Phototropic Dosage-Response Curves for Oat Coleoptiles [1,2]

Burke K. Zimmerman [3] & Winslow R. Briggs [4]

Biophysics Laboratory & Department of Biological Sciences,
Stanford University, Stanford, California

In 1934, Du Buy and Nuernbergk (9) compiled from a number of sources the first extensive phototropic dosage-response curve for oat coleoptiles. The various features of this curve, first positive curvature, first negative curvature, and second and third positive curvature, are now well known. The early dosage-response work in Avena was not done under controlled condition of light intensity, spectral purity of the light source, temperature, humidity, and growth regime. Hence many conflicting results were published. Intensities were usually measured in ft-candles or similar units, rather than in absolute units such as quanta per cm^2 per second at a particular wavelength. Combinations of intensity and length of exposure used to achieve a particular light dosage have rarely been reported. The importance of precise knowledge of light exposure conditions in interpreting dosage-response curves has been emphasized elsewhere (4) and will be considered in detail in the discussion.

Only very recently has the profound effect of red light on the phototropic response been appreciated (1,3,5,7,8). But here too the picture was confused. Some authors reported an increase in phototropic sensitivity (1,3,5) while others reported a decrease following exposure to red light (1,7,8). The manner in which red light was administered varied widely from one study to the next and it is therefore very difficult to evaluate these results or draw any valid conclusions from them.

The dosage-response work of Curry (7) is the first that may be considered reliable. The plants in his experiments were all given red light prior to phototropic induction with light of wavelength 4358 A. In general, however, Curry worked with high intensities and short exposure times, without specifying them in every case, so that in spite of his use of

a calibrated monochromatic source, the picture he obtained was incomplete. Under these conditions, first positive and first negative curvatures were obtained as well as an additional positive component occurring at very high dosages. Curry, however, designated the latter as base curvature. Thus, it is not clear that this can be equated with the second positive curvature of the present study which is strictly a tip response.

Thimann and Curry (11) have extracted approximate dosage-response curves at various intensities from the data of many workers. Thus they have clarified the confusing dosage-response picture somewhat, and have drawn attention to the fact that it is not simply the total amount of light received by the plant that is important, but the manner in which it is administered as well.

The present paper has three objectives. The first is to obtain a series of dosage-response curves for phototropic tip curvature with several intensities of monochromatic light and exactly defined conditions. The second is to clarify the effect of red light on phototropic sensitivity under a wide range of defined conditions of phototropic induction. The third is to provide the basis for a detailed kinetic analysis of phototropism of oat coleoptiles (13).

Materials & Methods

Oats (*Avena sativa* L., cv. Victory) were germinated following in detail a regime described elsewhere (5). The only light used during handling was from 15 w green fluorescent lights wrapped in one layer of green and two layers of amber cellulose acetate (Shades, Inc., San Francisco). The transmission of this combination of filtering material was determined with a Bausch and Lomb Spectronic 505 recording spectrophotometer. It was found to be greater than 0.1 % only in a narrow region of the spectrum from 5220 to 5630 A. When used during early stages of growth of the seedlings, the light described produced no noticeable phototropic effects. After the coleoptiles had reached a length of 0.5 cm, only a single lamp as above, wrapped with two layers of each type of cellulose acetate, was used, and then only for a short time. If extreme care was not taken even with this latter illumination, enough irregularity and curvature occurred to produce large variability

[3] Biophysics Laboratory. Present address: Committee on Biophysics, University of Chicago, 5640 Ellis Ave., Chicago 37, Illinois.
[4] Department of Biological Sciences.

from plant to plant in a given phototropic experiment. Restriction of the green illumination to a minimum was particularly important for plants given no red light treatment, since these were more sensitive to phototropic induction than were those given red light.

Approximately 70 hours after the beginning of soaking, plants were selected for straightness (but not length). Usually no more than 60 % were usable. Red light treatment, if any, was begun between 70 and 72 hours after the beginning of soaking, and phototropic induction between 72 and 74 hours. The plants were grown and selected in a room maintained at 25 ± 0.5 C and 92 ± 0.5 % humidity. Red and blue light exposure and curvature development occurred in a room maintained at 25 ± 1.0 C, but without humidity control.

For determining the effect of red light on phototropic sensitivity, two 40 w ruby red bulbs were used at a distance of 24 inches from the plants. Infra red was partially removed with a five cm water filter. The intensity at the level of the plants, as measured with a thermopile, was about 3,300 ergs cm^{-2} sec^{-1}. The spectral distribution of this source and far red reversal effects are currently under investigation (Mrs. H. P. Chon, personal communication). Exposures were always for exactly 2 hours, since it has been shown elsewhere that under these conditions, the red light effects on phototropic sensitivity of oats are at a maximum and have become stabilized (5).

The source of blue light was a 100 w high-pressure dc mercury arc (PEK Labs, Palo Alto, Calif.). The ac ripple was determined with a photomultiplier in conjunction with an oscilloscope and found to be ± 8 %. The lamp was mounted in a metal housing with an adjustable condensing lens, a diaphragm, and a shutter. This assembly was enclosed in a light-tight but ventilated box with an opening in front of the lens about two inches in diameter. A five cm water filter and a blue glass filter were placed immediately outside the opening to remove red and infra red radiation, both for protection of the interference filter, and to remove the longer wavelengths transmitted by the interference filter. Infra red leakage can cause serious errors in intensity measurements with a thermopile, since most interference filters transmit in the infra red region. A Baird-Atomic interference filter was used to isolate the 4358 A mercury line. Additional neutral filters were used to adjust the intensity. These were Kodak Wratten filters which had been calibrated at 4358 A.

Intensity was measured with a photomultiplier (Photovolt Corp.) calibrated at 4358 A against a thermopile (Eppley, eight junction, bismuth-silver). Due to the extreme sensitivity of the photomultiplier, the aperture was reduced to about 0.01 inches in diameter. Behind the aperture was placed a sheet of translucent plastic to scatter the light and make the orientation of the photomultiplier tube with respect to the direction of the light non-critical. A black tube was mounted on the photomultiplier to prevent stray light from the sides from affecting the reading of intensity from a particular source.

The exposure times were measured on a mechanical timer accurate to 0.25 seconds or with a stop watch. The shutter was operated manually. In this manner, exposures as short as 0.5 seconds could be accurately made.

The plants were assembled into rows of 10 to 12, and only the top three mm at most were exposed to light. The coleoptiles were arranged so that their narrow sides faced the light source. They were placed approximately six to eight feet from the arc, at which distance the light from the arc covered an area about two feet in diameter. Defocusing of the image of the arc is sufficient to insure uniformity of illumination to $\pm$ 10 % over an area about a foot in diameter in the center of the spot.

Curvatures were allowed to develop in the dark at 25 C for exactly 100 minutes, at which time shadowgraphs were taken. Curvature measurements reproducible to 1° were obtained with a goniometer. Averages were determined, and standard deviations and probable errors of the means (standard errors) were calculated on the Burroughs 220 digital computer at the Stanford Computation Center. Standard errors averaged about $\pm 1.5°$, and rarely exceeded $\pm 2.0°$.

Results

In figures 1a-f phototropic response in degrees curvature is plotted against $\log_{10}$ (I $\times$ t), where I is intensity and t is the length of exposure to blue light in seconds. For each curve, dosage was varied only by varying exposure time. Three intensities were used, 1.4×10^{-11}, 1.4×10^{-12}, and 1.4×10^{-13} einsteins cm^{-2} sec^{-1}. Figures 1a through 1c represent results obtained with these intensities and plants having no red light treatment other than mesocotyl suppression. [Mesocotyl suppression was previously shown to be without effect on phototropic sensitivity (5)]. Figures 1d through 1f represent results with the three intensities and plants having had in addition, 2 hours of red light treatment immediately before phototropic induction. For each blue light intensity, and with and without red light treatment, entire dosage-response curves were obtained on three to five separate occasions. Variation from one experiment to the next did not justify normalization to any standard curvature, so all points are included from each experiment as measured.

Where second positive curvature does not occur, the curves drawn are based on experiments at all three blue light intensities, both for the red-treated and untreated plants, since within this range, curvature appears dependent on dosage alone. Variation in curvature from one intensity to the next was not significant. Where a second positive component is present, each curve is based only on experiments done with the conditions specified, since curvature is clearly not a function of dosage alone.
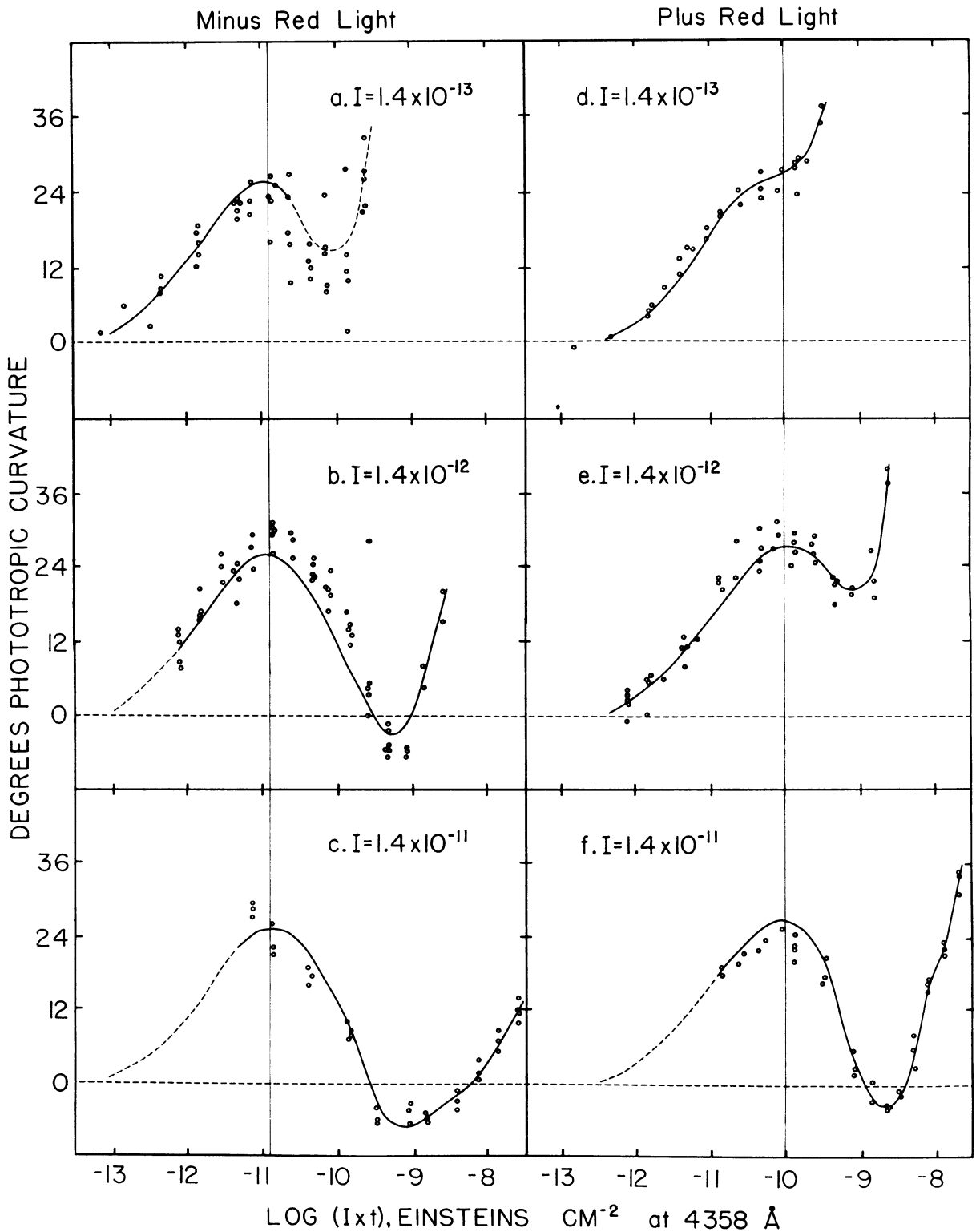
FIG. 1. Phototropic dosage-response curves for oat coleoptiles at three intensities of monochromatic light. Left-hand curves (a–c), no red light pretreatment. Right-hand curves (d–f), 2 hr red light pretreatment. The peaks for first positive curvature are denoted by the two vertical lines, showing the shift in sensitivity produced by red light treatment. Intensities (I) are in einsteins cm^{-2} sec^{-1} at 4358 A.

Apparently high humidity during growth is essential for reproducibility of phototropic curvatures from a given set of illumination conditions. If the humidity dropped below 90 % for more than a few hours, the curvature response was much reduced and variability was extreme.

Discussion

These experiments indicate that the kinetics of the phototropic response must be rather complex and may be the result of more than one independent system. With the plants given no red light, the low-dosage portion of the curve (first positive curvature) seems to depend only on the product $I \times t$. At higher dosages, however, such is not the case. Second positive curvature appears to be primarily a function of the length of exposure and independent of the intensity. Thimann and Curry (11, 12) first suggested the dependence of second positive curvature on exposure time only, from an analysis of the data of many workers.

The plants given 2 hours of red light exhibit much the same qualitative behavior. However, some striking differences are to be noted. Red light shifts first positive curvature (& first negative curvature when it occurs) toward higher doses, i.e. renders the system less sensitive to light. The sensitivity decrease is approximately fourfold. However, because the shapes of the log dosage-response curves differ slightly with and without red light treatment,

maximum first positive curvature occurs at light dosages differing by a factor of ten. The sensitivity changes described for first positive curvature were first noted by Curry (7) in 1957. The present observations for first positive curvature agree quantitatively with his. He did not study first negative curvature in this context. Second positive curvature, in contrast to first positive and first negative, is shifted toward lower dosages. Red light makes this system more sensitive to blue light by a factor of nearly three.

Briggs (5) has demonstrated that for a single dosage of unilateral white light (1,000 meter-candle-seconds), red light induces what appears to be an increase in phototropic sensitivity both for corn and oat coleoptiles, red light treated plants showing substantially more curvature than untreated plants. His results, however, are consistent with the present observations. Without red light, his dosage yielded curvature in the first negative range for oats and in a corresponding range for corn (whether or not first negative curvature occurs in corn has not been determined). With red light, this same unilateral light dosage yields substantial first positive curvature for both plants, as a consequence of the shifts for first positive and first negative curvature described above. The results described here also confirm those of Asomaning and Galston (1) (disregarding a reported increase, of doubtful significance, in the amount of first positive curvature obtained from several given dosages). Their observations can not
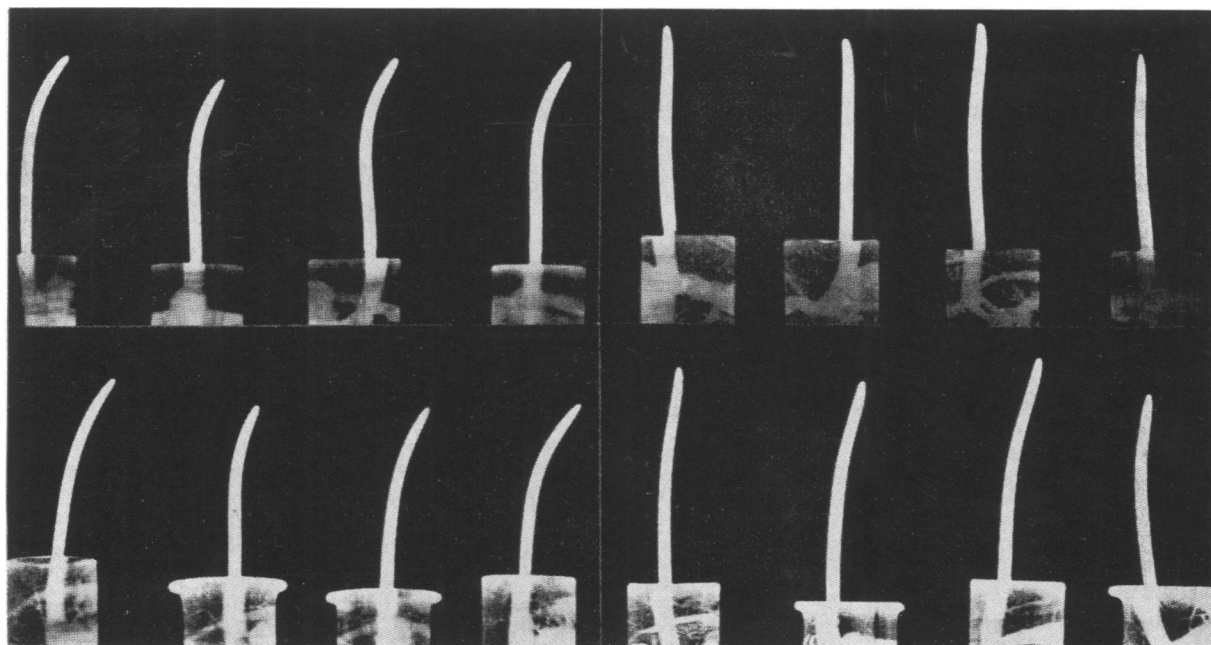

FIG. 2. Examples of shadowgraphs illustrating the various types of phototropic curvature in oat coleoptiles. Intensities (I) are in einsteins cm^{-2} sec^{-1} at 4358 A. Upper left, first negative curvature ($I = 1.4 \times 10^{-12}$, $t = 325$ sec, no red light). Note S-shaped plants resulting from combination of a positive and a negative component. Lower left, second positive curvature ($I = 6.6 \times 10^{-12}$, $t = 1710$ seconds, 2 hr red light). Lower right, curvature resulting from the sum of first negative plus second positive curvature ($I = 1.4 \times 10^{-13}$, $t = 1,000$ sec, no red light). Net curvature was about 14° positive.

be quantitatively compared with those in the present paper, since insufficient experimental detail was provided and their red light regime was different, but it is clear from their figure 3 that red light shifts the log dosage-response curve for first positive curvature toward higher dosages. Their observations with barley coleoptiles are of particular interest. They were only able to obtain phototropic curvature with very large unilateral light dosages, and the sensitivity of the barley phototropic system was substantially increased by pre-exposure to red light. They suggest that barley possesses only a mechanism for second positive curvature, lacking those for first positive and first negative. This latter suggestion is strongly supported by the red light-induced increase in the sensitivity of the second positive curvature mechanism in oat coleoptiles. Blaauw-Jansen's experiments (3) are not really comparable to the present ones, and therefore will not be considered further here. Briggs (5) discusses her experiments in detail.

It was suggested above that more than one independent phototropic mechanism is required to account for the dosage-response curves obtained. Two lines of evidence support this contention. First, red light decreases the sensitivity of the mechanisms for first positive and first negative curvature, but increases the sensitivity of the mechanism for second positive. Second, the appearance of curvature in various regions of the dosage-response curves suggests that possibly three separate mechanisms exist. Aside from first positive and first negative curvatures being opposite in direction, an important fact in itself, the nature of the responses are quite different. Negative curvature develops later than positive, and curvature is therefore more confined to the tip 100 minutes after induction. In some regions in which one would expect either positive (first or second) or negative curvature, depending upon the conditions of intensity, time, and red light treatment, distinctly S-shaped plants are found. These invariably have the negative component in the extreme tip and the positive component below (see fig 2).

Detailed studies of the time course of development of the three tip responses may provide further evidence for the existence of separate mechanisms. Such studies to date have not covered a sufficiently wide range of light conditions. In addition, care has not been taken to illuminate only the coleoptile tip, important at high light dosages where base curvature may occur. In order to prove conclusively that two or three separate tip curvature mechanisms exist, more direct studies are required in which a particular system can be isolated, perhaps by selective inhibition of the others. Action spectra of first negative and second positive curvatures would indicate pigment differences, if present.

It has long been assumed that the Bunsen-Roscoe law of photochemical equivalence applied to the phototropic responses (2, 10). The law states that response is a function of $I \times t$ only, regardless of

how each is varied. That this is not the case has been shown recently by Briggs (4) and Thimann and Curry (11, 12), although their ways of looking at reciprocity were somewhat different. Briggs considered the reciprocity law as failing at high intensities, concluding that if the intensity is low enough and the length of exposure long enough, reciprocity does indeed hold. Thimann and Curry suggested that for short exposures reciprocity appeared to hold, but that for long exposures, the response depended only upon the length of exposure. These would at first appear to be conflicting conclusions. However, it is clear how both conclusions could have been drawn on the basis of the results presented here. The dependence of second positive curvature on exposure time alone has been pointed out above. If in corn, physiological factors should limit the absolute amount of second positive curvature obtainable, then reciprocity would appear valid only for long exposures, regardless of dosage. Thus when Briggs decreased intensity and increased exposure time for dosages above those yielding maximum first positive curvature, he simply obtained progressively more second positive curvature until the physiological limit for second positive curvature was reached. It should be noted that when Briggs did comparable reciprocity experiments with oats, physiological factors never did become limiting, and therefore he never obtained reciprocity validity. Furthermore, for two dosages differing by a factor of ten (4, his figures 13 & 14), the amount of curvature obtained was a linear function of time and independent of dosage above the shortest exposures, precisely what one would expect from the present study. It is clear from the present study that, as Thimann and Curry suggested, the reciprocity law is valid for first positive and first negative curvature. Its apparent failure at low intensities is simply the consequence of introduction of a second positive component with increasing exposure time. The reciprocity law can not be valid for second positive curvature since response is a function of exposure time only. The present study appears to provide sufficient quantitative information for a detailed mathematical analysis of the phototropic responses of oat coleoptiles. Such a kinetic study is being presented in the following paper (13).

Summary

Phototropic dosage-response curves are presented for oat coleoptiles with and without red light pretreatment. Three different intensities of monochromatic blue light were used for phototropic induction. Red light decreases the sensitivity of the mechanisms for first positive and first negative curvature, while increasing the sensitivity of the mechanism for second positive curvature. The reciprocity law is valid for first positive and first negative curvature, but second positive curvature is a linear function of exposure time, and independent of intensity.

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A Kinetic Model for Phototropic Responses of Oat Coleoptiles [1,2]

Burke K. Zimmerman [3] & Winslow R. Briggs [4]

**Biophysics Laboratory & Department of Biological Sciences,
Stanford University, Stanford, California**

The present paper is an attempt to account accurately for phototropic tip curvature in the Avena coleoptile as a function of the intensity of unilateral illumination and duration of exposure to monochromatic light (4358 A). Detailed dosage-response curves on which this analysis is based are presented in the preceding paper (22). It has long been assumed that the direction and magnitude of the light gradient across the coleoptile tip determine respectively the direction and magnitude of the phototropic response (14, 16). Experiments by Buder (9) confirm that the direction of the gradient does indeed determine the direction of the phototropic response.

By using narrow light pipes and illuminating the interior of the tip as well as illuminating small portions of the outside of the tip with narrow beams of light, Buder found that the direction of curvature was always in the direction of the maximum light gradient, not in the direction of the incident radiation. His experiments also produced gradients many times larger than normally occur when the entire tip is placed in a beam of light, but approximately the same amount of curvature was always observed.

The role of the light gradient in determining the magnitude of the phototropic response has been questioned by Curry (12). He pointed out that the light gradient across the tip should be a function of the wavelength of the incident light. Thus if the magnitude of curvature depends upon the magnitude of the light gradient, it should also be a function of wavelength, something not observed (17). His attempt to account for the shape of the dosage-response curves on the basis of the light gradient was also unsuccessful.

Detailed studies of tip curvature based on the assumption that both the direction and magnitude of the gradient are necessary for determining the photo-

[3] Biophysics Laboratory. Present address: Committee on Biophysics, University of Chicago, 5640 Ellis Avenue, Chicago 37, Illinois.
[4] Department of Biological Sciences.