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Evidence for in vivo Light-Induced Synthesis of Ribulose-1,5-diP Carboxylase and Phosphoribulokinase in Greening Barley Leaves

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Summary. When actinomycin D, puromycin, streptomycin, chloramphenicol, and cycloheximide, known inhibitors of protein synthesis, were applied to leaves of intact seedlings or detached leaves of barley prior to their greening, the same general response resulted: the light-induced increase in activity of ribulose 1,5-diphosphate carboxylase was prevented while that of phosphoribulokinase was only partially suppressed; synthesis of chlorophyll was arrested. This is taken as preliminary evidence that de novo synthesis of protein may be responsible for the observed increase in ribulose-1,5-diphosphate carboxylase activity during greening. However, other factors may be involved with the light-induced stimulation of phosphoribulokinase.

Carbohydrate metabolites and substrates of the enzymes failed to induce the formation of ribulose-1,5-diphosphate carboxylase and phosphoribulokinase in the dark. No evidence was found for the presence of inhibitors in etiolated seedlings or activators in illuminated leaves of barley. Carboxylase activity almost equal to that of the illuminated water control was stimulated by $MgCl_2$ in the dark; $MgCl_2$ had no effect on the activity of the kinase.

The measured activities of ribulose-1,5-diP carboxylase and phosphoribulokinase increase markedly during greening of barley leaves (4). This light-induced increase may be a result of de novo synthesis or may be due to activation of enzymes already present. It was the purpose of this study to investigate the possibility of a light-induced de novo synthesis of the enzymes ribulose-1,5-diP carboxylase and phosphoribulokinase by application of known inhibitors of protein synthesis to leaves of barley. A preliminary report of this work has already appeared (5).

Earlier investigations showed that chloramphenicol inhibited the light induction of increased activity of enzymes in the carboxylative phase of photosynthesis (10). Recently, Chen et al. (2) reported that the light-induced increase in activity of ribulose-1,5-diP carboxylase was prevented by chloramphenicol and puromycin and that of phosphoribulokinase by chloramphenicol.

A variety of antibiotics differing in mode of action were chosen for this study in hope that results would be strengthened by mutual agreement in trend. The response of chlorophyll to treatment with inhibitors of protein synthesis was followed because there is evidence that such compounds arrest chlorophyll formation (1, 6, 9) and also because of evidence that ribulose-1,5-diP carboxylase and protochlorophyll holochrome may be intimately related (14). Attempts were also made to find inducers and/or activators which could cause the same increase in activities of ribulose-1,5-diP carboxylase and phosphoribulokinase in the dark as those observed during greening.

Materials and Methods

Plant Materials. Hordeum vulgare L. Var. Club Mariout was grown without light as previously described (4). Seven-day old plants were used in the study since at this stage growth of leaves is not significant during the test period. Thus, the increases in measured activities of the enzymes were due primarily to light and were not confounded by growth.

Assay of Enzymes. Assays of enzymes were carried out as previously described (4). The activity of phosphoribulokinase was assayed with a pH stat by following the rate of acid production concomitant with the formation of ribulose-1,5-diP. The reaction mixture (2 ml) contained the following in μmoles: ribose-5-P, 16; ATP, 18; $MgCl_2$, 30; EDTA, 2; GSH, 2; and 0.2 ml of cell-free extract from 1 g of leaf material in 3 ml of 0.2 M tris buffer, pH 8.0.

Ribulose-1,5-diP carboxylase was assayed by following the conversion of $KH^{14}CO_3$ into stable products. The reaction mixture (0.7 ml) contained the following in μmoles: ribulose-1,5-diP, 2; $KH^{14}CO_3$, 10, with a specific activity of 2×10^5 cpm per μmole; $MgCl_2$, 15; tris buffer, 80; and 0.2 ml of cell-free extract from 1 g of leaf material in 21 ml of 0.2 M tris buffer, pH 8.0. Assays were performed in duplicate or triplicate for each sample. Ribu-

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lose-1,5-diP was prepared as previously described (4). Activity of this substrate varied somewhat from preparation to preparation and diminished during storage. This resulted in variations in measured activity of ribulose-1,5-diP carboxylase among treatments run at different times during the course of these studies. Results within treatments, however, were consistent.

Each experiment consisted of a minimum of 3 replications. A standard error of the mean was calculated for each set of replications.

Preparation of Cell-Free Extracts. After treatment, detached leaves were prepared for assay by excising at the second joint. Unless otherwise indicated, when intact plants were used, first leaves from 9 or 10 seedlings 8 cm from the leaf tip were excised just prior to assay. Harvested leaves were ground with mortar and pestle in 3 ml of 0.2 M tris chloride buffer, pH 8.0, per g of leaf tissue and centrifuged at 37,000 $\times$ g for 15 minutes. The supernatant liquid was appropriately diluted and used as the source of enzymes. After harvest all procedures were carried out at 0°.

Chlorophyll and Protein. Chlorophyll was assayed as previously described (4). Soluble protein was precipitated with trichloroacetic acid and determined by the method of Lowry et al. (8).

Light Treatments. A bank of Sylvania VHO cool white fluorescent tubes, supplemented with 60-w incandescent bulbs provided a light intensity of 10,760 lux. Dark manipulation of plants was carried out in a dim green light.

Application of Inhibitors. In detached leaf studies, the seedlings were excised approximately 1 cm above the level of the vermiculite in the pot. The basal part of the blade was placed in the inhibitor solution and the leaf maintained in an upright position with the aid of a wire support. In studies of intact seedlings, blades were dipped for 5 to 10 seconds into graduate cylinders containing aqueous solutions of the inhibitors to which had been added 0.06 ml Tween 20 per 100 ml solution. Thirty minutes after the first treatment, plants were dipped again in the inhibitor solution and placed in darkness for 4 hours, then illuminated at 10,760 lux for 24 hours. Leaves of the light and dark controls were dipped for 5 to 10 seconds in an aqueous solution of Tween 20 (0.06 ml Tween 20/100 ml water) containing no antibiotics.

Sources of the Inhibitors. Cycloheximide was a gift of the Upjohn Company and actinomycin D was donated by Merck, Sharp and Dohme. Chloramphenicol was furnished by Parke, Davis and Company. Puromycin and streptomycin were obtained commercially.

Induction and Activation Studies. Detached leaves were allowed to imbibe extracts from both light-treated and dark-grown plants. The extracts were prepared by grinding 1 g leaf tissue in 3 ml water and recovering the supernatant fluid after centrifugation. The supernatant fluid was filtered to remove particles which failed to sediment. Extracts were then boiled ten minutes and the precipitate removed by centrifugation. Solutions administered were adjusted to pH 6.4.

Definition of Control Plants. In detached leaf studies both light and dark controls were maintained on water containing no additives. In inhibitor studies light controls and treated leaves received the same illumination; dark controls were maintained in the dark during the entire period the other leaves were illuminated. For chemical induction studies, light controls were illuminated for the periods shown in the results; dark controls and treated plants were kept in the dark for the same period of time.

For inhibitor studies on intact plants, light controls and treated seedlings received the same illumination. The dark controls for experiments in which inhibitor was applied prior to illumination were seedlings maintained in the dark during the entire period during which the other leaves were illuminated. For experiments in which inhibitor was applied subsequent to illumination, controls were seedlings which were harvested immediately following an eight hour period of illumination at 10,760 lux.

Moisture Content. For both detached and intact leaf studies, differences in moisture content among treatments were insignificant.

Gel Filtration of Cell-Free Extract. Gel filtration of the enzymatic extracts through Sephedex G-25 was employed to remove low molecular weight compounds which may influence the rates of the enzymatic reactions. Filtration through a 7 mm $\times$ 150 mm column, equilibrated with a 0.2 M tris-sulfate buffer, pH 8.0 containing 0.001 M EDTA, effectively separated compounds of a molecular weight of 1200 and less from fractions containing enzymatic activity.

Results and Discussion

The induction of increased enzymatic activity by light may be due to several causes. Among these are synthesis of the enzyme, activation of the enzyme by other protein or low molecular weight metabolites, or the disappearance of an inhibitor of the enzymatic reaction. To test for the possibility of de novo synthesis of the enzyme, several well known inhibitors of protein synthesis were employed.

Treatments of cycloheximide, puromycin and streptomycin resulted in almost complete suppression of the in vivo induction of increased activity of ribulose-1,5-diP carboxylase in detached leaves by light (table I). Actinomycin D at higher concentrations blocked light-induction of the carboxylase up to 85 %. The light-induced increase in activity of phosphoribulokinase was affected much less by inhibitors than that of the carboxylase. An increasing concentration of inhibitor which resulted in almost total inhibition of the carboxylase had no further effect on the kinase. In contrast, Chen et al. (2) reported that chloramphenicol completely blocked the light-induced increase in activity of phosphoribulokinase in detached

corn leaves. While all 4 antibiotics markedly decreased chlorophyll synthesis, cycloheximide was the most potent inhibitor (table II).

When applied foliarly to intact seedlings, actinomycin D, puromycin and streptomycin did not inhibit measured enzymatic activities or synthesis of chlorophyll. Since enzymatic activity and chlorophyll were markedly affected in detached leaves by all 3 compounds, apparently little or no penetration was achieved in the foliar application. To ascertain that response of detached leaves was not different from that of intact seedlings, leaves of intact seedlings were treated with 0.1 mg/ml of cycloheximide and 4.0 mg/ml of chloramphenicol. Both antibiotics almost completely inhibited light induction of increased activity of the carboxylase enzyme. Percent inhibition by chloramphenicol was 104.8 ± 16.4 and by cycloheximide, 95.6 ± 3.7. The light-induced increase in

Table I. *Inhibition of the Light-Induced Increase in Activity of Ribulose diP Carboxylase and Phosphoribulokinase in Detached Leaves*

Treatment mg/ml	RuDP Carboxylase activity				Phosphoribulokinase activity			
	Dark	Light	Treated*	Inhibition**	Dark	Light	Treated*	Inhibition***
	μmoles CO_2 (10 min)$^{-1}$ (g fr wt)$^{-1}$			%	μmoles H^+ min^{-1} (g fr wt)$^{-1}$			%
Cycloheximide								
0.0001	9.19	15.93	13.74	30.3 ± 5.9***	3.59	7.29	7.70	0***
0.001	8.84	15.93	12.86	41.8 ± 8.7	3.51	7.14	7.61	2.5 ± 4.3
0.005	8.80	15.76	12.27	49.2 ± 4.4	3.51	7.14	5.97	32.4 ± 9.6
0.010	9.19	16.95	9.45	98.5 ± 14.5	3.47	6.12	5.05	40.4 ± 4.0
Actinomycin D								
0.063	12.07	20.48	16.80	44.5 ± 5.5	3.54	7.01	5.16	52.6 ± 11.9
0.125	13.65	23.10	17.98	54.2 ± 6.9	3.37	6.69	5.58	32.3 ± 7.8
0.250	11.30	21.04	12.73	85.4 ± 10.4	3.76	7.17	5.55	49.1 ± 7.4
Puromycin								
0.125	12.07	20.48	18.63	20.2 ± 13.1	3.54	7.01	7.09	0
0.250	13.65	23.10	20.21	30.6 ± 8.3	3.37	6.69	5.17	45.8 ± 6.5
0.500	13.91	24.15	14.88	93.6 ± 16.2	3.87	8.40	5.40	48.7 ± 7.0
Streptomycin								
0.5	11.64	20.17	15.49	55.5 ± 13.3	4.02	8.42	6.42	46.3 ± 6.4
1.0	13.13	23.15	14.18	91.7 ± 12.7	4.15	8.79	6.55	48.7 ± 7.0
2.0	14.22	28.30	14.17	101.5 ± 8.5	4.38	9.34	6.87	50.3 ± 6.8

* Experimental conditions: inhibition treatments were applied for 12 hours in the dark followed by a 24 hour period of illumination at 10,760 lux.

** % Inhibition = $\frac{\text{light control-treated}}{\text{light control-dark control}} \times 100$.

*** Represents standard error of the mean.

Table II. *Inhibition of Synthesis of Chlorophyll in Detached Leaves*

Experimental conditions were the same as in table I.

Treatment mg/ml	Chlorophyll synthesis			
	Dark	Light	Treated	Inhibition
	mg chlorophyll (g fr wt)$^{-1}$			%
Cycloheximide				
0.0001	0.017	0.457	0.478	0
0.001	0.017	0.457	0.490	0
0.005	0.017	0.457	0.296	49.3 ± 19.4
0.010	0.016	0.406	0.019	99.0 ± 2.7
Actinomycin D				
0.063	0.006	0.337	0.149	57.0 ± 1.4
0.125	0.007	0.347	0.101	72.5 ± 4.3
0.250	0.014	0.393	0.092	79.4 ± 3.9
Puromycin				
0.125	0.006	0.337	0.275	18.7 ± 0.2
0.250	0.007	0.347	0.195	44.7 ± 0.9
0.500	0.007	0.423	0.200	52.3 ± 9.0
Streptomycin				
0.5	0.003	0.384	0.138	65.2 ± 6.2
1.0	0.005	0.418	0.070	84.5 ± 4.9
2.0	0.006	0.410	0.053	88.4 ± 3.9

activity of phosphoribulokinase was inhibited 62.5 ± 5.4 % by chloramphenicol and 33.0 ± 12.2 % by cycloheximide. Synthesis of chlorophyll was completely blocked by cycloheximide and inhibited 88.2 ± 1.8 % by chloramphenicol. Thus, there was close agreement between results of both intact seedlings and detached leaves.

The increase in activity of the kinase may be independent of chlorophyll formation. Treatment with cycloheximide before illumination resulted in complete suppression of chlorophyll synthesis, but inhibited the increase in kinase activity only about 50 %. Apparently the increased activity of this enzyme is not greatly dependent upon ATP from photophosphorylation either for substrate induction or as a source of energy for protein synthesis. Conversely, an increase in the activity of the carboxylase may be closely related to chlorophyll synthesis or both may be influenced by a third variable since no increase in activity of this enzyme occurred when chlorophyll formation was completely inhibited.

To determine if the light-induced stimulation of enzymatic activities could be arrested after initiation, chloramphenicol and cycloheximide were applied to intact seedlings following an 8 hour exposure to light (table III). Again the increase in measured enzymatic activities was markedly inhibited. The light-induced response of phosphoribulokinase seemed to be more severely inhibited when cycloheximide was applied following a period of irradiation (table III). Further synthesis of chlorophyll was completely prevented by application of cycloheximide under these conditions and greatly inhibited by chloramphenicol (table IV).

Table IV. *Effects on Chlorophyll Synthesis by Inhibitors Applied Following Illumination of Intact Seedlings*

Experimental conditions are the same as in table III.

Treatment	Chlorophyll synthesis: Dark control	Light control	Treated	Inhibition %
	mg chlorophyll (g fr wt)$^{-1}$			
Chloramphenicol (4.0 mg/ml)	0.190	0.707	0.269	84.3 ± 3.4
Cycloheximide (0.1 mg/ml)	0.190	0.707	0.164	105.2 ± 1.8

Enzymatic activity determined from reaction mixtures which were made 0.1 mg/ml in any of the antibiotics employed in these studies was the same as that of controls to which no inhibitors were added. Since this concentration exceeds that expected to be added to the reaction mixture from the diluted portion of the cell-free extract employed in this assay, these compounds probably had no directly inhibitory effects on ribulose-1,5-diphosphate carboxylase or phosphoribulokinase.

Concentrations of the antibiotics inhibitory to the light-induced increase of the 2 enzymes did not significantly decrease the content of soluble protein in detached leaves below the amount contained in the illuminated controls. During a period of illumination of 24 hours, the content of soluble protein in detached

Table III. *Effects of Chloramphenicol and Cycloheximide on the Measured Activities of Ribulose diP Carboxylase and Phosphoribulokinase in Intact Seedlings*

Inhibitor was applied following a period of illumination. Experimental conditions: seedlings were irradiated at 10,760 lux for 8 hours after which the 8 hour control was harvested and assayed. At this point inhibitors were applied and the treated and control plants were placed in the dark for 4 hours, then were subsequently illuminated for 16 additional hours at 10,760 lux. Eight centimeters of each leaf, measured from the tip, were harvested for assay.

Inhibitor treatment	RuDP Carboxylase activity: 8 Hr light control	Additional 16 Hr light control	Treated	Inhibition* %	Phosphoribulokinase activity: 8 Hr light control	Additional 16 Hr light control	Treated	Inhibition* %
	μmoles $^{14}CO_2$ (10 min)$^{-1}$ (g fr wt)$^{-1}$				μmoles H^+ min^{-1} (g fr wt)$^{-1}$			
Chloramphenicol (4.0 mg/ml)	29.40	41.13	29.14	102.8 ± 10.6**	8.37	12.72	9.64	71.8 ± 3.5
Cycloheximide (0.1 mg/ml)	29.40	41.13	30.19	91.0 ± 10.2	7.65	13.38	9.68	65.7 ± 3.3

* % Inhibition $= \dfrac{\text{additional 16 hr light control} - \text{treated}}{\text{additional 16 hr light control} - \text{8 hr light control}} \times 100.$

** Represents standard error of the mean.

leaves increased from the dark control value of 8.77 to 11.25 mg per g fresh weight in the light controls (L.S.D. $_{.05}$ = 1.77).

When applied to leaves of intact plants prior to illumination, both cycloheximide and chloramphenicol inhibited the light-induced increase in soluble protein (table V). When applied subsequent to illumination, only cycloheximide significantly inhibited the increase in soluble protein, although both inhibited the light-induced increase in enzymatic activity.

After gel filtration the measured activities of the enzymes were not significantly different from those in the crude extract (table VI). These results seem to rule out the disappearance of a naturally-occurring inhibitor of low molecular weight or the formation of a low molecular weight activator during the light treatment.

Degradation or addition products of the antibiotics as well as pools of metabolites that formed due to blocked protein synthesis may possibly act as inhibitors to the enzymes in the cell-free extract. After gel filtration of extracts from detached leaves treated with cycloheximide, the measured activities of the enzymes were the same as those in the crude extract. Thus the inhibition of enzymatic activity observed was probably not due to the accumulation of low molecular weight metabolites in the leaves treated with inhibitor.

When extracts of illuminated and etiolated leaves were mixed, activities were additive, indicating the absence of activators or inhibitors for either the carboxylase or kinase. To further investigate the possibility that a non-protein activator of enzymatic activity may be produced during illumination, extracts of both dark-grown and light-treated plants were deproteinized by boiling and supplied to detached leaves in the dark for a period of 16 hours. Since no differences were found between those seedlings nurtured on the extracts of illuminated leaves and those supplied with extracts of etiolated tissue, no light-produced activator was detected.

Metabolic Inducer and Activator Studies. Synthesis or activation of enzymatic protein during greening could be an indirect result of illumination. Other light-induced phenomena such as alteration in uptake and accumulation of ions in the chloroplast (11) or photophosphorylation could conceivably contribute to increased polypeptide formation. In addition, accumulation of photosynthetic products formed during the first few hours of illumination by the low levels of enzymes present in etiolated tissue may restore depleted supplies of carbohydrates required for amino acid skeletons; formation of increased amounts of ribulose-1,5-diP and ribulose-5-P (as well as ATP from photophosphorylation) may result in substrate induction. To investigate these possibilities, detached seedlings were maintained in the dark for 18 hours on each of the following: ATP; glucose; ribose-5-P; ribulose-1,5-diP and $MgCl_2$. When compared with dark and illuminated water controls, kinase activity in all cases was approximately the same as that of the dark control (table VII). Ribulose-1,5-diP and ribose-5-P at 0.005 and 0.02 M had no effect on carboxylase activity. Glucose (0.02 M) had no effect; however, there was some stimulation by 0.02 M ATP. $MgCl_2$ at 0.005 M had no effect, but at 0.03 M increased the activity 90 % over that of the dark control.

The moderate increase in carboxylase activity resulting from supplying exogenous ATP may indicate some dependence upon photophosphorylation as a source of this compound for polypeptide synthesis. This observation also supports the hypothesis that the increase in activity of the carboxylase may in some way be linked with chlorophyll synthesis. In these studies bacterial degradation of ATP during the

Table V. *Soluble Protein Content of Intact Leaves Treated with Inhibitors*

Treatment	Application of inhibitor: Prior to illumination*	Application of inhibitor: Subsequent to illumination**
	Soluble protein (mg per g fr wt)	
Light control	16.41	18.90
Dark control	12.93	14.09
Chloramphenicol (4 mg/ml)	13.92	16.20
Cycloheximide (0.1 mg/ml)	13.41	14.85
L.S.D. $_{.05}$	1.23	2.88

* Experimental conditions: see Materials and Methods.
** Experimental conditions: same as table III.

Table VI. *Gel Filtration of Enzymatic Extracts from Intact Barley Leaves*
The sample consisted of 7 leaves excised at the first joint. See Materials and Methods for gel filtration.

Treatment	Ribulose diP carboxylase: Crude extract	Ribulose diP carboxylase: Gel-filtered extract	Phosphoribulokinase: Crude extract	Phosphoribulokinase: Gel-filtered extract
	μmoles CO_2 (10 min)$^{-1}$ (g fr wt)$^{-1}$		μmoles H^+ min^{-1} (g fr wt)$^{-1}$	
7 Days dark	16.69	14.39*	2.74	3.69*
7 Days dark + 24 hours light	25.67	24.07*	15.08	15.83*

* No significant difference at the 5% level between crude and gel-filtered extracts.

Table VII. *Effects of Metabolites on Stimulating the Activity of Ribulose-1,5-diP Carboxylase and Phosphoribulokinase in the Dark*

Experimental conditions: detached leaves were supplied with various metabolites for a dark period of 18 hours and were then compared with controls maintained on water for the same period. One control received illumination at 10,760 lux while the remaining treatments were kept in the dark.

Metabolite	RuDP Carboxylase activity				Phosphoribulokinase activity			
	Dark	Light	Treated	Increase*	Dark	Light	Treated	Increase
	μmoles $^{14}CO_2$ (10 min)$^{-1}$ (g fr wt)$^{-1}$			%	μmoles H^- min^{-1} (g fr wt)$^{-1}$			%
ATP								
0.005 M	8.58	15.23	8.75	3.2 ± 2.6**	2.60	4.88	2.40	0
0.02 M	9.98	14.70	11.29	27.8 ± 4.2	2.90	8.56	3.10	3.8 ± 2.7
$MgCl_2$								
0.015 M	10.51	15.75	9.98	0	2.83	8.64	3.01	3.8 ± 2.0
0.03 M	10.15	16.98	16.63	93.1 ± 10.9	4.40	7.46	3.37	0

* $\%\ \text{Increase} = \dfrac{\text{treated} - \text{dark control}}{\text{light control} - \text{dark control}} \times 100.$

** Represents standard error of the mean.

inhibition period was not ruled out; thus it is possible that the increased activity may be due to degradation products of ATP. However, tests for ATP'ase activity in cell-free extracts from barley have been negative.

The increase in carboxylase activity with $MgCl_2$ (0.03 M) was of particular interest since phosphoribulokinase was unaffected by the salt at the concentrations employed. A precedent for this phenomenon of salt stimulation was reported by Steward and Preston (13) who found that potassium salts promoted increased protein synthesis in potato discs while calcium salts repressed protein formation. More recently, Zucker and Levy (15) found that a variety of salts promoted synthesis of chlorogenic acid. However, several factors may be involved in the enhancement of carboxylase activity by $MgCl_2$. Magnesium not only acts as cofactor for the carboxylase, but is essential for polyribosome formation. It is also required for light-induced accumulation of some ions in chloroplasts (11).

The results obtained from the variety of inhibitors of protein synthesis furnish preliminary evidence that de novo synthesis of enzymatic protein may be responsible for the observed increase in carboxylase activity. Recent studies indicate that actinomycin D has other actions in addition to inhibiting DNA-directed RNA synthesis (3, 7, 12); therefore, its interference with induction in our study is only suggestive evidence that RNA synthesis may be specifically required for the induction. Since all inhibitors employed essentially stopped the increase in activity whether they were applied before or during the light treatment, protein synthesis may be required during the entire period in which the carboxylase is showing an increasing rate of activity.

Since the increase in activity of the kinase was only partially affected by the inhibitors of protein synthesis, other activation mechanisms may be operative with this enzyme.

Literature Cited

1. Bogorad, L. and A. B. Jacobson. 1964. Inhibition of greening of etiolated leaves by actinomycin D. Biochem. Biophys. Res. Commun. 20: 114–19.
2. Chen, S., D. McMahon, and L. Bogorad. 1967. Early effects of illumination on the activity of some photosynthetic enzymes. Plant Physiol. 42: 1–5.
3. Garren, L. D., R. R. Howell, G. M. Romkins, and R. M. Crocco. 1964. A paradoxical effect of actinomycin D: the mechanism of regulation of enzyme synthesis by hydrocortisone. Proc. Natl. Acad. Sci. U. S. 52: 1121–29.
4. Huffaker, R. C., R. L. Obendorf, C. J. Keller, and G. E. Kleinkopf. 1966. Effects of light intensity on photosynthetic carboxylative phase enzymes and chlorophyll synthesis in greening leaves of *Hordeum vulgare* L. Plant Physiol. 41: 913–18.
5. Keller, C. J. and R. C. Huffaker. 1966. Evidence for in vivo light-induced synthesis of ribulose-1,5-diP carboxylase and phosphoribulokinase. Plant Physiol. 41: lvi.
6. Laloraya, M. M. and D. Banerji. 1965. Effect of chloramphenicol on the expansion of isolated cotyledons of *Curcurbita pepe* in light and in the presence of kinetin. Experimentia 21: 516–17.
7. Laszlo, J., D. S. Miller, K. S. McCarty, and P. Hochstein. 1966. Actinomycin D: inhibition of respiration and glycolysis. Science 151: 1007–10.
8. Lowry, O. H., N. J. Roseprough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265–75.
9. Margulies, M. M. 1962. Effect of chloramphenicol on light dependent development of seedlings of *Phaseolus vulgaris* var. Black Valentine, with particular reference to development of photosynthetic activity. Plant Physiol. 37: 473–80.
10. Margulies, M. M. 1964. Effect of chloramphenicol on light-dependent synthesis of proteins and enzymes of leaves and chloroplasts of *Phaseolus vulgaris*. Plant Physiol. 39: 579–85.

11. NOBEL, P. S. AND L. PACKER. 1965. Light-dependent ion translocation in spinach chloroplasts. Plant Physiol. 40: 633–40.

12. REVEL, M., H. H. HIATT, AND J-P. REVEL. 1964. Actinomycin D: an effect on rat liver homogenates unrelated to its action on RNA synthesis. Science 146: 1311–13.

13. STEWART, F. C. AND G. PRESTON. 1941. The effect of salt concentration upon the metabolism of potato discs and the contrasted effect of potassium and calcium salts which have a common ion. Plant Physiol. 16: 85–116.

14. TROWN, P. W. 1965. An improved method for the isolation of carboxydismutase. Probable identity with fraction I protein and the protein moiety of protochlorophyll holochrome. Biochemistry 4: 908–18.

15. ZUCKER, M. AND C. C. LEVY. 1959. Some factors which affect the synthesis of chlorogenic acid in discs of potato tuber. Plant Physiol. 34: 180–92.