

when inactive, denatured, virus RNA is incorporated into a reconstituted nucleoprotein.

It should be emphasized that the present data do not, in our opinion, permit a choice between the foregoing alternative explanations. The possibility that small amounts of otherwise inactive virus RNA components may, when incorporated into DNA-bearing nucleoproteins, render the polymer infectious is subject to experimental verification. The same is true of the alternative proposal that TMV protein may induce some biological activity in an indifferent nucleic acid incorporated into a reconstituted nucleoprotein. It is hoped that such investigations will permit an unequivocal explanation of the results just described.

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## REVERSAL OF THE LIGHT INHIBITION OF PEA STEM GROWTH BY THE GIBBERELLINS\*

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Gibberellin A (isolated by Yabuta and Hayashi<sup>1</sup>) and gibberellic acid (isolated by Cross<sup>2</sup>) represent a group of compounds which are rapidly coming to be recognized as of major importance in the physiology of higher plants. Phinney<sup>3</sup> has shown that the application of gibberellins will restore single-gene dwarf mutants of *Maize* to the normal phenotype, extending the results of Brian and Hemming,<sup>4</sup> who found that the application of gibberellic acid to dwarf varieties of *Pisum* resulted in growth rates equivalent to that of normal varieties. Lang<sup>5</sup> has shown that gibberellin will replace the vernalization requirement of biennial *Hyoscyamus niger* and, at higher doses, will replace the long-day requirement for flowering in this plant as well. Thus the gibberellins are active in promoting a response to at least two separate physiological phenomena which have, in the past, been inaccessible to chemical regulation.

Among other physiological responses which we are as yet unable to explain or reproduce experimentally are many of the responses of plants to light. Numerous

morphogenetic and growth responses in the plant are controlled by light, but the mechanisms of most of these responses are little understood. One of the striking effects of light on plant growth is the inhibition of stem elongation. The inhibition of stem growth in peas has been intensively studied, particularly in the case of dwarf varieties.<sup>6, 7</sup> It has been shown that radiation of approximately 650 m $\mu$  wave length is most effective, although radiation of any wave length throughout the visible spectrum is active to some extent in the inhibition of stem elongation.<sup>6, 8</sup>

The gibberellins have been shown to stimulate plant growth principally through their effect on cell elongation.<sup>9, 11</sup> Their activity is distinct from that of auxin, which also is effective in stimulating plant cell elongation.<sup>9, 12</sup>

Since light inhibits stem elongation and this inhibition cannot be reversed by auxins, the gibberellins, which also appear to promote stem elongation, were examined for their effect on the elongation of pea stems in light.

#### MATERIALS AND METHODS

Alaska pea seed (Ferry-Morse Seed Company, Los Angeles), a nondwarfed variety of *Pisum sativum*, were washed, soaked for 6–8 hours, and planted in moist vermiculite in stainless-steel trays. They were grown at 25° C. in complete darkness, except for brief exposures to weak blue or green light during watering and experimental manipulations. A standard light inhibition of growth was provided by passing the light from a 100-watt Mazda bulb through an orange glass filter (Corning No. 348). The plants were placed 50–60 cm. below the light. At the time of the experiment the plants usually were pulled gently from the vermiculite and transferred to small glass bottles containing tap water. The growth of the plants in the bottles appeared completely normal for at least 4 days.

The elongation of the second internode of the Alaska pea seedling is particularly sensitive to inhibition by light;<sup>6</sup> therefore, the experiments described here were concerned especially with the elongation of this region.

The gibberellins used in these experiments consisted of mixtures of gibberellin A and gibberellic acid.<sup>13</sup> For application to intact seedlings, the mixture was dissolved in 95 per cent ethanol and applied with a syringe and hypodermic needle. A No. 27 needle was found to present alcoholic drops of a volume of 0.004 ml. The concentrations of gibberellin usually used (100–250  $\mu$ g/ml) thus provided a dose of 0.4–1.0  $\mu$ g. per plant. Ten to 20 plants were used per treatment, and each experiment was repeated at least once.

#### EXPERIMENTAL

*The Reversal of Light Inhibition of Stem Elongation.*—Five-day-old dark-grown Alaska pea seedlings with the second internode just beginning to elongate were treated with 0.4  $\mu$ g. of gibberellin per plant. One-half of these treated plants were placed under light (orange filtered, as described above), and the rest remained in darkness. An equal number of untreated plants were placed in light and darkness. The average length of each internode of each of the four groups of plants was determined 2 and 4 days later. The results are presented in Figure 1. The plants which were placed in light are indicated by the light shaded bars, and those which remained in darkness by the darker shading. The breaks in the bars delineate the nodes.

It may be seen that the light treatment resulted in a marked inhibition of growth, and this inhibition is completely reversed by the gibberellin treatment. The treated plants, whether grown in light or in darkness, are almost identical in height, and the dark-grown controls are only slightly shorter. The gibberellin treatment, then, completely counteracts the light inhibition of stem elongation. In the numerous experiments which have been run, the gibberellin-treated plants in light and darkness grow to almost identical heights. The untreated, dark-grown plants show a greater variation in growth rate. Rates of elongation equal to those of the treated plants are observed, but often the growth is slightly less. The response of the dark-grown plants suggests that the level of "natural gibberellin activity" in the normal plants may sometimes limit the rate of growth in darkness as well as in light.

Another observation which may be made as a result of the findings described in this experiment is on the rate of node formation. The light-grown plants have formed more nodes than those plants grown in darkness, regardless of the rate of growth in height. This would indicate that, while the light inhibition of stem elongation may be reversed by the gibberellin, these compounds have no effect on the light control of node formation. It has, in fact, previously been impossible to separate the specific influence of light on node formation from the effect on the rate of stem elongation.

The expansion of leaves also is known to be promoted by red light.<sup>6</sup> In the experiments reported here, this phenomenon was confirmed, and it was found that the gibberellin treatment had no effect on the light-controlled expansion of leaves.

There are in this experiment three separate processes which are regulated by light. Gibberellin is shown completely to reverse one of these effects, namely, that on stem elongation, but to be without effect on the other two. It is clear, then, that the gibberellin is not active in this case in a reversal of the photochemical reaction but rather exerts its effect more or less directly on the stem-elongation process. The applied gibberellin, in effect, counteracts or circumvents the control mechanism through which light normally exerts its effect on stem growth.

*A Comparison of the Effects of Gibberellin and Indoleacetic Acid (IAA) on Light Inhibition of Stem Elongation.*—Since auxin is known to affect and presumably to control cell elongation in plants, it has been presumed in the past that auxin must be responsible for the effects of light on stem growth. Auxin has, in fact, been shown

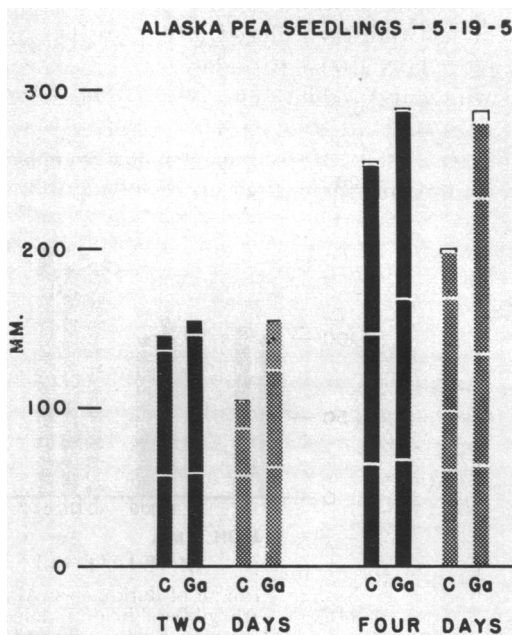


FIG. 1.—Growth of dark-grown Alaska pea seedlings 2 and 4 days following treatment with gibberellin ( $0.4 \mu\text{g}/\text{plant}$ ) in red light (*light shading*) or darkness (*heavy shading*). Plants were 5 days old at the time of treatment.

to reverse the light inhibition of the growth of pea stem sections.<sup>14</sup> It has, however, been impossible to reverse the light inhibition of the growth of intact seedlings by applied auxin. It was considered desirable, nevertheless, to repeat the experiments in which auxin was applied to light-inhibited seedlings and to compare the effects directly with the response to gibberellin. The results of such an experiment are illustrated in Figure 2. Single alcoholic drops of solutions of the two compounds, at the concentrations indicated, were applied to dark-grown plants, and the seedlings were then placed in light or kept in darkness. The measurements were made 2 days after treatment. The same convention for indicating light- or dark-grown plants used in Figure 1 is used here.

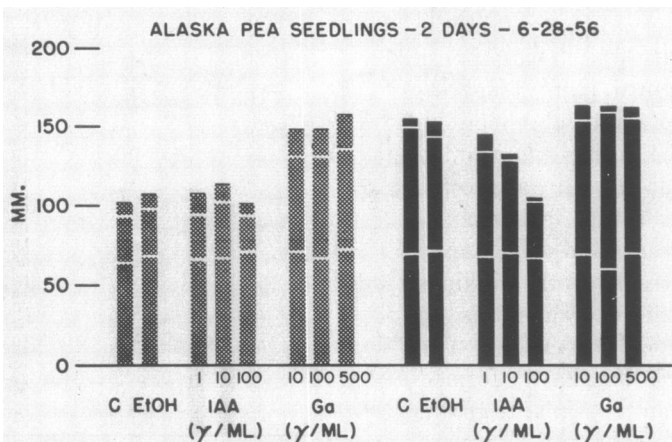


FIG. 2.—Growth of dark-grown Alaska pea seedlings 2 days after treatment with gibberellin or indoleacetic acid (0.01-ml. alcoholic drops). Plants were 5 days old at the time of treatment and transfer to red light (*light shading*). Heavy shading indicates plants which remained in darkness. C indicates the controls, while *EtOH* indicates plants treated with equivalent alcoholic drops.

On the light-grown plants the IAA has no significant effect, except a possible inhibition of growth at the highest concentration. On the dark-grown plants the IAA appears inhibitory at all concentrations. In all cases of inhibition by IAA the growth of the stem was abnormal; swelling of the growing region was observed approximately proportional to the auxin concentration. The gibberellin reversed the light inhibition, as previously described. The full effectiveness of the 10  $\mu\text{g}/\text{ml}$  concentration of gibberellin (0.04  $\mu\text{g}/\text{plant}$ ) indicates that the usual concentrations used (0.4–1.0  $\mu\text{g}/\text{plant}$ ) are optimal for this response. Controls to which an alcoholic drop was applied (*EtOH*) indicate that the alcohol alone had no effect.

*The Effect of the Gibberellins on Light- and Dark-grown Dwarf Peas.*—Six-day-old dark-grown dwarf pea seedlings (Morse's Progress No. 9, Ferry-Morse Seed Company) growing in plastic racks were selected for uniformity and treated with 0.01-ml. alcoholic drops of gibberellin at the concentrations indicated. Half the plants were then returned to darkness, while the other half were placed under the orange light. The length of each internode was measured 4 days later, and the average length of each internode for each treatment is illustrated in Figure 3. The results indicate that both the light-grown and the dark-grown plants respond to the gibberel-

lin treatments, and, further, at the higher concentrations the growth in light and darkness approach an identical maximum rate. Experiments with higher concentrations of gibberellin (1.0, 2.0, 4.0  $\mu\text{g}/\text{plant}$ ) have confirmed that a maximum stimulation is obtained with 1.0  $\mu\text{g}$ . gibberellin per plant, and the growth in light and that in darkness are equal at higher concentrations.

These results are consistent with the hypothesis that the growth rate of dwarf plants is limited by the availability of a physiological equivalent of the gibberellins, but they are not devoid of "gibberellin" activity—indicated by the fact that the growth rate of dwarfs is reduced in light compared to darkness, and this light inhibition is reversed by application of gibberellins. That is, the dark-grown dwarfs must still contain an active "gibberellin" factor, since their growth is further repressed by light, a repression which is reversed by the gibberellins.

#### DISCUSSION

The results presented here demonstrate that some factor in the plant, which may be replaced by gibberellin, controls the relative rate of stem elongation in light and darkness. In the non-dwarf Alaska peas the gibberellin factor is normally present at optimal amounts in darkness, allowing maximum growth within the limits of some other unknown limiting factor. This unknown factor may be seen to be independent of light, since the maximum growth rate with added gibberellin is identical in light and in darkness. In the dwarf pea variety used here, the same type of limiting factor seems to be operating, since here, too, when gibberellin is not limiting, the rate of growth is independent of light.

The inhibition of stem elongation by light might take place as a result of the operation of several alternative mechanisms: (1) light may inactivate, temporarily or permanently, the natural gibberellin factor; (2) light may render the elongating cells incapable of responding to physiological amounts of the natural gibberellin factor; or (3) light may interfere with the movement of the natural gibberellin factor from the area of production to the region of elongation. The application of gibberellin, then, would either replace the natural gibberellin, or it would flood the control mechanism, in either case restoring the growth rate to that of the nonlimiting condition. It is already known that light, to be effective, must fall on the region of the stem which is in the process of elongation,<sup>7, 14</sup> ruling out certain alternative explanations.

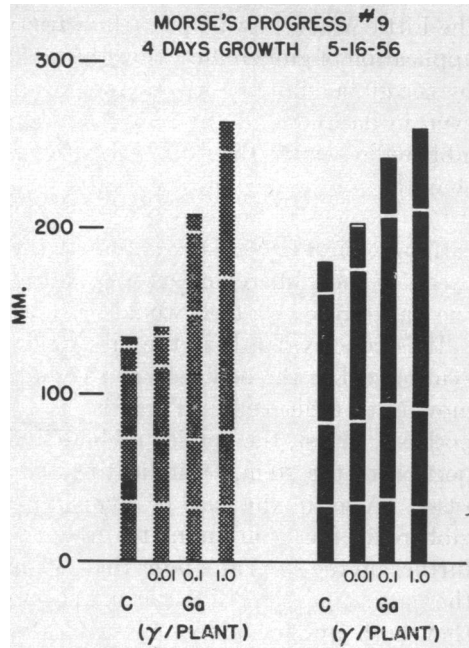


FIG. 3.—Growth of dark-grown Morse's Progress No. 9 dwarf pea seedlings 4 days after treatment with gibberellin at the doses indicated. Plants were 6 days old at the time of treatment. Heavy shading indicates growth in dark, and light shading indicates growth in red light.

The phenomenon of compensatory growth, described by Went<sup>6</sup> for a dwarf pea seedling, would seem to provide some evidence for the mechanism of the light inhibition of stem growth. Went observed that if the seedlings were given a single, rather brief exposure to light, their subsequent height was almost unaffected, although comparison of the lengths of the various nodes clearly showed that the nodes which were elongating at the time of the light treatment were considerably shortened, while subsequent nodes were longer than the controls. This demonstrates a compensating increase in growth rate after the light exposure, resulting in a recovery of the maximum height. Experiments by the author have shown that the Little Marvel dwarf pea (the variety used by Went) will respond fully to the application of gibberellin; thus the rate of growth of these seedlings is indeed limited by the gibberellin factor. Therefore, the rate of growth of these plants is limited, even in darkness, by the gibberellin; if the light were indeed destroying the natural gibberellin factor, the rate of growth on return to darkness could hardly be greater than that of the dark-grown plants. However, if the light were affecting the capacity of the cells to respond to the available gibberellin factor or were only temporarily interfering with the action of the gibberellin, then on return to darkness an excess of the gibberellin factor would be available and an increase of growth over the dark-grown controls would be expected, as is observed.

It is conceivable that the gibberellin factor does not move at all but rather is synthesized in the new cells as they are laid down, and exerts its function without moving to other cells. There is, however, considerable evidence against this hypothesis. First, the applied gibberellin is fully effective if applied to a leaf or a lower portion of the stem,<sup>3, 15</sup> indicating that it must be translocated, at least in these cases. Also, in the case of compensatory growth, it seems clear that the natural gibberellin factor accumulates when growth is inhibited by light and is effective further up the stem at a later time, strongly suggesting a translocation (upward) of the natural gibberellin factor in this case also. There is, then, evidence indicating that both applied gibberellin and the natural gibberellin factor may be translocated within the plant. Whether it normally does so is at present unknown, although results by the author<sup>16</sup> provide evidence that the natural gibberellin factor is produced in the tip of the stem and translocated downward, in much the same manner as auxin. If the tip of a dark-grown Alaska pea seedling is removed, elongation of the growing region soon ceases. This growth may be restored by application of gibberellin but not of auxin.

The stem elongation of tomato (*Lycopersicum*) has been reported to occur almost completely during the dark portion of the daily light-dark cycle.<sup>17</sup> It is interesting to speculate whether this inhibition of stem elongation by light represents the same mechanism as is reported here and whether it, too, may be reversed by gibberellin.

The light which causes the inhibition of stem elongation also is responsible for stimulating leaf expansion and increasing the rate of node formation. The action spectra for the promotion of leaf-expansion and stem-growth inhibition have been worked out in great detail,<sup>8</sup> while the effects on node formation have been observed but not studied.<sup>6</sup> The action spectra for these phenomena are consistent with the idea that they are all controlled by the same photochemical reaction, the red and far-red reaction first demonstrated by Borthwick et al.<sup>18</sup> This idea is strongly supported by the report of Downs<sup>19</sup> that leaf expansion and stem elongation in *Phaseo-*

*lus vulgaris* are promoted by red light, and this red-light effect may be reversed by irradiation with far-red. Assuming that this is in fact the case, it is clear at once that the gibberellin is not affecting the light reaction, since it does not reverse the promotion of leaf expansion; rather, the gibberellin is affecting some subsequent reaction in the chain between the photochemical reaction and the process of stem cell elongation. The photochemical reaction, then, must, directly or indirectly, control several independent processes—i.e., stem elongation, leaf expansion, and node formation. Stem elongation and leaf expansion are related only through being controlled by the same environmental stimulus and presumably through the same photochemical receptor. The same lack of direct relationship holds true for stem elongation and node formation, even though here it would have been most tempting in the past to suggest some physical-chemical relationship between these two phenomena. It is clear as a result of the experiments reported here that the rate of stem elongation and the rate of node formation (expressed per unit time or unit height) are completely independent so far as mechanisms of control are concerned.

## SUMMARY

Alaska pea seedlings grown in complete darkness show little or no response to applied gibberellin; but when seedlings whose growth is inhibited by red light are treated with gibberellin, the growth rate is restored to that in darkness. When dwarf peas are treated with gibberellin, their growth rate is increased even in darkness, but the growth of the dwarfs in light and that in darkness are also equal when treated with gibberellin. The rate of node formation is shown to be controlled directly by light. Both this process and the expansion of the leaves, which is also controlled by light, are unaffected by gibberellin treatment, in both dwarf and normal peas.

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## THE CONTINUOUS SUBMERGED CULTIVATION OF PLANT TISSUE AS SINGLE CELLS

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In the concept of tissue culture as introduced by Haberlandt at the turn of the century,<sup>1</sup> the original aim was cultivation of single cells. The achievement of this goal in the plant field has taken many decades. The progress in this direction and the important milestones passed were effectively described by White in his 1941 review.<sup>2</sup>

One of the projects in this laboratory is the development of efficient techniques for the culture of plant tissues to investigate certain biochemical processes and to study the production of various plant products and their potential usefulness in research and for commercial purposes. Since one of the most promising methods for answering problems of this type is the use of cells derived from multicellular plants growing under controlled conditions as individual cells,<sup>3</sup> the establishment of viable single-cell clones was one of the objectives.

Gautheret,<sup>4</sup> Reinert,<sup>5</sup> Nobecourt,<sup>6</sup> and other investigators have observed the dissolution of various tissue cultures into cell groups and sometimes into separate cells. We have gone further than this and have been successful in making a continuous subculture for the last four years of a clone of single pole bean (*Phaseolus vulgaris*) cells in liquid medium under submerged conditions.

This clone was isolated in our laboratory from the hypocotyl of pole bean in 1951 and maintained on a modification of White's medium with the addition of coconut milk (18 per cent by volume) and 2,4-dichlorophenoxyacetic acid (0.6 ppm). This is the same clone listed in Gautheret's "Catalogue of Plant Tissue Cultures."<sup>7</sup> The catalogue is a compilation as of 1954 of the plant cultures reported from the various laboratories throughout the world. After several subcultures on solid medium, transfers of tissue masses were made to the same medium without agar. Erlenmeyer flasks of 300-ml. capacity to which 50 ml. of the medium had been added and sterilized by autoclaving were used. Agitation was accomplished by several methods: reciprocal shaking, unidirectional rotary shaking, propeller stirring, and alternating rotary shaking with baffles. All these methods gave satisfactory results. Most of the work reported here was carried out using rotary shaking at 230 r.p.m.

Within several days after placing the pole bean tissue under these submerged conditions, many single cells were noticed in the medium upon microscopical examination. These cells were *not* sloughed off from tissue clumps, incapable of further