PHOTOREACTIONS IN PHYCOMYCES

Growth and Tropic Responses to the Stimulation of Narrow Test Areas*

By R. COHEN‡ and M. DELBRÜCK

(From the Division of Biology, California Institute of Technology, Pasadena)

(Received for publication, August 29, 1958)

ABSTRACT

Sporangiophores of *Phycomyces* in stage IV b have been stimulated by parallel light in test areas 0.2 mm. wide.

The growth responses to large stimuli are very large, owing probably to light scattered within the specimen. For medium stimuli the sensitive zone coincides with the growth response zone obtained previously and excludes the region of maximum stretch.

Sustained stimulations were used to elicit tropic responses. *The bends formed travel away from the sporangium at a speed equal to the growth speed.* Thus they remain very close to the stimulus when this is held at a constant level relative to ground but separate from it for stimuli programmed differently.

The existence of a protoplasmic structure, the "inner wall," with the following properties is postulated: it is attached to the lower, non-growing part of the sporangiophore and grows by addition above the sensitive zone. It neither stretches nor twists in the sensitive zone. It is the seat of the light receptors and gives growth and tropic responses. The cell wall follows its bends by elastic stretch.

Phycomyces has a sensory mechanism for light which looks appealingly simple when compared to those of other organisms, whether they be microorganisms, higher plants, or animals. The sporangiophore, acting both as a receptor and effector, is a single cell with perfect cylindrical symmetry, and is highly transparent; the vacuole is accurately axial and extends up into the columella; the protoplasm is confined to a thin peripheral zone. The growing zone, just below the sporangium, is short (2 to 3 mm.), and is of constant length for many hours during the main stage of development of the sporangiophore. Only light which falls on the growing zone itself evokes reactions. It seems as if receptor and effector are coincident in space without the intervention of a hormonal transport system which so enormously compli-

* Supported by a grant from the National Science Foundation.

‡ Present address: Service de Biologie, C.E.N. de Saclay, Gif sur Yvette, (S. et O.) France.

cates the analysis of stimulus response mechanisms in the case of higher plants, or of an electrical one, as in animals.

The Postulate of Local Autonomy

Theories attempting to account for the growth and tropic reactions in *Phycomyces* have assumed that the coincidence in space of stimulus and effect is valid even if extended to small elements of the growing zone. They have been based on a postulate of longitudinal and azimuthal autonomy, each element responding according to the illumination program it receives, the reaction of the whole growing zone being simply the sum of the reactions of its parts.

Is this postulate of autonomy valid? Are there, in fact, no integrative features which correlate the reactions of the various parts? There are considerations that make one suspicious. There is the fact that the tropic bend usually observed, when the whole growing zone is unilaterally illuminated, is confined to the lower half of the growing zone. If every portion of the growing zone reacted autonomously one would expect the bend to be more or less uniformly distributed over the growing zone. A second cause of suspicion derives from the spiral growth of the sporangiophore. In the absence of phototropic responses the several portions of the growing zone, while stretching, also continually twist relative to each other. Consider then a sporangiophore in the process of responding to a tropic stimulus. Suppose there is a bend a little below the middle of the growing zone resulting in a tilt of the tip through a certain angle. As growth continues, the portion of the growing zone below the tilt should twist, thus causing the tip to tilt in the wrong direction, displaced, looking from above, in a clockwise direction relative to the direction of the light source. While such deviations do occur, they are much smaller than might be expected from our detailed knowledge of the distribution of stretch and twist (Cohen and Delbrück, 1958).

The postulate of local autonomy contains an ambiguity, since the various parts of the cell move relative to each other. Below the growing zone the particulates in the protoplasm can be seen to stream in the following manner: there is a set of channels in which the particulates stream down with a speed of about 0.18 mm./min., and another set of channels in which the particulates stream upward with about ⅔ of this speed. These speeds are comparable to, but somewhat greater than the growth rate (0.05 mm./min.). Below the growing zone, both these sets of channels are accurately parallel to the axis of the sporangiophore. The channels are exceedingly narrow, of the order of 1 μ, and particles moving in opposite directions often seem to bump into each other as they pass. The nature of this unique convection system is obscure. In particular, it is not clear whether the channels are separated from each other by protoplasmic membranes. Pop (1938) thought that the streaming

was arranged in two concentric cylindrical sheets, the outside sheet carrying the downstream, and the inside sheet carrying the upstream. We have not been able to convince ourselves that this is correct, and believe that the question needs to be studied by modern methods for the analysis of ultrastructure.

In the growing zone the streaming is more complicated. Although there is here, too, a clearly bimodal distribution of upstream and downstream channels, the orientation of the channels is less uniform.

Keeping in mind these rapid motions of particulates in the protoplasm and the possible existence of protoplasmic membranes separating the channels of streaming, we must be prepared to find that if local autonomy exists it may be a local autonomy with respect to any one of the constituents of the sporangiophore. To be sure, the reactions we observe involve changes in stretch of the cell wall, but it is doubtful, indeed, whether the stimulus is received in the wall. If so, then stimulus and reaction might be truly coincident in space. If, however, the stimulus is received in some part of the protoplasm, then this portion of the protoplasm may move relative to the wall element in which the effect is ultimately seen. Since there is a delay of several minutes between input and output, the effect may seem to have been transported while, in reality, the cell wall may have moved relative to the receptor.

The studies reported in this paper were prompted by these considerations and constitute an attempt to investigate in the most direct manner the autonomy or interrelation of the sections of the growing zone in their responses to light by stimulating selected narrow sections and observing the responses, both as to *whether* and as to *where* they occur. Previous attempts in this laboratory to get at this problem through the study of the growth responses using relatively wide test areas, had not revealed any conflicts with the postulate of local autonomy. Our present studies, using a more accurate measuring technique, narrower test areas, and, above all, including tropic responses, have brought to light unexpected new features and have, in part, clarified the two paradoxical facts mentioned earlier.

Methods

General Considerations.—The use of narrow test areas entails a number of experimental difficulties. The responses are smaller than those to stimulations of the whole growing zone, in proportion to the dimensions of the test area. This is not a serious difficulty, as far as accuracy of measurement is involved, since it is relatively easy to improve the micrometric techniques to the point where responses from only $\frac{1}{10}$ of the growing zone might be measured reliably. The principal difficulty lies in the fact that the specimen itself, in the absence of stimulation, does not grow with absolutely constant speed and direction. Therefore, when a small section is stimulated and the effect on the whole specimen is observed, one is matching a small effect against the background noise in the whole growing zone. Several ways to get around this difficulty suggest themselves. One might put markers close to the stimulated section and

thus confine the measurements to this section. The difficulty in this case is the spiral growth which carries the markers around the sporangiophore, and the relative twist of markers located at different levels, causing the markers to be carried around at different speeds. We have found the positioning problems resulting from this feature insurmountable. A second approach might be to reduce or eliminate the background noise. In this direction we have not been able to go beyond very definite limits. The true growth speed may remain constant to within less than 1 per cent within one experiment, but slight tropic deviations occur even under optimal conditions, and these, for geometrical reasons, simulate variations in growth speed. It is then necessary to combine the choice of conditions giving optimal reduction in background noise with a careful study of the statistical properties of this noise, so that it can be partly eliminated from the experimental results.

In exploring the growing zone with stimuli applied to narrow test areas, the simplest question one may ask is whether a response occurs at all, without regard to the size or location of the response. This will tell us whether or not the test area is photosensitive. Using the growth response as a test, this is as far as we were able to go. Our measurements could tell us whether a stimulation confined to a certain section caused a growth response in the whole specimen, but we were unable to devise methods that would tell us where the response occurs, or to study the quantitative relations between stimulus and response.

Therefore, to localize the response, we turned to the tropic reaction. For this reaction it is believed that its initiation, more precisely its course during the first few minutes after the stimulus, is intimately related to the growth response. This belief is well founded, since under all conditions the latent period between stimulus and response is nearly equal to that between stimulus and tropic response. Further, the kinetics of dark adaptation and the action spectrum are similar for both responses. More specifically, the tropic response has been viewed as a *differential* growth response, a differential between the responses of the side proximal to the light source and the side distal to it. Blaauw (1909) conjectured that this differential was brought about by the dioptric properties of the sporangiophore, the sporangiophore acting as a cylindrical lens, concentrating the incident light near the midline on the distal side. Blaauw's supposition that this optic differential is responsible for the response differential was vindicated long ago by the classical experiment of Buder (1918) who immersed sporangiophores in a medium of high refractive index. Under these conditions the sporangiophore acts as a diverging lens. This alters the intensity distribution around the azimuth quite radically. The alteration is certainly not a simple reversal of the optical differential present in air. The principal characteristics of the alteration are that the bright focusing line on the distal side has been replaced by a fairly smooth distribution over the whole distal side, and that on the front side there now appear two almost completely dark marginal zones, due to total reflection of the incident light. Observation shows that under these conditions the sporangiophores display negative tropism, away from the light source instead of towards the light. This experiment demonstrates that the geometrical optics of the sporangiophore is important for the tropic reaction, but it leaves us a long way from having established a complete correlation between the tropic and the growth reaction. Particularly, as will be discussed in a later section of this paper, we lack an under-

standing of the fact that the tropic reaction continues far longer than the growth reaction.

Notwithstanding this gap in our theoretical understanding, we feel justified in using the *initiation* of the tropic response as an index for the localization of the area in which the transfer of the growth stimulus to the wall occurs.

Illumination for Adaptation.—A defect in previous work (Delbrück and Reichardt, 1956) on the stimulation of selected areas had been that during the stimulus of the test area the illumination of the rest of the growing zone had not been constant. In these experiments the test area had been blocked out by a removable collar. In the present experiments, adapting and stimulating light were entirely separate. The adapting light impinged from two sides at an angle of 60° with the vertical. As detailed studies of Dennison (1958) have shown, this arrangement is optimal for reducing spontaneous tropism. It is not perfect, though. The tropic control exerted by the two lights is apparently too weak to hold the specimen closer to the vertical than within a few degrees. We are dealing, therefore, with a *zone* of equilibrium of a few degrees around the vertical, rather than with a unique *direction* of equilibrium. Within this zone the specimen may shift from time to time from one direction to another.

In addition to these random shifts there occur frequently tropic oscillations with a period of 5 to 8 min., and an amplitude of about 2° (see Fig. 3 *b*).

When spontaneous tropisms occur the bend starts to be visible near 0.7 mm. from the sporangiophore. It then moves down the growing zone, the region previously bent straightening out. Eventually the upper 2 mm. are straight again, the bend now being localized in the lower portion of the growing zone. These characteristics of the bends permit in many cases the discrimination between spontaneous bends and those induced by the illuminations of test areas.

The detailed arrangements of the adapting light have been described previously (Delbrück and Reichardt, 1956). The intensities of illumination will be expressed on a logarithmic scale, taken to the base 2, such that 1 unit difference on this scale corresponds to a factor of 2 in the intensity. The 0 of this scale corresponds to about 100 erg/cm.^{2} sec. The light is filtered through a Corning 5–61 filter.

Illumination for Stimulation.—The filament of a General Electric galvanometer light bulb, set horizontally, was imaged, *via* a horizontal light path, slightly beyond the specimen (Fig. 1). The opening of the optical bundle at the image was 0.5°. At the specimen a vertical plane perpendicular to the direction of the beam intercepts a rectangle. The vertical width of the line is 0.20 mm. The horizontal width of the line is 5 mm. The intensity was constant along a 3 mm. length and the specimen was positioned near its center. This light was not filtered. The lamp was operated at a stabilized voltage of 3.7 volt, close to its rated voltage. Its intensity remained constant for long periods. Aging caused a slight reduction in intensity and necessitated a change after several months.

The unfiltered line intensity amounted to about 500 erg/cm.^{2} sec. at the specimen. From previous experience we may estimate that this amount of flux of unfiltered light corresponds in biological efficiency to about 100 erg/cm.^{2} sec. of light filtered by our Corning filters and thus to the 0 of our intensity scale.

The intensity of the stimulating light was never varied in the experiments here

reported. Changes in stimulus size were produced by varying the intensity of the adapting light.

Positioning and Measuring Procedures.—The culture vials were placed on a platform, movable by fine screws in two perpendicular horizontal directions. This platform, in turn, was mounted so that it could be rotated either by hand or by a synchronous motor drive, and this whole assembly in turn was attached to a micromanipulator permitting fine motions in three mutually perpendicular directions.

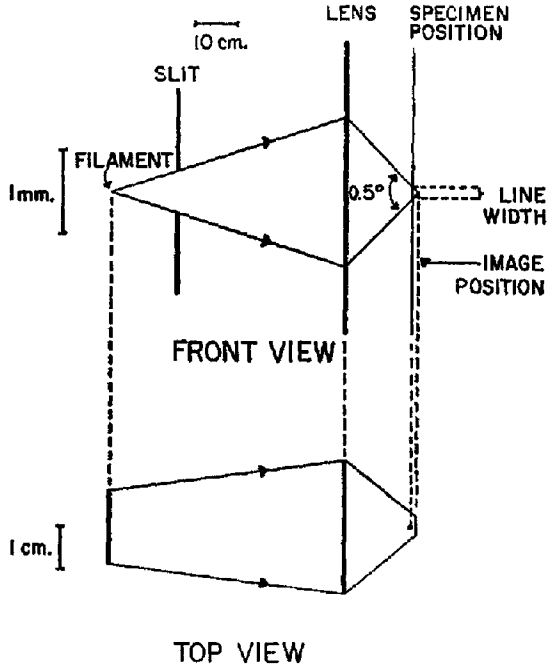

FIG. 1. Optical arrangement for illumination of narrow test areas. Schematic front and top view. Note differences in scale between horizontal and vertical coordinates and between the vertical coordinates in the two views.

The screw controlling the vertical motion was fitted with a large divided circle, whose position could be read against a fixed pointer. Vertical positions were read by means of an ocular micrometer containing a fixed hair-line and a movable narrow pair of hair-lines. Vertical positions were then read by bringing either the top of the sporangium or a marker attached to the sporangiophore to coincidence with one of the hair-lines, by moving the vertical screw of the micromanipulator. These readings were accurate to within less than 1 micron.

The positive phase of the growth response lasts for about 4 minutes. It is convenient to measure the growth speed at 1 min. intervals. The average growth speed is about 50 μ per min. Its variations during a maximal response of the whole growing zone is about 50 per cent. A maximal response of a section of the growing zone 0.2 mm.

long may be expected to be about $\frac{1}{10}$ this large, or 5 per cent. To get meaningful results several responses have to be averaged. A periodic illumination program with stimuli repeated every 5 or 6 minutes is convenient for this purpose. The stimuli are applied at a fixed distance from the sporangium. Measurements are begun after five or six cycles when the initial transients have died away and the response is truly periodic. The response is then averaged over 4 cycles.

Tropic angles were measured by means of an eyepiece fitted with a hair-line that could be rotated, the angle of rotation being read to 0.5° on a protractor coupled with the hair-line. By right-left motion of the micromanipulator and rotation of the eyepiece any given section of the specimen could be brought to tangency with the hair-line. Once a bend was visible, the angles made by portions of the sporangiophore above and below the bend, with respect to a reference direction, were measured. The tropic angle is the difference between these two angles, counted positive for bends towards the line. To locate the position of the bend, the hair-line was then set at an angle bisecting the directions of the specimen above and below the bend, the specimen was made tangent to the hair-line by right-left motion, and the distance of this point of contact from the bottom of the sporangium was measured on a scale running parallel to the reference hair-line in the protractor eyepiece. It was possible to measure tropic angle and bend center every minute.

The line stimuli had a duration of either 30 or 60 seconds. To make the stimulus symmetric the specimens were either rotated by hand or by a synchronous motor giving 2 R.P.M.

The lower the adapting intensity I the higher is the relative stimulus. For example, for $I = 0$ the stimulus is very weak, while for $I = -10$ it is very strong. Fig. 2 *a* shows the growth speed variations during a typical 4-cycle run, and Fig. 2 *b* the average cycle. The time difference between the signal and the maximum of the growth speed is constant to within ± 1 min. in all experiments except for $I < -11$, where the maximum occurs about 1 min. later.

We characterize the response by the relative amplitude A of the growth speed variation:

$$A = \frac{1}{2} \frac{\text{Maximum growth speed} - \text{minimum growth speed}}{\text{Average growth speed}}$$

The principal difficulty in the determinations of these amplitudes derives from spontaneous short period tropic oscillations. The specimens are rarely exactly vertical. Since the amplitudes of these oscillations are very small, the specimens in general will be on one side of the vertical during the entire cycle of oscillation. The oscillation then produces an apparent periodic variation of the vertical component of the growth speed, with a period similar to the true variation that may be produced by the periodic line illumination program. These "false" amplitudes can be recognized when their phase relation with the stimulus differs from the known phase relation of the true growth response. Statistically it must be expected, however, that a few cases will escape detection by this criterion.

For the study of tropic reactions the chief difficulty lies in the fact that *short* stimuli applied to narrow test areas give responses which are too small to be distinguished

from spontaneous tropisms. A 1 min. unilateral stimulus even if applied to the whole growing zone gives a final tropic angle of only 5–10°, occurring at so slow a speed as to be barely distinguishable from hunting. It is therefore necessary to use long stimuli, sufficient to produce a clearly visible and localizable bend, and to obtain the time and the place at which the bend first appears by back-extrapolation. Figs. 3 and 6 show how this works out for stimulations applied to the whole growing zone and to a narrow test area, respectively. For the whole growing zone the reaction time is 3.5 min., the same as for the growth response. For the narrow test area the reaction time

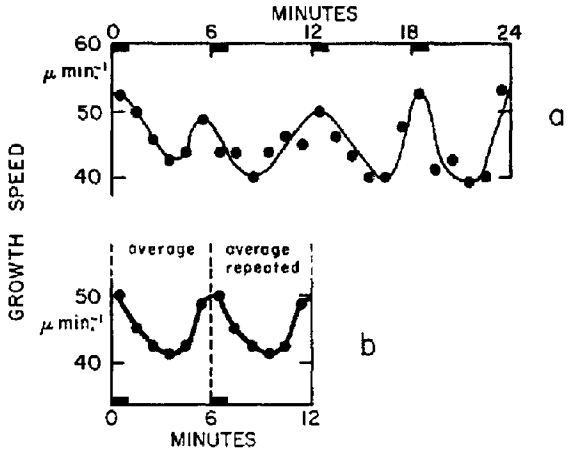

FIG. 2. Growth responses to periodic stimulation of a short section of the growing zone.

Width of test area 0.2 mm. Duration of stimulus 1 min. Period 6 min. Adapting intensity $I = -7$. Position of stimulus 1.5 mm. below sporangium.

Readings were started at the 6th stimulus. The upper graph shows 4 individual cycles; in the lower graph these 4 cycles are averaged. Average growth speed 45 μ/min. Amplitude of the growth speed variations 9.7 per cent. Blocks indicate timing of stimuli.

is 5.5 min. This difference in the reaction times is consistently observed. It is probably a result of the fact that the rate of rise of the tropic speed and its ultimate level are much lower (~1°/min.) in the case of narrow test areas than for the whole growing zone (~5°/min.) (Fig. 3).

It follows that the reaction time is a somewhat arbitrary quantity dependent on the accuracy of the measuring techniques, similar to "thresholds" often ascribed to biological effects, when in reality a gradual transition occurs and the threshold is conditioned by the measuring techniques. In our case the reaction time, under strictly standardized conditions of measurement, seems to be well reproducible as a linear back-extrapolate in angle-*versus*-time graphs. It is important in these measurements that the angle graphed is not simply the angle of the top section with the vertical, but the difference in angle between the section immediately above and below a localizable bend.

Material.—Phycomyces blakesleeanus strain 1555 (−) of the National Regional Research Laboratory, cultured on 5 per cent bacto potato dextrose agar in glass vials.

EXPERIMENTS

The Sensitive Zone for Growth Response.—In these experiments the specimens were equilibrated with a certain adapting intensity I, then subjected to a

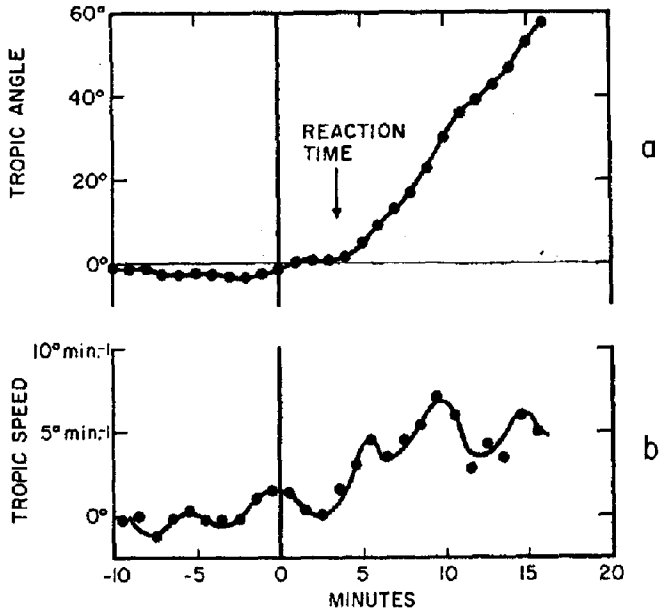

FIG. 3. Tropic angle (upper curve) and tropic speed (lower curve) *versus* time for a unilateral stimulation of the whole growing zone.

The specimen was bilaterally adapted to $I = -5$. At $t = 0$ the left channel was cut and the right channel raised to $I = -4$.

In the upper graph note the reaction time of 3.5 min. and some rather inconspicuous undulations in the curve. The lower graph is the derivative of the upper one. It shows that the undulations are quite regular with a period of 5 min. and a speed amplitude of 1° to 2°. This is the short period oscillation referred to in the text.

The lower graph shows in addition that the troping speed at 8 min. has reached its steady value of about 5°/min.

periodic stimulation by a line 0.20 mm. in vertical width, of effective intensity approximately corresponding to $I = 0$, this stimulation being applied at a fixed distance x from the bottom of the sporangium.

Fig. 4 shows the results of a series of experiments in which both x and I were varied.

The results show in the first place a region of sensitivity roughly coinciding

with the whole growing zone and a gradual increase of the amplitudes with decreasing level of adaptation. These are expected features. In addition, they show two unexpected features. In the first place, at low levels of adaptation the responses from the test areas are almost as large as those resulting from stimulation of the whole growing zone. The sum of responses of successive sections is about ten times larger than the maximum response of which the specimens are capable. In the second place, for a fixed x, say $x = 1.1$ mm., the response increases with decreasing I over a very wide range of I, far wider than in the case of stimulation of the whole growing zone. Extrapolating from

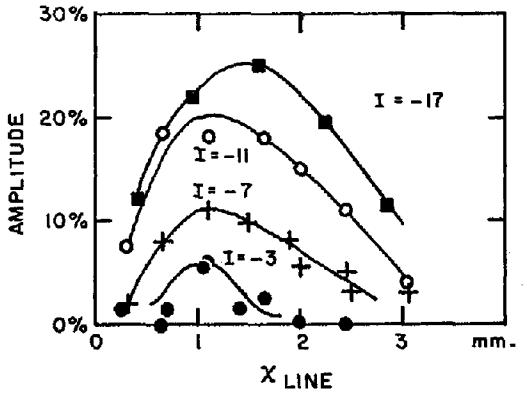

FIG. 4. Amplitudes of growth responses to periodic stimulations of short sections of the growing zone. Variable positions of stimulus and variable stimulus size.

The variations in (subjective) stimulus size were produced by varying the adapting intensity between $I = -3$ and $I = -17$.

the latter type of experiment we should have expected that the test line would give a saturating response for any I smaller than about -8. In actual fact, the response keeps on increasing down to $I = -17$.

The simplest way to explain these findings is to invoke light scattering within the specimen. It is certain that the light impinging on the test area will be distributed, with diminishing intensity, up and down the specimen, owing to scattering and internal reflections. Thus, if we stimulate directly one test area, we will indirectly stimulate and cause growth responses also in all those adjacent areas where the scattered intensity is appreciably above the adapting intensity. These adjacent areas will extend the wider the lower the adapting intensity. This explains both the large responses obtainable from an apparently narrow test area and the continued increase of the response with decreasing level of adaptation. This explanation remains hypothetical since we have not been able to measure the scattered intensities directly. It is clear, however, that the responses obtained at low I cannot be taken as indicating quantitatively the degree of sensitivity of a test area, or even

whether it is sensitive at all. By increasing I the effect of scattering light will be reduced, while that of the line will remain constant as long as the stimulus is nearly saturating in the test area. A reasonable compromise is an adapting intensity $I = -3$ with a stimulus duration of 1 min., or $I = -4$ and half-minute stimuli. Here the response amplitudes are around 5 per cent, are nearly

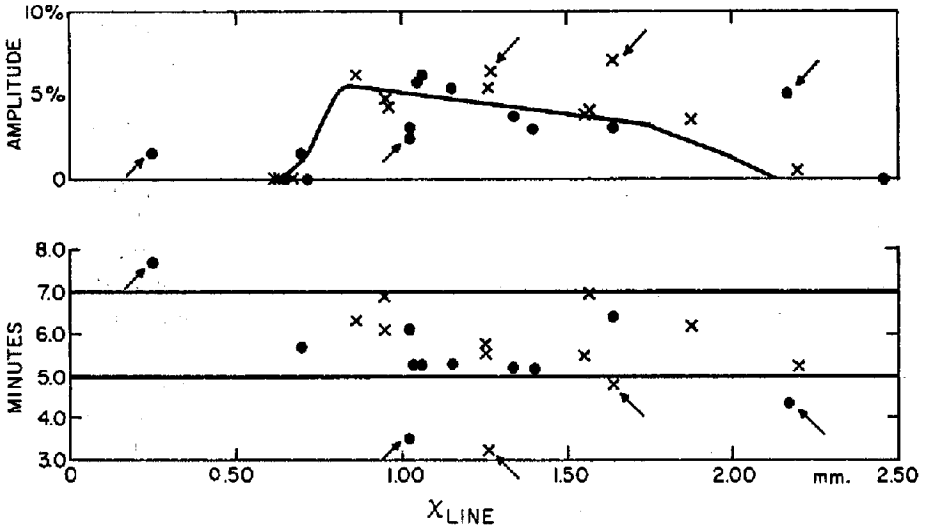

FIG. 5. Amplitudes and phases of growth responses to periodic stimuli of moderate size applied to short sections of the growing zone. Variable position of stimulus.

Width of test area 0.2 mm. Period 6 min.

●, $I = -3$, duration of stimulus 1 min.

×, $I = -4$, duration of stimulus 0.5 min.

Upper graph: amplitudes of the growth responses.

Lower graph: time difference between maximum of the growth speed and the midpoint of the preceding stimulus.

The arrows designate experiments with aberrant phase relations between stimulus and response. These are presumed to be artifacts, due to spontaneous short period tropic oscillations, as illustrated in Fig. 2.

saturating in the test area, but are in part affected by false response due to tropic oscillations, as discussed in the preceding section. Fig. 5 shows the results of two similar experimental series of this kind. The upper part shows the amplitudes, the lower part the phase of the maximum. It will be seen that most of the points showing abnormally large or small amplitudes also show an abnormal phase relation (time between stimulus and response maximum outside the range from 5 to 7 min.), and can be ignored. The rest of the points show a sensitive zone ranging from 0.65 mm. to some point beyond 1.8 mm. below the sporangium.

Tropic Experiments.—

(*a*) *Qualitative results*: In the section on procedures it was explained that long stimuli have to be used to obtain reliable measurements on the localization of bends. This results in a multiple choice as to the positioning of the specimen during the stimulus. We will begin with the choice that seemed to us the most natural one: The specimen is moved so that the stimulus falls continuously on the same marked wall element. This involves lowering the specimen continuously. Strictly speaking, to maintain a constant geometric relation between stimulus and wall element the specimen has to be rotated in addition to lowering, to compensate for twist below the test area. The case without rotation will be discussed first.

The bend first forms near the marker, but then moves away from the sporangium *faster than the marker*, so that it is soon located at the bottom of the growing zone, where it is not affected by twist. The direction of the tilt is towards the light source, even though, owing to twist below the line (and marker) the illuminated area often rotates during the course of the illumination through more than 180°.

If, in addition to lowering the specimen continuously to keep the line on a marked level, the marker is kept at a fixed azimuth relative to the stimulus then a bend develops as above, but the direction of tilt, which starts towards the stimulus, progresses around more and more towards the observer, during 30 to 50 min. In other words, the bend behaves as if the *onset* of the stimulus determines the direction of the tilt, relative to the foot of the growing zone, even though the continuation of the stimulus is necessary to maintain the bending speed.

We learn from these findings that the response does not stand in a fixed relation to a given *material element* of the wall, but moves away from the sporangium faster than the wall element. Therefore, the wall is not the locus of the primary effect of the stimulus. Instead, this effect must be located in some protoplasmic structure which moves relative to the wall. This conclusion is strengthened further by experiments, in which the stimulus is kept at a *constant distance from the sporangium*. Here, too, the bend which forms moves away from the sporangium faster than does a marked element of the wall.

These results suggest that it should be possible to move the stimulus away from the sporangium at the same speed at which the bend moves. It turns out that this condition is very nearly satisfied if the specimen is not moved at all. The stimulus then moves away from the sporangium at a speed equal to that of the growth rate, about 0.05 mm./min. Fig. 6 shows that in this case the bend center forms and at all times remains very close to the stimulated area. Fig. 7 shows a frequency distribution of the distance between the line center and the extrapolated position of the bend center, extrapolated to the time of its initiation. The mean of this distance in 18 experiments is 0.

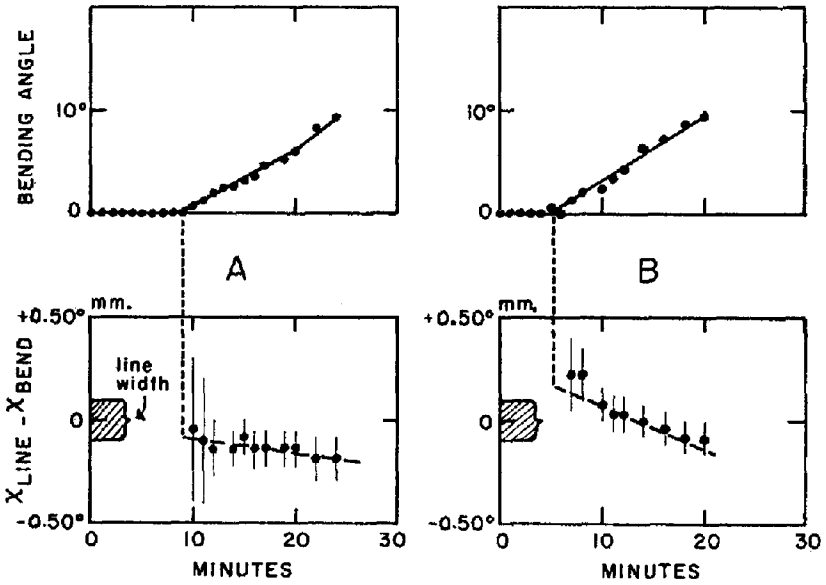

FIG. 6. Tropic responses to prolonged unilateral stimulation of a short section. Position of stimulus held at a constant level relative to ground.

Upper graphs: tropic angle *versus* time.

Lower graphs: location of bend relative to stimulus, *versus* time.

Initial distance x_0 of stimulus from sporangium.

Experiment A: $x_0 = 0.22$ mm.

Experiment B: $x_0 = 1.35$ mm.

Width of test area 0.2 mm. Adapting intensity $I = -5$.

Reaction times obtained by back-extrapolation: 8.8 min. for A, 5.5 min. for B.

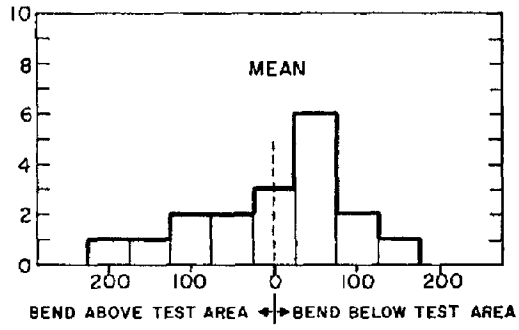

FIG. 7. Distribution of distances between center of test area and bend center at its first appearance. Eighteen experiments. The mean distance is 0. The standard deviation is 90 μ. It can be accounted for by errors of about 50 μ in the bend position, of about 30 μ in the line position, and by occasional spontaneous bends, undetected as such.

Qualitatively it could be seen that the bent region has the least extent in this last case. For any other positioning program the bend extends over a large part of the growing zone, whereas, if the specimen is not moved the bend seems to be limited to a region about 0.3 mm. long.

The conclusion that the stimulus is received in a structure with a definable kinetics, relative to the wall, is further strengthened by experiments in which the specimens were so positioned that the stimulus moved away from the

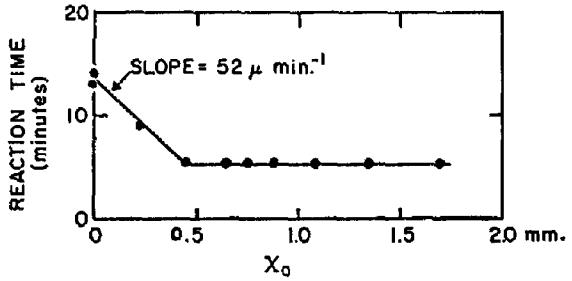

FIG. 8. Same experiments as Fig. 6. Lag between onset of stimulus and onset of tropic response versus initial position of stimulus. Onset of response obtained by back-extrapolation, as illustrated in Fig. 6.

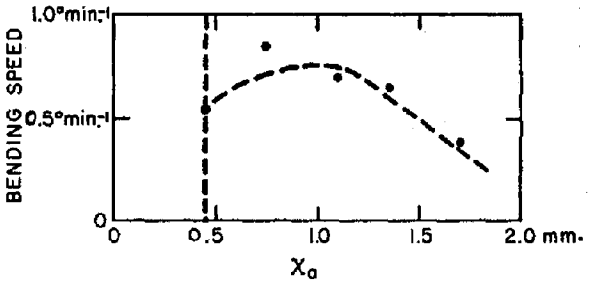

FIG. 9. Same experiments as Fig. 6. Bending speed *versus* initial position of stimulus. The bending speeds are obtained as the slopes of the curves given in Fig. 6.

sporangium faster than the growth speed (faster by 0.031 mm./min). In these experiments the bend first appeared *above* the line, approximately at a position predicted by the postulated kinetics of the receptor.

(*b*) *Stimulations at constant height relative to ground:* It is clear then, that in experiments involving long stimuli of short test areas meaningful results are most likely to be obtained when the specimen is not moved, so that the stimulus travels down the growing zone with a speed equal to the growth speed.

Figs. 8 and 9 show the results of such experiments, using levels of adaptation presumed to minimize the effects of internal scattering and varying the initial distance x_0 from the sporangium.

Fig. 8 shows the reaction time *versus* x_0. This time is the same (5.3 min.) for any $x_0 \geq 0.45$ mm. If the stimulus is started closer to the sporangium the reaction time increases by amounts equal to the time it would take the stimulus to reach the point $x = 0.45$ mm. In other words, illuminations above this critical point have no effect. The top half-millimeter of the growing zone is insensitive to tropic stimulation as it is to growth response stimulation. In a preceding publication (Cohen and Delbrück, 1958) we had shown (by marker experiments) that this zone also does not *respond* when the whole growing zone is stimulated. As far as stimulus and responses go it is therefore quite inert, even though it is the region of the most active stretch.

Fig. 9 gives the bending speed *versus* x_0. It should be kept in mind that this is not a measure of the sensitivity at x_0, because the bending speed is measured when it has reached its asymptotic value, about 10 min. after the beginning of stimulation. At that time the stimulus is at $x_0 + 0.5$ mm. The lower end of the sensitive zone is very ill defined. For $x_0 = 1.7$ mm. a measurable bending speed was barely ascertainable before the stimulus moved out of the growing zone. The method does not permit any conclusions as to how far the sensitive zone extends beyond this point.

DISCUSSION

The results of the present investigations may be briefly summarized as follows:—

1. The sensitive zone, on which light signals must fall to produce responses, is the same for growth responses and for tropic responses. It begins about 0.5 mm. below the sporangium, thus not including the zone in which the stretch rate is maximal.

2. The tropic responses to a steady stimulation of a narrow section remain sharply delimited in space only if this section is held at a constant position relative to ground. This means that it is moving away from the sporangium at a rate equal to the growth rate, and moving relative to a fixed material element of the outer wall with a speed which varies with the position in the growing zone, being maximal at the top and 0 at the bottom. Moveover, the response occurs as if, at least to a first approximation, spiral growth did not exist. In other words, the response occurs as if there existed in the sensitive zone an inner wall which is anchored to the lower non-growing zone of the plant and which does not stretch or twist in this zone. The responses which we *see* are, of course, responses of the outer wall. The outer wall may be presumed to be entirely passive and reversible in its responses and to slip over the postulated inner wall and to reflect the latter's distortions faithfully by elastic stretch.

In the remainder of this discussion we wish to explore whether these findings can help in establishing a coherent picture of the various types of growth and tropic responses.

Let us begin with a qualitative recapitulation of the principal effects to be explained.

1. *Growth responses* in situations of symmetric illumination. These are responses to *temporal* changes in the intensity. In the case of pulse-ups or step-ups or step-downs in the intensity, the responses are typically *transient*, with peaks around 5 to 8 min. and practically complete after 12 min. The final growth rate is the same as the initial growth rate, even if, as in the case of step-ups and step-downs, the final intensity is different from the initial intensity. This assertion is contrary to one of Blaauw (1918) who believed that he had found a dependence of the steady growth rate on the level of intensity. Measurements of Dennison (1958) have shown no indications of such a dependence.

In a previous publication (Delbrück and Reichardt, 1956) a formalism has been developed which accounts for the transient nature of these responses, by relating it to adaptation. There was postulated the existence of an internal variable, the *level of adaptation*, A, which follows the intensity of illumination according to a simple differential equation, identical with that of the charging of a condenser. The time constant of this adaptation is between 3 and 4 min., so that adaptation in typical growth response experiments is practically complete in about 10 min. Growth responses occur only as long as the specimen is not adapted to the illumination. They are determined by the subjective intensity $i = I/A$. Positive growth responses occur as long as $i > 1$, negative growth responses occur as long as $i < 1$.

This formalism predicts a sustained positive growth response under conditions when the intensity increases exponentially for a prolonged time. Under these conditions the level of adaptation keeps lagging behind the intensity and the subjective intensity is greater than unity during the entire course of the exponential rise. Under these conditions there are indeed observed sustained increases in growth rate (unpublished experiments by Rieser, Harm, and Cohen).

2. Tropic responses to unilateral illuminations. These are responses to *spatial* (azimuthal) differences in the intensity. In this case the response *persists*, being strong 15 min. after the establishment of the azimuthally unsymmetric distribution; *i.e.*, they persist strongly after the transient growth responses discussed above have died away. Herein lies the principal difficulty in relating tropic and growth responses. Blaauw attempted to get around this difficulty by invoking a dependence of the growth rate on the absolute intensity, even after adaptation had occurred. This approach, as pointed out above, is not supported by our observations.

A simple calculation may be given to support this contention. A bending speed da/dt, in which a is the angle of tilt, corresponds to a difference in growth speed between the proximal and the distal side equal to $b \cdot da/dt$, in which b is the diameter of

the specimen. For a steady bending speed of 5°/min. (Fig. 2), a diameter of 0.1 mm. and an average growth speed of 0.05 mm./min. this amounts to a difference in growth speed of about 0.008 mm./min.; *i.e.*, about 16 per cent of the normal growth speed. The intensity of illumination near the midline of the distal side is at most 2 units (a factor 4) higher than that of the proximal side (owing to focusing). If the growth speed in the steady state increased by 16 per cent for every 2 units of intensity it would have to increase by 80 per cent over the normal range ($-10 < I < 0$). This is entirely excluded by the experiments of Dennison, and even by Blaauw's original experiments.

Before discussing this difficulty further, we will describe an effect discovered by Reichardt and Varju (1958) which clearly shows that transient growth responses can produce transient tropic effects. The idea of the experiment of Reichardt and Varju is as follows: the usual tropic experiments suffer from the defect that at the onset of the stimulus both the general intensity level and the azimuthal distribution of intensity are altered. Thus, for instance, if we start with a bilaterally symmetric illumination and at time 0 cut off one channel, we alter the intensity in different azimuths by different factors, and the effect of this is difficult to evaluate theoretically. However, from the analysis of the growth responses, we should expect that after about 10 min. the transients have died away and every azimuthal part has a fixed level of adaptation. Experiments show that the specimen then bends at a fairly steady rate of about 5°/min. (Fig. 2). This steady bending rate we will leave unexplained for the moment. The experiment of Reichardt and Varju consists in raising the intensity of the open channel after this steady tropic state has been reached. This should raise, *by the same factor*, the intensity of illumination of every azimuthal section *which is illuminated*, and should produce a similar growth response in each such azimuthal section. The crucial point is now that only about ⅕ of the distal side of the sporangiophore is illuminated, while the whole of the proximal side is illuminated. The integrated response of the proximal side should, therefore, be much greater than that of the distal side, and a *negative, transient* response is expected, and indeed, found. Reichardt and Varju have investigated this effect under a variety of conditions and have found a nice qualitative and quantitative agreement with the predictions of the theory developed for growth responses.

These experiments show that *transient growth responses* can produce *transient tropic responses*, but they still leave the steady tropic responses unexplained and unrelated to the growth responses.

3. Our experiments on the tropic responses to unilateral stimulation of a fixed section of the inner wall show first of all the typical feature of persistence: the bend increases during the entire course of travel of the inner wall element through the growing zone, *i.e.*, for about 35 min. The total bend produced during this travel has been found to be roughly proportional to the

width of the test area. With the 0.20 mm. line this total bend amounts to about 20°. This finding can be related in a simple manner to the tropic experiments in which the whole growing zone is illuminated unilaterally. In this latter situation we must take into account that new elements of the inner wall are continually added at the top. If we give these elements a length of 0.2 mm., equal to the width of our test line, then there will be added a new element every 4 min. Each such element, then, as it travels down, will contribute a bending angle of 20°. This leads to a steady rate of 20°/4 min. = 5°/min., as observed under conditions of unilateral illumination of the whole growing zone.

A similar inference can be drawn from the results given in Fig. 9, in which the steady bending speeds in response to stimulation of a fixed inner wall element are plotted *versus* the initial position of this wall element. Of course these speeds are steady only for a limited time; *i.e.*, as long as the inner wall element remains in the growing zone. Still, one should expect that their sum is roughly equal to the steady bending speed when the whole growing zone is stimulated. Inspection of Fig. 9 shows that this is indeed the case.

This argument, while it relates the tropic line experiments with the usual tropic experiments in a satisfactory manner, still provides no clue as to the cause of the persistent tropism in either situation. The following suggestion, due to Jaffe (personal communication), may help. Jaffe supposes that in the case of an azimuthal inhomogeneity of the intensity the adaptation A is not strictly azimuthally autonomous, perhaps even completely uniform around the whole circumference, A taking a value corresponding to the average intensity. We consider such a complete averaging as rather unlikely for anatomical reasons, but will consider that in the vicinity of sharp azimuthal intensity gradients a certain smoothing out of the level of adaptation occurs. Consider, then, a unilateral illumination producing a fairly smooth intensity distribution over the proximal side, but sharp intensity gradients near the center of the distal side. If the adaptation is here smoothed out, then the bright center will continually see an intensity in excess of its level of adaptation, so that here, and only here, the subjective intensity will be greater than unity, leading to a sustained growth response and, therefore, to a sustained tropic response.

In other words, with respect to local (azimuthal) autonomy Jaffe assumes that it is perfect with respect to the growth response but imperfect with respect to adaptation. Actually, for geometrical reasons, the growth response must also be smoothed out azimuthally, but the two functions to be smoothed out are not identical and a net asymmetry between proximal and distal side may remain.

There are two difficulties with Jaffe's suggestion: in Buder's experiment (Buder, 1918) (specimen in mineral oil) there are no sharp azimuthal intensity gradients, and yet a strong steady tropism. Similarly, in Oehlkers' experiment

(Oehlkers, 1926) (illumination from all angles of one side, by placing the specimen near a large luminous screen) there is similarly no sharp gradient, yet good tropism.

Jaffe's suggestion, then, needs to be explored further by careful observations of the tropic responses under other conditions affecting the azimuthal gradients, such as in mineral oil, or under wide angle illumination, or under conditions when the angle of the incident light with the axis of the sporangiophore is varied.

Returning to the paradoxes mentioned in the introduction, the following statements can be made: the fact that the tropic bend appearing as a result of unilateral illumination of the whole growing zone is confined to the lower half of the growing zone finds a satisfactory explanation in the notion that the primary receptors and effectors are linked to the inner wall, travel down relative to the outer wall, and do not begin to show an effect until they are 1 mm. below the sporangium.

Similarly, the failure of spiral growth to affect the direction of tilt to the expected degree finds an explanation in the notion that spiral growth is a characteristic of the outer wall, and not of the inner wall, the outer wall reflecting the primary responses of the inner structure passively and reversibly by elastic stretch.

Our studies then have led us to postulate a new and hitherto unseen protoplasmic structure, the inner wall. Its identification by methods of microscopy remains as a challenge. If such a structure can be demonstrated, the reaction mechanism within it may exhibit almost perfect local autonomy.

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