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# NADPH/NADP$^+$ RATIOS IN PHOTOSYNTHESIZING RECONSTITUTED CHLOROPLASTS*

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Summary

Levels of reduced and oxidized triphosphopyridine nucleotides have been determined in reconstituted spinach chloroplasts and compared with levels in whole isolated chloroplasts during photosynthesis and darkness. The ratio of $NADPH/NADP^+$ reaches values slightly above 1.0 at the beginning of photosynthesis, less than half the ratio attained with whole chloroplasts. Nonetheless these lower ratios are sufficient to maintain high rates of photosynthetic carbon dioxide fixation and reduction which are comparable in the reconstituted chloroplasts to the rates found with whole chloroplasts. As with whole chloroplasts there is a decline in the ratio of $NADPH/NADP^+$ as a function of time of photosynthesis. The effect of addition of bicarbonate (6 mM) in causing a transient drop in the ratio of $NADPH/NADP^+$ is described and discussed in terms of the reversibility of the reduction of 3-phosphoglycerate to triose phosphate. The ratio $NADPH/NADP^+$ can be improved by the addition of more lamellae either before or during the course of photosynthesis, and this improvement in ratio is accompanied by an improved rate of $CO_2$ fixation or a more sustained rate of $CO_2$ fixation with time of photosynthesis. The importance of $NADPH/NADP^+$ ratio to not only the reduction of 3-PGA to triose phosphate but also to the activation of the ribulose-1,5-diphosphate carboxylase mediated step is discussed.

Introduction

Conversion of carbon dioxide to sugar phosphates by the soluble enzymes during photosynthesis requires ATP and NADPH which are regenerated by photoelectron transport and photophosphorylation reactions in the internal chloroplast membranes. Thus, these carriers of reducing equivalents and chemical energy are the direct links between the photochemical and the synthetic reactions of photosynthesis. Early experiments showed that isolated chloroplast membranes are capable of reducing $NADP^+$ photochemically [1-3]. Furthermore, illuminated chloroplasts could bring about the reduction of carbon dioxide to the level of sugar phosphates [4]. Also, $NADP^+$ could be reduced in broken chloroplasts in the dark when a source of electrons in the form of hydrogen, bacterial hydrogenase, and soluble ferredoxin was added [5].

From knowledge of the enzymatic reactions of the reductive pentose phosphate cycle [6], it was recognized that NADPH was required for the reduction of 3-phosphoglyceric acid (3-PGA) to glyceraldehyde-3-phosphate, which includes the step mediated by $NADP^+$-dependent triose phosphate dehydrogenase [7]. There are indications that NADPH and $NADPH/NADP^+$ ratios play an important role in the regulation of enzymes of the reductive and oxidative pentose phosphate cycle. With the oxidative cycle, the key regulated step, glucose-6-phosphate dehydrogenase (EC 1.1.1.49) is controlled by the ratio $NADPH/NADP^+$, as mediated by ribulose-1,5-diphosphate (Ribul-1,5-$P_2$) [8]. Moreover, studies with ribulose-1,5-diphosphate carboxylase (EC 4.1.1.39) have demonstrated that NADPH is a powerful effector for this enzyme [9,10]. Thus, it appears that the level of NADPH may play an important role in controlling the initial step for fixation of carbon dioxide.

Heber and Santarius [11] reported that in isolated spinach chloroplasts there is a rapid increase in the $NADPH/NADP^+$ ratio during the first 30 sec

of light followed by a return to its original level after three or four min and a further decline when the light was turned off. In contrast, we reported that NADPH/$NADP^+$ ratios in whole isolated spinach chloroplasts have steady values of 0.3 in the dark but reach values of up to 2.5 in the light and are maintained at those high values for as long as 10 to 20 min [8]. This difference can be mainly attributed to recent improvements in the techniques for the isolation of whole chloroplasts [12] and improvements in the method of determining the levels of reduced and oxidized triphosphopyridine nucleotides in biological materials.

During the past two years it has become possible to carry out complete photosynthetic carbon dioxide reduction in reconstituted chloroplasts [13]. The previously isolated chloroplasts are disrupted by lysis, and the separated membranes and soluble components are reconstituted with certain added co-enzymes which allow photosynthesis to proceed in vitro. In this preparation it is possible to add various cofactors and other chemicals without concern for a permeability barrier imposed by an outer chloroplast membrane. Therefore, it becomes possible to study directly the role of NADPH and $NADP^+$ in regulating the operation of the reductive pentose phosphate cycle of photosynthesis. It is necessary, however, to add additional ferredoxin and $NADP^+$ to these preparations in order to have sufficient concentrations of electron transport cofactors.

## Materials and Methods

Reconstituted Chloroplasts System. Spinach chloroplasts were isolated according to methods described previously [12]. The chloroplasts were disrupted by lysing. The membrane fraction and the soluble components were separated by centrifugation. The membranes were washed and were then recombined with soluble components in a suspension in which the soluble components were increased relative to the membrane components by

a ratio of 14 to 1 compared with whole chloroplasts [13]. In some experiments (described below) the amount of soluble components relative to the lamellae was reduced by a factor of 4 (to a ratio of 3.5).

Materials. ADP, $NADP^+$, 3-PGA, glucose and hexokinase were purchased from Cal-Biochem.

Fixation of $^{14}CO_2$. The incubation with $NaH^{14}CO_3$ (6 mM; spec. Act. 17 uCi/umole) and subsequent determination of rate of photosynthesis by incorporation of $^{14}CO_2$ into acid-stable compounds were as described previously [12,13].

Assay of NADPH and $NADP^+$. The samples for assaying NADPH and $NADP^+$ were removed by hyperdermic needle from the incubation flasks and were killed in HCl for determination of $NADP^+$, or in NaOH for determination of NADPH. They were then immediately assayed according to the previously described method [8].

Chlorophyll Determination. Chlorophyll was determined according to Vernon [14].

The detailed conditions for incubation of reconstituted chloroplasts are described in each case with the data.

## Results

### Comparison of Reconstituted System with Whole Chloroplasts

With photosynthesizing whole chloroplasts, the ratio of NADPH/$NADP^+$ typically rises above 2 and only slowly declines with time. When the light is turned off with whole chloroplasts, the ratio drops very rapidly to less than 0.5. When the light is turned on, the ratio again rises to high values (Fig. 1). The ratio is much lower with the reconstituted system, barely reaching values of 1.0. Addition of sodium bicarbonate (6 mM) results in very little change in the NADPH/$NADP^+$ ratio for whole chloroplasts but causes a substantial initial drop in the ratio for the reconstituted system. Towards the end of a 20 min period of

photosynthesis with a reconstituted system, values of the ratio may be as low as 0.5. It is important to note that the rate of $CO_2$ fixation is essentially as high on the basis of fixation per mg of chlorophyll in the reconstituted system as with the whole chloroplasts. For the experiment giving the data shown in Fig. 1, for example, the rate with whole chloroplasts was 129 umoles $CO_2$ fixed per mg Chl x h, whereas for the reconstituted system the rate was 117 umoles $CO_2$ fixed per mg Chl x h. Thus it seems clear that under the physiological conditions employed, the reconstituted system is able to carry out all of the reactions of the photosynthetic carbon reduction cycle at high rates despite the relatively lower $NADPH/NADP^+$ ratios.

Effect of Increasing the Amount of Lamellae Relative to Soluble Components

Since the ratio $NADPH/NADP^+$ was considerably lower in the reconstituted system than with chloroplasts, we investigated the effect of adding additional internal chloroplast membranes or lamellae in order to increase the rate of electron transport from the light reactions to the reduction of $NADP^+$. In an experiment employing glucose and hexokinase as primer, additional lamellae sufficient to increase the amount of chlorophyll by a factor of 4 were added 20 min after the initiation of photosynthesis by addition of bicarbonate (Fig. 2). With the reconstituted system under either an atmosphere of nitrogen or under an atmosphere of air (where lower ratios of $NADPH/NADP^+$ and rates of photosynthesis are observed) the addition of lamellae resulted in a substantial increase in the ratio, $NADPH/NADP^+$, over a period of time. Although the total fixation rate increased upon the addition of lamellae (Fig. 3), the actual fixation rate calculated on a basis of per mg chlorophyll would, of course, be much decreased since the amount of chlorophyll had been increased by a factor of 4. Thus, there might have been some dependence of the $CO_2$

fixation rate on the ratio, $NADPH/NADP^+$, although it must be noted that additional lamellae also increased the rate of photophosphorylation. When the lamellae were increased by a factor of 4 from the beginning of the experiment, then both the $NADPH/NADP^+$ ratio and the $^{14}CO_2$ fixation rate were maintained at higher levels throughout the course of the experiment (Fig. 4).

Effect of Glucose/Hexokinase or 3-PGA as Primers

In experiments with the reconstituted system, it is necessary to provide a primer of photosynthetic metabolites in order to achieve high rates of $CO_2$ fixation [13]. In our earlier work we have usually employed 3-PGA as a primer since this is a product of the carboxylation reaction. An alternative primer is glucose together with hexokinase. Photophosphorylation occuring in the light prior to the addition of sodium bicarbonate produces ATP, which in turn converts the glucose to glucose-6-phosphate. This compound is subsequently converted to various other sugar phosphates including Ribul-1,5-$P_2$, the substrate for the carboxylation reaction.

The sudden drop in the $NADPH/NADP^+$ ratio upon the addition of sodium bicarbonate (Fig. 1) was of interest since it does not occur significantly with whole chloroplasts, and since the only utilization of NADPH known to occur in the reductive pentose phosphate cycle is during the reduction of 3-PGA to glyceraldehyde-3-phosphate. In experiments with $^{14}C$-glucose as a primer, the 3-PGA concentration was less than 0.050 mM just prior to the addition of sodium bicarbonate. When 1 mM 3-PGA was used as a primer, its concentration was reduced prior to the addition of bicarbonate but is higher than when glucose was used as a primer. Addition of sodium bicarbonate to the reconstituted system with glucose as a primer should cause a larger relative change in PGA concentration, and in turn there might be a larger effect on the $NADPH/NADP^+$ ratio. However the $NADPH/NADP^+$ ratio actually declines somewhat less rapidly upon bicarbonate addition when

glucose was employed as a primer than with 3-PGA (Fig. 5).

This point was further investigated by carrying out an experiment (Fig. 6) in which in one case no primer at all was employed and in the other case 1 mM 3-PGA was employed. After 7 min light, 1 mM 3-PGA was added to the flask with no primer, but no bicarbonate was added. In the other flask with 3-PGA as a primer already present, 6 mM bicarbonate was added at 7 min. In both cases there was a sharp drop in the ratio $NADPH/NADP^+$ with the drop being about twice as much in the case with 3-PGA added. Therefore, a sudden increase in the concentration of 3-PGA does result in an increased utilization of NADPH for the reduction of the added 3-PGA. The question remains, however, as to why there is a sudden drop in $NADPH/NADP^+$ level upon addition of sodium bicarbonate to a reaction mixture which already contains a high concentration of 3-PGA.

The amount of $CO_2$ fixed during the first 30 sec following addition of bicarbonate produced about 20% as much 3-PGA as was already present, but the actual concentration of 3-PGA rose by only a small amount (Fig. 7). While we do not usually observe the concentration of 3-phosphoglyceraldehyde directly, owing to its very low concentration, we know from many experiments that the reaction mediated by triosephosphate isomerase (EC 5.3.1.1) is highly reversible and that we can estimate the concentration of glyceraldehyde-3-phosphate as 1/20th that of DHAP. It is interesting that the level of DHAP drops very rapidly upon the addition of sodium bicarbonate. Although the triose phosphates are formed much more rapidly following the addition of sodium bicarbonate, a larger effect on triose phosphate concentration is produced by the accelerated conversion of DHAP via the sugar phosphates of the cycle to the carboxylation substrate Ribul-1,5-$P_2$, which declines very rapidly in concentration upon the addition of 6 mM bicarbonate (Fig. 7). Since the reduction of 3-PGA to triose phosphates is a highly reversible (2 step) sequence [15], the accelerated conversion

of triose phosphates to Ribul-1,5-$P_2$ upon bicarbonate addition is in turn reflected in accelerated 3-PGA reduction. Thus, at 3-PGA concentrations of at least 0.05 mM, the rate of NADPH utilization depends more on this acceleration of the cycle (due to utilization of triose phosphate) than on the absolute concentration of 3-PGA. In other words, a change of 20% in 3-PGA concentration has a smaller effect on the net rate of reduction of 3-PGA than a 50% change in triose phosphate concentration.

## Discussion

Although the sustained ratio of NADPH/$NADP^+$ is lower in the reconstituted chloroplast system than in the whole chloroplasts, the supply of NADPH is sufficient to maintain high rates of $CO_2$ fixation during illumination of the system. We may conclude that the levels of NADPH in the reconstituted system are sufficient to fully activate the carboxylase enzyme [10]. We are unable to explain why the ratio of NADPH/$NADP^+$ appears to reach a saturation value of about 1.1 in reconstituted chloroplasts and 2.3 to 2.5 in whole chloroplasts in the absence of sodium bicarbonate addition. Presumably there is either some back reaction which oxidizes the NADPH to $NADP^+$, or some regulatory mechanism which limits the maximum extent to which the $NADP^+$ can be reduced. With respect to back reactions, it is noteworthy that with whole chloroplasts the maximum NADPH/$NADP^+$ ratio was relatively unaffected between an atmosphere of nitrogen and air whereas with the reconstituted system there is a large effect of nitrogen as compared to air. Of course, with air levels of $CO_2$ there is already some utilization of NADPH for photosynthesis due to the fixation of this $CO_2$ and the production of $NADP^+$. The difference in the NADPH/$NADP^+$ ratio remains high, however, and in fact increases after the addition of sodium bicarbonate (6 mM). Thus, it seems clear that the principle difference between the ratios with nitrogen and with air must be ascribed to oxidation of electron carriers

by $O_2$. Perhaps, even under nitrogen, the maximum ratio attained for $NADPH/NADP^+$ is limited by the oxygen produced by photosynthesis. It remains unexplained as to why the intact chloroplasts should be less susceptible to this phenomenon than the reconstituted system.

In comparing the ratios of $NADPH/NADP^+$ between whole chloroplasts and the reconstituted system, one needs to consider both the total amount of the cofactors as compared with the chlorophyll and also the actual concentration available to the enzymes. In whole chloroplasts, the internal volume of the chloroplast occupies about 0.01 of the total volume of the chloroplast suspension. This small volume contains the entire soluble components, including the enzymes. For whole chloroplast experiments in which the chlorophyll content is equivalent to 0.05 mg of chlorophyll per flask, although the amount of NADPH in the flask is very small, the concentration inside the chloroplast is estimated as 0.16 mM (Table 1). With the reconstituted system, where we initially add 0.1 umoles of $NADP^+$ per 1 ml of flask contents, and where just before addition of bicarbonate the ratio of $NADPH/NADP^+$ is 1, the concentration of NADPH in the flask is 0.066 mM. Thus, the actual concentration of NADPH available to the soluble components in the reconstituted system is about 0.4 that available in the intact chloroplasts at the time just before addition of bicarbonate.

In order to test the reversibility of the reactions leading to the reduction of 3-PGA to triose phosphate in this reconstituted system, we employed the methods described by Bassham and Krause [15] to calculate the actual free energy changes at various times during the course of the experiments which gave the data shown in Figs. 6 and 7. The physiological standard free energy change, $\Delta G^{0'}$, for the two-step reactions converting 3-PGA, ATP, and NADPH to glyceraldehyde-3-phosphate, $NADP^+$, ADP, and inorganic phosphate, is +4.3 Kcal. With *Chlorella pyrenoidosa* photosynthesizing under steady-state conditions, it was calculated that the

actual free energy change, $\Delta G^S$, was -1.6 Kcal. In the experiment with the reconstituted chloroplasts just described, $\Delta G^S$ was estimated as about -0.5 Kcal just prior to addition of bicarbonate (at 7 min). At the 30 sec point following addition of bicarbonate (7-1/2 min), the calculated value for the $\Delta G^S$ was almost zero, and then became about -0.5 Kcal at 20 min. Thus, it is clear that, as is the case with photosynthesizing Chlorella pyrenoidosa, the two reactions constituting the reduction of 3-PGA and mediated by 3-phosphoglycerate kinase (EC 2.7.2.3) and triosephosphate dehydrogenase (EC 1.2.1.12) are highly reversible and not rate limiting steps in the reconstituted chloroplast system. This does not mean that these steps could not be regulated as between light and dark as has been suggested elsewhere [16] but does indicate that they are not regulated steps in the light.

From the data given earlier, it seems clear that with the other metabolite concentrations found in the reconstituted system relatively low ratios of $NADPH/NADP^+$ (0.5) are adequate for the reduction of 3-PGA to triose phosphate, and subsequently regeneration of Ribul-1,5-$P_2$. In addition to its function as a reductant, NADPH is now known to be an important effector for ribulose-1,5-diphosphate carboxylase [9,10]. Thus, an adequate ratio of $NADPH/NADP^+$ is important for the activation of the carboxylation reaction.

From the results with addition of lamellae to the reconstituted system after a period of 20 min photosynthesis (Figs. 2 and 3) and the experiment where the amount of lamellae has been increased from the beginning, it is clear that with the levels of sodium bicarbonate which we have commonly employed (6 mM), the rate of reduction of $NADP^+$ to NADPH is sometimes unable to keep up with the needs of the soluble enzyme systems after some minutes of photosynthesis. By increasing the proportion of lamellae, this rate can be boosted and the rate of $CO_2$ fixation and the level of NADPH reduction

restored. Of course, when this is done, the actual rate of fixation as compared with chlorophyll content of the system will be much lower because of the greater chlorophyll content now employed. In other studies, where we have employed air levels of $CO_2$ throughout the course of the experiment, the ratio of lamellae to soluble components used in the present experiment has been sufficient to sustain a nearly constant $NADPH/NADP^+$ ratio and constant rate of photosynthesis for up to 1 h.

The decline in the $NADPH/NADP^+$ ratio with time in some experiments is the consequence of the deterioration of the thylakoid system during an extended period of in vitro photosynthesis. It has been reported [17] that with isolated spinach chloroplasts, photosynthesis in the light in the presence of a glutathione buffer leads to extensive swelling of the thylakoids accompanied by a decreased capacity for photophosphorylation. In examination of our thylakoid system by electronmicroscopy before and after a period of photosynthesis in the light, we have shown swelling of the thylakoids with time. In the experiments shown in Figs. 6 and 7, the ratio ATP/ADP declined from about 5:1 to about 2:1 during the 20 min of photosynthesis with sodium bicarbonate. Thus, both the decline in phosphorylation potential and the ratio $NADPH/NADP^+$ may be affected by the thylakoid swelling in the light. It would appear that in order to achieve even more steady-state conditions with the reconstituted system, including constant potentials of $NADPH/NADP^+$ and ATP/ADP, further improvements, perhaps in the buffer system or other conditions, will be required in order to overcome this problem of thylakoid swelling.

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FIGURE CAPTIONS

Figure 1. $NADPH/NADP^+$ ratios in whole chloroplasts and in a reconstitut chloroplast system during preillumination followed by a light-dark-light period. Addition of $NaH^{14}CO_3$ (6 mM, specific activity 17 uCi/umole) and the length of the dark period are indicated by an arrow and a dark bar, respectively. The experiment was run under nitrogen. 3-PGA (1 mM) was provided as primer of photosynthetic intermediates in the reconstituted chloroplast system. The total amount of triphosphopyridine nucleotides was 50 nmoles/mg Chl (whole chloroplasts) and 2.1 umoles/mg Chl (reconstituted chloroplast system).

Figure 2. The effect of $NaH^{14}CO_3$ (6 mM) addition and increased concentration of chloroplast lamellae after 20 min photosynthesis on the $NADPH/NADP^+$ ratio in a reconstituted chloroplast system. The system was provided with glucose (0.5 mM) and hexokinase (100 units) as a primer system instead of 3-PGA. Glucose was added just before the preillumination period; the time of addition of hexokinase and $NaH^{14}CO_3$ are indicated in the figure. The chlorophyll content for the first 32 min illumination was 53 ug/ml reaction mixture, and after 20 min photosynthesis it was increased 4 times. "1 x chloroplast lamellae" refers to our usual reconstituted system [13] in which the ratio of soluble components in the system to lamellae is 14 times greater than in the original chloroplasts. With "4 x chloroplast lamellae, this ratio is 3.5.

O $NADPH/NADP^+$ ratios under nitrogen (1x chloroplast lamellae)

x " " " " " (4x " " )

● NADPH/NADP$^+$ ratios under air (1x chloroplast lamellae)

Δ " " " " " (4x " " )

Figure 3. Kinetics of $CO_2$ fixation by a reconstituted chloroplast system under air and nitrogen and after increase of the chloroplast lamellae during photosynthesis. Samples for the determination of $CO_2$ fixation were taken from the same reaction mixtures used for the pyridine nucleotide measurements in Fig. 2. "1 x chloroplast lamellae" refers to our standard reconstituted system [13] in which the ratio of soluble components to lamellae is 14 times greater than in the original chloroplasts. With "4 x chloroplast lamellae" this ratio is 3.5. $^{14}CO_2$ fixation rate is given in terms of ug-atoms $^{14}C$ fixed per mg chlorophyll in the usual reconstituted system (14:1). The rate for "4 x chloroplast lamellae" is 1/4th that shown on a basis of $^{14}C$ fixed per mg chlorophyll.

O NADPH/NADP$^+$ ratios under nitrogen (1x chloroplast lamellae)

x " " " " " (4x " " )

● " " " " air (1x " " )

Δ " " " " " (4x " " )

Figure 4. Similar experiment to that described in Fig. 2; however the amount of chloroplast lamellae was increased 4 times at the beginning of the light period rather than later. $CO_2$ fixation was measured simultaneously with NADPH/NADP$^+$ ratios and is shown by dashed lines. Solid lines indicate NADPH/NADP$^+$ ratios. The experiment took place under a nitrogen atmosphere. "1 x chloroplast lamellae" refers to our standard reconstituted system [13] in which the ratio of soluble components to lamellae is 14 times greater than

in the original chloroplasts. With "4 x chloroplast lamellae" this ratio is 3.5. $^{14}CO_2$ fixation rate is given in terms of ug-atoms $^{14}C$ fixed per mg chlorophyll in the usual reconstituted system (14:1). The rate for "4 x chloroplast lamellae" is 1/4th that shown on a basis of $^{14}C$ fixed per mg chlorophyll.

O 1x chloroplast lamellae

● 4x " "

Figure 5. Kinetics of NADPH/$NADP^+$ ratios in a reconstituted chloroplast system with either 3-PGA (1 mM) or glucose (0.5 mM)/hexokinase (100 units) as primer. The 3-PGA primer system was preilluminated for 7 min and the glucose/hexokinase primer system was preilluminated for 12 min before the addition of $NaHCO_3$ (6 mM). The flasks were kept under nitrogen.

O 3-PGA

● glucose/hexokinase

Figure 6. Changes in the NADPH/$NADP^+$ ratios of an illuminated reconstituted chloroplast system upon addition of $NaHCO_3$ (6 mM) or 3-PGA (1 mM).

● 3-PGA (1 mM) present since beginning of preillumination period; addition of $NaHCO_3$ (6 mM) indicated by arrow.

O No 3-PGA present during preillumination period; addition of 3-PGA (1 mM) indicated by arrow; no addition of $NaHCO_3$.

Figure 7. Pool size changes of Ribul-1,5-$P_2$, 3-PGA and DHAP during preillumination and upon addition of $NaH^{14}CO_3$ (6 mM; specific activity 17 uCi/umole) in a reconstituted chloroplast system. $^{14}C$-3-PGA (1 mM, specific activity 51 uCi/umole) as the primer was introduced to the reaction flask prior to the preillumination

period. Since 3-PGA has three carbon atoms per molecule, the specific activity of carbon in 3-PGA was 17 uCi/ug-atom, which was the same as the specific activity of the $NaH^{14}CO_3$. Thus, $^{14}C$ contents of Ribul-1,5-$P_2$, 3-PGA and DHAP provide measures of pool sizes if we neglect endogenous, unlabeled carbon in these pools.

TABLE I

CONCENTRATIONS OF NADPH AND $NADP^+$ IN WHOLE ISOLATED SPINACH CHLOROPLASTS AND IN RECONSTITUTED CHLOROPLASTS.

Values for whole isolated chloroplasts in the light (Ref. 8) were NADPH: 32 nmoles $mg^{-1}$ Chl and $NADP^+$: 12 nmoles $mg^{-1}$ chlorophyll. One ml whole chloroplast suspension contained 50 ug chlorophyll, and an estimated 0.01 ml internal volume. Thus, there was 1.6 nmoles NADPH and 0.6 nmoles $NADP^+$ in 1 ml suspension, or 160 nmoles and 60 nmoles per 1 ml of internal volume, giving concentrations of 0.160 mM and 0.060 mM for NADPH and $NADP^+$, respectively, inside the whole chloroplasts.

In preparing the reconstituted system, the 0.16 + 0.06 = 0.220 mM endogenous NADPH + $NADP^+$ was diluted 6.94 fold, giving 0.0317 mM NADPH + $NADP^+$. An additional 0.1 mM $NADP^+$ was added before illumination, giving 0.1317 mM in all. After illumination under $N_2$, but before $H^{14}CO_3^-$ addition (see Fig. 6), the NADPH:NADP ratio was about 1.0, so the concentration of each was 0.0659 mM.

| | NADPH | $NADP^+$ |
|---|---|---|
| Whole Chloroplasts | 0.160 mM | 0.060 mM |
| Reconstituted Chloroplasts | 0.066 mM | 0.066 mM |

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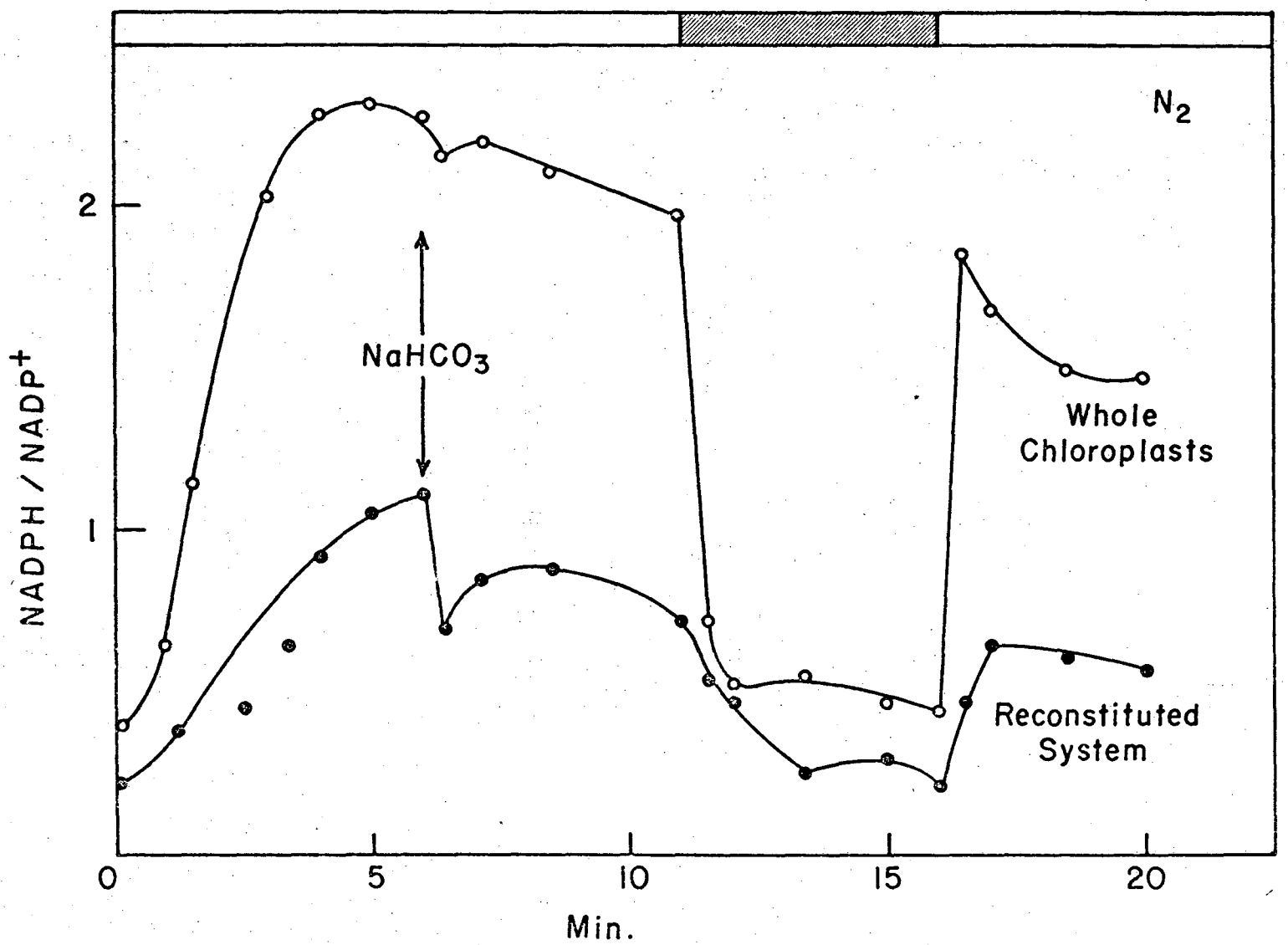


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Fig. 1

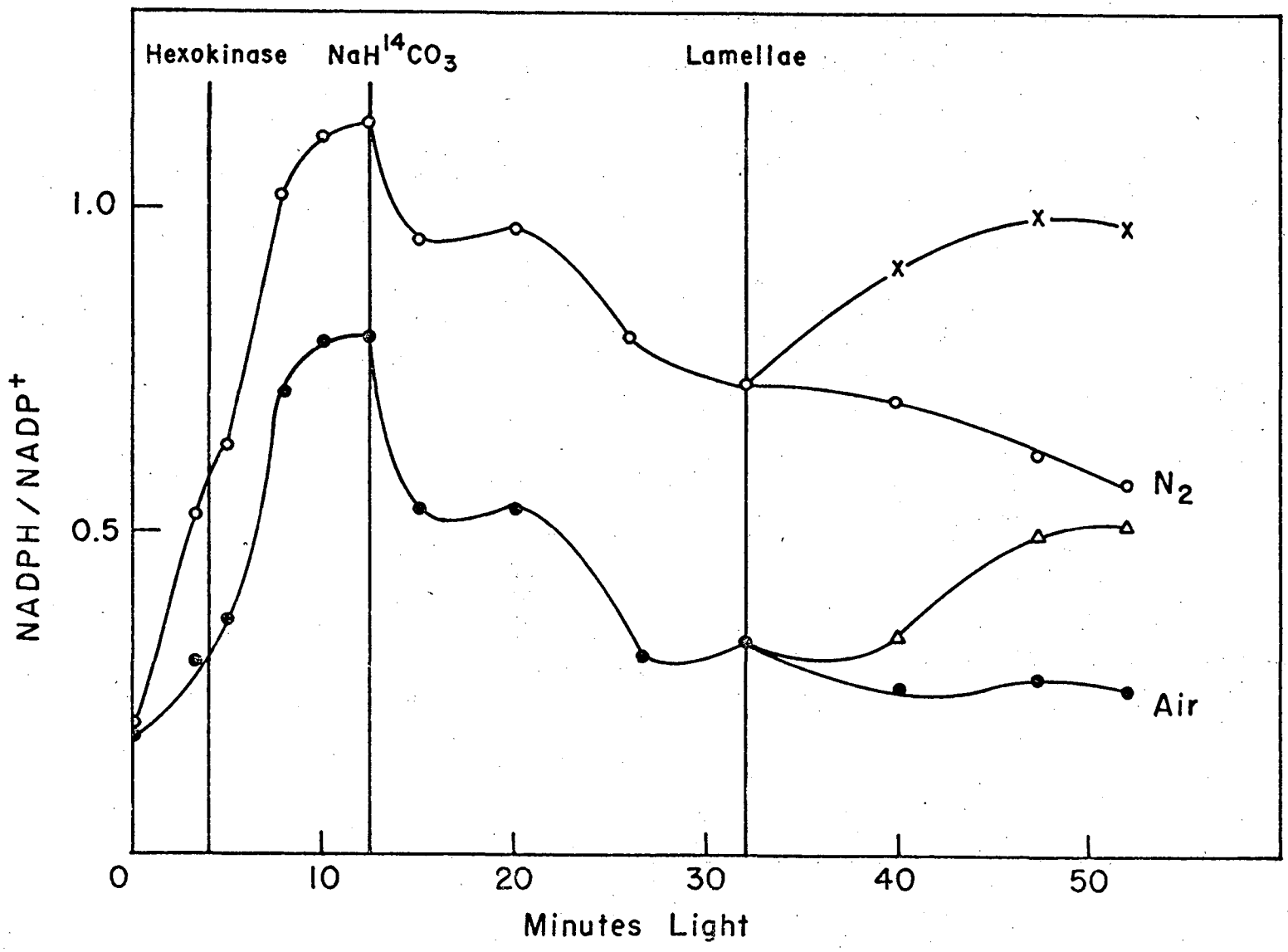


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Fig. 2

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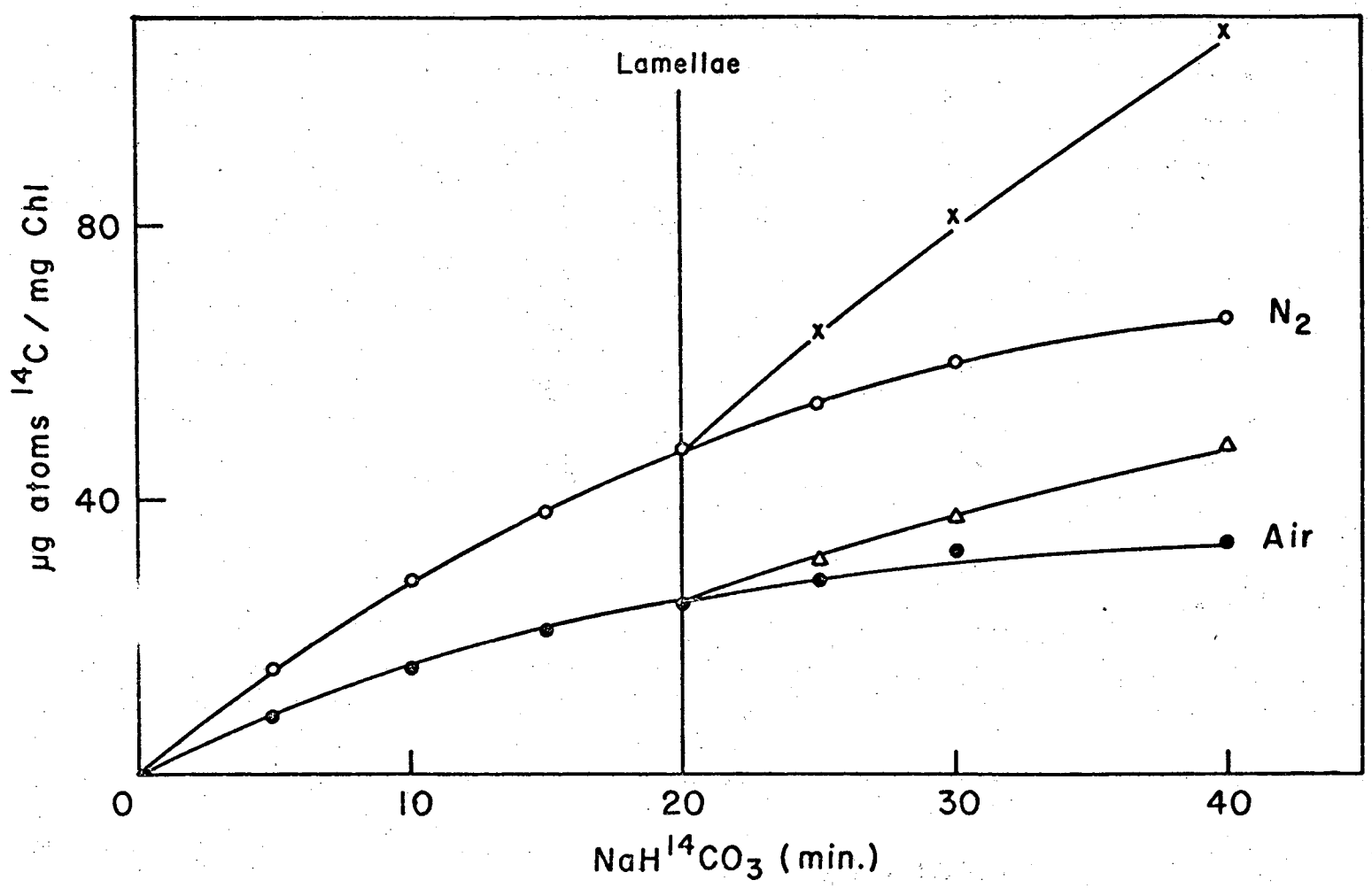


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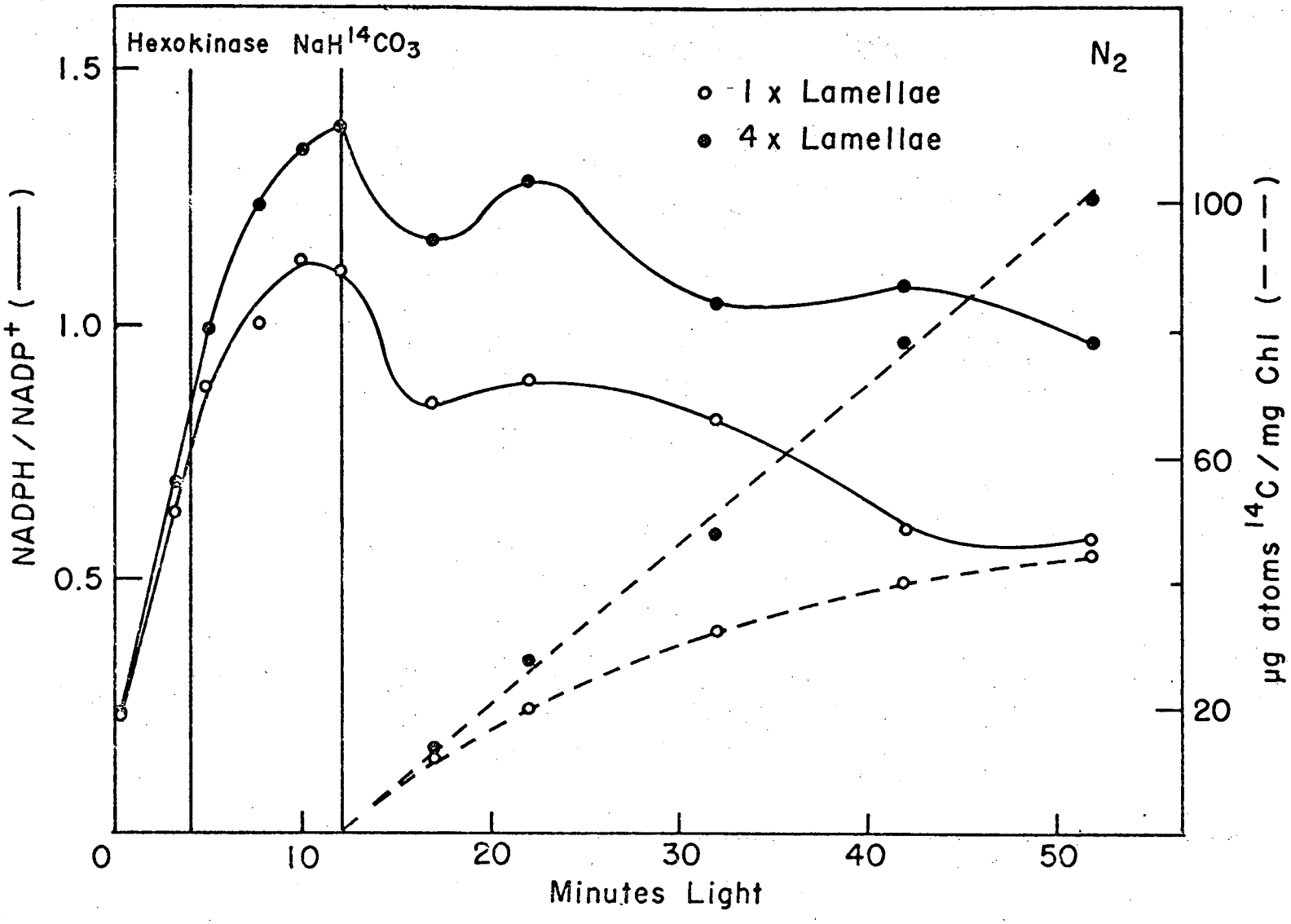
Fig. 3




XBL756-5272

Fig. 4

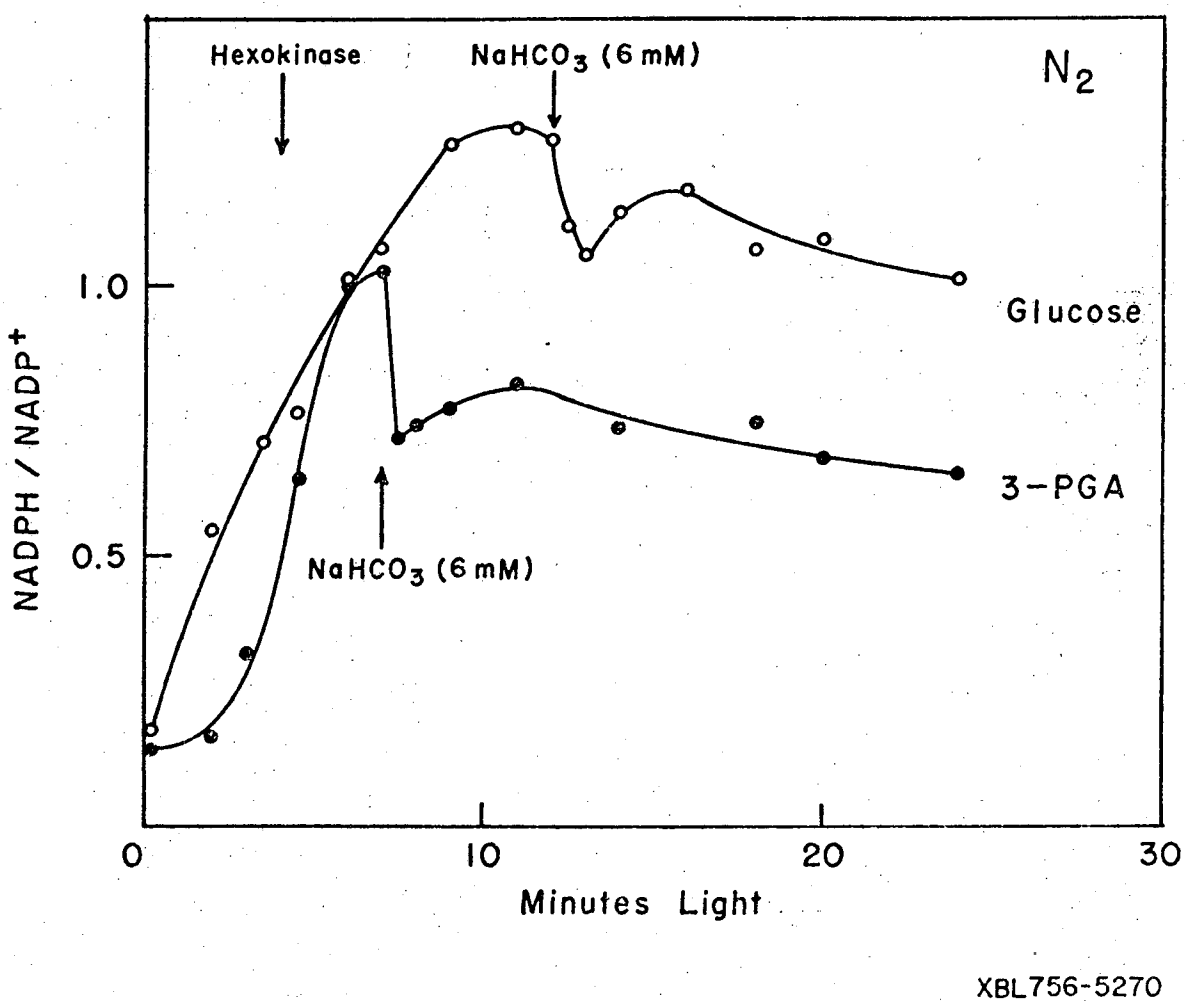

Hexokinase
NaHCO3 (6 mM)
N2
1.0
0.5
NADPH / NADP+
Glucose
3-PGA
NaHCO3 (6 mM)
0
10
20
30
Minutes Light
XBL756-5270

Fig. 5

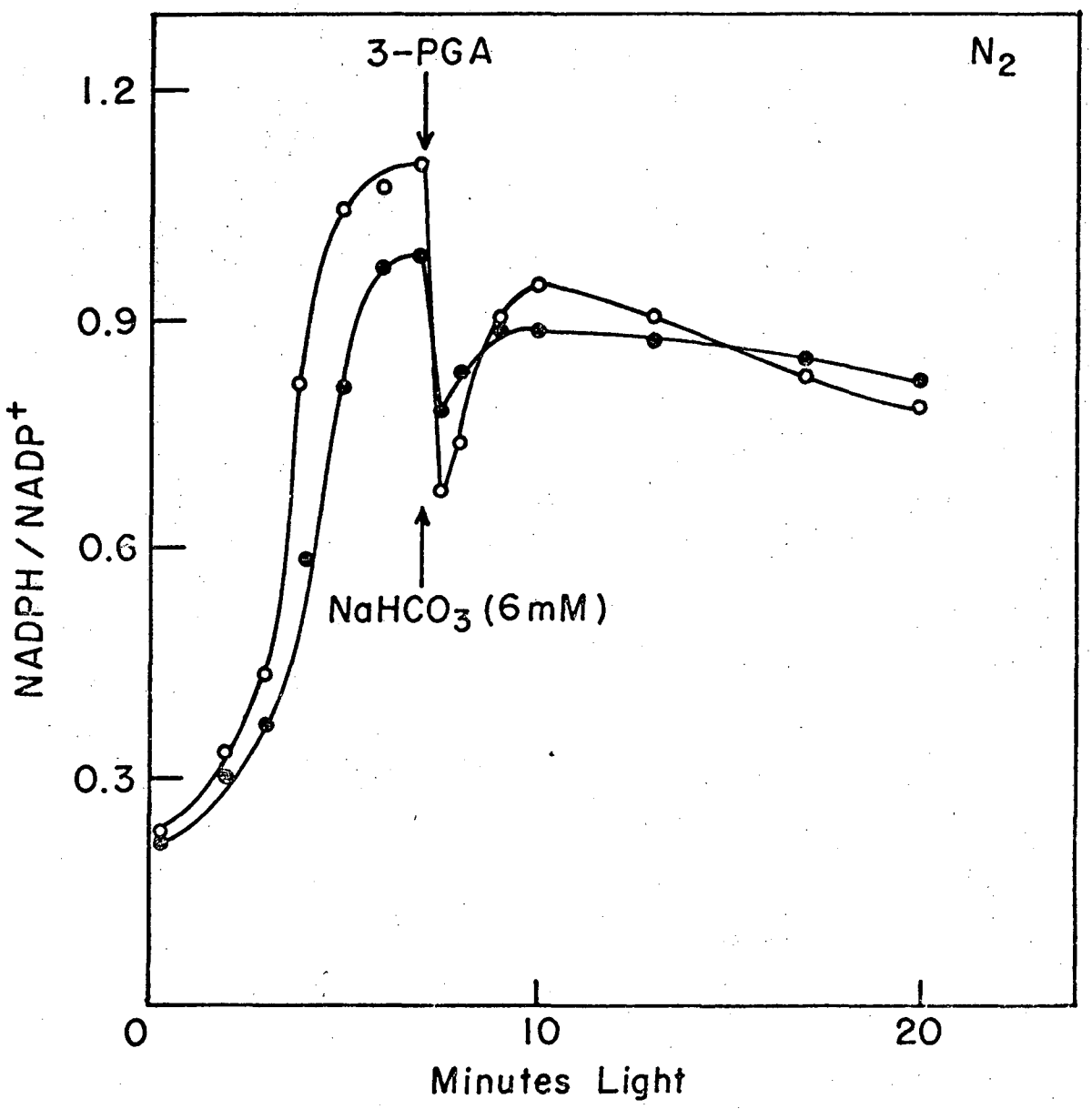



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Fig. 6

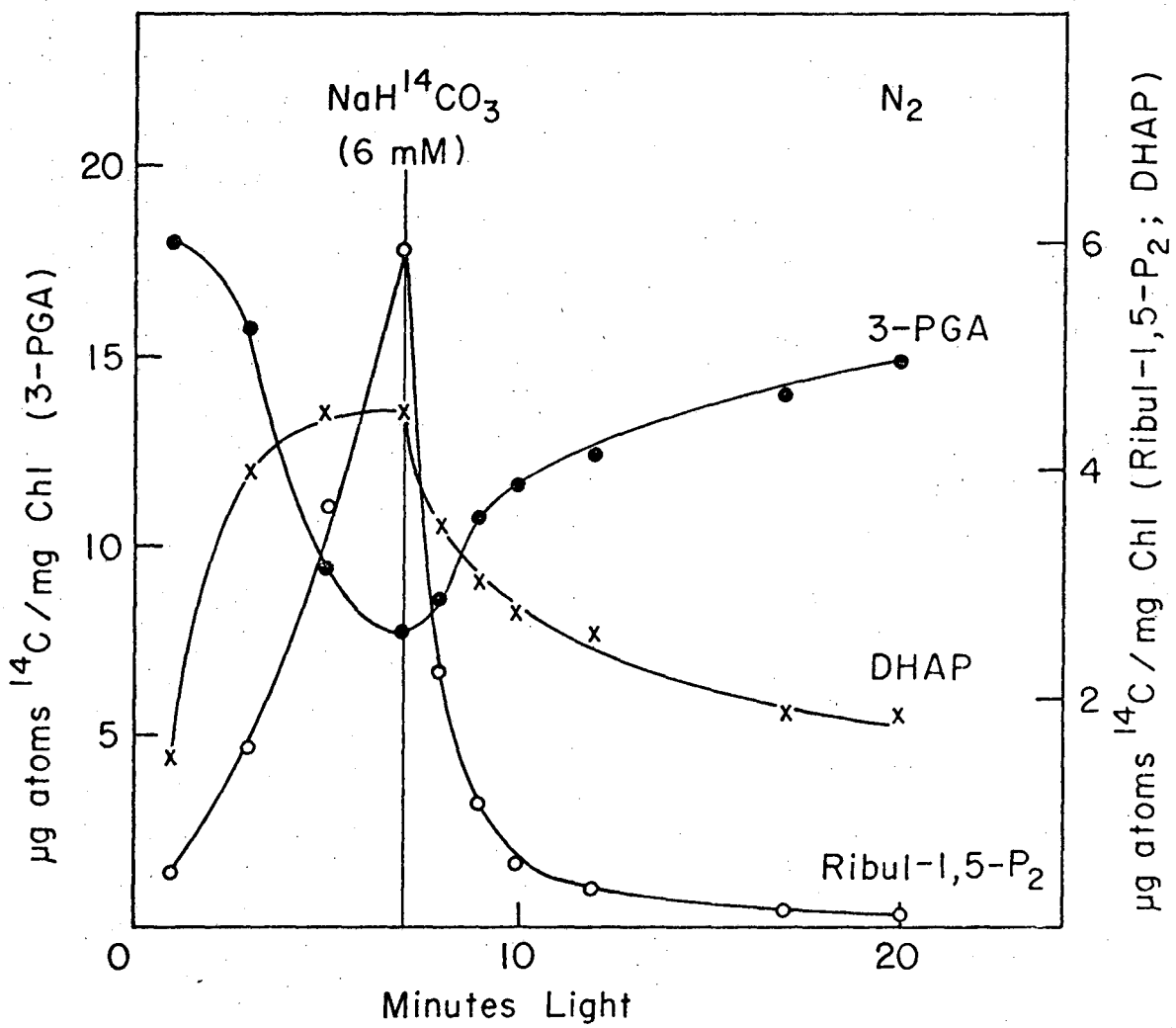


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Fig. 7

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