

# Lawrence Berkeley National Laboratory

## Recent Work

**Title**

IN VIVO CONTROL MECHANISM OF THE CARBOXYLATION REACTION

**Permalink**

<https://escholarship.org/uc/item/52764747>

**Author**

Bassham, James A.

**Publication Date**

1978-08-01

c.2

IN VIVO CONTROL MECHANISM OF THE CARBOXYLATION REACTION

James A. Bassham, Sheryl Krohne, and  
Klaus Lenzian

August 1978

RECEIVED  
LAWRENCE  
BERKELEY LABORATORY

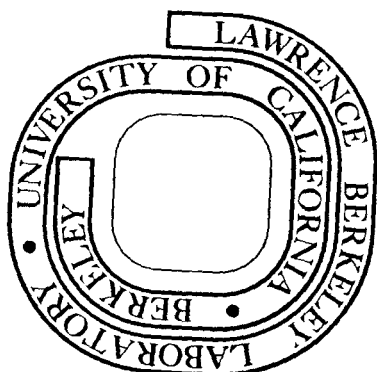
OCT 6 1978

LIBRARY AND  
DOCUMENTS SECTION

Prepared for the U. S. Department of Energy  
under Contract W-7405-ENG-48

**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. 6782



LBL-8154  
c.2

## **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.

LBK-

IN VIVO CONTROL MECHANISM OF THE CARBOXYLATION REACTION\*

for

Brookhaven Symposium in Biology, Number 30  
"Photosynthetic Carbon Assimilation (Ribulose 1,5-Bisphosphate Carboxylase/  
Oxygenase," May 30 - June 2, 1978

James A. Bassham, Sheryl Krohne, and Klaus Lenzian<sup>‡</sup>

Lawrence Berkeley Laboratory  
University of California  
Berkeley, California 94720

\*This research was supported by the Division of Biomedical and Environmental Research of the U. S. Department of Energy.

<sup>‡</sup>Present address: Institut für Botanik und Mikrobiologie der Technischen Universität München, D-8 München 2, Germany.

## INTRODUCTION

It is hardly surprising that both the synthesis and the activity of the enzyme ribulose 1,5-bisphosphate carboxylase (RuBPCase) are highly regulated. This most abundant enzyme on earth catalyzes the entry of  $\text{CO}_2$  into the reductive pentose phosphate pathway (Calvin cycle) (1), the pathway leading to the reduction of  $\text{CO}_2$  to sugar phosphates in all green plants (2), including those with a preliminary C-4 cycle (3) for  $\text{CO}_2$  accumulation. Such first reactions are often the sites of important metabolic regulation. The carboxylation reaction is one of four steps in the Calvin cycle unique to that cycle and not found in the oxidative pentose phosphate cycle (the other such reactions are the ones converting fructose and sedoheptulose bisphosphates to their respective monophosphates and the reaction converting ribulose-5-phosphate to RuBP) (Figure 1). All four of these reactions are inactivated or are less active in the dark, when the oxidative pentose phosphate cycle and the glycolytic pathway operate. The inactivation in the dark of these four reactions unique to the reductive cycle is required to prevent the operation of futile cycles.

In the light, the rates of the reactions catalyzed by RuBPCase and the bisphosphatases are balanced in order to keep the concentrations of Calvin cycle intermediates within acceptable ranges as carbon is withdrawn for biosynthesis from pools of both triose phosphates and hexose phosphates. The pools of these compounds are very small compared to the flux of carbon through the cycle, so very precise regulation is required.

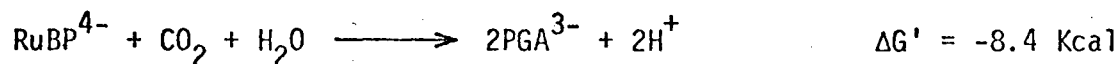
A further regulatory requirement is placed on the RuBPCase by its oxygenase activity. Given air levels of  $\text{CO}_2$  and  $\text{O}_2$ , with the level of  $\text{CO}_2$  further decreased inside the chloroplasts (at least in C-3 plants) by the

high rate of photosynthesis in bright light,  $O_2$  binds competitively with  $CO_2$  at the active site of the enzyme, after which  $O_2$  reacts with RuBP, giving as one product, phosphoglycolate, a substrate for photorespiration (4,5,6,7,8). This means that although a low  $K_m$  for  $CO_2$  is desirable for the efficient operation of the enzyme with air level  $CO_2$ , it would also be desirable (in order to minimize photorespiration) if the  $K_m$  for  $CO_2$ , and hence for  $O_2$ , could rise in the absence of  $CO_2$ .

It appears that the activity of the enzyme RuBPCase responds to all of these requirements in one way or another. Besides fast metabolic response, the amount of the enzyme RuBPCase increases and decreases in response to genetic and hormonal control, physiological adaptation, etc. The control of synthesis and degradation of the protein will doubtless be covered elsewhere in this Symposium and will not be further discussed in this paper. Principally, our discussion will be of results related to metabolic regulation obtained over the years in our laboratory.

#### FREE ENERGY CHANGE AND LACK OF REVERSIBILITY, STROMA CONCENTRATIONS

The carboxylation of RuBP and hydrolytic redox cleavage of the six-carbon intermediate to give two molecules of 3-phosphoglycerate (PGA) is one of the least reversible steps to occur along any important biochemical pathway. The Gibbs free energy change under physiological standard conditions



(all reactants with unit activity except  $H^+ = 10^{-7}$ ) is calculated to be  $\Delta G' = -8.4 \text{ Kcal}$  (9). With Chlorella pyrenoidosa photosynthesizing under air and saturating light, chloroplast concentrations of metabolites were estimated to be 1.4 mM for PGA and 2.04 mM for RuBP.

The method of estimation of concentrations was to allow the algae to photosynthesize with  $^{14}\text{CO}_2$  under conditions of steady-state photosynthesis with constant levels of  $\text{CO}_2$  and specific radioactivity until the intermediate compounds of the Calvin cycle were fully labeled with  $^{14}\text{C}$ . Samples of the algae were then killed, and the metabolites were separated by two dimensional paper chromatography. The  $^{14}\text{C}$  content of each compound could then be determined and used to calculate its concentration in the cells. This requires some assumption about the effective soluble volume of the chloroplasts or space in which the metabolite is dissolved. In the original calculations it was assumed that the stroma region of the chloroplast occupied about 1/4 the total volume of the algae, and that Calvin cycle metabolites were located only in that space. Also, a pH of 7.5 was assumed. Using the calculated concentrations as activities, steady-state  $\Delta G$  of -9.8 Kcal was calculated. At pH 8, the value would be  $\Delta G^S = -11.2$  Kcal. It should be noted, for later discussion, that the algae were grown for some days in air, not in  $\text{CO}_2$  enriched air.

In more recent experiments, (Paul et al., private communication) isolated mesophyll cells from Papaver somniferum were used in similar experiments to determine steady-state concentrations of metabolites. The amounts of metabolites were measured with respect to chlorophyll concentration which averaged 10 mg per ml packed cell volume in two experiments. If a stroma volume of 20  $\mu\text{l}$  per mg chlorophyll is assumed, the concentration of RuBP was calculated to be only 0.07 mM, or 70  $\mu\text{M}$ ! This was with cells that gave photosynthesis rates comparable to the rates of the leaves from which the cells were isolated (10). Note that in this calculation, stroma space, considered to be the exclusive location of RuBP in green cells, is taken

to be 0.2 ml, or 1/5 the total cell volume. The concentration of PGA, much of which might be outside the stroma, was very high, 0.307 moles per mg chlorophyll. Possibly, the inability of isolated mesophyll cells to export photosynthate, which results in an accumulation of sucrose in the cells (John Paul, private communication) also causes a buildup of PGA in the cytoplasm. Calculated stroma concentrations of other sugar phosphate intermediates of the Calvin cycle and of glucose-6-phosphate were generally two to ten times higher in the mesophyll cells than in Chlorella. If we arbitrarily assume that only 1/3 of this PGA is in the stroma, a concentration in the stroma of 5 mM is calculated. At these concentrations, a  $\Delta G^S = -8.38$  Kcal is obtained for the carboxylation reaction at pH 8. The more important point is the great difference in RuBP concentration between Chlorella and the mesophyll cells from poppy.

Of the chemical free energy expended by conversion to heat in the Calvin cycle, about 40% is used in the carboxylation reaction, another 40% in the other three regulated steps, and the remaining 20% is distributed among the reversible reactions (9).

#### LIGHT-DARK REGULATION IN VIVO AND IN CHLOROPLASTS

Although in Chlorella photosynthesizing in air the level of RuBP is high when the light is turned off, the concentration declines rapidly for the first two minutes and then reaches a concentration of about 5% of the light level from which it declines very slowly (Figure 2) (11). Since the  $K_m$  for RuBP for the fully activated enzyme is about .035 mM (12), and the  $\Delta G^S$  for the carboxylation reaction is -8.4 Kcal, this failure of the reaction to continue after two minutes of darkness means that the enzyme activity has greatly declined.



The light-dark inactivation of the RuBPCase is also evident with isolated spinach chloroplasts (13) where, following a period of photosynthesis with  $^{14}\text{CO}_2$ , the level of the RuBP in the dark declined to about one half the light value and then remained constant (Figure 3). When the light was again turned on, the level of RuBP rose very rapidly for 30 sec and then declined to a steady-state level. We attribute such transients to dark inactivation of the carboxylase, followed by light reactivation requiring 30 sec.

When the drop in RuBP level in the isolated spinach chloroplasts was prevented by addition of ATP to the suspending medium just after the light was turned off, very little uptake of  $^{14}\text{CO}_2$  occurred as long as the light was off (even though there was as much RuBP present in the chloroplasts as in the light). When the light was turned on again, high rates of  $^{14}\text{CO}_2$  uptake resumed (14). Although the rate of entry of ATP into whole chloroplasts may be low compared to the requirements of photosynthesis (15-17), this low rate is apparently sufficient to maintain the level of RuBP when it is not being consumed, once the RuBP carboxylase is inactivated.

#### LIGHT-DARK REGULATION OF RuBP CARBOXYLASE BY $\text{Mg}^{2+}$ AND OTHER FACTORS

The primary mechanism for light-dark regulation of the activity of the RuBPCase appears to be via changes in  $\text{Mg}^{2+}$  ion concentrations and pH. Other metabolites, particularly NADPH and 6-phosphogluconate may also contribute to both light-dark regulation and perhaps to regulation in the light. Isolated RuBP carboxylase is activated by preincubation with  $\text{CO}_2$  or bicarbonate and high levels of  $\text{Mg}^{2+}$  (e.g. 10 mM), before the enzyme is exposed to RuBP (18-21). Preincubation with physiological levels of RuBP in the absence of either bicarbonate or  $\text{Mg}^{2+}$  results in conversion of the enzyme to an inactive form with high  $K_m$  values for  $\text{CO}_2$ , and the enzyme does not recover its activity for

many minutes upon subsequent exposure to physiological levels of bicarbonate and  $Mg^{2+}$  (19,20). Full activation of the isolated purified enzyme requires that the preincubation with  $CO_2$  and  $Mg^{2+}$  also be carried out in the presence of either NADPH or 0.05 mM 6-phosphogluconate, each at physiological levels (19-21).

With respect to light-dark regulation, it seems clear that the changes in  $Mg^{2+}$  levels and pH in the chloroplasts result in changes in RuBP carboxylase activity, with the light-induced increases in pH and  $Mg^{2+}$  resulting in increased enzyme activity. The pH optimum of the isolated enzyme shifts towards the pH actually found in chloroplasts in the light (about 8) with increased  $Mg^{2+}$ , and the value of  $K_m$  for  $CO_2$  is lower at pH 8 than at pH 7.2 (22-24).

The activation of the isolated enzyme by NADPH seems to be another part of the light-dark regulation, but the activation by 6-phosphogluconate is at first surprising, since this compound appears in the dark. Kinetic studies show that the 6-phosphogluconate is still present during the first two minutes of light after a dark period (25), and it may be that a useful activation occurs then, while the level of NADPH is still being built up. In the dark, 6-phosphogluconate would not activate the carboxylase since the optimal conditions of pH and  $Mg^{2+}$  levels would not be met.

Presumably, any light activation of the enzyme via NADPH would involve the transfer of electrons to  $NADP^+$  via ferredoxin. This does not explain how oxidized ferredoxin could further increase the activity of the isolated RuBP carboxylase, as reported by Vaklinova and Popova (26), and confirmed by Popova in the author's laboratory (unpublished work).

CAN THE LOW  $K_m$   $CO_2$  FORM BE MAINTAINED  
OUTSIDE CHLOROPLASTS?

Although it appeared for many years that  $K_m$   $CO_2$  for RuDP carboxylase is too high to support the RPP cycle, a number of laboratories have found evidence in recent years that the  $K_m$   $CO_2$  is sufficiently low. In particular, Bahr and Jensen (27) found that a low  $K_m$   $CO_2$  form of the enzyme obtained from freshly lysed spinach chloroplasts could be stabilized with dithioerythritol, ATP,  $MgCl_2$ , and R5P. McC. Lilley and Walker (28) have shown that the activity and  $K_m$   $CO_2$  for the enzyme from spinach chloroplasts are more than adequate to support photosynthesis.

A common problem with some of the reported studies on the biochemical constants of RuBPCase outside the intact chloroplast, whether in the form of crude protein extract or as purified enzyme, is the changing value of  $K_m$   $CO_2$  and fixation rate during the time of the enzyme assays. Even with the purified enzyme preincubated with  $Mg^{2+}$  and bicarbonate in the presence of NADPH or 6-phosphogluconate as effectors, the most active rate of reaction was always during the first 3-5 min, followed by a decline to a slower rate. In some of the studies reported from other laboratories, linear fixation was obtained for less than 2 min, and  $K_m$  determinations were reported using only the rate during the first minute after the addition of RuBP to preincubated enzyme. While it may be common practice among biochemists to look only at initial rates of a reaction, when calculating kinetic constants, it would seem that subsequent behavior may be telling us something in the case of a large protein molecule with complex regulation including evident slow changes in state. We have therefore endeavored, with limited success, to discover conditions under which the enzyme, outside the intact chloroplast, might be able to exhibit prolonged activity and  $K_m$  values required for in vivo photosynthesis.

Lysed and subsequently reconstituted spinach chloroplasts can be made, under carefully chosen conditions, to carry on photosynthetic  $\text{CO}_2$  fixation at substantial rates linear up to 30 min (29). It should be mentioned that even after several years experience with this system, prolonged high rates cannot be guaranteed on any given day, presumably due to variability in biological material, minor impurities in reagents, or other uncontrolled variables. Nevertheless, we have succeeded often enough to be able on good days to carry out investigations on the kinetics of  $\text{CO}_2$  fixation.

An early result was that even though  $\text{Mg}^{2+}$  is maintained at 20 mM, and pH at 8.0 (the light chloroplast values),  $\text{CO}_2$  fixation in the originally reported reconstituted system is strictly light dependent. Since dithiothreitol (1 mM) and glutathione (50 mM) are used, the enzymes of the rate-limiting reactions are fully active, but of course in the dark there is no reduction of PGA and hence no cyclic regeneration of RuBP. Two kinds of experiments have been done in the past year: determination of the  $K_m \text{CO}_2$  in light, and determination of  $K_m \text{CO}_2$  in the dark with soluble enzymes only in the presence and absence of various additional effectors. In both types of experiments we make use of the gas handling steady-state apparatus (30) which has been adapted to allow connection to small round-bottom flasks used previously in our studies with isolated chloroplasts (31) and reconstituted chloroplasts (28). Each 15 ml flask has been fitted with 2 mm I.D. inlet and outlet tubes which are connected by small flexible tubing to gas manifold tubes in turn connected to the steady-state apparatus. We can thus control and monitor  $\text{CO}_2$  pressure,  $\text{O}_2$  pressure and  $^{14}\text{C}$  content during the course of the experiment. The  $^{12}\text{CO}_2$  and  $^{14}\text{CO}_2$  are supplied to the closed system by the pressurized cylinder and regulating system previously described (32).

For experiments with reconstituted chloroplasts, isolation of chloroplasts (31) and reconstitution were as described earlier (29) except that instead of the 14:1 ratio of soluble components to lamellae, a ratio of 7:4 was employed, in order to boost the concentration of RuBP to levels where the determined  $K_m \text{CO}_2$  would be meaningful. At this ratio, there is 14.5 mg soluble protein per mg chlorophyll, and for reproducibility, we adjust the reconstituted system to this ratio, rather than relying on volumetric measurement of thylakoid fractions. The rest of the assay mixture consists of 4 mM NADP, 2 mM ADP, 1 mM PGA, 0.05 mg ferredoxin, 4 mM Na-isoascorbate, and solution Z (29), all in a total volume of 0.5 ml in 15 ml round-bottom flasks.

Each pair of flasks was run at a separate gas concentration starting at the highest concentration. Five different concentrations were used to get the maximum number of data points possible using all the manifold inlets and outlets. Flasks not being used were clamped off until they were needed. They were unclamped and opened to the gas at time zero shown in the results. All flasks were in a nitrogen atmosphere previous to the assay and the assay mixture was put into the flask just before each separate experiment. Samples (50  $\mu\text{l}$ ) were taken during the assay and killed in 450  $\mu\text{l}$  methanol. From the resulting 500  $\mu\text{l}$  of mixture, aliquot samples were taken and counted in scintillation vials after acidification and drying. Carbon dioxide fixation in the 7:4 reconstituted system was linear from 5 to 20 min after the introduction of  $^{14}\text{CO}_2$  at levels ranging from 0.013% to 0.128% (Figure 4). Some time is required during the first five min after  $^{14}\text{CO}_2$  introduction to replace the gas initially in the flasks and to equilibrate gas and liquid phases.

When the reciprocals of the rate vs.  $\text{CO}_2$  concentrations are plotted (Figure 5) we obtain a value for  $K_m \text{CO}_2$  of 0.023% or 230 ppm. This is well

below the air level of 0.032% CO<sub>2</sub>. Moreover, this K<sub>m</sub> plot, unlike many that have been published, shows a truly linear character over a meaningful range of CO<sub>2</sub> pressures. The 1/v intercept at 1/s = 0 is 0.0175 and therefore V<sub>max</sub> = 60 μmoles CO<sub>2</sub>/mg chl·hr, or 4.2 μmoles CO<sub>2</sub>/mg soluble protein·hr (approximately 8.5 μmoles CO<sub>2</sub>/mg RuBPCase·hr). The rate at 0.036% CO<sub>2</sub> in air (slightly more CO<sub>2</sub> than in air) was 60% of V<sub>max</sub>. A Hill plot of the data (Figure 6) gives a slope of n = 1 which shows non-cooperativity of the CO<sub>2</sub> binding sites as previously reported by Bahr and Jensen (27).

The in vivo rate at air level should be about 75 μmoles CO<sub>2</sub> fixed per mg RuBPCase·hr. Thus, although we have obtained physiological K<sub>m</sub>CO<sub>2</sub> values that remain unchanged over 10 minutes, the reaction rates are only about 7% of the in vivo rates. One reason for the lower in vitro rates may be the fact that all the soluble components are considerably diluted in the reconstituted system as compared with in vivo concentrations. With the system used (14.5 mg protein per mg chl) this dilution is about 14:1. While this should not affect the usual assumptions made in enzyme kinetics as long as CO<sub>2</sub> and RuBP concentrations are maintained, it might affect the state of this enzyme, which we know is subject to a complex regulation.

Similar experiments to those just described were performed, but with <sup>14</sup>C-labeled PGA in which the specific radioactivity of each carbon position in the molecule was the same as that of the <sup>14</sup>CO<sub>2</sub> employed (44.7 μCi/μgram-atom). Small samples were withdrawn periodically during the experiment, killed in 80% alcohol, and subsequently analyzed by two-dimensional paper chromatography and radioautography (11). Since all carbon positions of the Calvin cycle intermediate compounds were fully labeled with <sup>14</sup>C of known specific radioactivity (except for small dilution by unlabeled intermediates initially present) it was possible to calculate the concentration of RuBP in the flasks

at each  $\text{CO}_2$  concentration (Table 1). Ideally, for a  $K_m \text{CO}_2$  determination, one would like saturating but not inhibitory concentrations of RuBP. In these experiments, the concentrations of RuBP at the four lower  $\text{CO}_2$  pressures (0.0116% to 0.0613%) are all well above the  $35 \mu\text{M}$  value for the  $K_m \text{RuBP}$  of the enzyme. Once again, a low  $K_m \text{CO}_2$  value was obtained (0.027%  $\text{CO}_2$ ).

One may at least conclude from these results that the RuBPCase can be maintained outside the intact chloroplast in the low  $K_m \text{CO}_2$  form, given cyclic regeneration of RuBP driven by cofactors from the illuminated thylakoids. The next question was whether the low  $K_m \text{CO}_2$  form could be maintained without thylakoids. To date we did not succeed in this, but have learned some interesting properties. We can obtain considerable stimulation of the fixation rate with the addition of several metabolites, including NADPH and ferredoxin (Figure 7).

The experiments are performed by preincubating the soluble enzymes with  $\text{Mg}^{2+}$  and  $^{14}\text{CO}_2$  for 10 min before addition of RuBP. Without effectors, we can obtain a " $K_m \text{CO}_2$ " of 0.031% by using the one min points as rates, but an examination of the kinetics (Figure 8) shows how misleading this is. Always, with the stroma enzymes and enough RuBP present initially to avoid using it up (10 mM in the case shown in Figure 8) there is a very rapid slowing of the rate. We attribute this to allosteric inactivation of the enzyme (18-20). Just as with the isolated enzyme, this inactivation can be partially overcome with added effectors such as 6-phosphogluconate (Figure 9) or NADPH, but not enough at these high concentrations of RuBP to give sustained linear rates characteristic of the low  $K_m \text{CO}_2$  form.

This inactivation by RuBP is probably the reason for the finding often reported by various workers that higher rates can be obtained when the RuBP is supplied by a RuBP generating system such as ribose-5-phosphate and ATP plus enzymes, instead of a high initial concentration of RuBP. As

reported earlier, levels of RuBP in whole photosynthesizing poppy leaf cells are quite low--around 70  $\mu\text{M}$ , or about double the  $K_m$  RuBP. Judging by the data in Table I, however, a concentration as high as 300  $\mu\text{M}$  RuBP is not seriously inhibitory with stroma enzymes at concentrations only several fold less than in intact chloroplasts.

If inhibition by RuBP concentrations of 0.5 mM or more is the cause of our failure to obtain linear kinetics with stroma enzymes, it might be possible to dilute the enzyme concentration and work with much lower RuBP concentrations, say 0.1 mM, without seriously depleting the RuBP as substrate during the course of the experiment. We have performed such experiments. Although a closer approach to linearity was obtained, some falling off in rate with time was still encountered (Figure 10). Perhaps all that can be concluded at present is that even relatively low concentrations of RuBP decrease the activity of the RuBPCase in diluted stroma enzymes over a period of time.

If this premise is accepted, we are left with the question of how Chlorella tolerates the high RuBP concentrations (2.0 mM) found in the measurements cited earlier. The reason for the inhibitory regulation of RuBPCase by RuBP in leaves is presumably to minimize photorespiration resulting from the competitive binding of  $\text{O}_2$  at the  $\text{CO}_2$  binding site. RuBP binding allosterically is thought to raise the  $K_m$  for both  $\text{CO}_2$  and  $\text{O}_2$ . Perhaps this protection is simply not so important for Chlorella. In shallow ponds and lakes, there is less light, more dissolved  $\text{CO}_2$ , no water stress, lower  $\text{O}_2$ , and consequently little chance for photorespiration. When Chlorella are exposed to high  $\text{O}_2$ , low  $\text{CO}_2$  and high light in the laboratory, huge amounts of glycolate are produced. Under such drastic conditions, the enzyme in Chlorella may be inactivated by a decrease in  $K_m$  for  $\text{CO}_2$  and  $\text{O}_2$ , since the rate of



phosphoglycolate formation appeared to decrease after about 30 seconds, even though RuBP concentration was still substantial (32). The regulatory behavior of RuBPCase from Chlorella might be worth further study.

#### Conclusion

The activity of RuBPCase is strongly regulated in light and dark, becoming inactive in vivo in the dark as part of the mechanisms of avoiding wasteful reactions. Changes in pH,  $Mg^{2+}$ , NADPH and other metabolites account for this change in activity. Probably these effectors also account for some fine control of RuBPCase activity in the light, permitting the rate of carboxylation to be balanced against rate of utilization of photosynthate. A major form of regulation of RuBPCase in leaves is the conversion of the enzyme to a high  $K_m CO_2$  form in the presence of high ratios of total RuBP to enzyme binding sites and in the presence of very low levels of  $CO_2$ . This regulation provides a measure of protection against photorespiration resulting from the attack of  $O_2$  on RuBP leading to phosphoglycolate formation. Steady-state concentrations of RuBP in the unicellular algae Chlorella are much higher than would appear to be tolerable in leaf cells, suggesting the possibility of a different degree of inhibition by RuBP in the algae and higher plants.

## REFERENCES

1. Bassham, J.A., Benson, A.A., Day, L.D., Harris, A.Z., Wilson, A.T. and Calvin, M. (1953) *J. Am. Chem. Soc.* 76, 1760-1770.
2. Norris, L., Norris, R.E., and Calvin, M. (1955) *J. Exptl. Bot.*, 6, 64-74.
3. Hatch, M.D. and Slack, C.R. (1966) *Biochem. J.* 101, 103-111.
4. Bassham, J.A. and Kirk, M.R. (1962) *Biochem. Biophys. Res. Commun.* 9, 376-380.
5. Bowes, G., Ogren, W.L., and Hageman, R.H. (1971) *Biochem. Biophys. Res. Commun.* 45, 716-722.
6. Bowes, G., Ogren, W.L., and Hageman, R.H. (1975) *Plant Physiol.* 56, 630-633.
7. Lorimer, G.H., Andrews, T.J. and Tolbert, N.E. (1973) *Biochemistry* 12, 18-23.
8. Richardson, K.E. and Tolbert, N.E. (1961) *J. Biol. Chem.* 236, 1285-1290.
9. Bassham, J.A. and Krause, G.H. (1969) *Biochim. Biophys. Acta* 189, 207-221.
10. Paul, J.S. and Bassham (1977) *Plant Physiol.* 60, 775-778.
11. Pedersen, T.A., Kirk, M. and Bassham, J.A. (1966) *Physiol. Plantarum* 19, 219-231.
12. Chu, D.K. (1974) *Biochemical and Physical Studies of Ribulose 1,5-Diphosphate Carboxylase from Spinach Leaves*. Ph.D. Thesis, University of California, Berkeley, California.
13. Bassham, J.A. and Kirk, M. (1968) in *Comparative Biochemistry and Biophysics of Photosynthesis*, pp. 365-378, K. Shibata, A. Takamiya, A.T. Jagendorf, and R.C. Fuller, eds. University of Tokyo Press, Tokyo, Japan.
14. Jensen, R.G. and Bassham, J.A. (1968) *Biochim. Biophys. Acta* 153, 227-234.
15. Heber, V.W. and Santarius, K.A. (1970) *A. Naturforsch* 256, 718-728.

16. Heldt, H.W., Sauer, F. and Rapley, F. (1971) in Proceedings of the 2nd Int. Congr. on Photosynthesis Res., Stresa, pp. 1345-1355, G. Forti, M. Avron, and A. Melandri. eds.
17. Stokes, D.M., and Walker, D.A. (1971) in Photosynthesis and Respiration, pp. 226-231, M.D. Hatch, C.B. Osmond, and R.O. Slatyer, eds. John Wiley & Sons, Inc., New York.
18. Pon, N.G., Rabin, B.R. and Calvin, M. (1963) *Biochemische Zeitschrift* 338, 7-9.
19. Chu, D.K. and Bassham, J.A. (1973) *Plant Physiol.* 52, 373-379.
20. Chu, D.K. and Bassham, J.A. (1974) *Plant Physiol.* 54, 556-559.
21. Chu, D.K. and Bassham, J.A. (1975) *Plant Physiol.* 55, 720-726.
22. Bassham, J.A., Sharp, P., and Morris, I. (1968) *Biochim. Biophys. Acta* 153, 901-902.
23. Sugiyama, T., Nakayama, N. and Akazawa, T. (1968) *Biochem. Biophys. Res. Commun.* 30, 118-123 (1968).
24. Lorimer, G.H., Badger, M.R. and Andrews, T.J. (1976) *Biochemistry* 15, 529-536.
25. Bassham, J.A., and Kirk, M. and Jensen, R.G. (1968) in *Comparative Biochemistry and Biophysics of Photosynthesis*, pp. 365-378, K. Shibata, A. Takamiya, A.T. Jagendorf, and R.C. Fuller eds., University of Tokyo Press, Tokyo, Japan.
26. Vaklinova, S. and Popova, L. (1971) in Proceedings of the 2nd Intl. Congr. on Photosynthesis, Stresa, pp. 1879-1874, G. Forti, M. Avron, and A. Melandri, eds., Dr. W. Junk N.V., The Hague.
27. Bahr, J.T. and Jensen, R.G. (1974) *Plant Physiol.* 53, 39-44.
28. McC. Lilley, R. and Walker, D. (1975) *Plant Physiol.* 55, 1087-1092.
29. Bassham, J.A., Levine, G. and Forger, J. III (1974) *Plant Science Letters* 2, 15-21.

30. Bassham, J.A. and Kirk, M.R. (1960) *Biochim. Biophys. Acta* 43, 447-464.
31. Jensen, R.G. and Bassham, J.A. (1966) *Proc. Natl. Acad. Sci.* 56, 1095-1101.
32. Platt, S.G., Plaut, Z. and Bassham, J.A. (1976) *Plant Physiol.* 57, 69-73.
33. Bassham, J.A. and Kirk, M.R. (1968) in *Comparative Biochemistry and Biophysics of Photosynthesis*, pp. 365-378, K. Shibata, A. Takamiya, A.T. Jagendorf, and R.C. Fuller, eds., University of Tokyo Press, Tokyo, Japan.

TABLE I  
Steady State Levels of RuBP in Reconstituted  
Spinach Chloroplasts

CO <sub>2</sub> (%)	mM RuBP	Rate--μmoles CO <sub>2</sub> fixed/mg chl·hr <sup>2</sup>
.1428	0.0224	90
.0613	0.0644	68.4
.0266	0.1262	50.4
.0202	0.2061	39.6
.0116	0.2998	25.2

Concentrations of RuBP were determined by <sup>14</sup>C content at steady-state with <sup>14</sup>CO<sub>2</sub> and <sup>14</sup>C-U-PGA (see text).

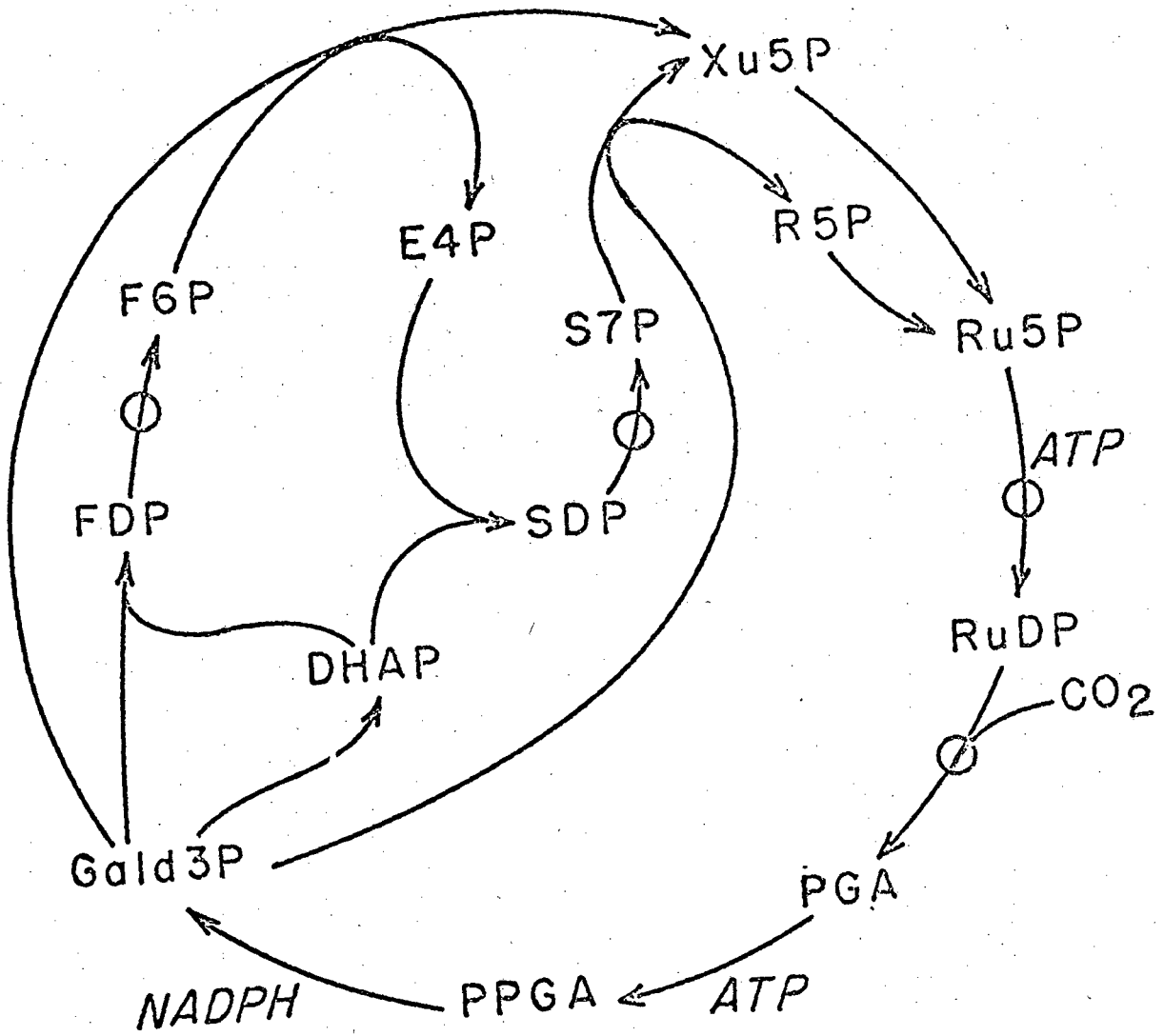
### FIGURE LEGENDS

- Figure 1. Rate Limiting Steps in the Calvin Cycle. RuDP = RuBP; Ru5P, ribulose-5-phosphate; Xu5P, xylulose-5-phosphate; S7P, sedoheptulose-7-phosphate; SDP, sedoheptulose 1,7-bisphosphate; E4P, erythrose-4-phosphate; FDP, fructose-7-phosphate; FDP = FBP, fructose 1,6-bisphosphate; DHAP, dihydroxyacetone phosphate; Gald3P, glyderaldehyde-3-phosphate; PPGA, phosphoryl-3-phosphoglycerate; PGA, 3-phosphoglycerate.
- Figure 2. Changes in RuBP Concentration in Chlorella pyrenoidosa in light and dark periods (from ref. 11).
- Figure 3. Changes in RuBP Concentration in isolated Spinach Chloroplasts in light and dark periods (from ref. 13).
- Figure 4. Steady-State CO<sub>2</sub> Fixation by Reconstituted Spinach Chloroplasts at Five Levels of CO<sub>2</sub>. Conditions described in text. Plot shown only for 5 to 20 min, since an initial equilibration time of 2 min is required.
- Figure 5. Lineweaver-Burke Plot:  $K_m$  CO<sub>2</sub> for RuBP. Plot based on data in Figure 4.
- Figure 6. Hill Plot of RuBP Carboxylase Activity with Varying CO<sub>2</sub> Pressure. Plot based on data in Figure 4.
- Figure 7. Stimulation of CO<sub>2</sub> Fixation in Diluted Stroma Enzymes by NADPH and Ferredoxin. Whole isolated spinach chloroplasts were lysed in HEPES buffer (25 mM, pH 8) thylakoids were removed by centrifugation at 20,000 x g for 10 min. The supernatant solution was diluted in HEPES buffer to give 50 µg soluble protein in each 0.5 ml of solution in a reaction flask. Reaction conditions as in text.

Figure 8.  $\text{CO}_2$  Fixation by Stroma Enzymes at Five Levels of  $\text{CO}_2$ . Whole isolated spinach chloroplasts were lysed in solution Z (28). Each 0.5 ml of reaction mixture in flask contained 940  $\mu\text{g}$  soluble protein. Reaction conditions as in text.

Figure 9.  $\text{CO}_2$  Fixation by Stroma Enzymes at Five Levels of  $\text{CO}_2$  with Added 6-phosphogluconate. Conditions same as in Figure 8.

Figure 10.  $\text{CO}_2$  Fixation with Diluted Stroma Enzymes and 0.1 mM RuBP. Whole isolated spinach chloroplasts were lysed in HEPES buffer (25 mM, pH 8), thylakoids were removed by centrifugation at 20,000 x g for 10 min. The supernatant solution was diluted in HEPES buffer to give 20  $\mu\text{g}$  soluble protein in each 0.5 ml of solution in a reaction flask. Reaction conditions as in text.



XBL 727-4703 A

Fig. 1



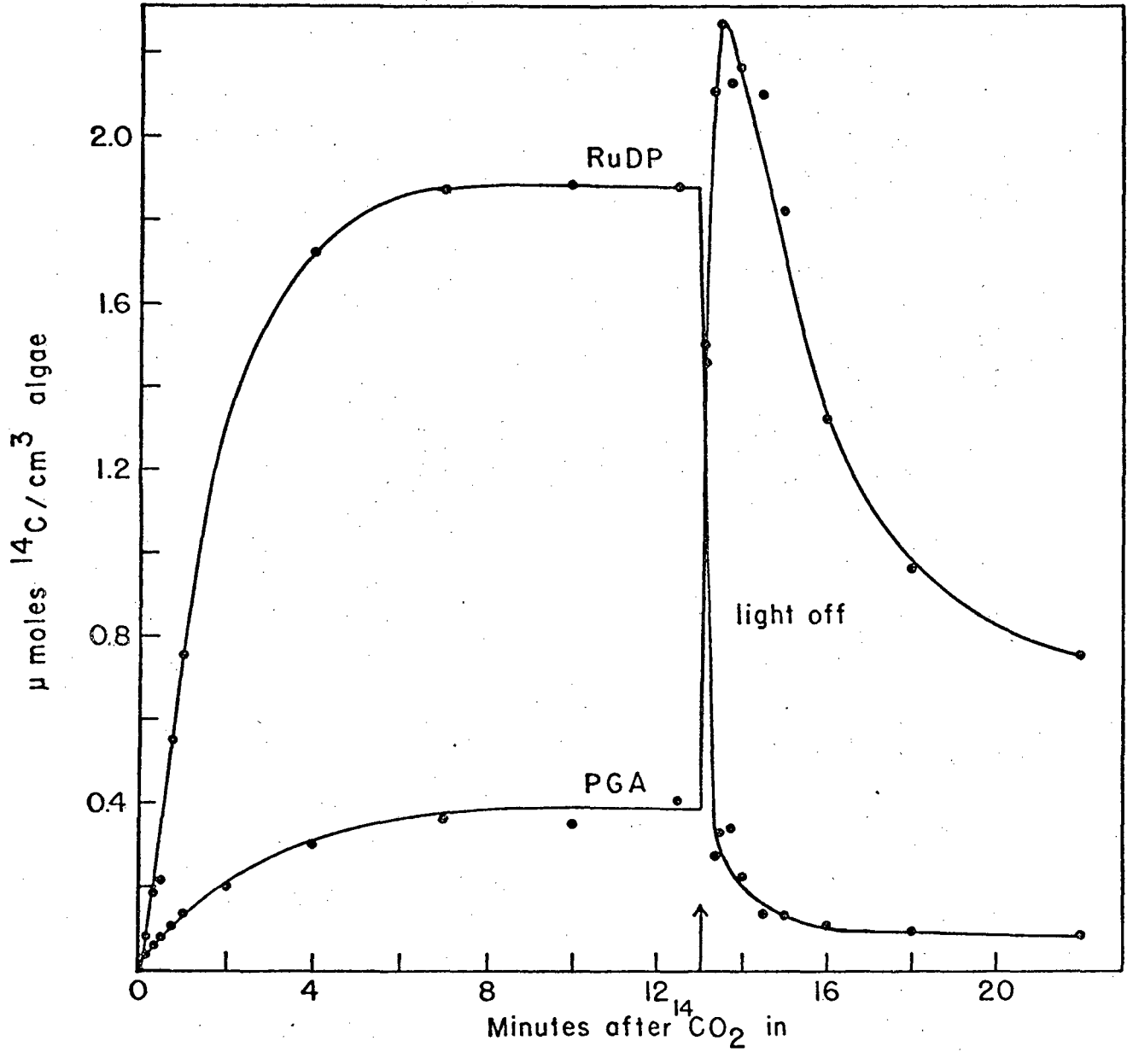


Fig. 2

MUB-4694

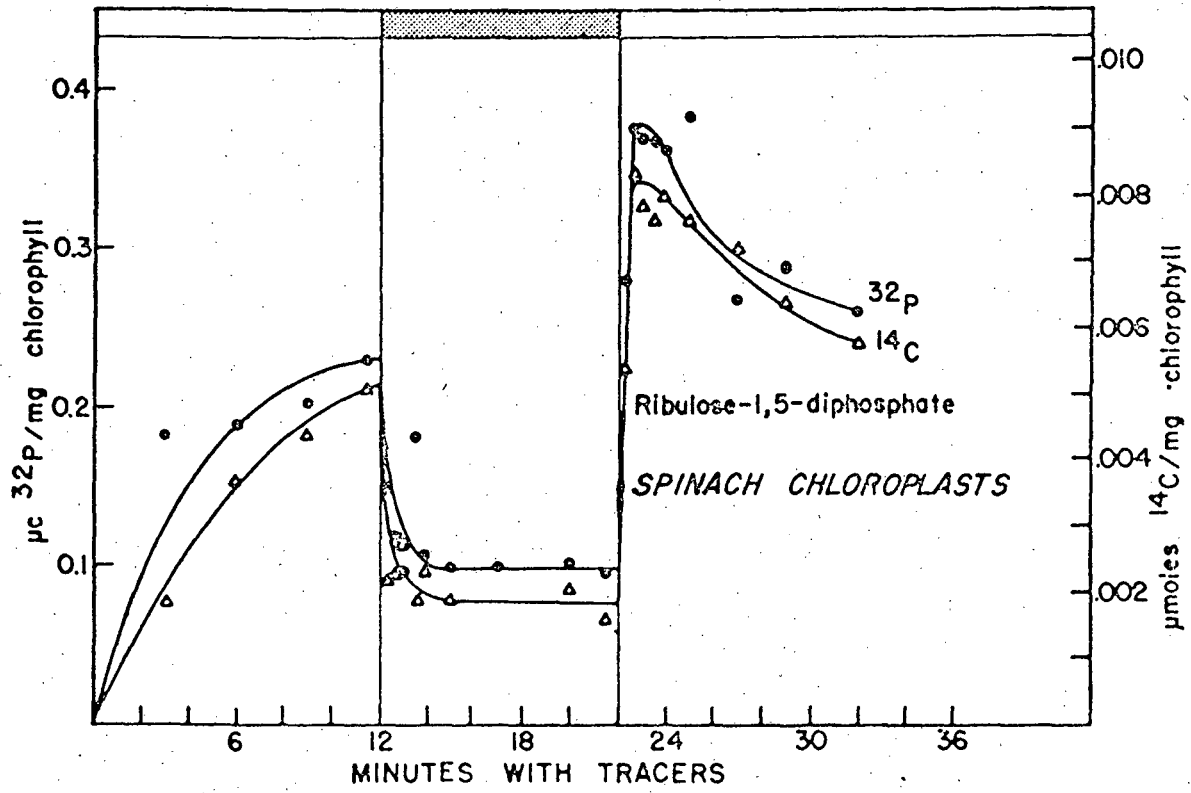
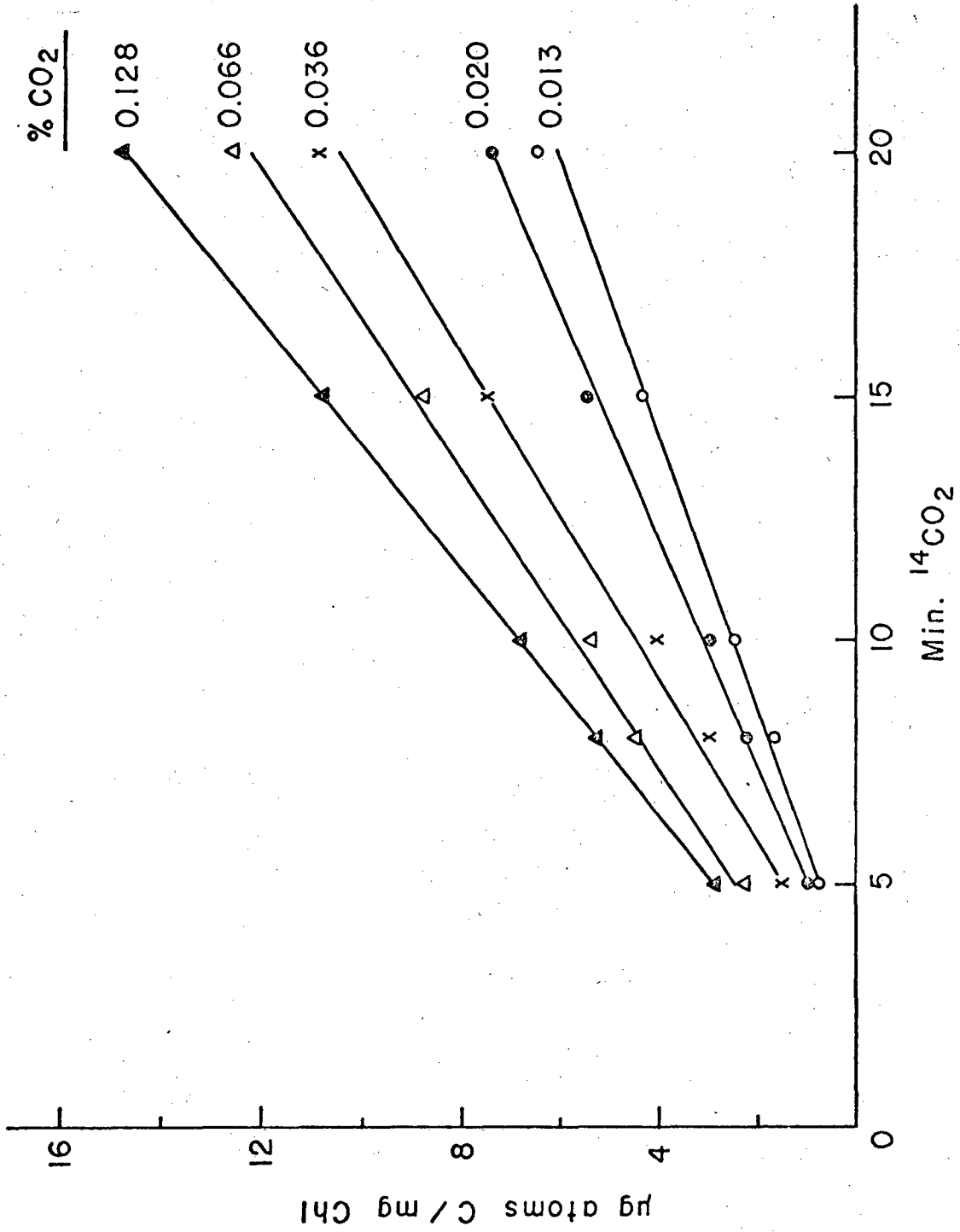


Fig. 3

MUB-9704



XBL 765-5902

Fig. 4

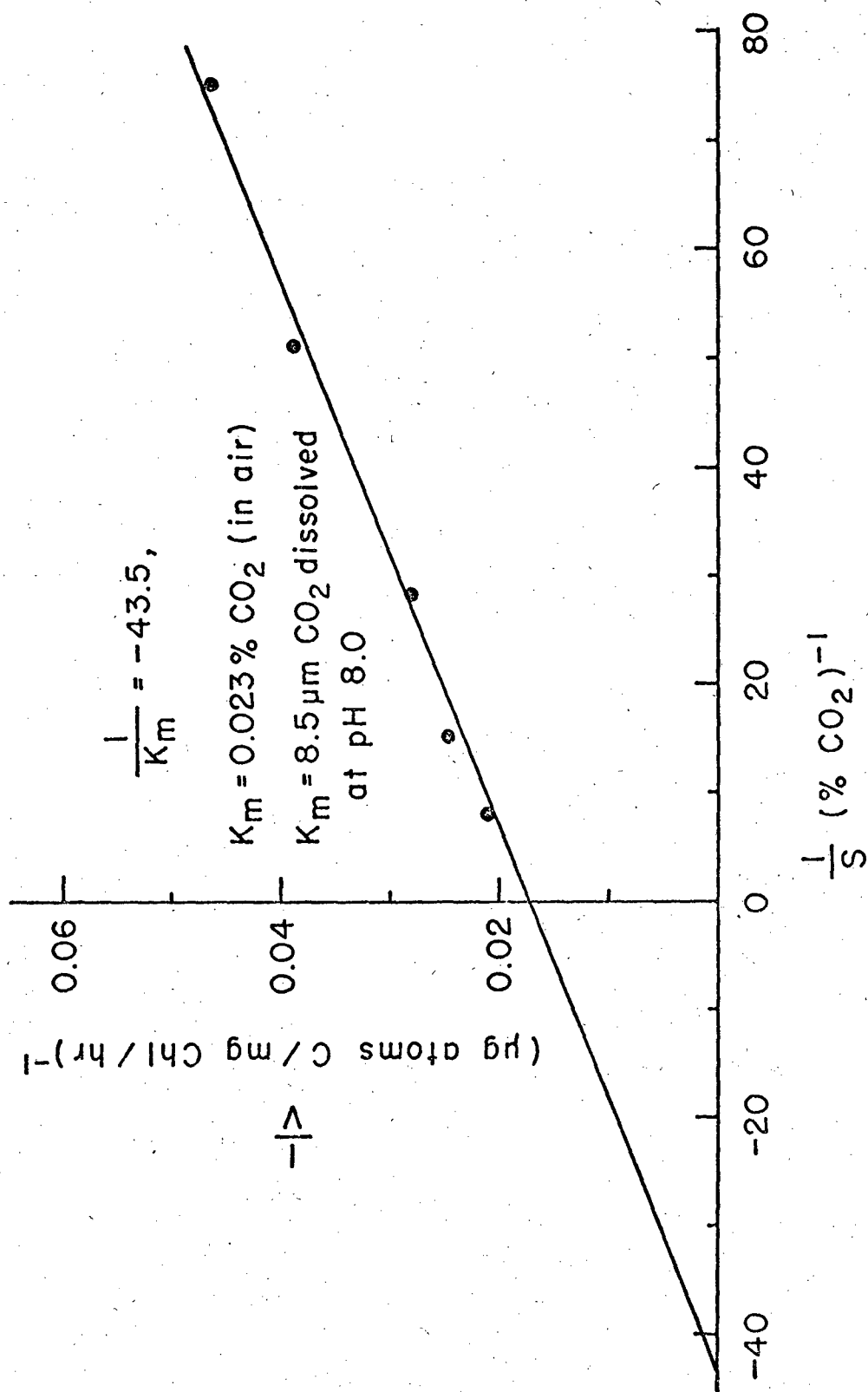
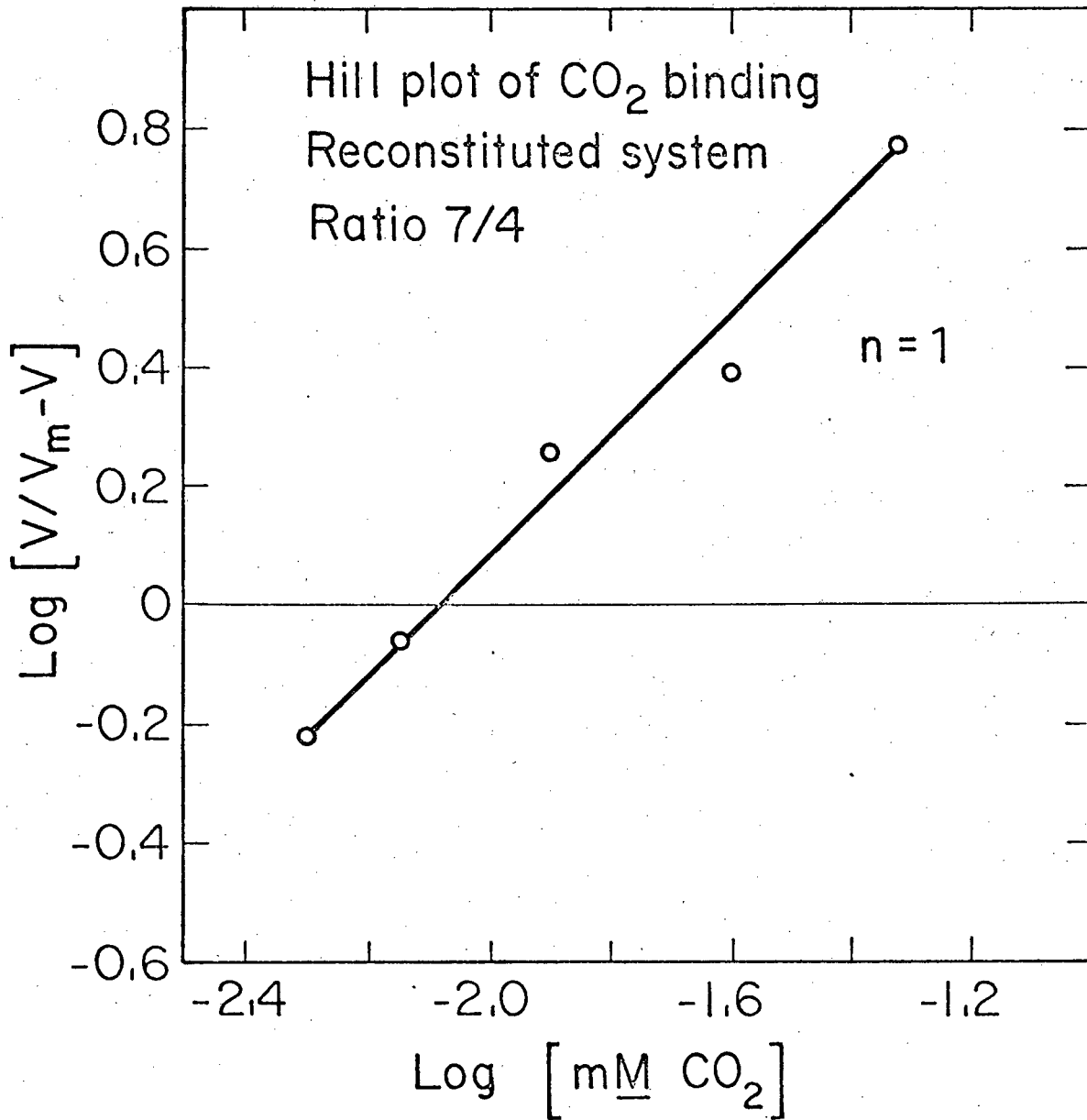


Fig. 5

XBL 765-5901



XBL 785-887

Fig. 6

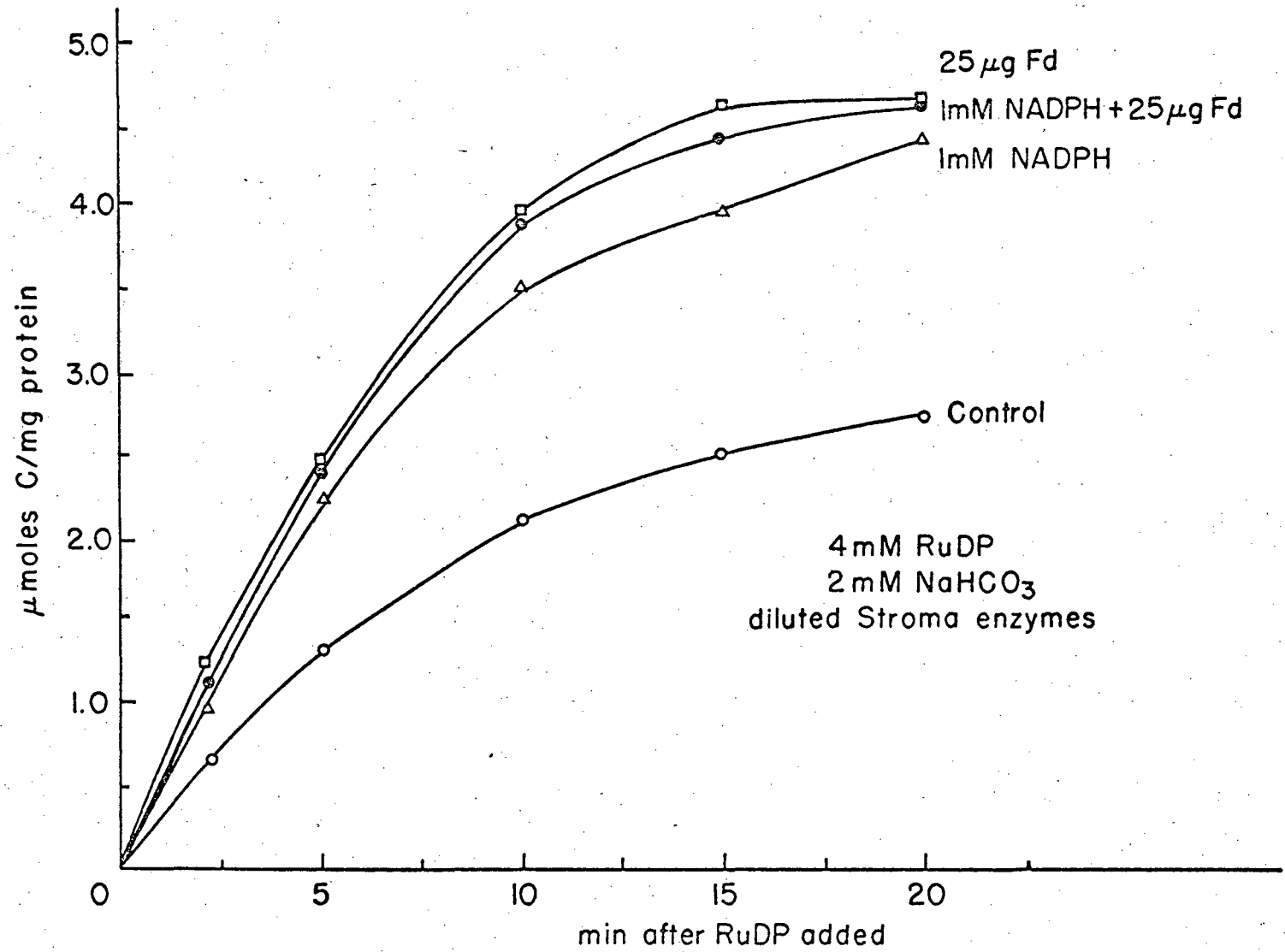
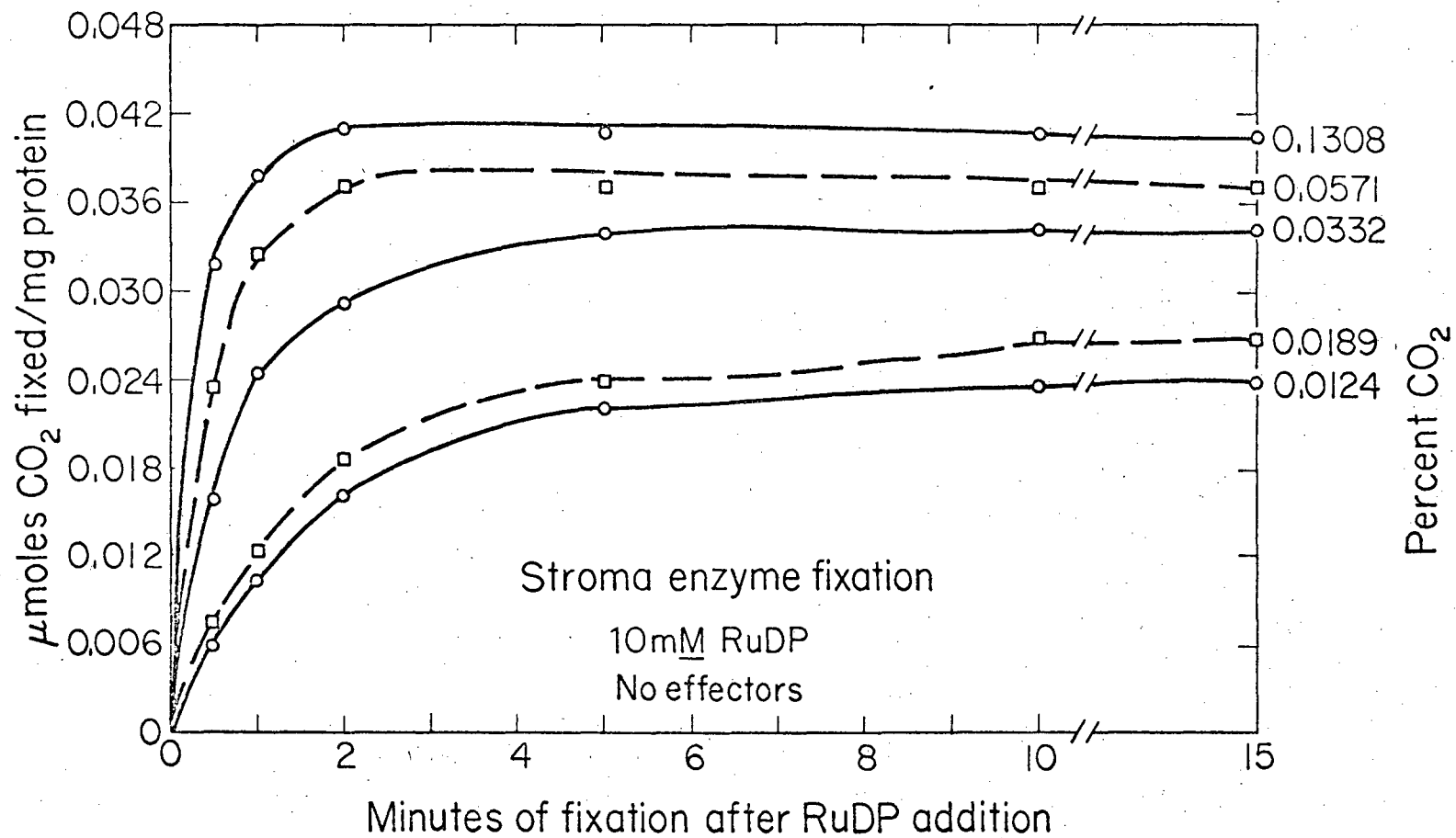


Fig. 7

XBL 767-6026



XBL 785-888

Fig. 8

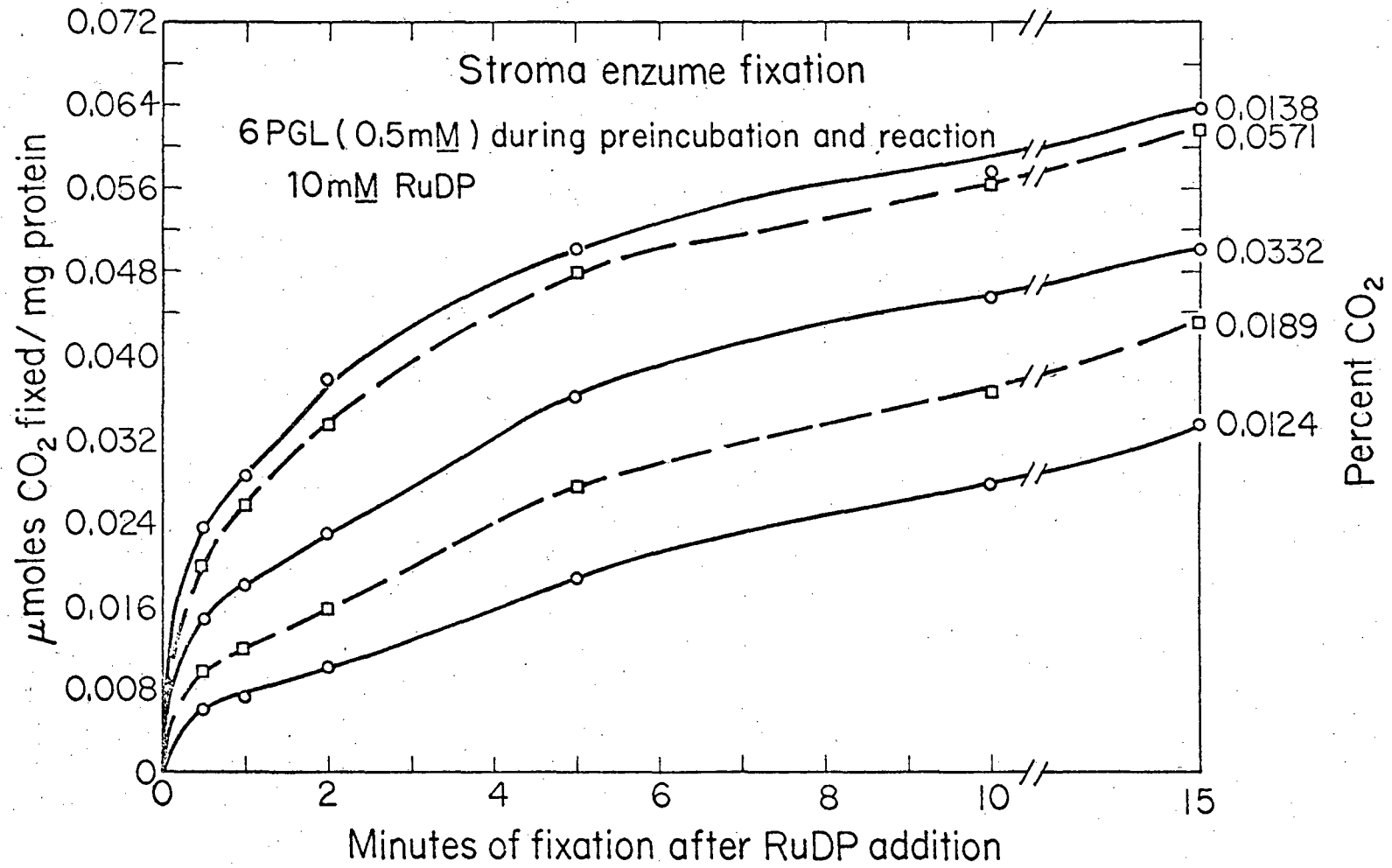


Fig. 9

XBL 785-889



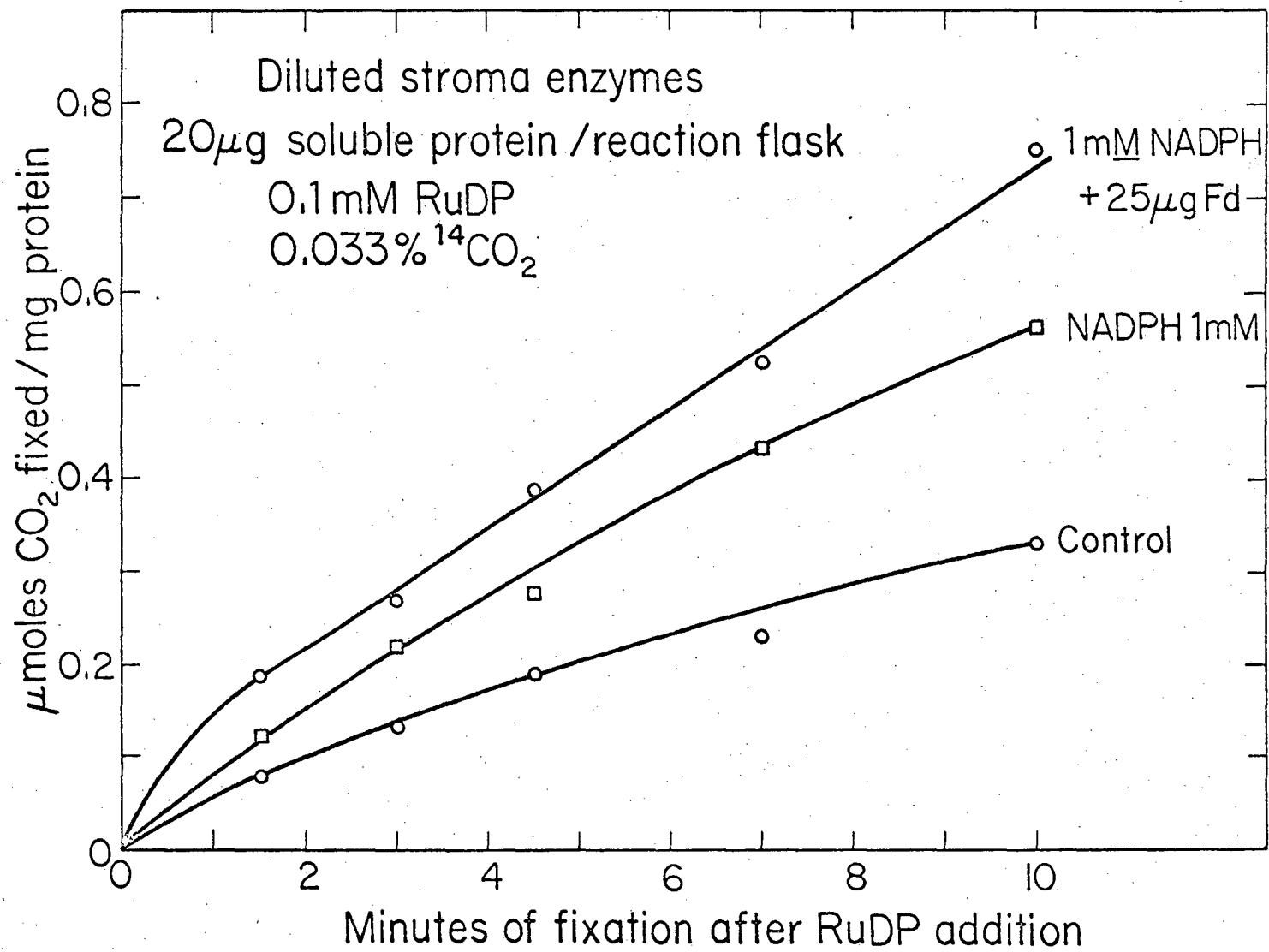


Fig. 10

XBL 785-890

This report was done with support from the Department of Energy. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the Department of Energy.

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