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Inhibitory Effect of Carbohydrate on Flowering in *Lemna perpusilla*

III. EFFECTS OF RESPIRATORY INTERMEDIATES, AMINO ACIDS, AND CO_2. GLUCOSE 6-PHOSPHATE DEHYDROGENASE ACTIVITY[1]

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ABSTRACT

A study was done on the effects of various factors on carbohydrate inhibition of flowering and on *in vitro* activity of glucose 6-phosphate dehydrogenase in *Lemna perpusilla* 6746 grown on dilute Hutner's medium in short days. Autoclaving decreased the flower inhibitory effect of sucrose but increased the effects of glucose and fructose. Sucrose inhibition was mimicked by CO_2 and was partially reversed by agitation of the cultures. Inhibition by sucrose was also partially reversed by ATP and intermediates of the tricarboxylic acid cycle, the glycolytic pathway, or the pentose phosphate pathway. Tartaric acid was inactive. Glycine, L-alanine, L-aspartate, L-asparagine, L-serine, L-glutamate, and L-glutamine were active, whereas L-cysteine, L-arginine, L-lysine, L-leucine, L-isoleucine, L-proline, L-tyrosine, L-tryptophane, and L-phenylalanine were not. Incubation of cultures on distilled water during a single inductive long night prevented flowering. This inhibition was partially reversed by L-alanine and glucose 6-phosphate. There was a correlation between carbohydrate inhibition of flowering and enhancement of glucose 6-phosphate dehydrogenase. Possible mechanisms for the carbohydrate inhibition of flowering are discussed.

Sucrose and other utilizable sugars inhibit an early stage in the flowering of *Lemna perpusilla* grown on dilute Hutner's medium in short photoperiods (11, 12). The inhibition requires the presence of ammonium ion (5) and is partially reversed by glycine, L-aspartic acid (13), Ca^{2+}, and P_i (12).

The following report describes attempts to identify the metabolic disturbances involved in carbohydrate-induced inhibition of flowering. The approach was to test various metabolites for the ability to prevent the inhibition. In addition, *in vitro* activity of glucose-6-P dehydrogenase was measured under a variety of conditions.

METHODS AND MATERIALS

Growth and Flowering. Cultures were grown on Hutner's medium as described elsewhere (12) or on the ammonium-free modification, KNO_3 medium (5). Carbohydrates were added prior to autoclaving (12 min, 15 psi) or by sterile filtration as indicated in the appropriate sections. Other supplements (Sigma) were added by sterile filtration.

Methods of culture and evaluation of flowering were as before (12) except that, in addition to tubes, 125-ml de Long flasks with stainless steel caps were used as noted. Unless otherwise indicated, Fl%[2] values are means based on five cultures.

In some experiments cultures were grown on a shaker which agitated them with about 150 3.5-cm strokes per min throughout the entire growth period.

For experiments in which the composition of the atmosphere was varied, cultures were grown in transparent Lucite chambers (volume of about 20 liters) through which the appropriate gas mixture was passed at a rate of about 1 liter/min.

Water Inhibition. Tubes planted with a single three-frond colony were grown on 0.5 strength Hutner's medium with 30 mM sucrose (autoclaved) for about 2 days under continuous cool-white light (about 40 ft-c). The fronds were then transferred to distilled water, to growth medium, or to water with a supplement and given an inductive long night (16 hr). The fronds were then transferred back to fresh 0.5 strength Hutner's medium with sucrose and replaced under noninductive, continuous cool-white light. The temperature was 24 to 26 C. Evaluation of flowering was on the 4th day after the long night (see Reference 13 for further details).

Chlorophyll. See Reference 13.

Glucose 6-Phosphate Dehydrogenase Activity. Cultures, started with about 20 three-frond colonies in 1-liter flasks containing 450 ml of medium, were grown for 1 week in an 8-hr photoperiod (12). Fronds were washed with tris buffer (50 mM), blotted, and weighed. The fronds were then homogenized in 50 mM tris buffer, filtered through four layers of cheesecloth, and centrifuged at 20,000*g* for 20 min, all at about 4 C. The supernatant was assayed for activity by a modification of methods described by Waygood and Rohringer (16). Cuvettes (1.0-cm light path) contained 0.1 ml of tris buffer (50 mM, pH 7.5), 0.2 ml of $MgCl_2$ (100 mM), 0.1 ml of 3 mM NADP (Sigma), and 1.0 ml of extract (about 10% on a fresh weight basis) in a volume of 1.9 ml. The reaction was started by adding 0.1 ml of 20 mM sodium glucose-6-P; the blank cuvette received 0.1 ml of water. The reduction of NADP was measured by increases in A_{340} for 5 min at about 25 C. Change in A_{340} per

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[2] Abbreviation: Fl%: flowering percentage.

Table I. *Effects of Types of Vessel, Volume of Medium, and Inoculum Size on Flowering and Frond Production of L. perpusilla*

Cultures were grown on 0.1 strength Hutner's medium without or with autoclaved sucrose at 30 mM, in an 8-hr photoperiod, with the use of 125-ml de Long flasks or 25- × 150-mm tubes. Plants − sucrose were dissected on day 9; plants + sucrose were dissected on day 7. Values are means ± SE of five cultures.

Vessel	Vol. of Medium	No. of Colonies in Inoculum	− Sucrose		+ Sucrose	
			No. of fronds	Fl%	No. of fronds	Fl%
	ml					
Tube	25	1	37.2 ± 2.3	48.1 ± 4.0	57.0 ± 4.3	37.8 ± 4.9
		2	59.6 ± 2.5	53.6 ± 3.5	95.6 ± 9.0	19.2 ± 1.6
Flask	25	1	36.2 ± 3.3	47.6 ± 6.1	51.0 ± 6.1	0.0
		2	59.4 ± 2.9	37.8 ± 4.9	103.6 ± 7.7	6.8 ± 3.7
Flask	50	1	37.0 ± 4.4	57.4 ± 4.5	60.0 ± 5.4	20.8 ± 8.6
		2	67.0 ± 5.6	52.7 ± 5.6	116.2 ± 8.6	26.0 ± 7.8

min was estimated from the 3- to 5-min portion of the linear rate curve. Activity is expressed as ΔA_{340} per min per g fresh weight.

RESULTS

As mentioned elsewhere (5), the inhibition of flowering by sucrose is extremely variable and has occasionally disappeared and reappeared under apparently constant conditions. The cause is not due to some condition peculiar to my laboratory since a similar variability has been observed by Dr. W. S. Hillman (personal communication) at Brookhaven National Laboratory. The experiments described in the four following sections were done to identify the possible sources of this variability.

Autoclaved *versus* Sterile Filtered Sugars. Day 8 Fl% values were higher for autoclaved sucrose than for sterile filtered (11 *versus* 0); in contrast, values were lower for glucose and fructose sterilized by autoclaving than by filtration. As examples, the comparable values were 3 *versus* 12 and 0 *versus* 6 for glucose and fructose, respectively. Thus autoclaving might have affected the variability in results. However, the occasional disappearance of sucrose inhibition has also been observed with sterile filtered sucrose, indicating the involvement of some factor.

The inhibitory effect of sucrose and glucose on chlorophyll levels, described earlier (13), also occurred with sterile filtered sugars. As examples, total chlorophyll values (ng/mg fresh weight) for cultures grown on 0.1 strength Hutner's medium without sugar, with autoclaved sugar, and with sterile filtered sugar were 815, 567, and 562, respectively, for 30 mM sucrose; and 744, 438, and 420, respectively, for 30 mM glucose.

Geometry of Growth Conditions. Cultures started with one or two colonies and grown as previously described (11, 12), *i.e.*, tubes with 25 ml of medium, were compared to those grown in 125-ml de Long flasks with either 25 or 50 ml of medium. Frond production in the absence or presence of sucrose was unaffected by the type of vessel and the volume of medium, and, as expected, doubling the inoculum size caused an approximate doubling in final frond number regardless of culture conditions (Table I). Flowering of cultures without sucrose was similarly unaffected, but with sucrose it was lower in flasks than in tubes; this latter difference was less pronounced if the flasks contained 50 ml of medium. Further, doubling the inoculum had little or no effect on the inhibition in flasks but seemed to increase the inhibition in tubes. Thus sucrose inhibition of flowering was affected by the geometry of the growth conditions, and although a number of factors may have been involved the possibility investigated further was the involvement of a component of the atmosphere.

Effects of Shaking. More direct evidence for the involvement of a gas were the results of experiments on the effects of agitation. In the absence of sucrose, shaking had little or no effect on flowering or frond production. Consistent with the previous experiment, cultures with sucrose that were not agitated had a slightly higher mean Fl% value in tubes than in flasks (Table II). This difference between tube and flask disappeared when the cultures were shaken. Both Fl% and frond production were enhanced by shaking.

Effects of CO_2. The results in Table III show that, in an atmosphere free of CO_2, frond production, even in the presence of sucrose, was reduced. However, the inhibitory effect of sucrose on flowering was also reduced. Frond production in an atmosphere of air supplemented with 1% CO_2 was promoted

Table II. *Effect of Agitation on Sucrose-induced Inhibition of Flowering of L. perpusilla*

Cultures were grown in vessels as in Table I containing 25 ml of 0.1 strength Hutner's medium with autoclaved sucrose at 15 mM. Shaking rate was 150 3.5-cm strokes per min. Samples were dissected on day 7. Values are means ± SE of five cultures.

Vessel	Without Shaking		With Shaking	
	No. of fronds	Fl%	No. of fronds	Fl%
Tube	78.6 ± 6.1	22.2 ± 5.0	97.0 ± 6.7	43.7 ± 2.7
Flask	75.8 ± 3.4	8.7 ± 4.4	96.8 ± 5.3	44.6 ± 2.2

Table III. *Effects of CO_2 on Flowering and Frond Production of L. perpusilla*

Cultures were grown in 125-ml de Long flasks containing 25 ml of 0.1 strength Hutner's medium in an 8-hr photoperiod. Sucrose was sterilized by autoclaving. Samples were dissected on day 8. Values are means ± SE of five cultures.

Atmosphere	Sucrose	No. of Fronds	Fl%
	mM		
CO_2-free air	0	No growth	No growth
	10	47.8 ± 3.5	42.9 ± 2.8
	30	70.6 ± 4.1	35.6 ± 3.6
Air	0	35.4 ± 2.0	38.6 ± 2.7
	10	83.2 ± 1.8	27.9 ± 2.9
	30	81.6 ± 1.8	9.2 ± 3.0
Air + 1% CO_2	0	52.2 ± 4.7	28.1 ± 4.9
	10	59.2 ± 3.4	7.7 ± 3.9
	30	70.6 ± 1.7	3.6 ± 1.7

in cultures without sucrose but inhibited in those with sucrose. Flowering was reduced by the CO_2 supplement in cultures with and without sucrose.

Since the sucrose inhibition required dilute medium (11, 12) and the presence of NH_4^+ (5), experiments were done to determine whether the CO_2 effect had similar requirements. With an atmosphere of air containing 0.25% CO_2, the day 7 mean Fl% values were 1 on 0.1 strength Hutner's medium and 54 on the ammonium-free modification, 0.1 strength KNO_3 medium. The comparable values for 0.5 strength media were 59 and 54. Thus the CO_2 and sucrose inhibitions appear to be closely related.

In summary, the results suggest that the variability noted in flowering on sugar-supplemented dilute medium involved a component of the atmosphere. Clearly, CO_2 affected flowering on dilute medium. The results with agitated cultures indicate that oxygen might also have been involved.

Amino Acids and Organic Acids. Earlier experiments showed that the amino acids glycine and L-aspartate partially overcame the sucrose inhibition (13). This approach was extended to include a variety of amino acids as well as respiratory intermediates.

Initially, supplements were tested at 150 μM. However, some amino acids markedly inhibited frond production at this concentration, thus making evaluation of any flowering effect impossible. Such compounds were therefore tested at lower concentrations, as noted.

The following had no detectable activity when tested at the indicated concentrations (μM) against 15 mM sterile filtered sucrose or 30 mM autoclaved sucrose in 0.1 strength Hutner's medium: L-cysteine (5), L-arginine (5, 50, 100), L-lysine (25, 50), L-leucine (25), L-isoleucine (25), L-proline (50), L-tyrosine (25), L-tryptophane (50), L-phenylalanine (100), potassium tartrate (100, 300).

The following showed activity at 150 μM: L-alanine, L-aspartate, L-asparagine, glycine, L-glutamate, L-glutamine, L-serine, potassium α-ketoglutarate, sodium pyruvate, potassium citrate, potassium succinate, potassium malate, potassium glyoxylate. The results in Table IV summarize a number of experiments comparing the activities of most of these compounds (60 μM). In general, the respiratory intermediates, especially at higher concentrations, not only partially reversed the sucrose inhibition on flowering but also promoted frond production; citrate was especially effective. In contrast, the active amino acids either inhibited or had no effect on frond production. Furthermore, amino acids were more active in reversing sucrose inhibition of flowering than were their respective keto acid precursors. The results in Table V show that L-glutamic acid and α-ketoglutaric acid had similar activities at the highest concentration tested, 240 μM, but at the lower concentration the amino acid was more effective than the keto acid. Similar results were obtained in a comparison of pyruvate and L-alanine.

Table IV. *Effects of Various Metabolites on Sucrose-induced Inhibition of Flowering of L. perpusilla*

Cultures were grown and dissected as in Table III. Supplements (60 μM) were sterilized by filtration. Sucrose was at 30 mM; in experiment B, it was sterile filtered; in experiment A, it was autoclaved. Values are means ± SE of five cultures.

Supplement	Experiment A		Experiment B	
	No. of fronds	Fl%	No. of fronds	Fl%
None	63.4 ± 2.7	9.6 ± 6.4	62.0 ± 2.7	9.0 ± 7.5
K citrate	71.2 ± 4.3	35.3 ± 9.2	...	...
K succinate	61.4 ± 2.2	41.4 ± 8.5	...	...
K malate	69.2 ± 0.9	25.5 ± 9.6	...	...
Glycine	59.4 ± 4.4	44.5 ± 5.2	61.0 ± 4.0	37.5 ± 4.0
L-Alanine	57.4 ± 3.4	46.6 ± 5.6	50.2 ± 3.1	47.9 ± 5.4
L-Aspartate	68.6 ± 3.1	20.4 ± 1.9	64.6 ± 3.7	31.8 ± 7.2
L-Asparagine	51.0 ± 4.0	45.9 ± 5.8	52.0 ± 4.9	40.2 ± 6.9
L-Serine	51.6 ± 2.6	47.2 ± 9.1	45.8 ± 3.6	46.1 ± 3.2
L-Glutamate	...	...	52.6 ± 2.3	48.0 ± 5.5
L-Glutamine	...	...	63.4 ± 3.1	27.6 ± 9.2

Table V. *Effects of L-Glutamate and α-Ketoglutarate on Sucrose-induced Inhibition of Flowering*

Cultures were grown and dissected as in Table III. Sucrose (30 mM) and other supplements were sterilized by filtration. Values are means ± SE of five cultures.

Supplement	Concn	No. of Fronds	Fl%
	μM		
None	...	65.6 ± 1.6	3.0 ± 2.2
α-Ketoglutarate	60	67.2 ± 2.6	22.7 ± 11.8
	240	70.4 ± 2.5	43.5 ± 4.0
L-Glutamate	60	62.4 ± 2.2	48.5 ± 3.3
	240	61.2 ± 1.4	58.4 ± 3.9

Table VI. *Effects of Various Phosphorylated Compounds on Sucrose-induced Inhibition of Flowering of L. perpusilla*

Samples were grown and dissected as in Table III. Sucrose was at 30 mM and was autoclaved. Other supplements were sterile filtered. Values are means ± SE of six cultures.

Supplement	No. of Fronds	Fl%
Experiment I (300 μM)		
None	51.0 ± 5.2	11.4 ± 4.2
Glucose-6-P	69.8 ± 2.5	66.8 ± 2.0
Fructose-6-P	73.7 ± 1.8	66.9 ± 1.8
Fructose-1,6-diP	74.2 ± 3.1	71.0 ± 2.2
3-P-Glycerate	74.0 ± 1.4	68.3 ± 1.1
Experiment II (300 μM)		
None	73.8 ± 1.5	8.4 ± 2.4
6-P-Gluconate	82.6 ± 1.4	67.1 ± 0.9
Ribulose-5-P	80.0 ± 1.2	62.8 ± 3.6
Experiment III (600 μM)		
None	50.2 ± 4.0	0.0
ATP	77.0 ± 2.4	25.7 ± 6.1

Phosphorylated Intermediates. Since various intermediates of the tricarboxylic acid cycle were active, their precursors—phosphorylated intermediates of the glycolytic pathway—as well as other phosphorylated compounds, including ATP, were also tested. In the absence of sucrose, these compounds had no effect on frond production or flowering. However, in the presence of sucrose, inhibition of flowering was partially reversed by all the compounds tested; frond production was also promoted (Table VI).

Water Inhibition. Halaban and Hillman (4) have suggested that the inhibition of flowering caused by transfer to distilled water during inductive long nights was related to the sucrose inhibition. It was of interest, therefore, to determine whether metabolites capable of preventing the sucrose inhibition were also active in preventing the water effect. A single inductive long night was given during which fronds were incubated on the indicated medium.

In a preliminary experiment, cultures were incubated on dis-

tilled water, 0.1 strength Hutner's medium, or 0.5 strength Hutner's medium; the last two contained 30 mM sucrose (autoclaved). The Fl% means ± SE on day 4 were 2.5 ± 0.8, 11.5 ± 1.7, and 20.3 ± 3.0, respectively. Thus inhibition occurred on dilute medium with sucrose and to a greater extent on distilled water.

Next, cultures were incubated on distilled water or the same supplemented with 100 μM L-alanine or glucose-6-P. The Fl% means on day 4 were 1.1 ± 0.7, 8.5 ± 2.5, and 12.4 ± 2.5, respectively. Frond production was unaffected by any of the treatments used.

Glucose 6-Phosphate Dehydrogenase. In a previous paper (13) it was suggested that enhancement of the pentose phosphate pathway might be involved in sucrose inhibition of flowering. Therefore, activity of glucose-6-P dehydrogenase was measured in extracts of cultures grown on four modifications of Hutner's medium: 0.5 strength medium, 0.5 strength medium with 30 mM sucrose, 0.1 strength medium, and 0.1 strength medium with 30 mM sucrose. Earlier work showed that flowering occurs in all but the last medium (11). The results in Table VII show that sucrose had little or no effect on enzyme activity in extracts from fronds grown on 0.5 strength medium. Fronds grown on the more dilute medium had low activity, and in this medium sucrose enhanced activity to levels higher than those observed in cultures grown on half-strength medium with or without sucrose.

Next, 0.1 strength medium and the same with autoclaved mannitol, fructose, or mannose were tested. It was shown previously that flowering is unaffected by mannitol but is inhibited by the last two (12). The results (Table VIII) show that fructose and mannose, but not mannitol, enhanced enzyme activity.

Table VII. Glucose-6-P Dehydrogenase Activity in Vitro from Cultures Grown in Various Modifications of Hutner's Medium

Cultures were grown in 1-liter flasks containing 450 ml of medium for 1 week in an 8-hr photoperiod. Sucrose was sterilized by autoclaving. See text for methods of extraction and measurement of enzyme activity.

Medium	Glucose-6-P Dehydrogenase Activity
	$\Delta A_{340}/min \cdot g\ fresh\ wt$
0.5 strength	0.305
0.5 strength plus sucrose (30 mM)	0.310
0.1 strength	0.100
0.1 strength plus sucrose (30 mM)	0.400

Table VIII. *Effects of Various Sugars on Glucose-6-P Dehydrogenase Activity*

Cultures were grown as in Table VII in 0.1 strength Hutner's medium. Sugars were at 15 mM, sterilized by autoclaving. See text for methods of extraction and measurement of enzyme activity.

Sugar	Glucose-6-P Dehydrogenase Activity
	$\Delta A_{340}/min \cdot g\ fresh\ wt$
Experiment A	
None	0.130
Fructose	0.910
Glucose	0.680
Sucrose	0.370
Experiment B	
None	0.110
Mannitol	0.098
Mannose	0.714

DISCUSSION

Glucose-6-P Dehydrogenase. The finding that sucrose supplementation of the growth medium increased the *in vitro* activity of glucose-6-P dehydrogenase confirms a similar finding with *Lemna minor* (15). The possible implications of this enhancement in the flowering inhibition will be discussed later. At this point it should be noted that the concentration of the mineral components of the growth medium also influenced activity: cultures grown on 0.1 strength medium had much lower activity than those grown on 0.5 strength. Thus the percentage of increase caused by the sugar was especially marked with the more dilute medium. This might have simply been the result of a general enhancement of protein synthesis, although there is the possibility that a mineral component of the medium was involved in the induction of glucose-6-P dehydrogenase.

Inhibition by CO_2. Carbon dioxide inhibited flowering on dilute medium. This inhibition required the presence of NH_4^+, as did the sucrose inhibition (5), suggesting a relationship between the two effects. Inhibition of flowering by CO_2 has also been reported for the long day plant *Lemna gibba* by Kandeler (7, 8), who suggested lowered levels of ATP as a possible mechanism. This possibility will be discussed later in relation to the sucrose inhibition.

Effects of Metabolites. It was previously suggested (13) that the inhibitory effect of sucrose on flowering might have been due to inadequate levels of amino acids. Some of the present results support this idea. For example, certain amino acids partially reversed the sucrose inhibition. The active amino acids were those believed to be produced primarily by amination or transamination reactions involving respiratory intermediates (2), whereas inactive amino acids were those requiring skeleton rearrangement or addition during their synthesis. Thus, one possibility is that any one of the active amino acids acted as a precursor for other required amino acids. Similarly, intermediates of the glycolytic and of the tricarboxylic acid cycle, also shown to partially reverse the sucrose pathway inhibition, might have acted as precursors of amino acids.

In regard to the mechanism by which growth on sucrose-dilute medium might have led to inadequate levels of amino acid, two results are of special interest. First there is the finding, reported elsewhere (5), that NH_4^+ is required for the sucrose inhibition. Possible mechanisms for NH_4^+ inhibition of flowering have been discussed by Kandeler (9). One not suggested is an acceleration of the oxidation of glucose-6-P via the pentose phosphate pathway, as described for glucose-oxidizing yeast (6). Such an effect in *Lemna*, together with the sugar-induced enhancement of glucose-6-P dehydrogenase, might have caused excessive diversion of glucose through the pentose phosphate pathway, resulting in an inadequate production of amino acid precursors. Possible examples of such an effect are the reports that intermediates of the tricarboxylic acid cycle reversed the growth inhibitory effect of NH_4^+ on the basidomycete *Schizophyllum commune* (14) and on cell suspensions of soybeans (3). Nitsch (10) has also suggested that flowering might be affected by the degree to which glucose is channeled through the pentose phosphate pathway, but in his scheme stimulation of the pathway is conducive to flowering.

The ability of intermediates of the pentose phosphate pathway to partially reverse the sucrose inhibition (Table VI) is inconsistent with the hypothesis. However, since increasing the levels of P_i in otherwise dilute Hutner's medium also prevented the sucrose inhibition (12), the activity of phosphorylated compounds might have merely been due to the release of P_i. An-

other possibility is that these supplements reduced flow through the pathway by mass action or by a feedback mechanism.

An alternative explanation for the sucrose effect with which many of the data are consistent is that of inadequate levels of ATP. Kandeler (8) has suggested such a mechanism to explain sucrose inhibition of flowering of *L. gibba*. For sugar inhibition of flowering in *L. perpusilla,* low levels of P_i were required (12). Under such conditions, excessive synthesis of starch via the phosphorylase reaction might have occurred (1), resulting in a reduction in ATP. In the present study, respiratory intermediates, amino acids readily convertible to respiratory intermediates, aeration, and ATP overcame the sucrose inhibition. These factors might have increased endogenous levels of ATP.

However, two additional findings should be considered in this connection: amino acids, in contrast to respiratory intermediates, reversed the inhibition of flowering without promoting frond production, and, at low concentrations, amino acids were more active than their keto acid precursors in reversing the sucrose inhibition. These results are more consistent with the idea of amino acid inadequacy than with the ATP hypothesis, although differences in the rates of uptake might have accounted for some of the differences in activity.

Water Inhibition. The report by Halaban and Hillman (4) that flowering of strain 6746 was inhibited by incubation on distilled water during inductive long nights was confirmed in the present study. Recent evidence (R. Halaban, personal communication) suggests that during incubation leakage of a substance or substances occurred and that this substance might have been required for a reaction critical to photoperiodic induction. Halaban and Hillman (4) have also shown that the water effect was reduced when cultures grown without sucrose were used; further, the addition of Ca^{2+} or P_i to the water or the substitution of NO_3^- for NH_4^+ in the medium partially prevented the inhibition. Since these factors also modify the sucrose inhibition (5, 12), Halaban and Hillman (4) suggested that the two phenomena might be related. Additional evidence in favor of this idea is the finding in the present study that inhibition by either sucrose or water was partially reversed by L-alanine and glucose-6-P.

Thus, the sucrose effect might also involve the postulated substance. In this view, there are a number of ways in which supplements could prevent the sucrose inhibition: either by replacing the substance, by preventing the loss of the substance, or by obviating the requirement for the substance in photoperiodic induction.

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