STUDIES ON THE PHYSIOLOGY OF LIGHT ACTION. IV. LIGHT ENHANCEMENT OF AUXIN-INDUCED GROWTH IN GREEN PEAS

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(WITH SEVEN FIGURES)

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In the first paper of this series (**3**), it was demonstrated that the auxin-induced growth in length of sub-apical sections of etiolated pea stems is greatly inhibited by light. This light-growth inhibition was subsequently (**1, 2**) interpreted in terms of a riboflavin-sensitized photoinactivation of indoleacetic acid (IAA). Such an interpretation derives support from the fact that light, concurrent with its inhibition of growth, increases the rate of disappearance of IAA from the external medium.

In the course of similar experiments with stem sections of light-grown peas, we noticed that light enhances, rather than inhibits, the auxin-induced growth. This response was unexpected and of such great magnitude that it was considered desirable to investigate it more fully. The present paper describes the phenomenon in some detail and presents an explanation of it which seems consistent with all the facts at our disposal. The simple section growth test herein described may, incidentally, be of some use to those interested in utilizing green tissues for auxin or herbicide studies.

Materials and methods

Alaska peas were thickly sown in coarse gravel in plastic boxes 4 by 4 by 3 inches, and were watered twice daily with half-strength Hoagland's solution. In order to permit drainage of the excess nutrient solution from the box, several small holes were drilled in the bottom. The boxes were exposed, in the "phytotron" (**4**), to the following constant environmental conditions: temperature, 20° C; light, eight hours per day at 800 foot-candles of mixed "Daylight" and "White" fluorescent light augmented by incandescent bulbs. Under these carefully controlled conditions in the phytotron, the growth of the plants is extremely regular and reproducible. At the age of 12 days, the plants had five nodes visible on the stem. The subterminal portions of the stem (designated "A" on figure 1) were then utilized for section growth tests. In the selection of plants to be used in these tests, care was taken to reject any in which the sixth internode had started to elongate. In such plants, active growth is largely restricted to this sixth internode, and sections excised from the fifth internode will not grow rapidly. Sections of young petioles and tendrils may also be used in growth tests but are not as convenient for this purpose as the stem sections.

After the stems had been severed at the ground level, the expanded fifth leaf and apical bud were cut off. The stems were then inserted into the "coleoptile microtome" previously described (**3**) and two successive sections, each 5.34 millimeters long, cut from the apical region. The cut sections were placed in a Petri dish containing a shallow layer of distilled water, and randomization in the tests insured by thorough mixing of the sections prior to their insertion into any experimental flask. In the tests, 18 sections were floated on the surface of 5 ml. of nutrient medium contained in a 50-ml. Erlenmeyer flask. For the "dark" treatment, the flasks were kept in an absolutely dark growth chamber; for the light treatment, they were exposed to about 400 fc of mixed "Daylight" and "White" fluorescent illumination. The temperature was maintained at about 25° C in both growth chambers.

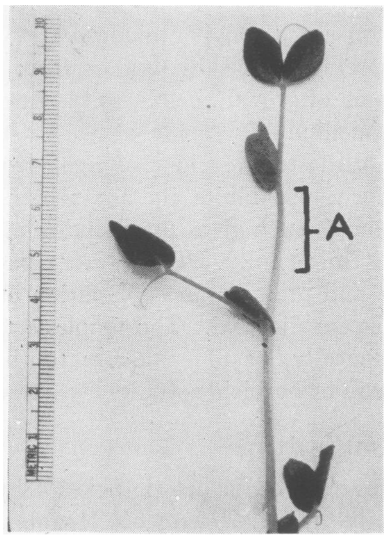

FIG. 1

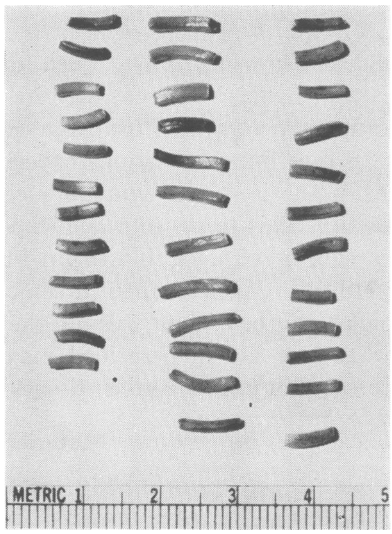

FIG. 2

FIG. 1. A typical 12-day-old plant utilized in the section growth experiments. "A" designates sub-apical region from which the sections were cut.

FIG. 2. The interaction of light and auxin in the growth of green pea stem sections. Left: light, no auxin; center: auxin and light; right: auxin, no light.

The sections were harvested after 16 to 24 hours, lined up on a microscope slide, and their length measured to the nearest 10th of a millimeter with an ocular micrometer. Group averages and standard errors were then computed.

The appearance of the sections and their variability within any one group may be seen from figure 2. In general, growth is not as uniform in this test as in the test involving etiolated sections. In addition, green sections sometimes exhibit contorted growth, making straight-growth measurements difficult. Despite these limitations, the test gives statistically valid data because of the great elongation accomplished by the sections under favorable conditions.

Experimental

Response of green sections to auxin

Previous experiments with etiolated pea sections had shown that good growth could be obtained in a medium containing 2% sucrose, M/60 pH 6.1 phosphate buffer and 0.1–1.0 mg./l. IAA. In the preliminary tests on the growth of green sections, similar media were employed, with the IAA being varied over a wide concentration range, from 0 to 100 mg./l. Representative results from such growth tests are shown in figures 2 and 3. It is

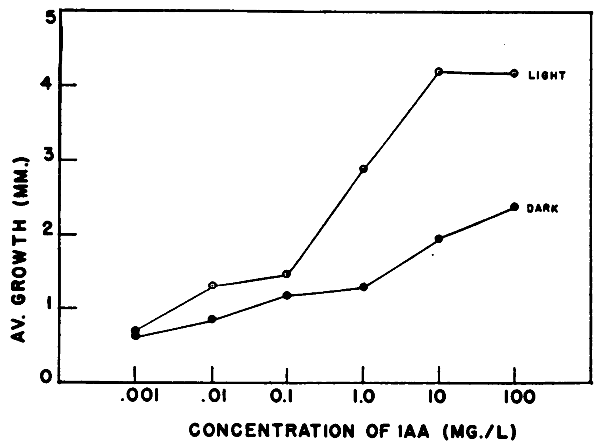

Fig. 3. The effect of auxin concentration on growth of green pea stem sections in light and dark.

apparent that practically no elongation occurs in the absence of IAA. In the presence of IAA in the dark, there is somewhat greater elongation, but when both light and IAA are supplied, the sections may elongate 80% or more in 16 hours. The optimum IAA concentration at this level of sucrose appears to be in the range 10–30 mg./l., a figure at least 100 × higher than

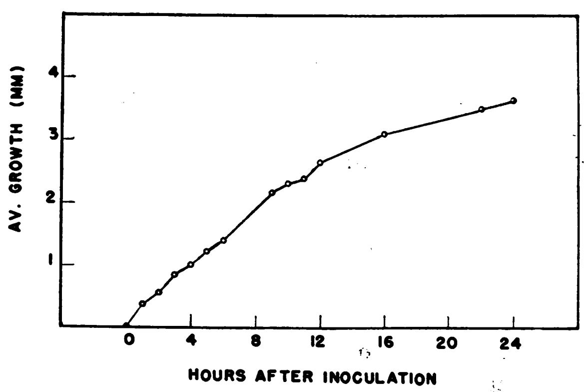

Fig. 4. The time course of growth of green pea stem sections.

the optimal IAA level for etiolated sections. The time course of growth of sections exposed to 10 mg./l. IAA in the light is shown in figure 4. Unlike the etiolated sections, which had practically completed their growth at the end of 12 hours, the green sections continue their growth up to (and presumably beyond) the 24-hour point on the curve. The effect of the pH of the medium on the growth of sections is shown in figure 5. A rather broad

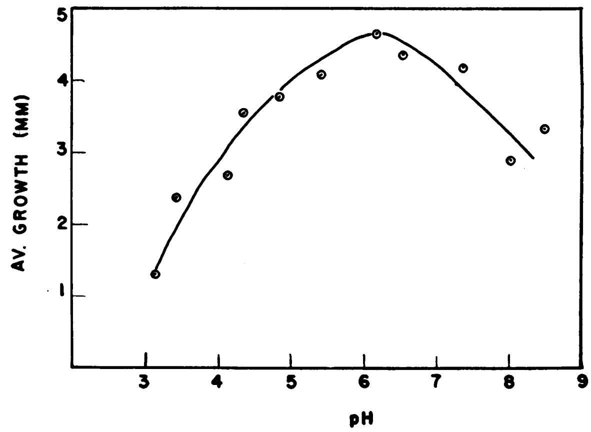

FIG. 5. The effect of pH on the growth of green pea stem sections.

optimum for growth exists in the region of pH 6, thus justifying the continued used of M/60 pH 6.1 KH_2PO_4–Na_2HPO_4 buffer.

The nature of the light effect

It will be recalled that periods of illumination as short as one minute or less are effective in causing marked inhibition of growth of etiolated sections (**3**). Obviously, the photochemical reactions involved in such a growth inhibition are consummated by small quantities of light energy. For the sake of convenience, we can refer to such an effect as "microquantic," in contrast with photo-reactions like photosynthesis which require much higher amounts of light energy, and which may therefore be referred to as "macroquantic." Our next experiments were designed to discover whether the light-growth stimulation of these green sections in the presence of 10 mg./l. of IAA is a "microquantic" or "macroquantic" type of effect.

Sections were cut and placed in the dark into media lacking sucrose. Individual flasks were then permitted to receive from three seconds to 24 hours of illumination, being removed to the dark chamber at the conclusion of the light period. At the end of 24 hours, all sections were harvested and measured. Typical results are shown in figure 6. In general, it was found that the stimulatory effect of light on growth increases with increasing exposure time, no saturation effect of light having been observed. This differs from the light dosage relations observed with etiolated tissues (**3**) and probably justifies our calling this a "macroquantic" type of effect.

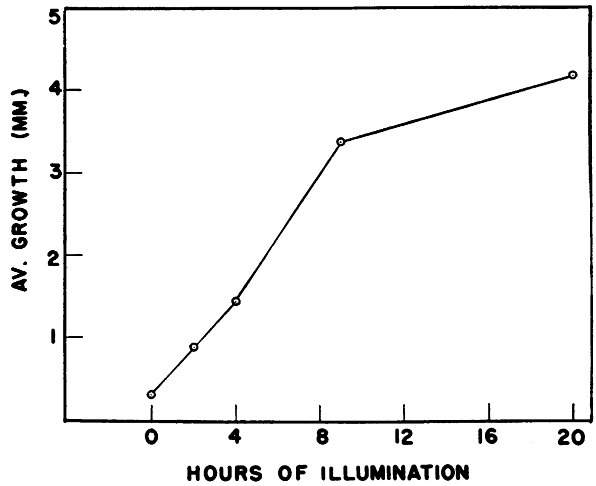

FIG. 6. The effect of duration of illumination on the growth of green pea stem sections provided with auxin. Light intensity was approximately 400 fc.

In subsequent experiments, it was found that the addition of sucrose to the medium compensates in part for the action of light. This suggests that the "macroquantic" effect of light on growth is exerted through photosynthesis. Typical results (fig. 7) show that sections grown in the light are not benefited by the addition of sucrose, being in fact inhibited by concentrations in excess of 1%. Sections grown in the dark, on the contrary, are stimulated by sucrose, the maximum growth being attained at a level of 4%. Despite the addition of sucrose, the best growth attained in the dark is not as good as that attained in the light. This indicates that most, but not all of the light stimulation of growth may be interpreted in terms of a photosynthetic production of substrates essential for respiration and growth.

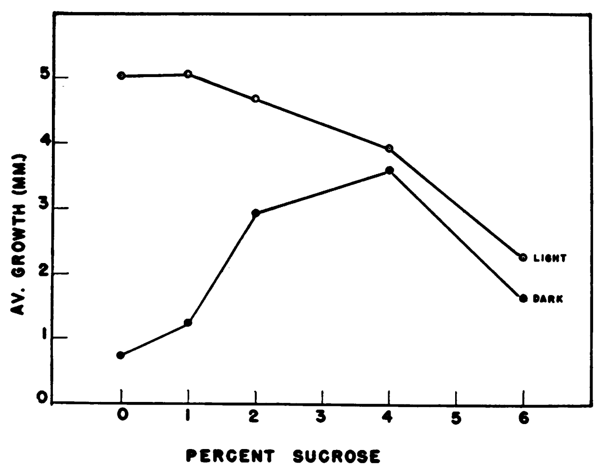

FIG. 7. The effect of sucrose concentration on the growth of green pea stem sections in light and dark.

Discussion

A comparison of the results obtained in growth studies on etiolated (**3**) and green pea stem sections reveals some interesting differences. The growth of stem sections of etiolated peas is inhibited by light, this effect being most probably due to auxin destruction in the medium. Green pea stem sections will also destroy more auxin in the light than they do in the dark, yet the overall effect of light is to stimulate their growth. The data of the present paper indicate that this effect can best be interpreted in terms of a photosynthetic production of sugar. Therefore, sugar is paradoxically more limiting to the growth of green tissue than to corresponding etiolated tissue.

It is also noteworthy that dark-grown pea sections are much more sensitive to auxin than are light-grown sections. The former have an optimal IAA concentration of about 0.1 mg./l.; the latter about 10 mg./l. This difference can probably be attributed to auxin photoinactivation in the green plant. The fact that the addition of auxin increases the growth of green sections raises the problem of why application of auxin to intact green plants does not similarly stimulate growth. This and related problems are under investigation.

Summary

1. Excised stem sections of green pea plants will grow rapidly in fairly intense light if they are provided with 10–30 mg./l. of indoleacetic acid. Even at this optimal concentration of auxin, the sections grow poorly if kept in the dark. The light effect is probably due to photosynthesis, since illumination can be replaced by 4% sucrose.

2. The differences in behavior between stem sections of etiolated and green peas may be summarized as follows: Light inhibits the growth of etiolated pea epicotyl sections by inactivating auxin, which limits the growth of this tissue; light stimulates the growth of green pea stem sections by causing a photosynthetic production of sugar, which limits growth in this tissue. Thus, these green tissues are apparently more limited by sugar than are the corresponding etiolated non-photosynthetic tissues.

3. The green pea section growth test is suggested as a convenient screening procedure for testing the growth-regulatory effects of various compounds.

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LITERATURE CITED

1. GALSTON, A. W. and BAKER, R. S. Studies on the physiology of light action. II. The photodynamic action of riboflavin. Amer. Jour. Bot. **36**: 773–780. 1949.
2. GALSTON, A. W. and BAKER, R. S. Studies on the physiology of light action. III. Light activation of a flavoprotein enzyme by reversal of a naturally occurring inhibition. Amer. Jour. Bot. (In press).
3. GALSTON, A. W. and HAND, M. E. Studies on the physiology of light action. I. Auxin and the light inhibition of growth. Amer. Jour. Bot. **36**: 85–94. 1949.
4. WENT, F. W. The Earhart Plant Research Laboratory. Chronica Botanica **12**: Reprint No. 2. 1949.