenance is accomplished by fine control of key enzymes of the cycle.

Along pathways of biosynthesis leading from the cycle, but usually within two or three steps from the beginning, other regulatory sites are required to control the rate of flow along each pathway. For example, the conversion of phosphoenolpyruvate to pyruvate, three steps removed from an intermediate compound of the cycle, controls the rate of conversion of photosynthetic products to precursors of amino acid and fatty acid synthesis, and energy metabolism in the dark.

Besides the regulation of activities of soluble enzymes at key points, there is control of the movement of metabolites through the outer chloroplast double membrane. This movement is highly selective with respect to compounds, and appears to depend very much on physical and chemical conditions both inside and outside the chloroplasts.

0 0 0 3 8 0 0 9 7 2

The present discussion will be limited to a consideration of rapid metabolic regulation resulting from modification of the activities of already existing enzymes and cellular structures. Another important type of control of metabolism is that regulating the synthesis and degradation of enzymes and membranes. Such regulation is no doubt of great importance in the control of metabolism of the cells during their life cycle.

THE INVESTIGATION OF METABOLIC REGULATION *in vivo*

In the study of metabolic regulation in some other systems, much knowledge has come from a determination of which isolated enzymes exhibit capabilities for activity modification by changes in concentrations of metabolites. In the case of photosynthetic carbon metabolism, much of the early progress has been made through *in vivo* studies (for review, see Bassham, 1971). This is due, in part, to the ease with which some green plants such as unicellular algae can be used for such study. However, the method has applicability to any system where whole cells can be grown and uniformly sampled in the presence of labelled substrate. For example, we are currently applying these techniques to the investigation of metabolic regulation of animal cells in tissue culture.

With green cells, the basic experiment is to permit the cells to photosynthesize in the presence of $^{14}CO_2$ and/or ^{32}P-labelled phosphate. The concentration and the specific radioactivity of the labelled substrates are maintained at essentially constant conditions throughout the experiment (except in those cases where substrate concentration is deliberately varied). Photosynthesis with labelled substrate is continued until the metabolites to be measured are as fully labelled as the substrate. At that point the total amount of radioactivity in each

metabolic pool is a measure of the pool size, that is, the concentration in the cells. Samples of the cells are taken and killed. After subsequent analysis, the pool sizes can be determined by dividing the radioactivity of the compounds by the specific radioactivity of the substrate.

The information about pool sizes under a specified condition of steady-state metabolism is itself useful in assessing sites of metabolic regulation. Assuming that the physiological free energy changes of the reactions along the pathway are known (free energy changes at pH = 7, $\Delta G^{o\prime}$), the concentrations of metabolites may be used to calculate the actual free energy changes, ΔG^{s}, accompanying the reactions under the given steady state (Bassham & Krause, 1969). Those reactions with large negative ΔG^{s} values (-4 to -10 kcal) are nearly "irreversible"; that is, the forward reaction rate exceeds the back reaction rate by three to six orders of magnitude. Such reactions were expected on theoretical grounds to be sites of metabolic regulation (Yates & Pardee, 1956). In photosynthetic cells independent experimental evidence shows that such reactions are regulated.

Once the metabolites are "saturated" with the label from the substrate under a given steady-state condition, the system may be perturbed by imposing some environmental change which alters the steady state. Transient changes are followed by rapidly sampling the system for subsequent analysis. If the system comes to a new steady state, this can be determined by further sampling after the transient period is over. Finally, in the case of reversible perturbations, it may be possible to return to the original steady state, once again following the transient changes.

Samples from such experiments are analyzed by two-dimensional paper chromatography, radioautography, and measurement of the radioactivity in each compound (Pedersen, Kirk & Bassham, 1966a). Because of the different energies of their beta particle emissions, ^{32}P and ^{14}C can be separately determined in those experiments where both isotopes are employed.

POOL SIZE CHANGES *in vivo*

When the steady state is perturbed, and changes in pool sizes occur, it is frequently necessary to bring together several kinds of evidence to interpret the changes. Obviously, the size of a pool responds to changes in the rates of reactions following and preceding it. Fortunately, the majority of steps in a metabolic sequence are not regulated, but are highly reversible. Consequently, one commonly finds that two or more successive pools in a metabolic sequence undergo similar transient changes, and that subsequent steps in the sequence undergo different transient changes. For example, when photosynthetic membranes are reversibly altered by low concentrations of fatty acids in the light (Pedersen, Kirk & Bassham, 1966a), pools of both dihydroxyacetone phosphate (DHAP) and fructose-1,6-diphosphate (FDP) suddenly increase, whereas the pools of fructose-6-phosphate (F6P) and glucose-6-phosphate (G6P) decrease. Clearly the pool changes are due to a decreased rate of conversion of FDP to F6P.

Some of the commonly seen types of changes in pool sizes *in vivo* are illustrated by the idealized curves in Figure 2. In each case, curve B represents the pool size of the reactant; where shown, C is the pool size of the product.

Figure 2a represents reactant and product pools for a rate-limiting step (regulated reaction) which is increased in rate by the environmental change. Thus the pool of B drops to a new steady-state level, while the pool of C rises. An example is the addition of 1 M NH_4^+ to photosynthesizing algae, leading to a decrease in the pool size of PEPA and an increase in the pool size of pyruvate (Kanazawa, Kirk & Bassham, 1970; Kanazawa _et_ _al._, 1972).

When environmental change results in a more rapid formation of reactant, but its conversion to product is activated only after some time, an overs'oot results as in Figure 2b. This occurs, for example, with the FDP pool in green cells when the light is turned on after a period of darkness. During darkness the FDP pool had been maintained by a pathway different from the photosynthetic pathway, but the conversion of FDP to F6P was low, due to low activity of fructose diphosphatase (Pedersen, Kirk & Bassham, 1966b). As soon as light is turned on, reduction of PGA to triose phosphates and condensation of triose phosphates to give FDP commences. Since the FDPase is apparently not yet fully active, there is a rapid increase in pool size in FDP. Then, as FDPase is activated, the pool drops, the pool of F6P rises, and all pools come in time to the light steady-state level. The oscillation is due to the fact that once the FDPase is activated, the surge of carbon must pass all the way around the cycle before it returns via PGA and triose phosphates, and the steady-state rate of photosynthesis is achieved.

The curve for B in Figure 2c is identical to the curve for B in 2a, but is intended to depict a quite different situation based on

0 0 0 0 3 8 0 0 9 7 4

other knowledge about the reaction. In the case of 2a it was known that the reaction was accelerated because metabolites made from the product were much more rapidly labelled after the transition. The curve in 2c represents a case in which the environmental change results in an immediate cessation of the reaction forming B, together with a slower inactivation of the reaction converting B to C.

An example of this situation occurs when the light is turned off with green cells. The formation of the carboxylation substrate, RuDP, stops quickly because the supply of ATP, needed to form RuDP from ribulose-5-phosphate (Ru5P), is momentarily stopped due to cessation of photophosphorylation and delay in the onset in oxidative phosphorylation (Pedersen, Kirk & Bassham, 1966b). Later the phosphoribulokinase becomes inactive so that RuDP formation remains blocked. Since the carboxylation reaction is highly irreversible ($\Delta G = -10$ kcal), the RuDP should be completely used up. However, due to inactivation of the RuDPCase, the carboxylation reaction stops after 1 to 2 minutes' darkness while there is still a measurable level of RuDP. The contention that the RuDPCase is inactive in the dark is further borne out by the fact that CO_2 fixation completely ceases in the dark, even though the RuDP level is high enough to support carboxylation. While it might be argued that in algae a small rate of CO_2 fixation is masked by respiratory CO_2 evolution, the same cessation of fixation of CO_2 in the presence of measurable RuDP in the dark is seen with isolated spinach chloroplasts, where there is no respiration (Jensen & Bassham, 1968b).

A different case of inactivation of reactions both preceding and following a pool B (again RuDP) is seen in Figure 2d. In this case,

observed with the RuDP pool when lipoic acid was added to photosynthesizing green cells (Pedersen, Kirk & Bassham, 1966a), the carboxylation began to be inhibited before the phosphoribulokinase-mediated reaction was affected. This caused the pool of RuDP to rise momentarily. Then the kinase reaction stopped, before the RuDPCase was completely inactive, and RuDP concentration fell as it did in case 2c. This is a rather special kinetic effect, and we have not seen it often.

A reversible effect is shown in Figure 2e. As mentioned earlier, addition of octanoate at pH 4 to photosynthesizing _Chlorella pyrenoidosa_ causes a rapid rise in the pool sizes of FDP and sedoheptulose-1,7-diphosphate (SDP). This is probably due to the entry of the undissociated acids into the hydrophobic part of the chloroplast membranes, resulting in some disruption of membrane function. One such function is the light-stimulated pumping of ions through the membrane, reported by Neuman & Jagendorf (1964), Dilley and Vernon (1965) and many others. The direction of the pumping in the light is such as to raise both the pH and the Mg^{++} ion concentration in the stroma region (the space containing FDPase and other soluble enzymes outside the thylakoidds). Both RuDPCase and FDPase are activated by high levels of Mg^{++} ions (10 mM or more) and with both enzymes, the pH optima shift from alkaline to neutral with such high Mg^{++} ion concentrations (Preiss, Biggs & Greenberg, 1967; Bassham, Sharp & Morris, 1968).

The inhibition of photosynthesis and the inhibition of these specific enzymes _in vivo_ can be reversed to some extent by raising the pH of the external medium to 7. Presumably, dissociation of the octanoic acid results in its loss from the hydrophobic region of the

0 0 0 0 3 8 0 0 7 7 5

membranes, and a restoration of membrane functions earlier disrupted by the acid.

Another example of the kind of transient changes shown in Figure 2e is seen with the addition of small amounts of vitamin K_5 to photosynthesizing Chlorella pyrenoidosa (Krause & Bassham, 1969). In this case, we believe that the vitamin K_5 accepts electrons directly from the photo-electron transport chain after first being oxidized in air and light. The result is that the ratio $NADPH/NADP^+$ drops rapidly, and the most immediate effects are somewhat different than in the case of octanoic acid addition. In time, the vitamin K_5 is converted to an inactive colored product, and the photosynthetic process is partially restored.

Upon the addition of vitamin K_5, there is an immediate increase in the pool of 6-phosphogluconic acid (6-PGluA) and in the pools of the pentose monophosphates, Ru5P, ribose-5-phosphate (R5P) and xylulose-5-phosphate (Xu5P). The appearance of 6-PGluA is an indication of the activation of glucose-6-phosphate dehydrogenase, which is ordinarily inactive in the light in the chloroplasts. The formation of 6-PGluA from G6P in the presence of $NADP^+$ is accompanied by a ΔG of -11.4 kcal (Bassham & Krause, 1969), so that this reaction could not be prevented solely by a high ratio of $NADPH/NADP^+$ which is present in the chloroplasts in the light until the vitamin K_5 is added. A ratio of 10^8 would be required if mass action were to prevent G6P oxidation in the light. Such a ratio of $NADPH/NADP^+$ is at least six orders of magnitude greater than the actual in vivo ratio. However, it is entirely possible that the ratio or the concentration of either $NADP^+$ or NADPH does affect the enzyme allosterically and control its activity.

The sudden increase in pentose monophosphate pool sizes upon addition of vitamin K_5 suggests that the ratio $NADPH/NADP^+$ may play a part in controlling the activity of phosphoribulokinase, since the level of ATP did not drop. With this inhibitor, the inactivations of RuDPCase and FDPase were also seen, but were delayed, suggesting a different regulatory mechanism for these controlling enzymes than for phosphoribulokinase and glucose-6-phosphate dehydrogenase.

Comparison of the transient increases in 6-PGluA and pentose monophosphates illustrates the necessity for supplementary information in interpreting transient changes in pool si:es. Both transient increases were of the type shown in Fig. 2e. Examination of other data and the known reactions of metabolic pathways, indicates that whereas 6-PGluA concentration increased due to increased rate of formation, the concentrations of pentose monophosphates increased due to decreased utilization. While it is true that the oxidation of 6-PGluA provides an additional pathway forming pentose monophosphates, other effects of vitamin K_5 addition (conversion of FDP and SDP to monophosphates) block the much greater rate of formation of pentose monophosphates via the photosynthetic pathway.

Two further facts provide important evidence for the light-dark switch from reductive to oxidative pentose phosphate cycle (OPP cycle) in the chloroplasts of green cells. 1. When the light is turned off after photosynthesis in <u>Chlorella pyrenoidosa</u>, 6-PGluA appears immediately. 2. With the addition of vitamin K_5 to isolated spinach chloroplasts, there is an immediate appearance of 6-PGluA. This shows that the OPP cycle can operate in chloroplasts. We have not seen 6-PGluA when the isolated

chloroplasts are darkened. Perhaps this is because there is no large pool of electron acceptor, such as is provided by vitamin K_5 in the light, or *in vivo* in the dark by the shuttle mechanism discussed later.

To summarize, the sites of metabolic regulation in the photosynthetic reductive pentose phosphate cycle are the carboxylation reaction (mediated by RuDPCase, $\Delta G^s = -10$ kcal), the conversion of FDP and SDP to monophosphates (mediated by FDPase, $\Delta G^s = -6.5$ kcal), and the conversion of Ru5P to RuDP (mediated by phosphoribulokinase, $\Delta G = -4$ kcal). When the light is turned off, each of these reactions stops within 1 to 2 minutes, and glucose-6-phosphate dehydrogenase is activated, permitting an oxidative pentose cycle to operate. During steady-state photosynthesis, the RuDPCase- and FDPase-mediated reactions are, of course, rate-limiting, since they are accompanied by large negative ΔG^s values. The balance between these rate-limiting steps is needed to control the flow of carbon within the cycle and to branching biosynthetic paths. The mechanisms of these activations and inactivations probably includes changes in Mg^{++} concentration, pH, and ratios of reduced to oxidized cofactors in the chloroplasts. However, there may be other, more specific mechanisms, to be discussed later.

PHOTOSYNTHETIC AND RESPIRATORY METABOLISM

The purpose of the light-dark regulatory mechanisms just described clearly is to permit the green cells to switch efficiently from light driven metabolism to oxidative energy metabolism. The chloroplasts lack a pathway for oxidative phosphorylation. Kinetic studies with algae (Pedersen, Kirk & Bassham, 1966b) showed that when the light is turned off, the level of ATP in the cells dips momentarily but is

restored after a few minutes in the dark to about the same steady-state level as in the light, presumably due to oxidative phosphorylation in the mitochondria. Recent kinetic studies of algae in light and dark, and with added NH_4^+ (Kanazawa et al., 1972), have provided new information about the light-dark transition, and the mobilization of photosynthetic products for energy metabolism in the dark.

In Chlorella pyrenoidosa the principal storage of photosynthate is in starch, with a lesser but significant amount as sucrose. When the light was turned off, with cells that had been deprived for a short time (one hour) of a nitrogen source, there was an immediate utilization of starch which continues linearly for at least 30 minutes at a rate (1.65 μmoles of carbon per minute per cm^3 algae) which is about the total respiratory rate for cells under optimal physiological conditions. The level of 6-PGluA immediately rose as usual with the light off, and other transient changes typical of the switch from reductive to oxidative metabolism were seen.

The level of adenosine diphosphoglucose (ADPG) dropped very rapidly to below detectable limits, despite the fact that glucose monophosphates (G6P and G1P) remained high in the dark. Since the level of ATP is also high in the dark, this indicates that the enzyme, ADPG pyrophosphorylase, which mediates the formation of ADPG from G1P and ATP, must become inactive in the dark. This had been predicted by studies of the isolated enzyme (Ghosh & Preiss, 1965) which was found to be subject to allosteric regulation.

Carbon flowing from photosynthesis to the tricarboxylic acid cycle (TCA cycle) must pass through PEPA and pyruvate. It is significant the

0 0 0 0 3 8 0 3 9 7 6

levels of PEPA and pyruvate were the same in the dark as in the light, although there was a transient upsurge in the pool sizes of both compounds (Figure 3a,b). The steady-state level of citrate was somewhat higher in the dark than in the light (Figure 3c).

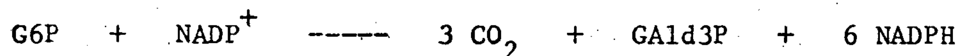
The synthesis of sucrose stopped in the dark, but there was no immediate utilization of sucrose. However, when 1 mM NH_4^+ was added to the medium 10 minutes later, there was an immediate utilization of sucrose which continued linearly for the duration of the experiment (20 minutes) at a rate of 0.85 μmoles per minute per cm^3 of algae. The addition of NH_4^+ resulted in greatly increased amino acid synthesis. The ATP level dipped momentarily (Figure 4a) and then came back to a little less than the light and dark steady-state level. The steady-state level of PEPA dropped while the steady-state level of pyruvate rose, indicating that NH_4^+ had stimulated pyruvate kinase. The level of 6-PGluA rose even more rapidly than it had when the light was turned off (Figure 4b).

We interpret these observations as follows: Since starch is in the chloroplasts, its mobilization is activated in the dark by a mechanism in the chloroplasts, presumably starch phosphorylase. Perhaps this is due to a rise in the concentration of inorganic phosphate inside the chloroplasts, but there may be some additional mechanism.

Phosphofructokinase activity is missing from chloroplasts (unpublished observation), so oxidation of the G6P can occur only via the OPP cycle. The lack of change in the levels of PEPA and pyruvate (on darkening) suggests that the amount of carbon flowing through the pyruvate kinase step to energy metabolism via the TCA cycle in the dark was no greater than the amount of carbon flowing through the same step for

biosynthesis of fats and proteins in the light. The slight rise in citrate does indicate some increased TCA operation in the dark.

With the OPP cycle operating (and no phosphofructokinase), at least one-half of all CO_2 released from glucose comes from the oxidation of 6-PGluA.



$$\text{G6P} + \text{NADP}^+ \longrightarrow 3\ CO_2 + \text{GAld3P} + 6\ \text{NADPH}$$

The glyceraldehyde-3-phosphate (GAld3P) either could be transported out of the chloroplasts, converted to pyruvate, and oxidized via the TCA cycle, or it could be converted back to G6P via aldolase, FDPase and the triose and hexose phosphate isomerases.

Although the kinetic evidence shows that FDPase is much less active in the dark than in the light, there is no evidence that FDPase is completely inactive in the dark. From the known photosynthesis rate in the light (about 18 μmoles CO_2 per minute) the stoichiometry of the RPP cycle, and the fact that the FDPase mediated steps are rate limiting, we calculate that 15 μmoles of phosphate were released per minute from FDP and SDP during photosynthesis. The utilization of starch via the oxidative cycle at 1.65 μmoles of carbon per minute would produce only 0.28 μmoles of FDP per minute, if all the triose phosphate made by the oxidative cycle were converted to FDP. Thus less than 2% of the FDPase activity needed in the light would be required for conversion of triose phosphate back to G6P allowing complete combustion of starch to CO_2 via the oxidative cycle.

The fact that FDP, SDP and DHAP levels, following an initial drop in the dark, build back up to levels comparable to those in the light

0 0 0 0 3 8 0 0 9 7 7

suggests that triose phosphate produced by the oxidative pentose phosphate cycle in the chloroplasts is not rapidly oxidized in the chloroplasts, but may be partly converted back to G6P and partly transported out of the chloroplast. In any event, substantial amounts of NADPH are produced inside the chloroplasts. Some of this reducing power may be used in the chloroplasts for biosynthesis (Bassham & Kirk, 1968). However, from the relatively large amounts of NADP produced, and the evidence for active oxidative phosphorylation, it is now clear that most of the NADPH must be used for energy metabolism. The double, limiting membrane of the chloroplasts appears to be a barrier to the free diffusion of NADP and many other metabolites (Heber & Santarius, 1965; Bassham, Kirk & Jensen, 1968). At least two shuttle mechanisms have been found to permit the movement of NADPH out of chloroplasts. One of these in the transport of triose phosphate out of the chloroplasts, oxidation of triose phosphate to PGA, with generation of NADPH and ATP, and reentry of PGA into the chloroplasts (Heber & Santarius, 1965; Stocking & Larson, 1969). Earlier studies of the movement of metabolites out of isolated chloroplasts during photosynthesis with $H^{14}CO_3$ had shown that PGA and DHAP are among the metabolites which rapidly appear in the suspending medium. This mechanism seems suitable for shuttling ATP and NADPH produced in the chloroplasts in the light to the cytoplasm, but not for movement of NADPH only out of the chloroplasts in the dark. In the dark, ATP must be transported into the chloroplasts, whereas the triose phosphate-PGA shuttle would have the opposite effect. A malate-oxalacetate shuttle (Heldt & Rapley, 1970; Heber & Krause, 1971), would be suitable for the movement of NADPH out of the chloroplasts, and perhaps into the mitochondria. Another possibility would be a DHAP-glycerol phosphate shuttle.

Whatever the mechanism, it appears that the principal energy metabolism of Chlorella pyrenoidosa is via the OPP cycle, with transport of the resulting NADPH out of the chloroplasts and into the mitochondria via a shuttle mechanism. Presumably, the oxidative leg of the shuttle mechanism in the mitochondria employs an NAD-specific enzyme, so that NADH is produced directly for oxidative phosphorylation.

Viewed this way, the principal role of the TCA cycle in Chlorella pyrenoidosa appears to be for amino acid synthesis. It is not known whether this is also true for green cells of higher plants. In Chlorella, addition of NH_4^+ does not affect starch utilization, but causes immediate utilization of sucrose for amino acid synthesis. The most obvious regulatory effect is the increased pyruvate kinase activity resulting in a greatly increased rate of flow of carbon into the pyruvate pool.

The mechanism of this pyruvate kinase stimulation in green cells is not yet known. The addition of NH_4^+ caused an immediate dip in the ATP level, which never quite returned to its former steady-state level, if we allow for the tilted base line due to slow saturation of the phosphate group adjacent to ribose (Figure 4a). Thus the initial effect might be a slight uncoupling of oxidative phosphorylation, leading to a decreased "energy charge" (Atkinson, 1966) and resulting stimulation of pyruvate kinase. Another possibility is that NH_4^+ ions and FDP exert a direct effect on pyruvate kinase, as has been reported for pyruvate kinase in yeast (Hess & Haekel, 1967).

There must be a prior activation of the reaction which hydrolyzes sucrose, the reaction of sucrose with UDP to give fructose and UDPglucose. We could speculate that the availability of UDP is increased due to the

0 0 0 0 3 8 0 0 1 7 8

observed drop in ATP level, but we have no direct evidence on this point. There is also the question of the intracellular location of the OPP cycle involved in sucrose breakdown. Key enzymes of the OPP cycle are found both inside and outside the chloroplasts in green cells (Heber, Hallier & Hudson, 1967). We suspect that sucrose breakdown, in contrast to starch breakdown, may occur in the cytoplasm.

COMPARISON OF CHLOROPLAST AND BLUE-GREEN ALGA METABOLISM

In view of the theories that the chloroplasts of eucaryotic cells are derived from the more primitive blue-green algae (Ris & Plaut, 1962), it is of interest to compare their metabolism. Like chloroplasts of Chlorella pyrenoidosa, the oxidative metabolism of blue-green algae relies entirely on the OPP for energy metabolism, and exhibits the same regulatory mechanisms, insofar as they are known (Pelroy & Bassham, 1972; Pelroy, Rippka & Stanier, 1972). Blue-green algae, being procaryotic cells, have no mitochondria, and no complete TCA cycle, since there is a block at the oxidation of alpha ketoglutarate to succinate and CO_2 (Smith, London & Stanier, 1967; Pearce, Leach & Carr, 1969). Unlike isolated chloroplasts, blue-green algae do have an oxidative electron transport system, and carry out oxidative phosphorylation, utilizing electrons from the OPP cycle. Assuming that the primitive blue-green algae were in fact incorporated into eucaryotic cells and became chloroplasts, the ability to carry out oxidative phosphorylation would have been no longer needed, if we also assume that the host cells already contained mitochondria.

REGULATION OF THE RPP CYCLE IN THE LIGHT

As suggested in the introduction, regulation of the RPP cycle is

required because RPP cycle intermediate compounds are withdrawn from the cycle at more than one point for biosynthesis. Synthesis of starch takes place solely in the chloroplast, and begins with G6P which is made from F6P. Sucrose synthesis also begins with G6P and F6P, but we do not know yet whether sucrose synthesis occurs in the chloroplasts or outside the chloroplasts. Photosynthesizing isolated spinach chloroplasts make starch but usually very little sucrose. Isolated chloroplasts transport, or allow to diffuse out of the chloroplasts, large amounts of triose phosphates (Bassham, Kirk & Jensen, 1968), but very little hexose and heptose monophosphates. It is possible that in whole cells, triose phosphates are converted to F6P and G6P outside the chloroplasts, and that sucrose is then made in the cytoplasm. As already indicated, there is reason to think that sucrose is metabolized in the dark outside the chloroplasts. In any event, the triose phosphates which come out of the chloroplasts, as well as PGA, are starting points for the synthesis of numerous amino acids and fatty acids, once the PGA is converted to pyruvate via the metabolically-regulated, pyruvate kinase-mediated step. Also, DHAP can be reduced to glycerol phosphate for the synthesis of phospholipids and fats.

The removal of these intermediate compounds from the cycle could lead to unbalance and collapse of the cycle in the absence of regulatory mechanisms in the cycle to keep the concentrations in balance. Studies of the flow rates of carbon from the cycle in synchronized cell cultures of *Chlorella pyrenoidosa* showed that there is a relatively large shift in products with the changing stages in the life cycle of the cells (Kanazawa *et al.*, 1970). Sucrose and protein synthesis especially varied

and in a somewhat reciprocal manner. However, fat and starch synthesis also varied, and the ratio of sucrose plus protein plus fat synthesis to starch synthesis varied appreciably.

Despite these variations in products, the rate of CO_2 uptake remained remarkably constant, falling a little only in the case of cells about to divide. The levels of the carboxylation substrate, RuDP, varied by a factor of 2.5, suggesting that in this rate-limiting step the RuDP substrate was saturating the enzyme at all times except in the predivision case. Thus the activity of RuDPCase was the principal rate-limiting factor for photosynthesis in these experiments.

The ratio of FDP/F6P, which could be taken as some indication (though admittedly not an accurate one) of <u>total</u> FDPase activity in the cell, was smallest when the sucrose synthesis was large and protein synthesis small and was largest when there was little sucrose synthesis and much protein synthesis. Given the uncertainty as to the site of sucrose synthesis, and the fact that FDPase, FDP and F6P occur in both chloroplasts and cytoplasm, interpretation is somewhat risky, but the changes observed are generally consistent with an adjustment in FDPase to accommodate changing amounts of carbon withdrawn as triose phosphate as compared with carbon withdrawn as F6P and G6P. Thus, when more protein synthesis was required, FDPase activity may have just slightly declined to a point where FDP and triose phosphates increased in concentration and F6P and G6P decreased. Of course, the cycle would continue to run just as fast as before, since the increased withdrawal of carbon as triose phosphate would be compensated for by a decreased withdrawal of carbon as F6P and G6P and since the prime rate-limiting

step, the carboxylase reaction, is unchanged. If sucrose is made outside the chloroplasts, some of this regulation would be of FDPase outside the chloroplasts.

To summarize, we have proposed that control of the levels of intermediate compounds of the cycle used for biosynthesis is achieved by a delicate balance between the activities of the rate-limiting steps mediated by RuDPCase and FDPase. The activity of RuDPCase, under conditions of light saturation, appears to be rather constant and is rate-limiting for the overall process. The FDPase (we propose) is subject to fine adjustment, decreasing slightly when more triose phosphates are needed for protein and fat synthesis, and increasing slightly when carbohydrate synthesis is preeminent.

STUDIES WITH ISOLATED SPINACH CHLOROPLASTS

Isolated spinach chloroplasts can, under appropriate conditions, carry out photosynthesis with $^{14}CO_2$ at rates approaching those of intact leaves (on a per mg chlorophyll basis) for 10 to 20 minutes (Jensen & Bassham, 1966). One of the first observations to be made with these chloroplasts was that they make practically nothing except intermediate compounds of the RPP cycle, plus G6P, starch, and glycolate. Isolated chloroplasts are capable of the synthesis of a great variety of complex molecules when fed appropriate substrates (such as acetate and amino acids). Thus it appears that the conversion of PGA to acetyl CoA must occur in whole cells outside the chloroplasts, although we cannot entirely rule out the possibility of some block with the isolated chloroplasts which does not exist *in vivo*. The ready export of triose phosphates and PGA by isolated chloroplasts, and their ready uptake of

0 0 0 0 3 8 0 0 7 8 0

acetate and amino acids, further reinforces the concept of an external formation of acetate and amino acids (or their carbon skeletons).

The shuttle mechanism whereby reducing power is exported in the form of triose phosphate has already been mentioned. An early observation with the isolated chloroplasts is that triose phosphates and FDP appear in the supernatant solution as compared with retention in the chloroplasts about 100 times faster than hexose and heptose monophosphates (Bassham, Kirk & Jensen, 1968). The movement of compounds out of isolated chloroplasts can be greatly influenced by the factors in the medium. Significantly, some of these factors greatly influence the rate of CO_2 uptake by chloroplasts (Bassham _et al._, 1970).

Even though there is evidence that the level of free Mg^{++} inside chloroplasts may be higher than 8 mM (Lin & Nobel, 1971), levels of Mg^{++} in the suspending medium of the chloroplasts as high as 5 mM can cause greatly increased loss of intermediate compounds to the medium and simultaneous decrease in CO_2 uptake rate. This effect of Mg^{++} depends on the level of inorganic pyrophosphate (PP_i), which is commonly used in chloroplast media, since PP_i stimulates the fixation rate (Jensen & Bassham, 1968a), and by itself reduces the loss of cycle intermediates to the medium. In general, Mg^{++} ion concentration must nearly equal PP_i concentration before it begins to inhibit fixation and increase compound loss. With 5 mM PP_i, and no initial added Mg^{++}, addition of 1.0 mM Mg^{++} stimulates fixation. With enough Mg^{++} to just begin to inhibit fixation, addition of an as yet unidentified protein factor from spinach leaves causes severe inhibition (Table 1). The same amount of protein factor added without Mg^{++} causes stimulation of the rate. To further add to

the complexity of this apparent regulatory phenomenon, PP_i must be present to give these stimulatory and inhibitory effects, even though higher levels of PP_i abolish the inhibitory effects, presumably by complexing with the Mg^{++} (Bassham et al., 1970).

For a time we thought that the protein factor was FDPase. However, we now have separated it from FDPase activity by gel filtration. So far, we have been unsuccessful in purifying and concentrating the activity, due to abnormal behavior under ordinary separation techniques.

It might appear that the inhibitory effect of Mg^{++} plus the protein factor is due to the increased loss of cycle intermediates from the chloroplasts. This is not the reason, for with amounts of factor which cause both inhibition and loss of intermediate compounds from the chloroplasts, the level of the carboxylation substrate is in some cases even higher than in the control. It may be that the observed effect of Mg^{++} plus protein factor on movement of cycle intermediates out of the chloroplasts is matched or exceeded by an unobserved effect on bicarbonate movement into the chloroplasts. Otherwise, the factor must in some other way directly affect the activity of the RuDPCase.

Aside from PP_i, the only other factor which has been found to significantly increase the rate of CO_2 fixation in isolated spinach chloroplasts, is antimycin A (Champigny & Gibbs, 1969; Champigny & Migniac-Maslow, 1971; Schacter, Champigny & Gibbs, 1971). It was proposed that this factor might stimulate photosynthesis in isolated chloroplasts by increasing the rate of transport of bicarbonate or CO_2 into the chloroplasts. Recent studies (Schacter & Bassham, 1972) showed that this is not the case. Increased transport of HCO_3^- into the chloroplasts should result

0 0 0 0 3 8 0 0 9 8 1

in a decreased level of RuDP. Such a decreased RuDP level is observed when the level of bicarbonate in the medium is increased. However, with antimycin A, the level of RuDP increases, even as the rate of CO_2 uptake increases. As indicated earlier, carboxylation rate does not depend on RuDP level within wide limits but does depend strongly on both RuDPCase activity and CO_2 concentration.

In the case of added antimycin A, there must be an increase in RuDPCase activity. At the same time, levels of FDP and SDP were lower with added antimycin A, in spite of greater CO_2 uptake. This suggests that the general light activation mechanism, responsible for the activation of RuDPCase and FDPase, is somehow enhanced by the addition of antimycin A.

A further effect noted was a large increase in the rate of starch synthesis, with antimycin A addition. The light activation of ADPglucose pyrophosphorylase has aalready been mentioned in connection with the light-dark studies with algae. The stimulation of starch synthesis upon addition of antimycin A to chloroplasts suggests that the general light activation mechanism responsible for RuDPCase and FDPase activity in the light also serves for ADPglucose pyrophosphorylase.

MECHANISMS OF ENZYME REGULATION OF RuDPCase AND FDPase

The possibility that regulation of the activities of the control enzymes of the RPP and OPP cycles is due in part to the redox state in the chloroplasts as well as Mg^{++} concentration and pH has already been mentioned. Other regulatory mechanisms have been reported, although the physiological significance of some of them is still in doubt.

The physical, chemical and biochemical properties of RuDPCase have been reviewed recently (Kawashima & Wildman, 1970; Siegel, Wishnick &

Lane, 1972) and will not be extensively discussed. The protein from green plants varies somewhat from one species to another, but is large (around 500,000 daltons). It is composed of a complex quaternary structure, containing perhaps 32 subunits, 16 each of molecular weights at least 10,000 and 20,000, or perhaps 8 subunits of 56,000 daltons and 8 subunits of 14,000 daltons. There may be 8 binding sites for RuDP, under certain conditions. The enzymes require about 10 mM Mg^{++} for maximum activity, and the pH optimum shifts from 8.5 to 7.8 when the Mg^{++} concentration is raised from 1 mM to 40 mM. Also, it is reported that the apparent binding constant for HCO_3^- is lowered from 20 mM to 2.5 mM when the Mg^{++} concentration is so raised (Bassham, Sharp & Morris, 1968).

A small protein, containing a chromophore and called light-activating factor (LAF) has been isolated from tomato leaves by Wildner and Criddle (1971). When the purified RuDPCase is illuminated in the presence of this factor, as much as fourfold activation of the enzymic activity was observed. The LAF has an absorption peak at 325 nm, with very little absorption at wavelengths beyond 500 nm. The reported action spectrum for the activation has a peak at 325 nm, but surprisingly, there is reported to be about twofold activation even at 750 nm.

The physiological role, if any, of this factor in regulating carbon metabolism in photosynthesis is not yet established. Since the activation is reportedly seen with visible light of all wavelengths, and since the activation may be effected by light intensities lower than those required to saturate photosynthesis *in vivo*, the physiological role is difficult to test. The effect is reportedly variable with species of plants from which the RuDPCase was isolated. The existence of a photodynamic effect produced by one molecule isolated from leaves when

0 0 0 0 3 8 0 0 / 8 2

placed in solution with a complex, regulated enzyme does not prove a physiological role.

Activation of RuDPCase by F6P and inhibition by FDP has been reported (Buchanan and Shürmann, 1972). With 10 mM Mg^{++}, a threefold activation was observed with 0.5 mM F6P. This effect was largely abolished by equal amounts of FDP, and FDP alone inhibited the enzyme about 50%. The presence of F6P reduced the K_m for bicarbonate from the control value of 25 mM to 4 mM; this effect also was abolished by FDP. If such effects occur in vivo, they could provide a link between the two key regulated enzymes of the RPP cycle. Increased FDPase activity, by increasing the steady-state ratio of F6P/FDP could increase the RuDPCase activity. However, before taking this possibility too seriously, it is advisable to consider the assay problems described below and encountered with another activation of the isolated enzyme.

In the course of a survey of the effects of metabolites on RuDPCase activity, it was found that of all the intermediates of the RPP cycle tested, none (including F6P) caused stimulation, while FDP caused some inhibition and 6-PGluA caused a large inhibition, with a K_i of only 50 μM (Chu & Bassham, 1972). Since 6-PGluA is the one intermediate of the two pentose phosphate cycles unique to the OPP cycle (not in the RPP cycle), this finding could represent an important physiological mechanism for completing the inactivation of RuDPCase in the dark. It will be recalled that in green cells, 6-PGluA appears in the dark and disappears in the light (during photosynthesis).

In the course of trying to reconcile the lack of F6P activation with the work of Buchanan and Shürmann, we found that much depends on

the assay method. We had been assaying the enzyme by adding the enzyme last to the reaction mixture. When the enzyme is preincubated with Mg^{++} and bicarbonate, plus the metabolite being tested, and the reaction started by adding RuDP, 6-PGluA actually caused a stimulation at low HCO_3^- concentrations. Lesser amounts of stimulation were observed with a number of other metabolites, including F6P.

Such results suggest the possibility that some of the many binding sites (perhaps both allosteric sites and active sites) of this complex enzyme must be occupied by phosphate sugars for it to become active. When no RuDP is present, this function can be partly fulfilled by other phosphate compounds, with varying degrees of effectiveness. If some of the sites are already occupied by the substrate (RuDP), then binding of a specific allosteric regulator can cause an inhibitory effect. It is clear that more detailed kinetic studies with substrate and suspected regulators are required before physiological significance can be assessed.

In addition to the effects of Mg^{++} and pH on FDPase activity already mentioned, Buchanan, Schürmann and Kalberer (1971) have reported the activation of FDPase by reduced ferredoxin and another small protein factor. The activation was relatively small at the high levels of Mg^{++} which are present in chloroplasts in light. If the Mg^{++} levels drop in the dark, as suggested by Lin and Nobel (1971), Mg^{++} and reduced ferredoxin could work together to effect the light activation indicated by the *in vivo* experiments.

ACKNOWLEDGMENT

This work was supported, in part, by the U. S. Atomic Energy Commission.

U U U U 3 8 U U 7 3 3

REFERENCES

Atkinson, D. W. (1966). Regulation of enzyme activity. Annu. Rev. Biochem., 35, 85-124.

Bassham, J. A. (1971). Control of photosynthetic carbon metabolism. Science, 172, 526-34.

Bassham, J. A., Benson, A. A., Kay, L. D., Wilson, A. T. & Calvin, M. (1954). The path of carbon in photosynthesis. XXI. The cyclic regeneration of carbon dioxide acceptor. J. Amer. Chem. Soc., 76, 1760-70.

Bassham, J. A., El-Badry, A. M., Kirk, M. R., Ottenheym, H. C. J. & Springer-Lederer, H. (1970). Photosynthesis in isolated chloroplasts. V. Effect on fixation rate and metabolite transport from the chloroplasts caused by added fructose-1,6-diphosphatase. Biochim. Biophys. Acta, 223, 261-74.

Bassham, J. A. & Kirk, M. (1968). Dynamic Metabolic regulation of the photosynthetic carbon reduction cycle. In Comparative Biochemistry and Biophysics of Photosynthesis, 365-78 ed. Shibata, K., Jagendorf, A. T. & Fuller, R. C.: University of Tokyo Press.

Bassham, J. A., Kirk, M. & Jensen, R. G. (1968). Photosynthesis by isolated chloroplasts. Diffusion of labeled photosynthetic intermediates between isolated chloroplasts and suspending medium. Biochim. Biophys. Acta, 153, 211-18.

Bassham, J. A. & Krause, G. H. (1969). Free energy changes and metabolic regulation in steady-state photosynthetic carbon reduction. Biochim. Biophys. Acta, 189, 207-21.

Bassham, J. A., Sharp, P. & Morris, I. (1968). The effect of Mg^{++} concentration on the pH optimum and Michaelis constants of the spinach chloroplast ribulosediphosphate carboxylase (carboxydismutase). Biochim. Biophys. Acta, 153, 901-2.

Buchanan, B. B. & Schürmann, P. (1972). A regulatory mechanism for CO_2 assimilation in plant photosynthesis: Activation of ribulose-1,5-diphosphate carboxylase by fructose-6-phosphate and deactivation by fructose-1,6-diphosphate. FEBS Letters, 23, 157-59.

Buchanan, B. B., Schürmann, P. & Kalberer, P. P. (1971). Ferredoxin activated fructose diphosphatase of spinach chloroplasts. J. Biol. Chem., 246, 5952-9.

Champigny, M. L. & Gibbs, M. (1690). Photosynthesis by isolated chloroplasts in the presence of antimycin A and ascorbic acid. In Progress in Photosynthesis Research, Vol. III, 1534-7 ed. Metzner, H., Tubingen City.

Champigny, M. L. & Migniac-Maslow (1971). Relations ntre l'assimilation photosynthetique de CO_2 et la phosphorylation des chloroplastes isoles. I. Stimulation de la fixation de CO_2 par l'antimycine A, antagoniste de son inhibition par le phosphate. Biochim. Biophys. Acta, 234, 335-43.

Chu, D. K. & Bassham, J. A. (1972). Inhibition of ribulose-1,5-diphosphate carboxylase by 6-phosphogluconate. Plant Physiol., in press.

Dilley, R. A. & Vernon, L. P. (1965). Ion and water transport processes related to the light-dependent shrinkage of spinach chloroplasts. Arch. Biochem. Biophys., 111, 365-75.

0 3 0 3 8 0 0 3 8 4

Ghosh, H. P. & Preiss, J. (1965). The biosynthesis of starch in spinach chloroplasts. J. Biol. Chem., 240, 960-1.

Hatch, M. D. & Slack, C. R. (1966). Photosynthesis by sugar cane leaves. A new carboxylation reaction and the pathway of sugar formation. Biochem. J., 101, 103-11.

Hatch, M. D. & Slack, C. R. (1970). Photosynthetic CO_2-fixation pathways. Annu. Rev. Plant Physiol., 21, 141-62.

Heber, U., Hallier, U. W. & Hudson, Z. (1967). II. Lokalisation von enzymen des reductiven und dem oxydativen pentosephosphat-zyklus in den chloroplasten und permeabilität der chloroplasten-membran gegenüber metaboliter. Z. Naturforsch., 226, 1200-15.

Heber, U. & Krause, G. H. (1971). Transfer of carbon, phosphate energy, and reducing equivalents across the chloroplast envelope. In Photosynthesis and Respiration, 218-35 ed. Hatch, M. D., Osmond, B. C. & Slayter, R. O., New York: Wiley Interscience.

Heber, U. W. & Santarius, K. W. (1965). Compartmentation and reduction of pyridine nucleotides in relation to photosynthesis. Biochim. Biophys. Acta, 109, 390-408.

Heldt, H. W. & Rapley, L. (1970). Specific transport of inorganic phosphate, 3-phosphoglycerate and dihydroxyacetone phosphate, and of dicarboxylates across the inner membrane of spinach chloroplasts. FEBS Letters, 10, 143-8.

Hess, B. & Haekel, R. (1967). Interaction between potassium-ammonium and fructose-1,6-diphosphate activation of yeast pyruvate kinase. Nature (London), 214, 848-9.

Jensen, R. G. & Bassham, J. A. (1966). Photosynthesis by isolated chloroplasts. Proc. Nat. Acad. Sci. U.S., 56, 1095-1101.

Jensen, R. G. & Bassham, J. A. (1968a). Photosynthesis by isolated chloroplasts. II. Effects of addition of cofactors and intermediate compounds. Biochim. Biophys. Acta, 153, 219-26.

Jensen, R. G. & Bassham, J. A. (1968b). Photosynthesis by isolated chloroplasts. III. Light activation of the carboxylation reaction. Biochim. Biophys. Acta, 153, 227-34.

Kanazawa, T., Kanazawa, K., Kirk, M. R. & Bassham, J. A. (1972). Regulatory effects of ammonia on carbon metabolism in Chlorella pyrenoidosa during photosynthesis and respiration. Biochim. Biophys. Acta, 256, 656-69.

Kanazawa, T., Kanazawa, K., Kirk, M. R. & Bassham, J. A. (1970). Regulation of photosynthetic carbon metabolism in synchronously growing Chlorella pyrenoidosa. Plant & Cell Physiol., 11, 149-60.

Kanazawa, T., Kirk, M. R. & Bassham, J. A. (1970). Regulatory effects of ammonia on carbon metabolism in photosynthesizing Chlorella pyrenoidosa. Biochim. Biophys. Acta, 205, 401-8.

Kawashima, N. & Wildman, S. G. (1970). Fraction 1 protein. Annu. Rev. Plant Physiol., 21, 325-57.

0 0 0 0 3 8 0 0 7 8 5

Kortschak, H. P., Hartt, C. E. & Burr, G. O. (1965). Carbon dioxide fixation in sugar cane leaves. Plant Physiol., 40, 209-13.

Krause, G. H. & Bassham, J. A. (1969). Induction of respiratory metabolism in illuminated Chlorella pyrenoidosa and isolated spinach chloroplasts by the addition of vitamin K_5. Biochim. Biophys. Acta, 172, 553-65.

Lin, D. C. & Nobel, P. S. (1971). Control of photosynthesis by Mg^{+2}. Arch. Biochem. Biophys., 145, 622-32.

Neuman, J. & Jagendorf, A. T. (1964). Light-induced pH changes related to phosphorylation by chloroplasts. Arch. Biochem. Biophys., 107, 109-19.

Norris, L., Norris, R. E. & Calvin, M. (1955). A survey of the rates and products of short-term photosynthesis in plants of Nine-Phyla. J. Exp. Bot., 6, 64-74.

Pearce, J., Leach, C. K. & Carr, N. G. (1969). The incomplete tricarboxylic acid cycle in the blue-green algae Anabena variabilis. J. gen. Microbiol., 55, 371-8.

Pedersen, T. A., Kirk, M. & Bassham, J. A. (1966a). Inhibition of photophosphorylation and photosynthetic carbon cycle reactions by fatty acids and esters. Biochim. Biophys. Acta, 112, 189-203.

Pedersen, T. A., Kirk, M. & Bassham, J. A. (1966b). Light-dark transients in levels of intermediate compounds during photosynthesis in air-adapted Chlorella. Physiol. Plant., 19, 219-31.

Pelroy, R. A. & Bassham, J. A. (1972). Photosynthetic and dark carbon metabolism in unicellular blue-green algae. Archiv. Mikrobiol., in press.

Pelroy, R. A., Rippka, R. & Stanier, R. Y. (1972). Metabolism of glucose by unicellular blue-green algae. Arch. Mikrobiol., in press.

Preiss, J., Biggs, M. L. & Greenberg, E. (1967). The effect of magnesium ion concentration on the pH optimum of the spinach leaf alkaline fructose diphosphatase. J. Biol. Chem., 242, 2292-4.

Ris, H. & Plaut, W. (1962). Ultrastructure of DNA-containing areas in the chloroplast of Chlamydomonas. J. Cell Biol., 13, 383-91.

Schacter, B. & Bassham, J. A. (1972). Antimycin A stimulation of rate-limiting steps of photosynthesis in isolated spinach chloroplasts. Plant Physiol., 49, 411-6.

Schacter, B., Champigny, M. L. & Gibbs, M. (1971). Antimycin A and the rate of CO_2 fixation in isolated spinach chloroplasts. Plant Physiol., 48, 443-6.

Stocking, C. R. & Larson, S. (1969). A chloroplast cytoplasmic shuttle and the reduction of extraplastid NAD. Biochem. Biophys. Res. Commun., 37, 278-82.

Smith, A. J., London, J. & Stanier, R. Y. (1967). Biochemical basis of obligate autotrophy in blue-green algae and thiobacilli. J. Bacteriol., 94, 972-83.

Siegel, M. I., Wishnick, M. & Lane, D. M. (1972). Ribulose-1,5-diphosphate carboxylase. In The Enzymes, Vol. 5, 169-92 ed. Boyer, P. D., New York and London: Academic Press.

Wildner, G. F. & Criddle, R. S. (1969). Ribulose diphosphate carboxylase I. A factor involved in light activation of the enzyme. Biochem. Biophys. Res. Commun., 37, 952-60.

Yates, R. A. & Pardee, A. B. (1956). Control of pyrimidine biosynthesis in E. coli by a feedback mechanism. J. Biol. Chem., 221, 757-70.

TABLE 1

Effects of Mg^{++} and stimulating protein factor on rates of $^{14}CO_2$ fixation by isolated spinach chloroplasts

Protein factor added µg	Mg^{++} mM	Rate (µmoles ^{14}C per mg chlorophyll per hr)	% Stimulation (+) or inhibition (-)
0	0	101	—
21	0	126	+25
0	2.0	110	+10
21	2.0	17	-83
0	5.0	48	-52
21	5.0	2.3	-98

5 mM PP_i was present in all flasks. (From Bassham et al., 1970).

FIGURE CAPTIONS

Figure 1. The Reductive and Oxidative Pentose Phosphate Cycles and Related Metabolic Pathways.

The reductive pentose phosphate cycle (RPP cycle) and metabolic paths leading to starch synthesis and acetyl CoA are indicated by solid lines; the oxidative pentose phosphate cycle (OPP cycle) and triose phosphate oxidation are indicated by dashed lines. Sites of activation in the light are indicated by open circles; a site of activation of glucose-6-phosphate dehydrogenase in the dark is indicated by the solid circle; the site of NH_4^+ activation of the conversion of phosphoenolpyruvate (PEPA) to pyruvate is indicated by the open square.

Other abbreviations used in this figure and in the text are: RuDP, ribulose-1,5-diphosphate; Ru5P, ribulose-5-phosphate; PGA, 3-phosphoglycerate; 6-PGluA, 6-phosphogluconate; PPGA, phosphoryl-3-phosphoglycerate; GAld3P, glyceraldehyde-3-phosphate; DHAP, dihydroxyacetone phosphate; FDP, fructose-1,6-diphosphate; F6P, fructose-6-phosphate; G6P, glucose-6-phosphate; ADPG, adenosine diphosphoglucose; E4P, erythrose-4-phosphate; SDP, sedoh ptulose-1,7-diphosphate; S7P, sedoheptulose-7-phosphate; R5P, ribose-5-phosphate; Xu5P, xylulose-5-phosphate.

Figure 2. Steady-state and Transient Changes in Pool Sizes of Metabolites in *Chlorella pyrenoidosa* $\longrightarrow$ A $\longrightarrow$ B $\longrightarrow$ C

a. Change in steady-state levels due to increase in rate of conversion of B to C (see text).

b. Transient overshoot due to increased rate of formation of B, before enzyme catalyzing conversion of B to C is activated.

c. Change in B when conversion A to B stops suddenly but conversion B to C stops after a short delay.

FIGURE CAPTIONS (Cont.)

d. Change in B when conversion B to C gradually stops, while conversion A to B stops suddenly after conversion B to C has begun to slow but before conversion B to C has completely stopped.

e. Reversible change in B when conversion B to C first stops, and later starts again.

Figure 3. Changes in the Levels of Metabolites in _Chlorella pyrenoidosa_ upon Darkening and Later with Addition of NH_4^+ (Kanazawa _et al._, 1972).

a. PEPA

b. Pyruvate

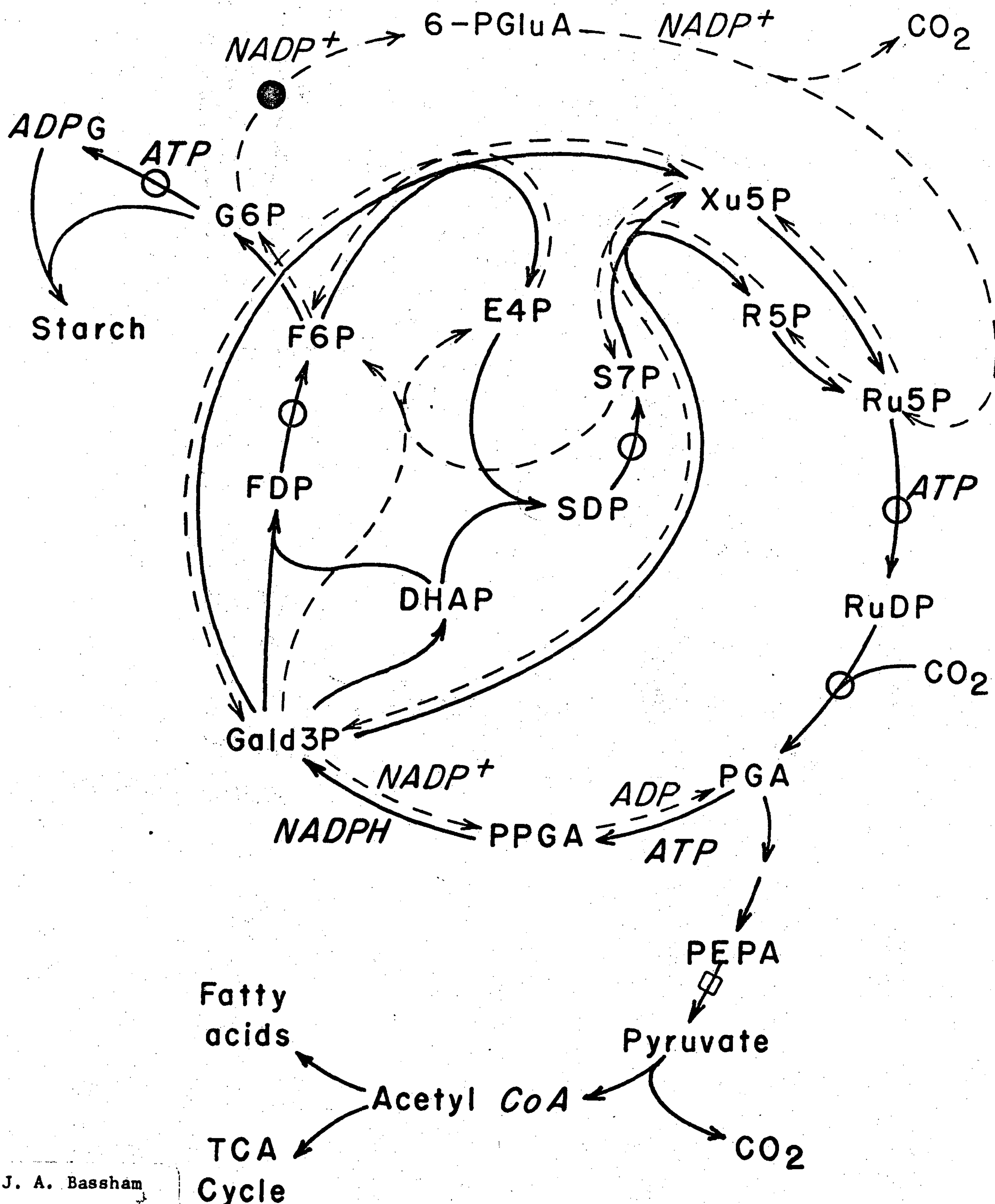
c. Citrate

Figure 4. Changes in the Levels of Metabolites in _Chlorella pyrenoidosa_ upon Darkening and Later with Addition of NH_4^+ (Kanazawa _et al._, 1972).

a. ATP and ADP

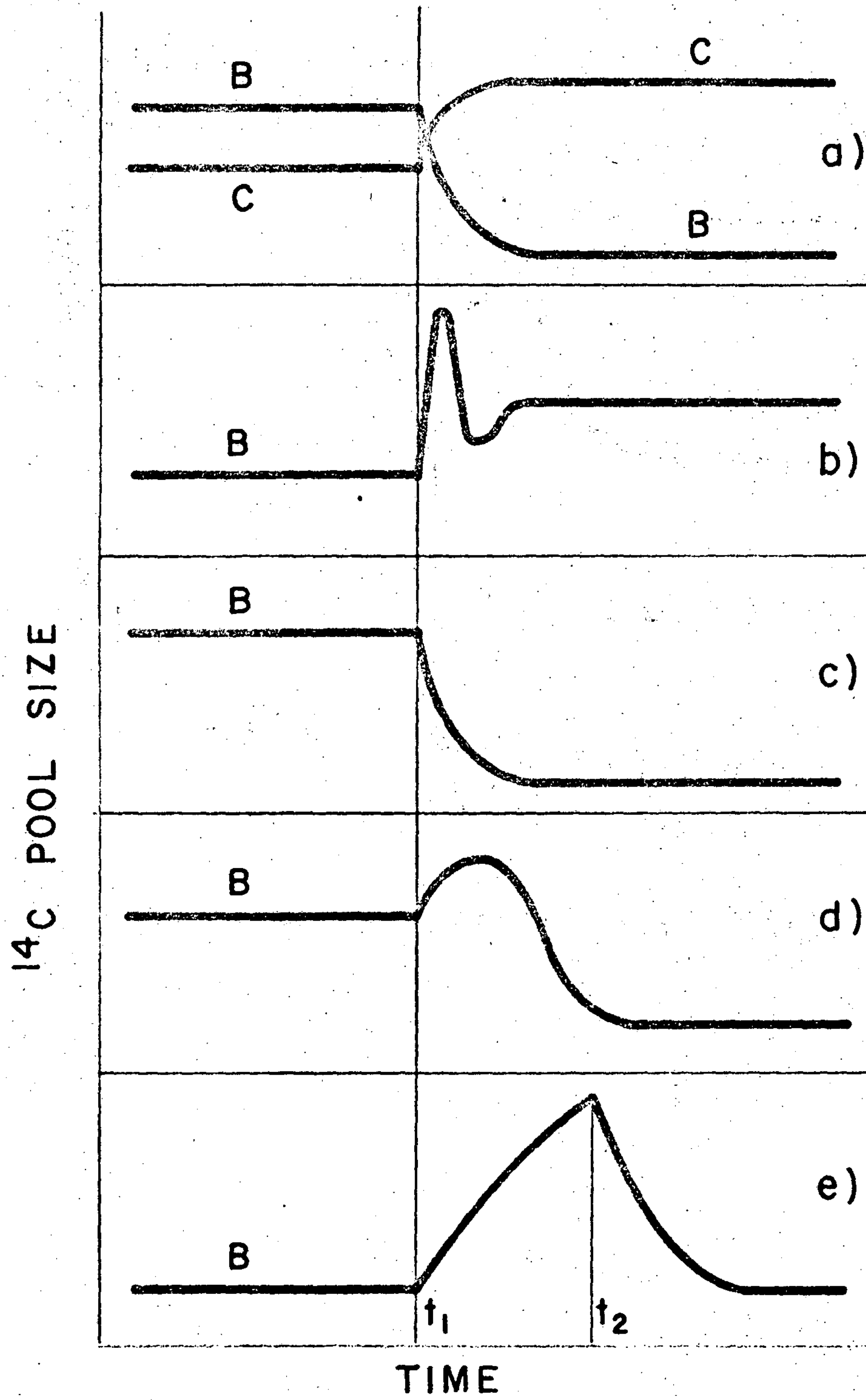
b. 6-PGluA

0 J U 0 3 8 0 3 7 8 7



J. A. Bassham

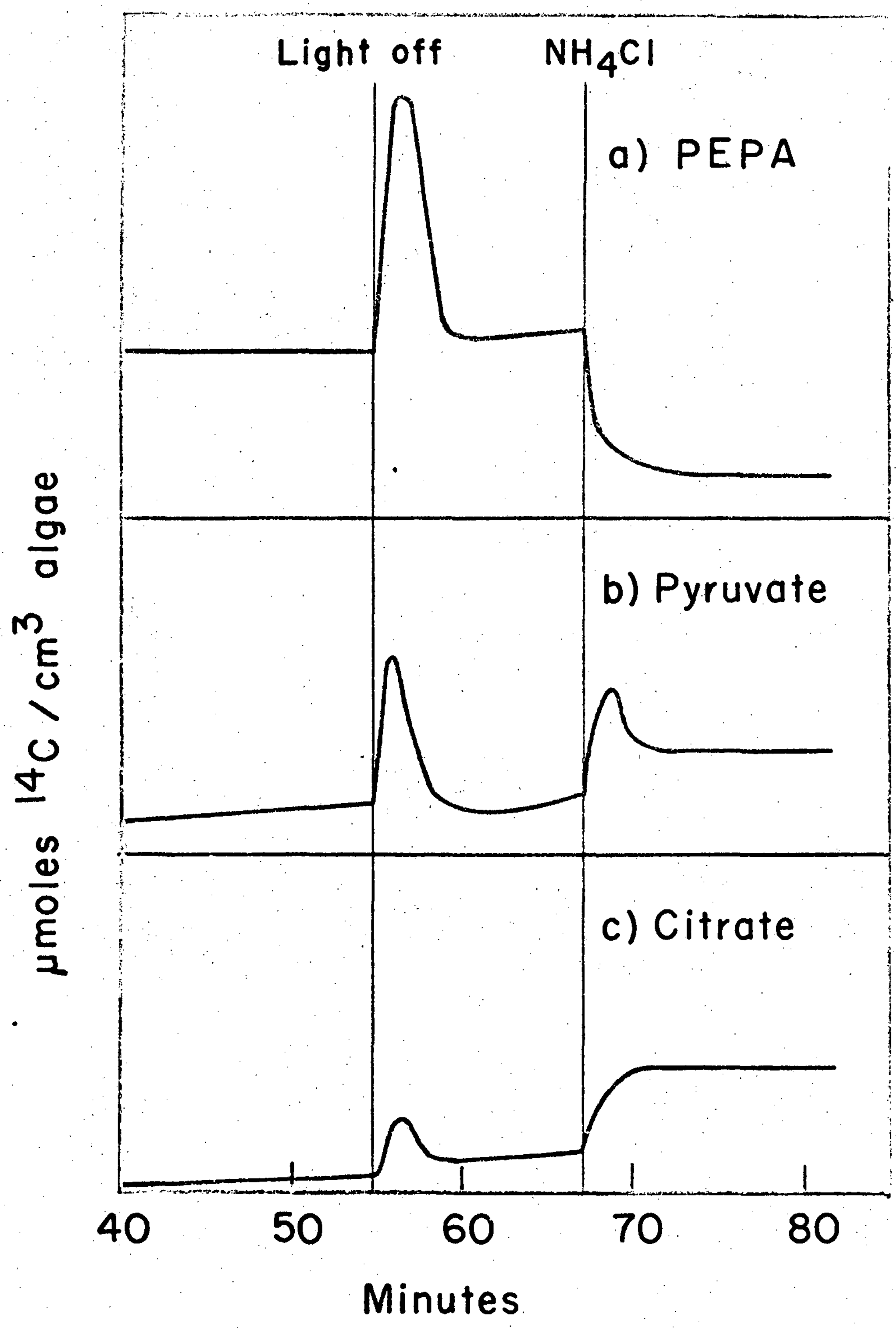
Fig. 1



J. A. Bassham

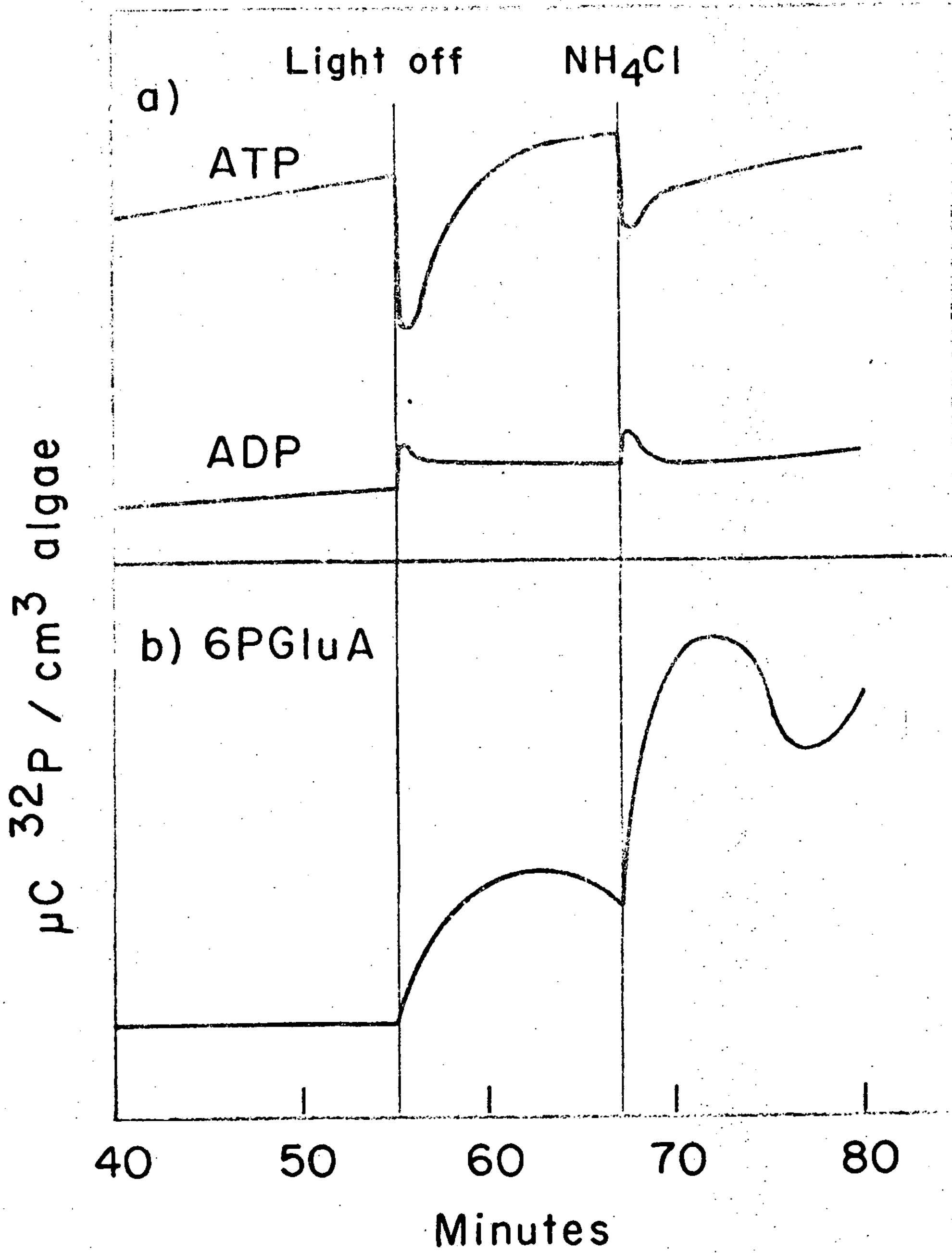
Fig. 2

0 0 0 0 3 8 0 0 7 8 8



J. A. Bassham

Fig. 3



J. A. Bassham

Fig. 4

LEGAL NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or 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.

TECHNICAL INFORMATION DIVISION
LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720