

## Control by Phytochrome of $^{14}\text{C}$ -Sucrose Incorporation into Buds of Etiolated Pea Seedlings<sup>1</sup>

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**Summary.** When etiolated pea epicotyls are excised immediately above the cotyledons and dipped basally into  $^{14}\text{C}$ -sucrose, their terminal buds respond to red light by increased growth (IG) and enhanced incorporation of sucrose (EIS). Both phenomena are phytochrome controlled, showing typical kinetics, reversal by far-red light, escape from photochemical control and limitation to leaf tissue. EIS is of greater magnitude, occurs more rapidly and is saturated by lower energies of red light than IG, suggesting its possible importance as a controlling reaction in phytochrome-mediated growth. Both IG and EIS are best shown in the presence of a long epicotyl derived from a 5 to 6-day-old seedling in the presence of about 0.1 M unlabeled sucrose in the medium.

Enhanced incorporation is most dramatic with sucrose; lesser effects are shown with fructose, glucose, maltose and ribose in that order. Both level of incorporation and red light effects are poor for labeled tyrosine, phenylalanine, valine, acetic acid, cinnamic acid and  $\alpha$ -ketoglutaric acid. The possible connection between carbohydrates and phytochrome-mediated photomorphogenesis is considered.

Several photomorphogenic responses of etiolated pea seedlings seem to be under the control of phytochrome (2, 14) which has also been shown to be abundant in the growing regions of the seedling (6, 10). Red light also causes pea plumules to initiate the synthesis of several quercetin derivatives which they form in addition to the kaempferol derivatives found in dark-grown seedlings (3, 4, 22). This change from kaempferol to quercetin involves the introduction of an additional hydroxyl group in the 3' position of the flavonoid nucleus. In view of the fact that others have noted similar biochemical changes upon irradiation of sorghum (26) and gherkin (8) seedlings, the biochemical mechanisms involved in phytochrome-controlled hydroxylations were deemed worthy of further investigation.

As one approach to an investigation of this problem, we have fed labeled precursors of various portions of the flavonoid molecule (15) to excised portions of etiolated pea seedlings. We have thus been led to the discovery of a phytochrome-mediated control of  $^{14}\text{C}$ -sucrose incorporation into the growing bud. Because of the apparent specificity of this reaction and its extremely rapid kinetics, it appears

to have some relevance for the mechanism of photomorphogenesis.

### Materials and Methods

**Plants.** Seeds of *Pisum sativum* L., variety Alaska, obtained from Asgrow, Incorporated, of Orange, Connecticut, were grown as previously described (14).

Six-day-old etiolated seedlings were used for the experiments. Only plants with recurved hooks (2) were selected. At this age, the length of the third (youngest) internode in the selected plants was 10 to 20 mm. The plants were removed from the flats, individually cut immediately above the cotyledons in water and transferred into 50-ml Erlenmeyer flasks containing 10 ml of 0.03 M  $\text{KH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  buffer, pH 6.4, 0.1 M sucrose and  $^{14}\text{C}$ -sucrose, 15,000  $\pm$  1500 cpm/ml. When other labeled compounds were used, the radioactivity level was the same, and carrier sucrose was always present. Solutions were freshly prepared for each experiment using stock solutions of buffer kept at low temperature. The standard experimental dark period after light treatment was 22 to 24 hours. When only the plumule was being considered, the terminal buds, including all tissue at and above the lowest node bearing a foliage leaf (12), were harvested. When internodes were also sampled, 10-mm sections were excised from the middle of the first and second internodes, and the 10-mm section from the third internode was cut from the top so as to include part of the apical hook.

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**Light Source and Treatments.** The red light source ( $2.3 \times 10^4$  erg  $\text{cm}^{-2}$   $\text{min}^{-1}$ ) consisted of 4 red fluorescent tubes (Sylvania 15 w) wrapped with 5 layers of Du Pont red cellophane, at a distance of 42 cm from the top of the plants. The far-red light source ( $8.0 \times 10^4$  erg  $\text{cm}^{-2}$   $\text{min}^{-1}$ ) consisted of 5 waterproof 300 w internal reflector incandescent flood lamps at a distance of 22 cm from the top of the plants. The radiation from the lamps was filtered through 15 cm of water and 6 layers each of red and blue Du Pont cellophane. In both cases the energy levels were measured at a distance equal to that of the plant tops from the light source.

The standard red (R) or far-red (FR) light treatment consisted of 15 minutes of irradiation. In experiments where FR reversibility was studied, the experimental system included a final dark control, R followed by FR, and FR followed by R. Single R and FR controls could be omitted, since experience showed that they were identical, respectively, with FR→R and R→FR, and the experimental system had the virtue of exposing all plants to the same total irradiation, with only the order of the treatments being altered.

**Radioactivity Measurements.** Radioactivity was measured by means of an Anstron liquid scintillation counter. The counting medium consisted of the following mixture: toluene (1 liter) to which 2,5-diphenyloxazole (PPO) (5 g/liter), 1,4-bis-(2,4-dimethyl-5-phenyloxazolyl)-benzene (dimethyl-POPOP) (0.3 g/liter) and Cab-O-Sil (34.5 g/liter) were added. The quenching caused by the use of intact plant material was specially tested, and 2 essentially equivalent counting methods were finally adopted as satisfactory. In the first, plant material was harvested, put into vials contained in a dry-ice bath, lyophilized overnight, ground and the radioactivity of the powder measured in toluene-Cab-O-Sil solution. In the second method, fresh plant tissue was put directly into the scintillation solution. The lyophilized and powdered material did not show any significant change in its radioactivity during a counting period of 38 hours, but the radioactivity of the fresh material was initially only 75% of the level attained after 16 to 18 hours, after which time both methods gave equivalent counts. Thus, for convenience, we used the second

method, placing freshly harvested material directly into the toluene-Cab-O-Sil solution and storing it at  $27 \pm 1^\circ$  for 24 hours before counting. A plug-in  $^{14}\text{C}$   $\beta$ -discriminator was used, and counting proceeded for 4 to 10 minutes with 1 recount. Three cycles were counted for each experiment, and the mean of 6 recordings for each sample was calculated. The background was 25 to 34 cpm. Radioactivity values less than twice the background were recorded as traces.

**Radioactive Compounds and Stock Solutions.** The following compounds were used: sucrose- $^{14}\text{C}$ , 12 c/mole and cinnamic acid- $^{14}\text{C}$ , 0.82 c/mole (International Chemical and Nuclear Company, California); D-glucose- $^{14}\text{C}$ , 22.3 c/mole, D-fructose- $^{14}\text{C}$ , 70.0 c/mole,  $\alpha$ -ketoglutaric acid- $^{14}\text{C}$ , 6.95 c/mole and acetic acid- $^{14}\text{C}$ , 12 c/mole (Calbiochem); L-phenylalanine- $^{14}\text{C}$ , 360 c/mole, L-tyrosine- $^{14}\text{C}$ , 154 c/mole and L-valine- $^{14}\text{C}$ , 200 c/mole (Schwarz); D-ribose- $^{14}\text{C}$ , 2.0 c/mole and D-maltose- $^{14}\text{C}$ , 4.45 c/mole (Nuclear-Chicago).

Radioactive compounds were dissolved in sterilized phosphate buffer (0.03 M, pH 6.4), transferred into serum bottles covered with rubber stoppers and stored as stock solutions in a deep freeze. For each experiment aliquots were removed by a sterile disposable syringe.

Each experiment was repeated at least 2 to 3 times, and each treatment included 3 to 5 replicates consisting of 10 plants. Data are presented as the mean for each type of experiment, and when needed, standard errors were calculated. The radioactivity was calculated on a fresh weight basis, and is tabulated together with the fresh weight of the tissue studied.

## Results

**R and FR Effects on Plumule Growth and  $^{14}\text{C}$ -Sucrose Uptake.** In seeking to develop an appropriate system for the study of the uptake and incorporation of labeled compounds, we excised epicotyls at the level of the cotyledons and at various points between the cotyledonary node and the terminal bud. Such excised epicotyls were then placed in the solutions and exposed to the various irradiation regimes.

Table I. Reversible Effects of Red (R) and Far-red (FR) Illumination on Plumule Growth and  $^{14}\text{C}$ -Sucrose Uptake

No of cycles	Light treatments	mg fr wt/plumule	% of Dark control cpm/mg fr wt of plumule
0	Dark control	100	100
1	FR→R	131.7	142.7
	R →FR	94.8	112.2
2	FR→R →FR	97.5	94.4
	R →FR→R	147.8	191.1
3	FR→R →FR→R	140.4	168.3
	R →FR→R →FR	109.5	115.5
4	FR→R →FR→R →FR	106.3	114.4
	R →FR→R →FR→R	149.0	180.0

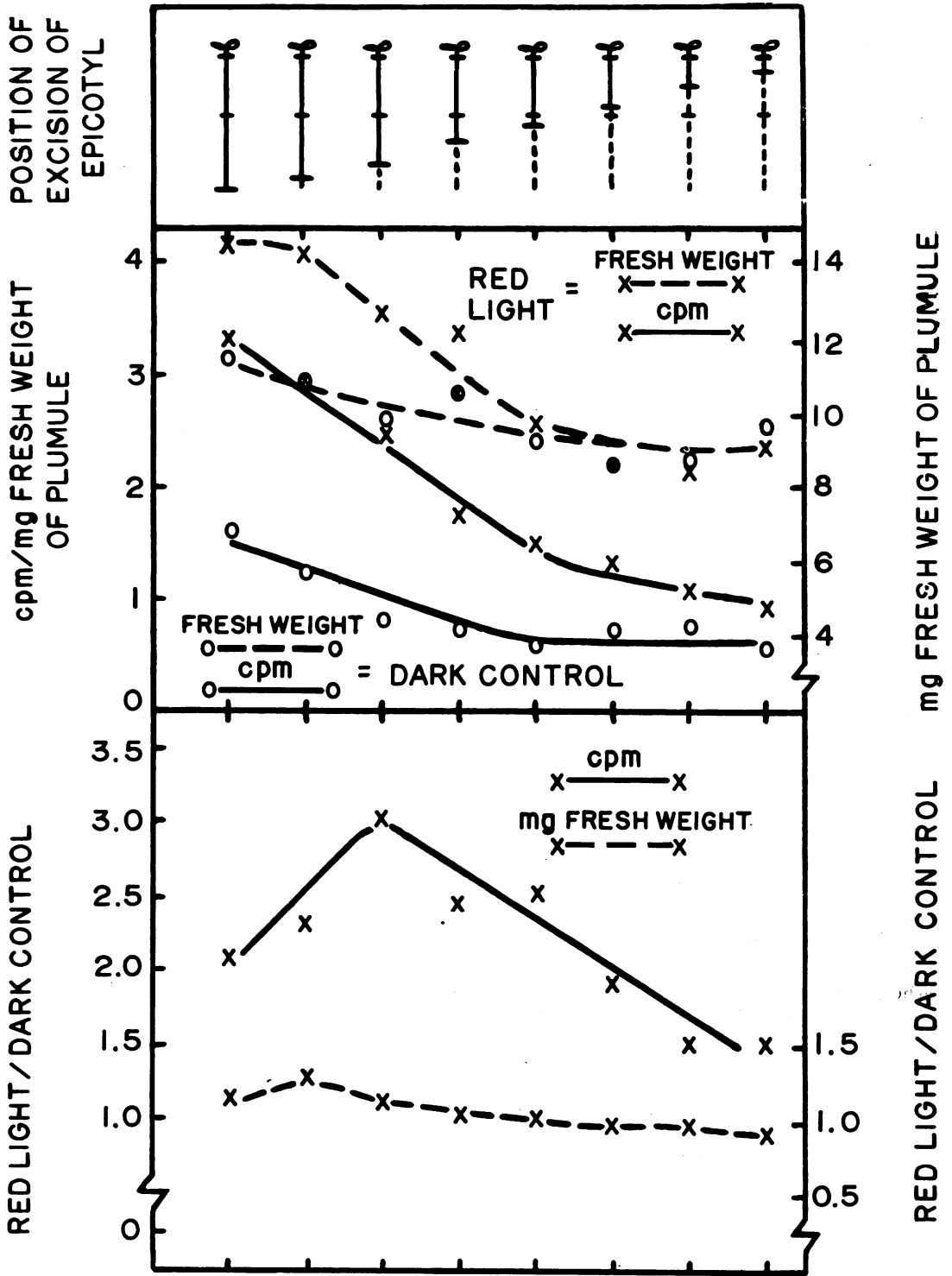


FIG. 1. The effect of epicotyl length on <sup>14</sup>C-sucrose incorporation and plumule growth. The portion of the epicotyl below the cut (shown in dotted lines) was discarded and the remainder of the epicotyl dipped basally into the sucrose solution. The small cross lines on the epicotyl show the positions of the nodes.

Terminal buds of plants with long epicotyls responded to R treatment both by increased growth (IG) as measured by fresh weight, and by enhanced incorporation of labeled sucrose (EIS). The EIS effect was about  $3\times$  as great as the IG effect. In the short epicotyls, growth,  $^{14}\text{C}$ -sucrose incorporation and response to R were much lower than in the long epicotyls. Repeated R and FR treatments administered to long epicotyls produced expected results in that the last light treatment determines the final effects. Typical results are presented in table I.

The differences in behavior of long and short epicotyls were further investigated, in view of the fact that the anatomy of the first internode is completely different from that of the internodes above it (16). Figure 1 presents the results of an experiment in which the epicotyl was severed at 8 different levels, i.e. at 0, 10, 25, 40 and 55 mm above the cotyledons (all in the first internode), 10 mm above the first node, in the middle of the second internode and 10 mm below the second node. It is obvious that (1) the greater the length of epicotyl, the greater is growth and sucrose uptake of the bud and (2) the part of the epicotyl most influencing the response to red light is the first internode. Buds devoid of epicotyl show no response to R at all. We adopted excision immediately above the cotyledon as the standard procedure for further experiments, since the fresh weight of the terminal bud and its accumulated radioactivity were highest in this treatment.

Hopkins and Hillman (19) found that excised tissue can be validly employed for the study of the kinetics of phytochrome changes in etiolated peas. We administered R and FR both before and after excision, and tested the effects both on plumular growth and  $^{14}\text{C}$ -sucrose incorporation. Both R-induced promotion and FR-induced reversion were identical in both cases, ruling out any excision artifacts.

The possible role of the cotyledons was studied further by excising epicotyls with cotyledons attached, with cotyledons removed, and with cotyledons removed but added back to the nutrient medium in the presence or absence of carrier sucrose.

Cotyledons in the absence of sucrose permit a maximal plumular growth response to red light, since the R/D ratio of plumular fresh weights is 1.31, as compared with 1.42 for intact plants. The addition of 0.1 M carrier sucrose depresses growth somewhat, presumably due to osmotic effects, but the R/D ratio remains the same. Contrariwise, carrier sucrose enhances both  $^{14}\text{C}$ -sucrose incorporation and the R promotion of this phenomenon, both in the presence and absence of cotyledons. We decided that the best experimental system is the cotylectomized epicotyl, which shows the promotive effects of sucrose and red light on both growth and accumulation of  $^{14}\text{C}$  from labeled sucrose. Epicotyls retaining their cotyledons show a promotive effect of R on growth of the plumule, but are not

satisfactory for  $^{14}\text{C}$ -sucrose uptake studies, presumably due to competitive transport from the cotyledons. Roots were removed, since they were found to retard uptake and not to promote growth over 24 hour periods.

*Dose-Response to Carrier Sucrose.* The importance of carrier sucrose in the phytochrome-mediated  $^{14}\text{C}$ -sucrose incorporation led us to examine the quantitative aspects of this effect. Figure 2 shows that the addition of carrier sucrose promotes both IG and EIS in the terminal bud. The optimum

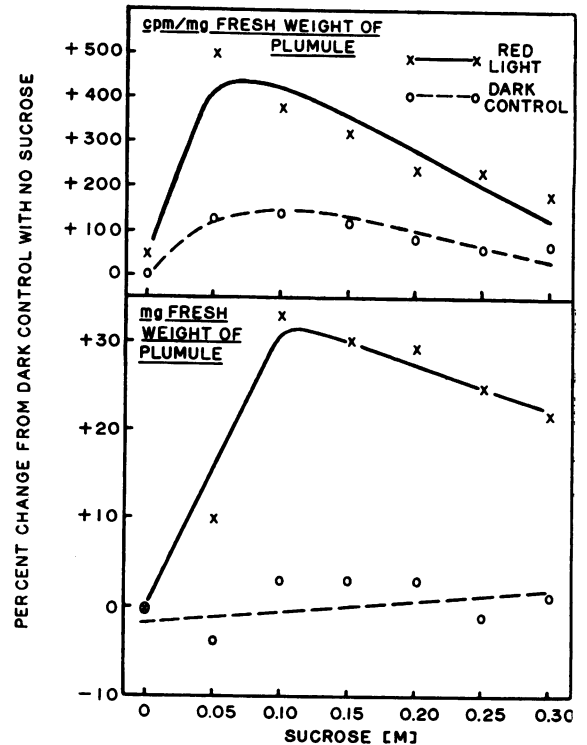


FIG. 2. The effect of concentration of carrier sucrose on  $^{14}\text{C}$ -sucrose incorporation and plumule growth.

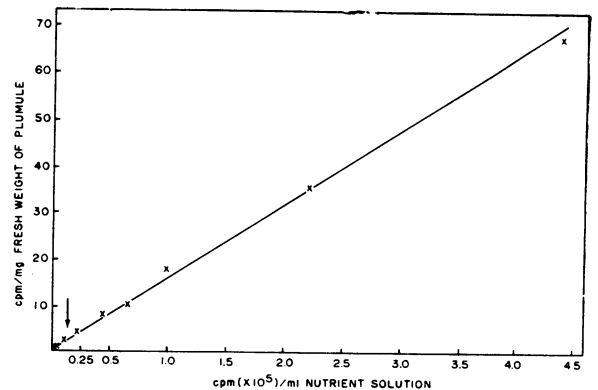


FIG. 3. The effect of level of  $^{14}\text{C}$ -sucrose in the solution on radioactivity of the plumule. (Arrow indicates radioactivity level in the standard nutrient solution).

concentration for EIS was 0.05 M and for IG was 0.10 M. It should be noted that in the absence of carrier sucrose there is no significant effect of R on plumule growth, but there is still an effect on EIS. One-tenth molar carrier sucrose was adopted as a standard since it caused maximal growth response of the plumule and near maximal <sup>14</sup>C-sucrose incorporation.

*Dose Response to <sup>14</sup>C-Sucrose Concentration.* We next investigated the effect of increasing levels of <sup>14</sup>C-sucrose on incorporation in the presence of an optimal concentration of carrier sucrose. Into a 0.1 M solution of carrier sucrose, we added increasing levels of <sup>14</sup>C sucrose from 0 to 4.5 × 10<sup>5</sup> cpm/ml. (Even the highest level of radioactive sucrose in this experiment can still be considered as weightless.) The results presented in figure 3 show a linear relationship between the radioactivity in the solution and in the bud.

*Effect of the Age of the Seedlings.* It has previously been reported (10), that the concentration of phytochrome in 10-mm sections of etiolated peas excised 3 mm below the apical hook is constant up to 10 days of age. Because of interference by phytochlorophyll in the determination of phytochrome, no equivalent data are available for the bud. Figure 4 shows the effect of seeding age on the R promotion of plumule growth and <sup>14</sup>C-sucrose uptake. The best IG effect occurs between the fifth and the sixth day, while the greatest EIS occurs on the fifth day. Since the highest values for fresh weight and <sup>14</sup>C-sucrose incorporation were obtained on the sixth day, this age was selected as standard in the present study.

*Dose Response to Red and Far-red Light.* Bottomley et al. (3) reported that about 30 Kergs/cm<sup>2</sup>

of R will saturate the growth response of the terminal bud. We were interested in determining whether the uptake of <sup>14</sup>C-sucrose shows a similar energy dependence. Typical results of a dose-response study are presented in figure 5. It is obvious that there is a mirror-image relationship between R and FR effects. While the R effect on IG is not completely saturated after 64 minutes

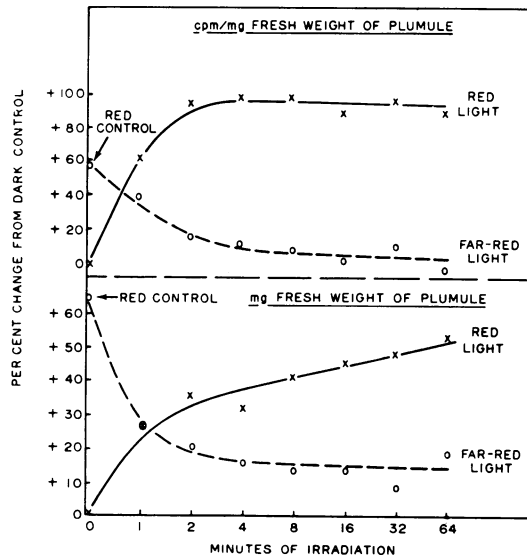


FIG. 5. Dose-response to red and far-red light. Upper curve refers to <sup>14</sup>C uptake; lower curve refers to growth.

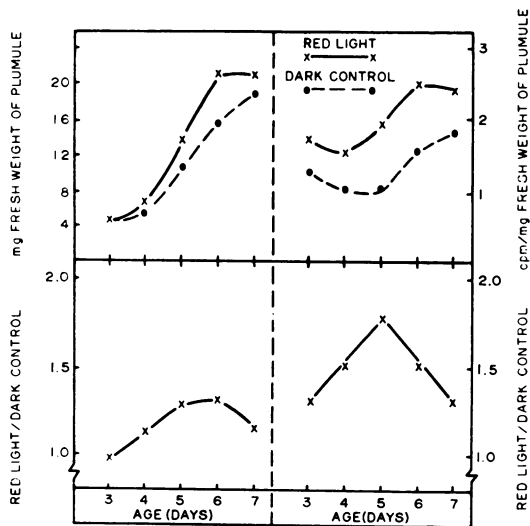


FIG. 4. Effect of seedling age on response to red light. Left column refers to plumule growth, right column refers to <sup>14</sup>C uptake.

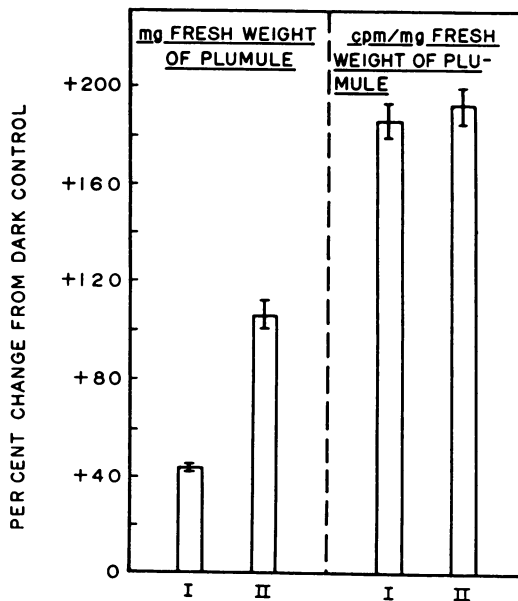


FIG. 6. The effect of a 15-minute treatment with red light (I) and continuous red light (II) on <sup>14</sup>C-sucrose uptake and plumule growth.

of irradiation (150 Kergs/cm<sup>2</sup>), only 2 minutes of red light (48 Kergs/cm<sup>2</sup>) were sufficient to saturate EIS. Two minutes of FR (160 Kerg/cm<sup>2</sup>) completely reversed the effects of R on both growth and incorporation. For both processes, 3 × as much FR energy is required to get reversal as the R initially given. This is in agreement with the results of others on lettuce seed germination (5) and for inhibition of flowering of *Pharbitis nil* (17).

In a separate experiment, seedlings were exposed to continuous R and, for comparison, to an irradiation of 15 minutes. The results presented in figure 6 show that 24 hours of R more than doubled the fresh weight increase of the plumule induced by a 15 minute percent irradiation. However, <sup>14</sup>C-sucrose incorporation was identical in both light

treatments, showing once again that the promotion of <sup>14</sup>C-sucrose incorporation is saturated at lower R levels than growth itself.

*Kinetic Studies.* It has previously been reported (3, 14) that the promotion of terminal bud growth starts about 4 hours after a bright R flash and reaches a peak after 12 hours. At lower energy levels, there is a steady increase in growth for the full 24-hour experimental period. In order to study the kinetics of <sup>14</sup>C-sucrose incorporation we repeated experiments on the kinetics of plumule growth in the dark and also after R and FR treatment. The energy level of 368 Kergs/cm<sup>2</sup> used in the present study is close to that used by Bottomley et al. (4). Results in figure 7 show that the IG induced by R is significant after about 8

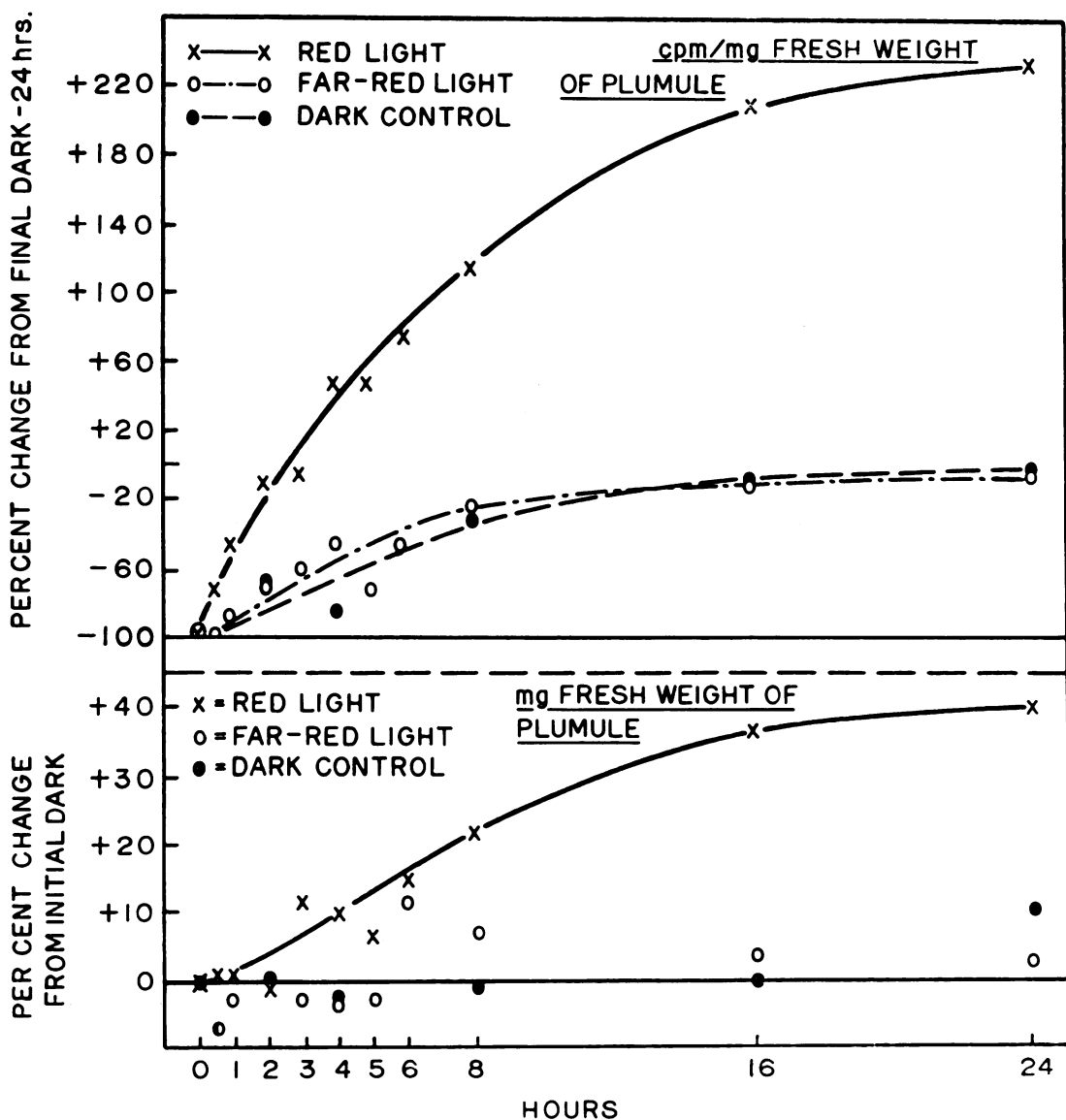


FIG. 7. Kinetics of <sup>14</sup>C-sucrose incorporation and of plumule growth.

hours and ceases after 16 hours. As for EIS, a significant R effect is obtained as early as 2 hours after irradiation, with a peak effect also at about 16 hours. The incorporation of  $^{14}\text{C}$ -sucrose in the dark is the same as after FR treatment. The radioactivity level is much less variable than growth, which is affected by the differences in initial weights of the plumules selected for the experiment.

*Escape from FR Reversibility and Decay of the R Effect.* It has been shown (10) that in stem sections of etiolated peas, the dark reversion of  $P_{FR}$  to  $P_R$  is complete after 4 to 6 hours, with about 55% of the phytochrome being lost. Similar results were obtained for the plumule, but the determination of phytochrome was complicated by the conversion of protochlorophyll to chlorophyll. In a more recent study (11) it has been shown that in *Pisum*  $P_{FR}$  may either revert to  $P_R$  or may decay to an inactive, nonphotoreversible form.

The nonphotochemical transformations of phytochrome and their effects on growth and  $^{14}\text{C}$  accumulation were studied in 2 ways. The first was to follow the escape from FR reversibility by spacing various intervals of dark time between the R and FR treatments. Parallel measurements of a typical experiment on the kinetics of R effects served as a basis for calculation of the escape from photochemical control. The results, presented in figure 8, were calculated as follows:

$$\text{percent inhibition by FR light} = 100 - \left( \frac{FR_n - R_n}{R_{24} - R_n} \times 100 \right)$$

where:

$FR_n$  = Value (plumule weight or cpm/mg fr wt of plumule) obtained after  $n$  hours between red and far-red light treatments.

$R_n$  = Value obtained  $n$  hours after red light treatment.

$R_{24}$  = Value obtained 24 hours after red light treatment.

If  $P_{FR}$  is the only effective molecule involved in these responses, we should expect complete escape from reversibility after about 6 hours, and indeed only about 15% reversal of the R effect by FR is evident for  $^{14}\text{C}$ -sucrose incorporation at this time. Results for fresh weight are less definite because of greater fluctuations in the data, but the trend is roughly the same.

In a further experiment we measured the decay of the R induced effect on growth and  $^{14}\text{C}$ -sucrose incorporation. Plants were irradiated in buffer, and after varying experimental periods (up to 8 hrs) in the dark, were transferred for an additional 24 hours into buffer containing the usual concentrations of carrier and labeled sucrose. The parallel dark controls were transferred simultaneously after equivalent periods of storage in buffer. To correct for plumule growth in dark and R treatments after the varying time intervals, plumules

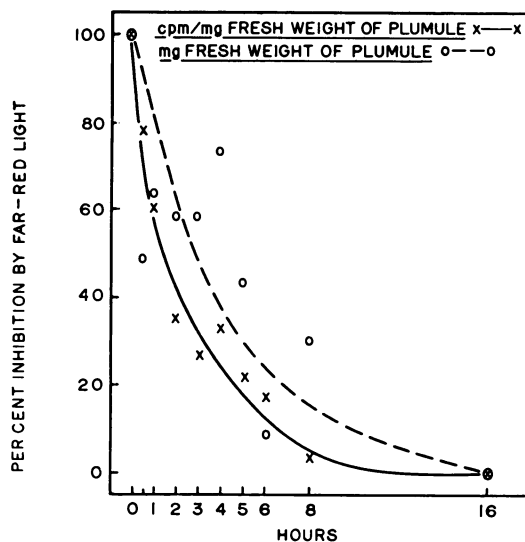


FIG. 8. Kinetics of escape from FR reversibility. Abscissa indicates interval between R and FR.

from both treatments were harvested at intervals for growth determinations. As a red light control, we employed seedlings irradiated in the usual way, i.e. directly in the nutrient solution containing  $^{14}\text{C}$ -sucrose. Typical results for EIS are shown in figure 9 and it is clear that the longer the time lapse between R irradiation and insertion into the nutrient solution, the less is the EIS effect. The data for IG (not shown) show a similar trend. These results indicate the occurrence of a dark reversion of  $P_{FR}$  to  $P_R$  or decay of the unstable

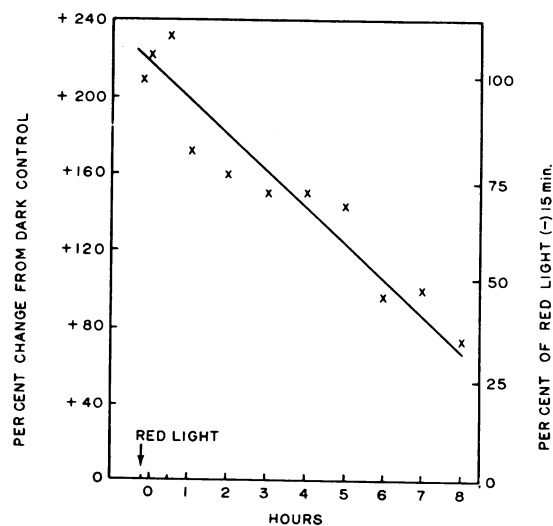


FIG. 9. Decay of the R effect on  $^{14}\text{C}$ -sucrose incorporation, as studied by spacing various periods of darkness between red light and insertion into the  $^{14}\text{C}$ -sucrose.

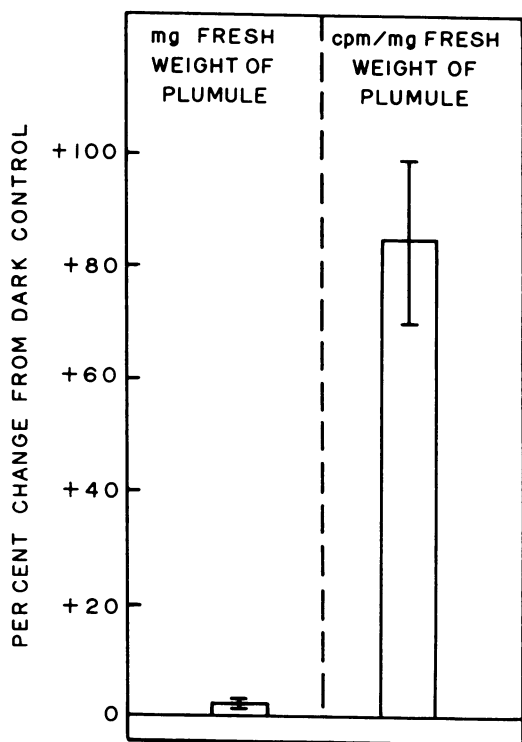


FIG. 10. Effect of R on  $^{14}\text{C}$ -sucrose incorporation in the absence of effects on plumule growth. The lines at the top of the bars indicate  $\pm$  standard error.

$P_{FR}$  to some inactive form of phytochrome. The kinetics agree with previous data on photoreversibility (10).

*Variations in Light Effects.* It has been previously noted (14) that marked variations in morphology and behavior can be observed from day to day in standard seedlings grown under apparently

identical conditions. In 5 out of about 90 experiments in the present investigations the plumule was unaffected by red light. The radioactivity of these R-exposed plumules was nevertheless measured and compared with dark controls. The results, presented in figure 10, point out again that the effect of phytochrome conversion on  $^{14}\text{C}$ -sucrose incorporation can occur whether or not the subsequent growth changes are permitted to manifest themselves.

*A Survey of Light Effects on the Uptake of Other Substances.* To investigate the specificity of the  $P_{FR}$ -promoted incorporation of  $^{14}\text{C}$ -sucrose, many other compounds belonging to various biochemical classes were surveyed. Maltose, fructose, glucose and ribose were selected as carbohydrates, L-phenylalanine and L-tyrosine as possible amino acid precursors of the cinnamic acid and *p*-coumaric moiety of flavonoid compounds (15), L-valine as an aliphatic amino acid, acetic and cinnamic acids as direct precursors of the A and B rings of the flavonoid compounds (15) and  $\alpha$ -ketoglutaric acid as a member of the tricarboxylic acid cycle.

Concomitant with its stimulatory effect on plumule growth, R inhibits the growth of the stem of the etiolated pea (2). To investigate the possibility that a competitive relationship between terminal bud and third internode might be involved in the incorporation of substrate and in growth, samples from all 3 internodes (see Materials and Methods) were harvested in addition to the terminal buds. The results are presented in table II.

The most striking fact evident from the data is that the photomorphogenic effects are most closely related to  $^{14}\text{C}$ -sucrose, which also shows a much higher level of radioactivity in the terminal bud and third internode than all other compounds tested. A slight promotive effect of R and lowered reversibility by FR was detected for all other sugars, the order being fructose > glucose > maltose

Table II. *Effect of Red Light on the Uptake and Distribution of Various Labeled Compounds.*

The data are given as cpm/mg fresh weight of plumules or of 10 mm sections taken from the middle of the first, second, and third internode.

Compound	Dark Control				FR $\rightarrow$ R				R $\rightarrow$ FR			
	Internode				Internode				Internode			
	1st	2nd	3rd	Plumule	1st	2nd	3rd	Plumule	1st	2nd	3rd	Plumule
Sucrose	0.40	0.84	6.00	1.80	0.33	0.58	5.50	3.40	0.38	0.61	6.00	1.90
Maltose	0.44	0.40	1.20	0.50	0.48	0.32	1.10	0.80	0.48	0.33	1.20	0.50
Fructose	0.58	0.58	1.50	0.90	0.57	0.55	1.30	1.10	0.62	0.57	1.51	1.00
Glucose	0.40	0.41	1.10	0.80	0.39	0.34	1.10	0.90	0.42	0.46	1.10	0.80
Ribose	0.41	0.24	0.60	0.50	0.41	0.27	0.60	0.60	0.44	0.28	0.70	0.50
L-Phenylalanine	0.24	0.11	0.32	0.33	0.26	0.11	0.39	0.44	0.30	0.15	0.36	0.41
L-Tyrosine	0.37	0.44	0.65	0.64	0.49	0.44	0.68	0.65	0.39	0.52	0.63	0.67
L-Valine	0.33	0.13	0.44	0.26	0.33	0.11	0.57	0.53	0.38	0.15	0.47	0.41
Cinnamic acid	0.20	tr.	0.15	tr.	0.16	tr.	0.13	tr.	0.21	tr.	0.16	tr.
Acetic acid	0.17	tr.	0.19	tr.	0.20	tr.	0.16	tr.	0.18	tr.	0.18	tr.
$\alpha$ -Keto-glutaric acid	0.20	0.23	0.60	0.30	0.20	0.21	0.60	0.60	0.24	0.22	0.70	1.00



> ribose. In each case, the highest level of radioactivity in stem was detected in the third (youngest) internode.  $^{14}\text{C}$ -sucrose was present in increasing amount from the first to the third internode, irrespective of light treatment, while the other sugars tested showed the same radioactivity in the first and second internodes. In no case involving carbohydrates was there any R-induced difference in the accumulation of  $^{14}\text{C}$  in the third internode; the R-induced incorporation seems to be specific for the plumule.

Replacing labeled sucrose by labeled amino acids greatly reduced the radioactivity detectable in the buds.  $^{14}\text{C}$ -Tyrosine moves faster and is accumulated more in all tissues than the other 2 amino acids. However, neither tyrosine nor phenylalanine incorporation was affected by red light, but the effect on valine was significant. The uptake of  $^{14}\text{C}$  from the organic acids was poor, only traces being found in the second internode and plumules, and a little more in the first and third internodes.

### Discussion

These experiments show that irradiation with red light greatly increases the incorporation of  $^{14}\text{C}$ -sucrose into the plumules of etiolated peas. The effect is almost totally restricted to plumular tissue, and is greatly reduced or absent when other substances are substituted for sucrose. This response occurs more rapidly than R-induced growth responses and is saturated by lower energy levels. The kinetics of loss of the R effect and of its escape from FR reversibility indicate control by the  $P_{FR}$  form of phytochrome.

The relative specificity of sucrose for this effect is not understood, but corresponds with similar reports for other photomorphogenic phenomena (2, 20). In a possibly related series of observations it has recently been shown (24) that red light enhances the utilization of starch and sugars in etiolated maize leaves.

The role of the cotyledons and first internode in the R effect on plumule growth and  $^{14}\text{C}$ -sucrose incorporation are also difficult to explain completely. Bonner et al. (1) found that more than 40% of the total dry weight of pea cotyledons is sucrose, and about 5% other carbohydrates. Wanner (27) concluded that sucrose is the transport form of carbohydrate in the pea plant and that glucose and fructose found in the roots result from cleavage of the sucrose transported from the cotyledons. Various authors (2, 13, 25) have found that about 2% sucrose is optimal for auxin-induced growth and photomorphogenic effects in etiolated pea epicotyl stem sections. For maximal IG we found a higher optimum concentration of sucrose (0.1 M = 3.42%), but for EIS the optimum concentration of carrier sucrose was 0.05 M or 1.71%. From figure 2, it is obvious that the concentration of carrier sucrose greatly affects the uptake of labeled sucrose, while increasing the concentration

of  $^{14}\text{C}$ -sucrose in the presence of optimal carrier sucrose yields a linear increase in the radioactivity found in the terminal bud (fig 3). These results could be interpreted in terms of the pressure-flow hypothesis, the high sucrose concentration at the source being necessary to generate a sufficient pressure to transport en masse to the sink.

Studying the escape from far-red reversibility (fig 8, 9) is one way to determine whether  $P_{FR}$  alone is responsible for the R effects reported in the present paper. The typical escape kinetics shown in these figures agree fully with *in vivo* studies on phytochrome disappearance (10). As  $P_{FR}$  in *Pisum* disappears both due to decay and reversion to  $P_R$  (11), it is difficult to conclude whether the intermediate formed during reversion (18) influences the phenomena studied in the present work. Irradiation with continuous R should prevent any decrease in  $P_{FR}$ , but figure 6 shows that the same response to red light is nevertheless obtained, at least as far as  $^{14}\text{C}$ -sucrose incorporation is concerned. On the other hand, in the experiment where red light was separated in time from  $^{14}\text{C}$ -sucrose administration (fig 9) 35% of the red light effect was still detected after 8 hours. At this time,  $P_{FR}$  is no longer present (10), contrary to the results of the experiments on escape from photochemical control in which FR was already ineffective after 6 hours. R still promotes the growth of the plumule and of  $^{14}\text{C}$ -sucrose incorporation. These results suggest that unknown intermediates in the decay process of  $P_{FR}$  may be also involved.

The known effects of cytokinins (21) and auxins (23) in causing a local accumulation of translocated organic materials suggests that they may play a role in the phenomena reported here. The second paper of this series will include experiments bearing on this and related questions.

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