RELATIONSHIP OF THE COBALT AND LIGHT EFFECTS ON EXPANSION OF ETIOLATED BEAN LEAF DISKS[1]

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(WITH THREE FIGURES)

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The expansion of etiolated bean leaf disks in the dark may be promoted by briefly exposing the disks to light. Expansion may also be increased by placing the disks on media containing cobaltous or nickelous salts (**1**) and the author has suggested a close relationship of the light and cobalt effects. Investigations concerning this relationship are reported herein.

A full description of the test utilizing the etiolated leaf disks has been published (**1**) and is recorded elsewhere (**2**). It is summarized here. Disks, 5.0 mm. in diameter, are cut with a cork borer from the two simple leaves immediately above the cotyledons of etiolated Burpee Dwarf Stringless Greenpod bean plants. A section of a main lateral vein approximates a diameter in each disk. The plants are used seven to nine days after the sowing of the seeds in flats of sand in a darkroom maintained at a temperature of $25 \pm 1°$ C. All manipulations involving the disks are performed in dim green light. Five milliliters of a test solution are added to a Petri dish containing three 9 cm. sheets of Whatman no. 1 filter paper. Ten disks, lower epidermis up, are placed on the pad of filter paper wetted with the test solution. The basal solution contains 3% D-glucose by weight and KNO_3 at an 0.08 M concentration. The pH of each solution is adjusted to 5.6. The Petri dishes are kept in a dark cabinet at $25 \pm 1°$ C. The diameters, perpendicular to the segments of heavy veins, are remeasured at the desired times.

In figure 1, the values for diameters of disks two days after light exposures are plotted against the length of time the disks were exposed to incandescent-filament light at an intensity of 200 foot-candles. The greatest increase per amount of light energy occurs with exposures of five minutes or less. Actually, as found in other experiments, the sharp rise of the curve occurs with exposures of one minute or less. The curve does not level off at a maximum value. It probably would continue upward with longer exposures since expansion of disks exposed to light at the beginning of several experiments was considerably further increased by another exposure the next day. Thus, the limiting condition affected by light is not completely eliminated by a single exposure to light. After all light treatments, expansion was nearly uniform in all directions.

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As previously reported (**1**), the expansion of disks on a cobalt-containing medium was nearly uniform along all diameters. The optimal concentration was about 5 p.p.m. of the cobaltous ion.

The mechanics of the disk expansion after both light and/or cobalt treatments was investigated. Cell size measurements were made in sections prepared from material mounted in paraffin. Practically all expansion was apparently a result of cell enlargement. Therefore, both cobalt and light influence processes which lead to cell enlargement.

If the cobalt and light actions are distinctly different, it should be possible to find plant material in which one effect could be observed and the other could not be. In search of such material, disks from leaves of plants growing in the greenhouse were employed as test objects. The basal medium already described was used. The expansion of disks from green primary bean leaves having areas of about 8 sq. cm. increased in the presence of cobaltous ions; however, the expansion also increased after the disks were exposed to light (200 fc) for five minutes one day following the start of the tests. The same appeared to be true of leaf disks from young green pea

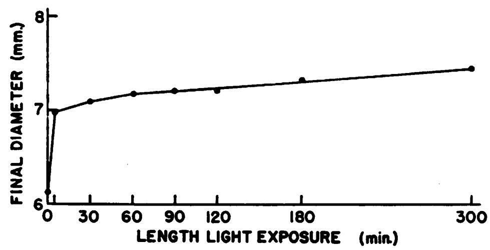

FIG. 1. Final diameters of etiolated bean leaf disks after two days in darkness on basal medium. At the beginning of the experiment disks exposed to light (200 fc) for the time indicated. Standard errors ranged from ± 0.03 to ± 0.07 mm.

plants (var. Little Marvel) although the results were not very clear-cut since not much growth occurred. The expansion of disks from green leaves of *Chenopodium album* and of radish (var. French Breakfast) was unaffected by cobalt and by exposure to light. This was true of *Chenopodium album* leaf disks even if coumarin was present in the medium (**3**). Thus, no separation of the two effects was obtained by use of green leaf material.

Shortly after germination, a hook is evident in the hypocotyl of an etiolated bean seedling. Under conditions of these experiments, as the seedling grew, this hook moved morphologically upward from the hypocotyl into the first internode of the stem and finally into the petioles of the primary leaves. Petioles of both primary leaves became hooked. Exposure of the seedlings to light caused the disappearance of the hooks. In several experiments, hooked portions of hypocotyls, stems, and petioles were excised from the seedlings and used as test objects. Although exacting techniques were not developed, it was obvious that the elongation and straightening of the hooks could be increased by exposing them briefly to light or by adding cobaltous salts to the basal medium. The same was true of any excised portions taken from above but not from below the bend. However, these latter portions grew very little; therefore, the tests made to detect light and cobalt effects may not have been valid. This is a further example of association of the cobalt and light effects. In no tissue has only one of the effects been observed.

Distinctly different causes, if existent, of the cobalt and light effects might be reflected in the time-expansion curves. In figure 2, time-expansion curves are presented for disks exposed for 15 min. to incandescent-filament light at an intensity of 200 foot-candles and placed on a basal medium containing 5 p.p.m. Co^{++} (as $Co(NO_3)_2 \cdot 6\,H_2O$), for disks not exposed to light

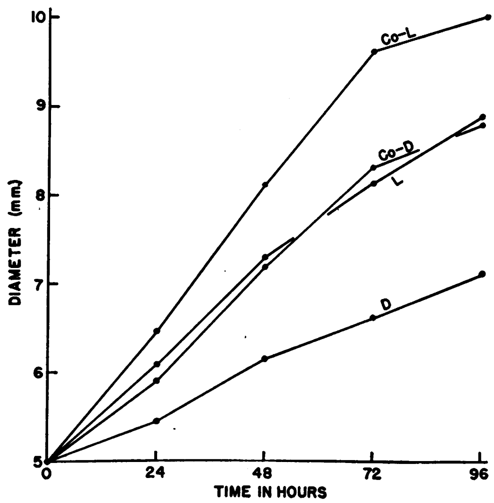

FIG. 2. Time course of expansion of etiolated bean leaf disks given the following treatments: D—no light exposure, basal medium; CoD—no light exposure, Co^{++} (5 p.p.m.) in basal medium; L—15 min. light exposure, basal medium; CoL—15 min. light exposure, Co^{++} in basal medium. Standard errors ranged from $\pm$ 0.03 to $\pm$ 0.07 mm.

but placed on the cobalt salt medium, for disks exposed to light and placed on basal medium, and for unexposed disks placed on basal medium. Except for the steepness of the slopes, there are no characteristic differences among the four curves; they are in phase. Quantitatively, the cobalt-dark (Co-D) and light (L) curves were similar in other experiments but not so identical as those presented in figure 2. The curves in figure 2 bring out no distinction between the cobalt and light effects.

The disks given both cobalt and light treatments, however, expanded more than disks given either treatment alone even though the optimal concentration of cobaltous salt was used. Obviously, the action of Co^{++} does not replace the action of light. From the curves it seems the two effects might be additive. This possibility was tested.

A wide range of values for expansion of disks given one or both treatments was obtained by measuring diameters of disks at various times after the start of experiments, by varying the length of light exposure, and by varying the concentration of cobaltous salts in solution. The amount of expansion resulting from any particular treatment was obtained by subtracting the computed areas of the control disks (given neither cobalt nor light treatment) from the computed areas of the treated disks. In figure 3, values along the ordinate represent the increases in area of disks given both cobalt and light treatments. The values along the abscissa represent the sums of area increases resulting from the cobalt and light treatments being given separately to different disks. If the effects are strictly additive, the points should fall on the theoretical line which has been drawn in the figure.

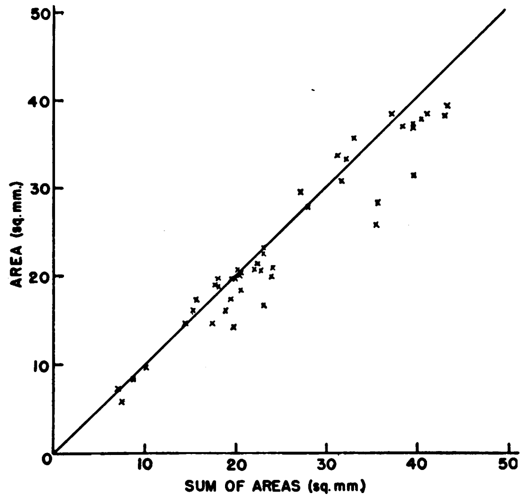

FIG. 3. Increases in etiolated bean leaf disk areas resulting from Co^{++} and light treatments given simultaneously to same disks vs. the sums of area increases resulting from treatments given separately to different disks. See text for explanation of the curve.

The deviations are not great. The cobalt and light effects, at least under the conditions of the experiments reported here, are essentially additive.

The cobalt and light effects are similar in that both occur in the same plant materials, both increase cell enlargement, both cause uniform leaf disk expansion, and both influence some property apparently common to cells in many different tissues. These relationships support the idea that both cobalt and light affect the same limiting condition. It might be argued that the fact that the two effects are additive is evidence against such a possibility. This argument would not necessarily be valid. The limiting condition affected by light was only partially removed by single exposures of the disks to light, and addition of cobaltous ions might further diminish the same condition. It seems quite possible that cobalt and light reduce the same limiting condition but perhaps by different pathways.

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