Metabolism of Oat Leaves during Senescence

V. SENESCENCE IN LIGHT

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ABSTRACT

A comparison has been made of the progress of senescence in the first leaf of 7-day-old oat plants (Avena sativa cv. Victory) in darkness and in white light. Light delays the senescence, and intensities not over 100 to 200 ft-c (1000-2000 lux) suffice for the maximum effect. In such intensities, chlorophyll loss and amino acid liberation still go on in detached leaves at one-third to one-half the rate observed in darkness; however, when the leaves are attached to the plant, the loss of chlorophyll in 5 days is barely detectable. Transfer of the leaves from 1 or 2 days in the low intensity light to darkness, or vice versa, shows no carryover of the effects of the preceding exposure, so that such treatment affords no evidence for the photoproduction of a stable substance, such as cytokinin, inhibiting senescence. Light causes a large increase in invertase-labile sugar and a smaller increase in glucose, and application of 100 to 300 mm glucose or sucrose in the dark maintains the chlorophyll, at least partially. Correspondingly, short exposure to high light intensity, which increased the sugar content, had a moderate effect in maintaining the chlorophyll. However, 3-(3,4-dichlorphenyl)-1,1-dimethylurea (DCMU) completely prevents the increases in sugars and yet does not prevent the effect of light on senescence, whether determined by chlorophyll loss or by protein hydrolysis. Light causes a 300% increase in the respiration of detached oat leaves, and kinetin lowers that only partly, but unlike the increased respiration associated with senescence in the dark, the increase in the light is fully sensitive to dinitrophenol, and therefore cannot be ascribed to respiratory uncoupling. The increased respiration in light is prevented by DCMU, parallel with the prevention of sugar formation. It is therefore ascribed to the accumulation of soluble sugars, acting as respiratory substrate. Also, 1-serine does not antagonize the light effect. For all of these reasons, it is concluded that the action of light is not mediated by photosynthetic sugar formation, nor by photoproduction of cytokinin. Instead, we propose that light exerts its effect by photoproduction of ATP. The action of sugars is ascribed to the same mechanism but by way of respiratory ATP. This hypothesis unifies most of the observed phenomena of the senescence process in oat leaves, and helps to explain some of the divergent findings of earlier workers.

The four preceding papers of this series (4, 22-24) have been concerned with the senescence of the first leaf of the 7-day-old oat plant, held in darkness, either detached or attached to the whole plant. However, as a practical matter, leaves do senesce even in full light, and a few investigators (e.g. 2, 7, 25) have studied the process in daylight. Light has been found to delay senescence in leaves of the oat (3, 25, 26), wheat (8), potato (14), tobacco (20), rice (16), and Hibiscus (17); in the last instance, a long photoperiod was found more effective than a short one. In rice, red light was effective in delaying senescence and far red had the opposite effect (16). In the presence of EDTA, however, white light was reported to promote bleaching (11), and although this effect has twice been confirmed (9, 23), its relationship to normal senescence in light is not clear. Recently, Takegami (20) has found light to prevent the decrease in total RNA which occurs in senescing tobacco leaves. The effect differed in the different fractions, the fall in 16S and 23S components being somewhat inhibited, whereas the 18S and 25S fractions were actually increased by white light.

Several investigators have ascribed these effects of light to the photosynthetic production of sugars. Applying sucrose to detached leaves did delay senescence, at least for 48 hr, and to about the same extent as light (5, 14); it also inhibited the hydrolysis of RNA (5, 25). Our own experiments, reported below, show a moderate effect of glucose, or of endogenous sugar, on the senescence of the first leaves of oats, and glucose plus nitrate has been reported to exert some retarding effect on senescence of the fourth leaf of the same cultivar (27). However, the effects of sugars on senescing bean and wheat leaves were small (3, 7); indeed both in wheat and in Xanthium leaves, sucrose tends to promote the bleaching of Chl in the light (7, 10). In 1937, Vickery et al. (26) found that glucose was much less effective than light in decreasing protein loss in tobacco leaves. In detached cucumber cotyledons, in which light delays the Chl loss, 3% glucose imitates the effect of light on Chl loss but not its effect on proteolysis (12). Also, in the somewhat artificial system of wheat leaf segments treated with aminotriazole, light delayed Chl loss, and neither sugars nor DCMU had much influence (8). Thus, to explain the action of light on senescence as being due to sugar formation is certainly open to question. The present series of experiments has been carried out to make a more thorough study of the differences between senescence in light and in darkness and to elucidate the somewhat confusing comparisons between the effects of white light and those of sugars. Some interactions between attachment to the plant, exposure to light, and treatment with cytokinin have also been explored, and an attempt has been made to bring the effects of light into harmony with the concepts of the senescence process arrived at in preceding papers and by other workers. As a result, a general hypothesis is proposed to explain the influence of light and of some other factors on senescence.

MATERIALS AND METHODS

The experimental material consisted of the first leaves of oat seedlings (Avena sativa cv. Victory) being grown for 7 days under cool white fluorescent light (200 ft-c at the base of the plants), as used in the preceding papers (22-24). In most experi-
ments, the leaves or leaf segments were floated on the test solutions as described in ref. (22). Where infusion was desired, the 3-cm leaf segments were evacuated and vacuum-infiltrated. In some experiments, either the apical or basal halves of the leaves were shaded with a black Bakelite cap. For these experiments, the leaf segments were 4 cm long, and were maintained on three layers of wet gauze in a Petri dish.

DCMU (K. and K. Laboratories) was purified by dissolving in 1-propanol, clearing with activated charcoal, distilling off the propanol to the saturation point, and then precipitating by adding water at 0 C.

Sugars, DNP, and kinetin were of the highest purity commercially available.

The cool white fluorescent light was measured by a Weston light meter calibrated against an Evans electro-selenium photometer.

**RESULTS**

**General Course of Senescence in Light.** Table I shows that white light (about 150 ft·c) powerfully decreases the rate of senescence, as measured by Chl disappearance. The decrease is not total, for about 20% of the initial Chl content disappears in the light in 4 days (at 25 C), but this contrasts with a disappearance of about 85% in the same period in the dark. In attached leaves (exp. 3), the effect is indeed total (cf. Fig. 2C below). For detached segments, the maintenance of Chl would have been no greater at higher light intensities—indeed even somewhat less, for the reason shown in Figure 1. Here, the relationship between Chl content after 3 days and intensity of white light is plotted. It will be seen that saturation is reached at the very moderate value of about 150 ft·c or 1500 lux. At intensities six or seven times this level, the effect remains about the same, but at still higher intensities the Chl content tends to decrease, possibly due to photooxidation. Preliminary measurements at a series of different wavelengths have indicated that the red and blue regions are about equally effective in this respect, green and far red having lesser, though positive, effects. This is in contrast to the behavior of rice leaves, in which far red light has been reported actually to accelerate senescence (16).

Figure 2, A and B shows the time course of loss of Chl, and the increase in free amino acids, in light (200 ft·c) and in the dark. In both cases, the retardation of senescence is clear; but the effects are both about equally incomplete; in 6 days the Chl content falls to 50% of the initial, while the amino acids rise to a little over half of the dark value. Up to the 3rd day, Chl is well retained (cf. Table IV below).

It was shown in part II (24) that attachment to the plant brings about a moderate delay of senescence in the dark. Figure 2C shows that the combined retardations due to light and to attachment result in almost complete cessation of senescence. The free amino nitrogen, which in the dark shows a rise and then a fall, due to translocation into the roots (cf. 24), shows no rise at all in the light, during the period studied (Fig. 2D). Although light merely retards senescence in detached leaves, it prevents it altogether, for at least 5 days, in leaves attached to the plant.

**Durability of the Light Effect.** If light acts by synthesis of a cytokinin, or conversely if darkness acts by allowing the synthesis of a “senescence factor,” some carrier of the effect of light into a subsequent dark period, or vice versa, would be expected. Experiments on transferring leaves from 1 to 2 days of exposure to light into darkness (Fig. 3) do not show such an effect. If the detached leaves are held in light for a time and then transferred to darkness, the progress of senescence in the dark period is not retarded by the preceding light exposure, even if high light intensity has been used (Fig. 3A). Correspondingly, the senescence rate in light is not accelerated by preexposure to darkness, at least for the first 2 days (Fig. 3B). After the 2nd day in the dark, the progress of senescence seems to have become essentially irreversible, in the same way as senescence appeared to have “escaped” from control by kinetin after 2 days in the dark (see Fig. 6A of ref. 22). We conclude that if the action of light were due to the photoproduction of a senescence-retarding substance other than sugars (see under “Role of Sugars”), then such substance cannot remain in the leaf for as long as 1 day.

**Transportability of the Light Effect.** A number of experiments similar to those described in part II (24) were carried out to explore the extent to which the light effect was localized. For this purpose, apical segments 4 cm long were cut and the apical and basal 2-cm halves analyzed after senescence with one or both halves illuminated. Table II gives one example; two other series of experiments gave very similar results. In the table, A = apical segment, B = basal segment. Although there are some slight inconsistencies, the following conclusions are justified. (a) The action of light in retaining protein is partly transmitted from A to B, but much less so from B to A. (b) When B is dark and A light, much more amino-nitrogen moves from A to B than when A is dark and B light. Thus the amino-nitrogen here is subject to the strongly polar basipetal transport already described (24). (c) When A is lighted, Chl retention in B (in darkness) is marked, but when B is light, the retention in A is only about half as great. (d) The transport of sugars from light to dark is somewhat greater from A to B than from B to A, so that the sugars do show a slight polarity in transport. (e) When both segments are treated in the same way (whether both in light or both in dark), the sugars show no sign of movement in either direction.

Another similar group of experiments shows that the application of kinetin in light has only a small further effect in maintaining the protein, but does lower the reducing sugar by about 50%. This is in line with the general conclusion previously reached both by us and others (e.g. 18) that a major effect of the cytokinins is to inhibit hydrolytic processes of several kinds.

**Table I**

<table>
<thead>
<tr>
<th>Initial</th>
<th>After 4 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>In dark</td>
<td>In light</td>
</tr>
<tr>
<td>Expt. 1</td>
<td>0.875</td>
</tr>
<tr>
<td>Expt. 2</td>
<td>0.690</td>
</tr>
<tr>
<td>Expt. 3</td>
<td>0.895</td>
</tr>
</tbody>
</table>

**Fig. 1.** Chl contents of detached 3-cm oat leaf segments after 3 days in white light of a series of intensities.

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2 Abbreviation: DNP: 2,4-dinitrophenol.
Role of Sugars. The first point to establish in regard to the sugars is whether light does cause the expected formation of sugars in these detached leaves. Figure 4 shows that indeed white light causes rapid formation and accumulation both of reducing sugars and of "sucrose." (The term "sucrose" is used to denote the invertase-liberated reducing sugars. Much of this material is actually oligosaccharides of fructose, since the storage material in these leaves is mainly fructan [21]. It is evidently mobilized with great readiness.) Kinetin, 3 \( \mu \text{g/ml} \), consistently decreases the accumulation of reducing sugars by about one-third of the control value, but it has little clear effect on the invertase-liberated sugar. Other data, obtained gravimetrically, show that kinetin has no effect on the total photosynthetic dry matter production. The large sugar accumulation in these leaves is doubtless the result of their being detached so that sugars cannot be transported out. In this respect, they are not closely comparable with discs cut out of dicotyledonous leaves, such as have often been used in past senescence studies, for those preparations do lose considerable quantities of substances to the ambient solution. As seen in Table II, sugars produced in oat leaves, when they are evenly illuminated, show very little transport either way.

The second point, however, and the critical one, is that nevertheless the accumulation of sugars can hardly be the primary mechanism of the light-induced retention of Chl. The reason is that the sugar accumulation can be almost wholly prevented by DCMU, which is well known to inhibit photosynthetic electron transport, though not cyclic photophosphorylation. Table III shows the effect of 0.1 mm DCMU on the sugar and Chl content of leaf segments after 3 days in white light. The reducing sugar has been maintained at almost its initial low value, and the sucrose is even lowered from its initial value, yet the Chl retention is nearly complete. In dark controls, this concentration of DCMU has virtually no effect on the loss of Chl, nor (Fig. 6) does it affect the loss of protein and gain of free amino nitrogen. Similar behavior is shown in more detail and compared with the action of kinetin in Figure 5. Here, DCMU was used at a series of concentrations, white light was present throughout, and a duplicate series was floated on kinetin as well. Clearly DCMU at about \( 2 \times 10^{-6} \) M prevents the formation of reducing sugar and has a smaller, perhaps indirect, effect on the sucrose. The effects of DCMU and kinetin in this respect are apparently additive.

Figure 6, A and B, shows the effects of DCMU and of kinetin on the proteolysis. For comparison, the behavior in the dark is plotted in dashed lines; DCMU, as expected, does not influence the proteolysis that occurs in the dark. (Rather surprisingly, DCMU at concentrations above \( 10^{-6} \) M interferes with the action of kinetin; at the highest concentration tested \( [5 \times 10^{-4} \text{ M} \), which is a solution saturated with DCMU], there is about a 20% loss of protein in spite of the presence of kinetin. These are doubtless secondary effects, comparable to those which have been reported elsewhere for such high concentrations.) In general, DCMU does not affect proteolysis at levels where it does drastically inhibit sugar formation. But the main point is that light in the presence of DCMU is about as effective in preventing

In general, then, the results of illumination are to a moderate extent transportable. The polarity of movement of amino acids is as clearly shown when light is the controlling factor as it was in the earlier experiments (24) in which kinetin was the controlling factor. In those experiments, the presence of kinetin was able to induce acropetal movement of amino acids against the normal basipetal polarity, but in Table II, light has no such effect. Evidently, the actions of light and kinetin are by no means identical.

![Figure 2](image_url) Changes in Chl (left) and \( \alpha \)-amino nitrogen (right) in light (○) and dark (●). Above: detached leaves; below: leaves attached to the plant.

![Figure 3](image_url) Loss of Chl in continuous darkness (○), continuous light (●), or after transfer from one to the other on day 1, day 2, or (from light to dark) day 3.

In general, then, the results of illumination are to a moderate extent transportable. The polarity of movement of amino acids is as clearly shown when light is the controlling factor as it was in the earlier experiments (24) in which kinetin was the controlling factor. In those experiments, the presence of kinetin was able to induce acropetal movement of amino acids against the normal basipetal polarity, but in Table II, light has no such effect. Evidently, the actions of light and kinetin are by no means identical.

### Table II

<table>
<thead>
<tr>
<th>Pair No.</th>
<th>Treatments</th>
<th>Chl in ml/seg</th>
<th>( \alpha )-amino N</th>
<th>Protein N</th>
<th>Reducing Sugar</th>
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<tbody>
<tr>
<td></td>
<td>Initial A</td>
<td>0.640</td>
<td>251</td>
<td>277</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td>Initial B</td>
<td>0.738</td>
<td>256</td>
<td>376</td>
<td>137</td>
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<tr>
<td>1</td>
<td>Light A</td>
<td>0.564</td>
<td>691</td>
<td>272</td>
<td>996</td>
</tr>
<tr>
<td>1</td>
<td>Light B</td>
<td>0.629</td>
<td>837</td>
<td>368</td>
<td>1067</td>
</tr>
<tr>
<td>2</td>
<td>Dark A</td>
<td>0.515</td>
<td>650</td>
<td>278</td>
<td>959</td>
</tr>
<tr>
<td>2</td>
<td>Dark B</td>
<td>0.414</td>
<td>23.56</td>
<td>355</td>
<td>329</td>
</tr>
<tr>
<td>3</td>
<td>Light A</td>
<td>0.305</td>
<td>1560</td>
<td>190</td>
<td>335</td>
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<tr>
<td>3</td>
<td>Light B</td>
<td>0.469</td>
<td>1586</td>
<td>243</td>
<td>973</td>
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<tr>
<td>4</td>
<td>Dark A</td>
<td>0.188</td>
<td>1728</td>
<td>140</td>
<td>261</td>
</tr>
<tr>
<td>4</td>
<td>Dark B</td>
<td>0.187</td>
<td>1035</td>
<td>146</td>
<td>261</td>
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</tbody>
</table>
Effect of DCMU, 0.1 μM, on sugar accumulation and chlorophyll retention after 3 days in white light, 200 ft-c (and dark control). The effects of kinetin and light are here similar and partly additive. It can be concluded from these data that the main effect of light in retarding senescence is not exerted via the production of carbohydrate. That it could be exerted via the production of a cytokinin is not excluded by these data; the fact that high levels of DCMU inhibit the actions both of light and of kinetin might be considered suggestive in this connection. But the general lack of carryover of a stable senescence-retardant from light to dark-proteolysis as kinetin is. Haber et al. (8) also noted that light could exert its effect in the presence of DCMU. The effects of kinetin and light are here similar and partly additive.
ness, discussed above, seems to exclude such formation of cytokinin.

The conclusion that light does not delay senescence through sugar formation seems at first to be at variance with the findings of earlier workers mentioned in the introductory paragraphs, who found that treatment with sugars delays senescence much as light does. We have, therefore, studied the effects of treatment with sugar solutions on the Chl (Fig 7). After 3 days in the dark at 26 C, the Chl content of controls has fallen to less than half of the initial value, and 0.1 mM glucose restores some two-thirds of this loss. Sucrose is less effective. The action is not ascribable to osmotic effects because mannitol is inactive. It had previously been shown (22) that both glucose and sucrose are readily oxidized in these leaves, 0.3 mM glucose increasing the O₂ consumption by 80%.

Preliminary experiments show that high light intensities, e.g., 1500 ft-c, for periods of not over 12 hr, can increase the sugar content of the leaves to the point where it is about as effective in delaying senescence, in a subsequent dark period, as exogenous 0.3 mM glucose. These intensities are some 10 times higher than is needed for the maximum light effect in delaying senescence. Thus, glucose can imitate at least a good part of the action of light, even though, paradoxically, light itself seems to act through another mechanism.

Changes in Respiration during Senescence in Light. The data on respiration just mentioned showed that when the leaf segments are infiltrated with glucose, not only does the O₂ consumption increase, but the respiratory quotient (R.Q.) comes up from its normal low value to approach the theoretical 1.0. For example, in one of the present experiments, 0.3 mM glucose raised the O₂ consumption rate from 3.3 to 5.3 µl/segment/hr and raised the R.Q. from 0.84 to 1.04. The respiration is evidently quite sensitive to substrate concentration. Table IV shows that while after 3 days in the light the respiration rate has more than doubled, DCMU has held the rate down to its initial level. As was seen in Table III, the reducing sugar and sucrose have been correspondingly held down to about their initial levels. (We have found that by preincubating with DCMU the sugar content can even be brought below the initial level.) Table IV shows that the R.Q., which had risen in 3 days on water in parallel with the obvious rise in sugar oxidation, was wholly prevented from increasing by the DCMU.

Figure 8 shows the effect of kinetin on the respiration when applied after 1 full day in the light. During the light period, the respiration rate has increased greatly (in line with the increase in respirable sugars) but the effect of kinetin in stopping further increase follows closely its effect on the respiration during senescence in darkness (Fig. 5 of ref. 22). Figure 9 shows that in continuous light, kinetin still lowers the respiration, but only to a slight extent, consistent with the decrease in monosaccharides which the kinetin causes.

It seems justified to infer that the increase of respiration in light is due to the accumulation of respirable sugar; the inhibition of polysaccharide hydrolysis in the dark by cytokinin—one either prevents the respiratory increase completely.

In Part I of this series (22), we deduced that the increase in respiration shown by leaves senescing in darkness could be due only in small part (perhaps some 25%) to the liberation of respirable sugars and amino acids. The remainder of the increase was ascribed to respiratory uncoupling. Treatment with kinetin not only lowered the respiration rate but rendered it much more sensitive to DNP. Thus we proposed that kinetin maintains or even tightens the respiratory coupling. It follows that the respiratory rise caused by light has a different basis from that caused by dark senescence, and that for the rise in light, the accumulation of respirable substrate, i.e., sugars, provides an adequate explanation.

Support for this conclusion is provided in a striking manner by Figure 10. Here the leaf segments were floated on water or 500 mM glucose (together with 0.1 mM penicillin G to minimize infection) both with and without 3 µg/ml kinetin. The respiration rate on water increases to about 2.5 times the initial, as was previously recorded (Fig. 5 of ref. 22). Kinetin represses this completely. A rise comparable to that on water is caused by glucose, yet kinetin, although in a concentration which has maximal effect on senescence, scarcely lowers this glucose respiration at all. Thus, the increased respiration due to glucose is kinetin-insensitive, while the increased respiration due to dark senescence is fully sensitive to repression by kinetin.

That the respiratory rise in the light has a different basis from that which occurs in the dark has been confirmed by a number of experiments with DNP. Typical results with DNP are shown in Table V. The figures from a simultaneous dark experiment are included for comparison. It may be seen that, as was previously found (Table V of Ref. 22), after senescence in the dark, respiration has become so uncoupled that DNP cannot increase it much more, even though applied at the optimum concentration and pH. Kinetin counteracts this effect, lowering the absolute respiration rate and maintaining DNP sensitivity. However, senescence in light presents a contrast. Although the respiratory rate is about as high as in the dark, it remains tightly enough coupled that DNP can still produce a large increase. The absolute values of the increase caused by DNP in light, namely 2.70 in water and 2.61 in kinetin, are actually greater than the increase of 1.92 due to DNP in the presence of kinetin in the dark. Also, kinetin exerts only a slight effect in lowering the respiration of the light-senesced leaves and it does not significantly increase their sensitivity to DNP (Fig. 10).

Apparent Parallelism between the Effects of Light and of Cytokinin. It is clear that in general, light and kinetin act in the same direction, both apparently maintaining an effective supply of ATP. The main difference between the effects of light and kinetin is that kinetin lowers the respiration rate while light increases it. The latter result is now explained by the photosynthetic formation of sugars, which kinetin acts to polymerize (or to maintain in the polymerized state). That kinetin prevents the liberation of reducing sugars from the polysaccharide in the leaf has now been amply documented.

An additional test of the above is furnished by the reaction of the leaves to L-serine. In these leaves, L-serine rather specifically antagonizes the action of kinetin, strongly promoting senescence in the dark (15); 50 mM L-serine even completely nullifies the effect of 5 µg/ml (0.025 mM) kinetin. The action of L-serine in the light is different.

Figure 11 shows that the effect of serine in the light is minimal. Even at 50 mM L-serine, the Chl loss after 3 days in the light is only 10%. If light were producing a cytokinin, it would have to be at an effective concentration many times the equivalent of 3 µg/ml kinetin, in order to be so insensitive to serine. Such a concentration is quite excluded by several of the experimental findings above. We must conclude that light achieves its cytokinin-
Figs. 8 and 9. Respiration and the effects of light and kinetin on it. Figure 8 (left): respiration of the 3-cm oat leaf segments in the dark after 1 full day in white light (1500 lux). Figure 9 (right): respiration of 3-cm oat leaf segments in continuous light (○) or darkness (●) and the influence of kinetin 3 μg/ml in lowering it.

Fig. 10. Showing that the increase in respiration due to darkness alone is repressed by kinetin, while the increase in respiration due to exogenous glucose (which causes partial retention of the Chl) is not repressed by the same concentration of kinetin (3 μg/ml).

### Table V

<table>
<thead>
<tr>
<th>Pre-Treatment</th>
<th>Then Infused</th>
<th>Chlorophyll a (μg/mg)</th>
<th>O2 Uptake (μl/mg/hr)</th>
<th>Increase due to DNP: as percent of water control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Water</td>
<td>0.055</td>
<td>5.88</td>
<td>+0.21 + 3.6</td>
</tr>
<tr>
<td>Water</td>
<td>Kinetin</td>
<td>0.783</td>
<td>5.51</td>
<td>+1.92 +53.3</td>
</tr>
<tr>
<td>Water</td>
<td>Kinetin + Glucose</td>
<td>0.816</td>
<td>5.90</td>
<td>+2.61 +44.2</td>
</tr>
</tbody>
</table>

nin-like effect without actually forming a cytokinin. Both act to promote the synthesis, or more probably to inhibit the hydrolysis, of polysaccharides, proteins, and RNA.

The most probable remaining explanation of the effect of light is that light provides ATP by cyclic photophosphorylation. The ATP is used for the continuous resynthesis of polymers. Cytokinin, acting in the dark by tightening the coupling between oxidation and phosphorylation, would enable the smaller amount of mitochondrial ATP to exert its full effect, and thus have the same ultimate effect as light. Sugars would operate in the same direction also, by way of the mitochondria.

This concept would explain the relatively low light intensity needed for saturation of the light effect (Fig. 1), since only an
amount of ATP about equal to that yielded by mitochondrial oxidation appears to be required. Cyclic photophosphorylation, which is largely immune from inhibition by CMU and DCMU (6), is generally considered to be small in amount compared to the noncyclic type. Also, if the photoassimilation of glucose is considered to be a measure of the cyclic process, then it is highly suggestive that that process reaches light saturation at low light intensity (13). Thus, while this is still a hypothesis, it is the only one that has survived our numerous experimental tests, and it brings together a number of widely different types of experiments.

Two additional studies in the literature may be mentioned; one (1) is apparently in conflict with this view; the other (19) is parallel in our findings. In the first, intense light has been found to cause a partial uncoupling in isolated mitochondria, which would be an effect in the opposite direction from that found here. But the use of isolated mitochondria entails quite different conditions from those prevailing in the leaf; e.g. isolated chloroplasts senesce at a quite different rate from chloroplasts in the leaf (4). Furthermore, the very high light intensities (8000 ft-c) used in those experiments fall far to the right of our highest intensity. The second system, which may have closer parallelism to senescence, is that of "glucose-bleached" cells of *Chlorella*. These cells are stimulated to form Chl by white light; the effect saturates at about 1000 lux, and CMU does not inhibit it (1). Since sugar is provided, there would be every reason to consider this reaction limited by ATP. All in all, we feel that our interpretation is well supported.

**Fig. 11.** Action of L-serine in opposing the action of kinetin in the dark, but not opposing either the effect of light, or the effect of kinetin in the light.