TROPIC RESPONSES OF *FUNARIA* SPORES TO RED LIGHT

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ABSTRACT The tropic responses of *Funaria hygrometrica* spores to continuous illumination with red light (610 to 690 $m\mu$) have been studied over the intensity range from 10^{-5} through 10^{+6} erg/cm² second, using both plane polarized light and partial illumination with unpolarized light. From the relative frequency of outgrowth origin in different directions, the following is inferred. (1). The germination direction of chloronemal filaments is directly influenced by red light over this whole intensity range, while that of rhizoids tends to be opposite the chloronema. (2) Three photoreceptor systems direct chloronemal primordia: (*a*) A low intensity system acting from 10^{-5} to $10^{-0.5}$ erg/cm² second. It favors their growth from a cell's brightest part(s). Its photoreceptors are disoriented, excited by the electric vector, and probably are dispersed phytochrome molecules. (*b*) A medium intensity system which acts largely alone only at $10^{0.5}$ erg/cm² second but is influential from 10^{0} to 10^{5} erg/cm² second. It likewise favors growth from a cell's brightest part(s); its receptor molecules are also excited electrically, but they are tangentially oriented. (*c*) A high intensity system which acts alone from 10^{5} to 10^{6} erg/cm² second and is influential down to 10^{1} erg/cm² second. It favors growth of the chloronemas from a cell's darkest part. Its receptors probably are magnetically excited and tangentially oriented. The polarotropic responses of the chloronemas resemble those directing their origins. One new feature is that under intense (10^{6} erg/cm² second) plane polarized and vertically directed light, many soon grow to form tight helices.

INTRODUCTION

Cellular phototropism may be defined as the localization and hence orientation of cell expansion by light. Generally, little change in the average rate of such growth is linked to the spatial redistribution which defines the phenomenon. In some cases light redistributes expansion between two sides of an elongating structure while in others, particularly those of oriented germination, it determines the site of initiation of a new outgrowth. We include both under phototropism since we feel that the difference lies in the consequences rather than the mechanism of growth localization.

These consequences serve to guide the growing cell towards regions of optimal illumination, but here our concern is with mechanism not function.

The phenomenon was found in a variety of organisms in the 1880's, was first studied intensively by Blaauw (21) in the *Phycomyces* sporangiophore, and has received considerable study in this and to some extent other materials ever since. Useful recent reviews include those by Banbury (18), Haupt (19), and Shropshire (20). A good reivew of the related phenomenon in multicellular forms, is that of Briggs (25).

Briefly, these investigations show a great similarity between phototropism in unicellular and multicellular forms. In both, much is known of the relations between the stimulus wavelength, intensity, and duration, and the response measured as a degree of growth orientation. Some major inferences from these relations are that the tropic photoreceptor molecules are usually yellow pigments (probably carotenoids and/or flavines) and that the blue-sensitive systems they mediate show marked and varying outputs over a very wide range of input duration and intensity; this broad responsiveness often being brought about by at least two relatively independent subsystems, one dominating the responses to brief stimuli, the other to long, intense ones. However, until recently, relatively little was known of the critical relationship between the *spatial* pattern of light absorption and the resultant pattern of growth localization. Moreover, very little is known of the intermediate mechanisms in unicellular forms; the best clue may be the important fact that a photoinduced lateral transport of auxin is a link in the process in multicellular ones.

Another aspect of the phenomenon of phototropism, discovered in 1956 with the egg of the brown alga, *Fucus* (22) and soon found to be general (26) is that of *polarotropism.* When unilaterally illuminated with plane polarized light, phototropically responsive cells *either* grow out in directions sharply concentrated in the polarization plane[1] *or,* depending upon the species, among directions perpendicular to this plane. In an effort to interpret polarotropism, means were developed to impose known intensity patterns upon large numbers of cells (2). Thus it became possible to determine how large a transcellular intensity difference of unpolarized light must be to orient growth as strongly as does polarized illumniation.

This *equivalent difference* was determined for two dissimilar cells: a fungal spore which tends to grow out from its brightest part and a fern spore which tends to grow out from its darkest part. In both, the equivalent difference proved to be very large—about 60 to 90 per cent.[2]

Assisted by this key measurement, one could infer the general mechanism of

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[1] Here as elsewhere in the biological literature the plane of vibration or polarization refers to that of the electric vector.

[2] Such a difference is defined as the quotient of the maximum difference of intensities imposed upon a cell divided by the *highest* of these. Thus if one part of a cell receives a relatively high intensity, I_H and the rest, the low one, I_L, then the difference is given by $(I_H - I_L)/I_H$.

polarotropism; the photoreceptor molecules themselves are highly dichroic and oriented with respect to the nearby cell surface, hence they convert polarization into a large absorption gradient. None of the other mechanisms considered could give equivalent differences in this size range, at least, not in the cases so far studied. Among these excluded alternative mechanisms are ones dependent upon the action of dichroic filters in front of the receptor molecules; polarized fluorescence, scattering or reflection; and the direct creation of molecular orientation as by differential synthesis, destruction, or solution.

Specifically it was inferred in some kinds of cells that expansion tends to center in the brightest region; in others, the darkest; in some, the photoreceptor molecules are tangentially oriented; in others, radially (2). Moreover, in all cases it could be directly inferred that the photoreceptor molecules are excited by the electric rather than the magnetic vector in the light (4).

All this refers to exclusively blue-sensitive systems, but ones which also respond tropically to red light are known (23, 26). Hence we felt it appropriate, using the new polarization and controlled gradient methods, to extend the investigation, and thus the possible generality of the findings, to such a panchromatic system.

The easily obtained spores of the common moss, *Funaria* are known from preliminary study to be such a one (1). Moreover, as Heitz long ago pointed out, these cells are generally excellent material for the study of cellular tropisms (24). They are *easily stored* in a dry, dormant state for many years without change in their behavior when activated by water, thus they are available with the ease and reliability of a stable, off-the-shelf, chemical. From our viewpoint, they are *highly generalized* plant cells, since they are sensitive to the main tropic stimuli, *i.e.,* blue and red light, gravity, and other cells; they seem to bear all of the chief photoreceptor systems, *i.e.,* the photosynthetic, phytochrome, and blue-sensitive tropic ones; they are germ cells which immediately generate the two chief primitive filament types, *i.e.,* the rhizoidal and chloronemal, and ultimately generate a complex, multicellular plant. They are *spherically symmetrical* which greatly simplifies inferences of structure from behavior.

In addition to their panchromatic sensitivity, *Funaria* spores were found in a preliminary investigation to exhibit two other poorly studied aspects of the phenomenon; they tend to grow out *perpendicular* to the vibration plane of polarized light,[1] and their responses involve the origins of both rhizoids and of chloronemas (1). In the present, more extended study, the responses to several types of continuous red illumination were used.

(*a*) We used two patterns of partial illumination with unpolarized light which are illustrated in Fig. 1 and Fig. 2. Under unipolar illumination, only one polar segment of each cell (not necessarily a hemisphere) is illuminated; under bipolar, two equal and opposite ones are. These were used to find the pattern of growth control, that is, to determine whether and to what degree both the chloronemas and

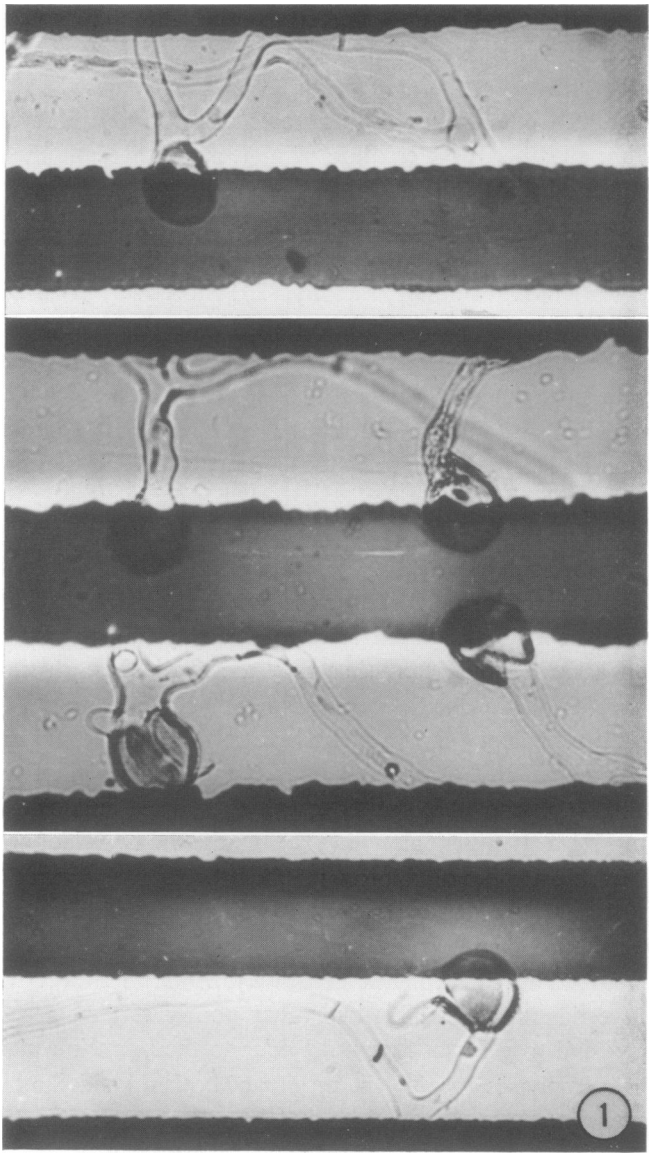

FIGURE 1 Cells grown under unipolar illumination. Subjected (for 48 hours after being wet) to 0.1 or 1 ergs/cm^2 second of unpolarized red light coming from below. Photographs of seven spores which happened to lie upon a boundary are shown. All long outgrowths are chloronemas. Note tendency of chloronemas, not only to originate from the bright part of the spore, but to subsequently bend or branch so as to remain in an illuminated region.

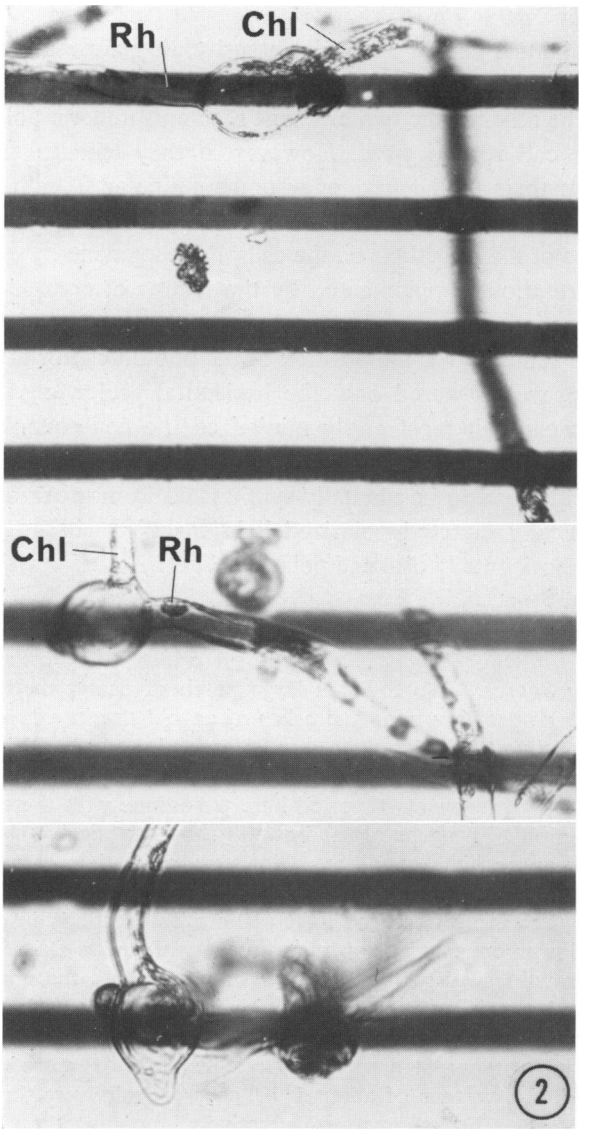

FIGURE 2 Cells grown under bipolar illumination. Subjected (for 44 hours after being wet) to 100 ergs/cm^{2}second of unpolarized red light coming from below. Photographs of three spores which happened to be centered upon an opaque band are shown. *Chl* = Chloronema; *Rh* = Rhizoid.

the rhizoids tend to originate from the brightest, from the darkest, opposite the brightest part, *etc.* (The need to investigate antipodal growth control possibilities—ones not considered before—was suggested by the obvious tendency of spores to generate a chloronema and a rhizoid from opposite poles. Thus if the chloronemas grow from the brightest part at a certain intensity, then we reason that they should tend to start in the center of the bright segment under unipolar illumination and the center of one or the other of the two brightest segments under bipolar illumination—all directions we call zero degrees. However, if they tend to grow opposite the darkest part, then their modal frequency under unipolar illumination should likewise be at 0°, but under bipolar illumination they should grow most frequently from 90°, *i.e.,* one of the directions on the cells' dark segments.

(*b*) This information, supplemented by the results of complete illumination of cells with unidirectional plane polarized light, indicates the orientation, mode of excitation, and to some degree the position of the photoreceptor molecules (2-4).

(*c*) As before, we measured only the horizontal projections of the outgrowth directions; this because it most easily minimized the consequences of orientation by gravity and the substratum. Hence the three dimensional response to unidirectional polarized light had to be mentally synthesized from measurements upon cells treated with vertically directed polarized light together with measurements upon cells treated with horizontally directed polarized light.

MATERIALS AND METHODS

Obtaining Spores. We found a large patch of nearly mature sporophytes of *Funaria hygrometrica* growing upon the ashes of an old fire in a sparsely wooded field in Waltham, Massachusetts. These were dug up, potted, and placed in a greenhouse. A month later the now mature spore-bearing capsules were harvested and then stored in the dark at 3°C. The experiments reported here were done with these spores when they were 2 to $4\frac{1}{2}$ years old. Five-year old spores still show practically 100 per cent normal germination under appropriate conditions. However, some time between 2 and 4 years after harvesting, the spores come to need a cell concentration of at least 30 cells/mm^2 when grown under relatively weak red light (10 erg/cm^2 second) in order to germinate normally; for at lower concentrations, many then exhibited a striking syndrome characterized by outgrowths swollen to up to two or more times the normal diameter and large inclusion bodies staining very darkly with iodine. All experiments reported here were done with cells sown at sufficiently high concentrations to develop normally.

For each experiment, about ten randomly selected capsules were dissected open and their spores pooled. An average of about $1 \cdot 10^5$ cells/capsule were thus obtained.

Sowing Spores. Dry spores were effectively immobilized on the desired surface by means of a very thin film of agar, one containing about 1×10^{-5} gm/cm^2. This was done by spreading, over 6 cm^2 of substratum, one drop of a freshly prepared suspension of cells in a solution of 0.1 per cent agar in water, and then quickly drying this film in vacuo. The spores were thus redried less than 30 minutes after being wetted. This process had no apparent effect upon their subsequent development when later rewetted by the culture solution. Surface concentrations of 4 to 80 cells/mm^2 were obtained as

desired by adjusting the volume concentration of cells in the suspension. The method gave well dispersed cells; about two thirds of them were found to lie more than one cell diameter away from any neighbor.

Various simple and obvious methods gave suspensions for the above procedure in which the cells were badly clumped, so cells were dispersed as follows: the inside of a 5 cm Petri dish bottom was thinly coated with platinum[3] to make it electrically conductive and then with polystyrene[4] to make it hydrophobic. Dry spores were evenly scattered in this dish,[5] the dish was gently breathed on several times to fog it and 4 cc of 0.1 per cent agar poured in. The spores were now found to be loosely attached to the bottom under the solution and well dispersed. They were then easily brought into suspension with gentle brushing.

To spread the spore suspension so thinly the substratum was first primed. (1) It was covered with freshly prepared 0.5 per cent gelatine at 23°C for a few minutes and then rinsed with water. (2*a*) If the substratum was a glass cuvette (used for unilateral illumination) it was then filled with freshly prepared 0.1 per cent agar for ten minutes, drained to leave a thin wet film and dried in vacuo .(2*b*) Quartz squares striped with chromium (used for partial illumination) were dipped in boiling 0.1 per cent agar and slowly withdrawn so as to leave an agar coating thin enough to show interference colors.

Medium. The medium was a modified 0.05 per cent Knop solution concocted after recipes of Fitting (5) and of Kofler (6). It was made up in glass distilled water, filtered through a type HA millipore filter, and had a pH of 6.5. Its composition follows:

1.8×10^{-3}M	$Ca(NO_3)_2$		2×10^{-5}M	H_3BO_3
7×10^{-4}M	KNO_3		4×10^{-6}M	$ZnSO_4$
6×10^{-4}M	$MgSO_4$		6×10^{-7}M	$MnSO_4$
3×10^{-4}M	$Fe_2(SO_4)_3$		1×10^{-7}M	$CuSO_4$
2×10^{-4}M	K_2HPO_4		6×10^{-8}M	KI
1×10^{-4}M	KH_2PO_4			

The cells were grown under a 4 mm deep layer of this medium.

Control of Temperature and Bacteria. The culture medium was kept at 23 $\pm$ $\frac{1}{2}$°C during the experiments. At irradiances of $\leq 10^3$ erg/cm^2 second, control of the ambient air temperature sufficed; at 10^4 to 10^6 ergs/cm^2 second, it was supplemented by pumping thermostated water through the culture dish supports.

Bacterial growth was kept low enough by measures including removal of pieces of the spore capsule before making a spore suspension, ultraviolet treatment of the culture dishes, autoclaving the sowing medium, and milliporing the culture medium.

Light Control and Measurement. The light source was either a 1000 watt tungsten projector lamp or a 1600 watt high pressure xenon arc lamp. Up to twelve cultures per experiment received beams radiating from the same centrally placed source.

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[3] *Via* "Liquid Bright Platinum, #05-X," Hanovia Chemical Co., East Newark, New Jersey.

[4] Applied by filling the dish with a solution of 0.3 per cent ground Falcon Petri dishes in benzene, draining, and evaporating the residual solvent.

[5] Done by freeing the spores in a Petri cover, inverting the coated bottom in this top, flipping this covered dish so as to slap it right side up on the table top and shake the spores into a cloud which settled on the coated bottom, and thus flipping it over four more times.

Each beam was filtered so as to leave only a broad but sharply bounded band of red light; one falling to 1/2 maximum intensity at wavelengths of 620 and 680 mμ; 1/10 at 610 and 690 mμ; 1/100 at 605 and 700 mμ. An interference filter plus 4 cm of water served to remove long wavelengths; and absorption filter or filters, short wavelengths. (We were very careful to rigorously exclude significant traces of blue or of ultraviolet light.) Coarse intensity control was obtained with lenses and Wratten neutral density filters; fine by varying the lamp-to-culture distance. For polarization, we used polariod type H sheets placed last in the optical train. Their transmission is practically independent of wavelength from 600 to 700 mμ.

For unilateral illumination the cells were fixed to the bottom of horizontally placed rectangular cuvettes filled with culture medium and illuminated with a beam coming in through one side. In some experiments, this beam descended through the medium at 7° to the horizontal; in others, involving higher cell concentrations, at 14°. In either case the beam directly illuminated the chamber bottom and then was totally reflected by it. Thus each spore was illuminated by two equal beams whose average direction was exactly horizontal, yet the shadowing of cells by each other was negligible.

Unipolar illumination of the spores was made possible by immobilizing them upon substrata of fused quartz striped with 40μ wide opaque bands (of 0.1μ thick chromium) 40μ apart. (See Fig. 3 for diagram, Fig. 1 for illustration, (2) for further details.) Bipolar illumination of the 25μ diameter spores was made possible by immobilizing them upon similar substrata striped with 11μ wide opaque bands 29μ apart. (See Fig. 3 for diagram, Fig. 2 for illustration.) A cell was considered to have been subject to bipolar illumination if, after germination and iodine fixation, it (or really its outline imagined without outgrowths) was sufficiently centered upon an opaque stripe for the thickness of its smaller illuminated segment to be more than half that of its larger one. All cells defined thus, in a given area, were selected for counting by carefully scanning along one group of a few opaque stripes after another with the aid of a Leitz 23 × water immersion objective.

Irradiances were measured with a photronic cell calibrated against a thermopile in turn calibrated against a United States Bureau of Standards lamp.

Identification of Outgrowth Types and Duration of Illumination. Outgrowths were identified as rhizoids rather than chloronemas if they were relatively clear-tipped, tapered, and straight. From 10^{-5} through 10^{3} erg/cm² second, almost all outgrowths more than a few cell diameters long fell into two sharply distinct classes on this basis; but from 10^{4} through 10^{6} erg/cm² second, numerous and confusing intergrades appear. Here the necessary and sufficient method for categorizing outgrowths was to count only those in *heteropolar* forms, *i.e.*, individual germlings having two outgrowths differing by the same criteria used in dimmer light. In practice, half or more of the outgrowths were thus identifiable.

The duration of illumination was picked to yield numerous identifiable outgrowths. Thus spores under 10^{-5} through 10^{-3} erg/cm² second were illuminated for about 3 days before being killed; 10^{-1} through 10^{3}, for about 2 days; 10^{3} through 10^{5}, for 1 to 1½ days; 10^{6}, about 2 days.

Determining Growth Orientation. Before counting, the germlings were usually fixed by a solution of 1 per cent I_2 + 1 per cent KI in water. We counted only germlings arising from spores separated by a gap of more than one spore diameter from any other spore. The direction in which an outgrowth originated was defined as the horizontal component of the direction running from the spore's center to the outgrowth's base.

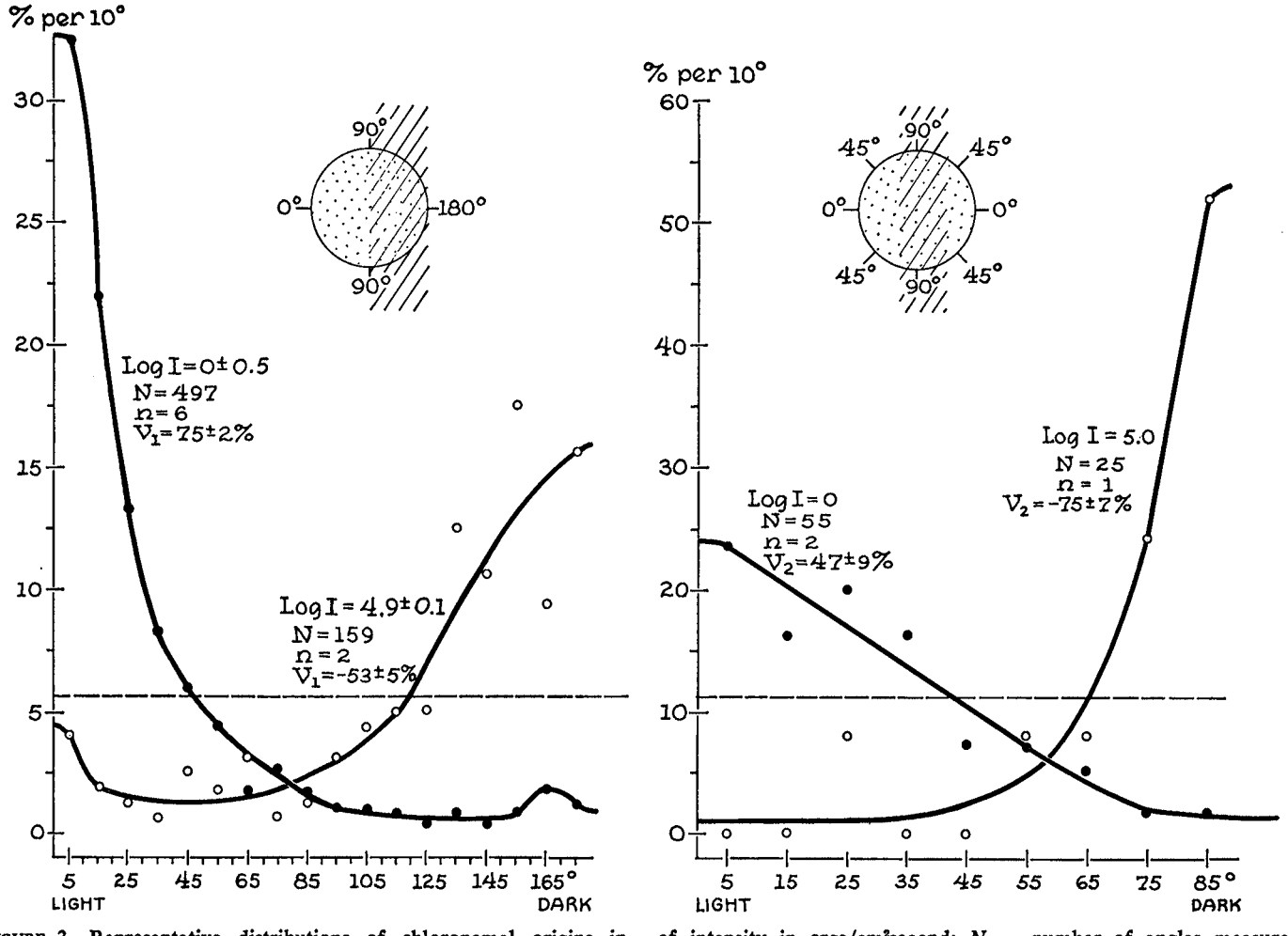

FIGURE 3 Representative distributions of chloronemal origins in response to unipolar illumination (left) and bipolar (right). Dashed horizontal line represents a uniform distribution. Log I = logarithm of intensity in ergs/cm²second; N = number of angles measured; n = number of experiments pooled; V_1 or V_2 = per cent orientation.

The measurement of large numbers of outgrowth directions was speeded by an electric goniometer (7). Statistical techniques have been described before (2, 3).

RESULTS AND ANALYSIS

Pattern of Growth Control in the Spores. In determining the response to partial illumination (Fig. 1, 2) we measured 4925 outgrowth angles to obtain 77 angular distributions. Each of those distributions which showed significant orientation either peaked in the direction marking the center(s) of each cell's illuminated segment(s), *or* (depending upon light intensity and outgrowth type) in that marking its dark part's center. This fact is illustrated by the representative distributions plotted in Fig. 3.

Since this is true, the degree and sign of each distribution's orientation is well described by the simple parameters previously used (3).

$$V_1 = \sum p \cos \theta \qquad V_2 = \sum p \cos 2\theta$$

where V_1 or V_2 is the *per cent orientation* in response to unipolar or bipolar illumination, respectively.[6]

p is the per cent of outgrowths originating in the direction θ. θ varies from 0°, the central direction of the bright part(s), to 180° or 90°, the central direction of the dark part under uni- or bipolar light respectively (see Fig. 3).

Hence we have thus characterized all the distributions determined in response to partial illumination and plotted the results in Fig. 4*a* (chloronemas) and 4*b* (rhizoids). Under both unipolar and bipolar illumination, and from about 10^{-4} to $10^{4.2}$ erg/cm^{2}second, the *chloronemas* tend to grow from each cell's brightest part(s); while from about $10^{4.7}$ to 10^{6} erg/cm^{2}second, from its darkest part. The response of the *rhizoids* is weaker and more complex. Roughly, the rhizoidal response to unipolar illumination at all intensities tends to be the reverse of that to bipolar illumination. In good part, then, the rhizoids' origin is controlled by a tendency to be opposite the cell's brightest part from about 10^{-3} through $10^{4.5}$ erg/cm^{2}second and opposite the cell's darkest part from about 10^{5} to 10^{6} erg/cm^{2}second.

Polarotropic Control of Chloronemal Origin. In determining the response to vertically directed polarized light, we measured 3188 chloronemal outgrowth angles to obtain 32 distributions. Each non-random distribution showed a single peak normal to the polarization plane, an example being shown in Fig. 5. Hence we characterized each of these distributions by V_2 and plotted the results (joined by the curve labeled "vertical") in Fig. 6.[7] Orientation begins at about $10^{-0.2}$ erg/cm^{2}second, rapidly rises to a maximum at about $10^{1.5}$ to $10^{3.5}$ erg/cm^{2}second, then slowly declines up to 10^{6} erg/cm^{2}second, the highest intensity used.

[6] Note that if all outgrowths form in the center of the cell's bright or dark parts, then the per cent orientation is +100 per cent or −100 per cent respectively, while 0 per cent orientation characterizes a uniform distribution.

[7] Note that negative orientation indicates alignment normal to the polarization plane.

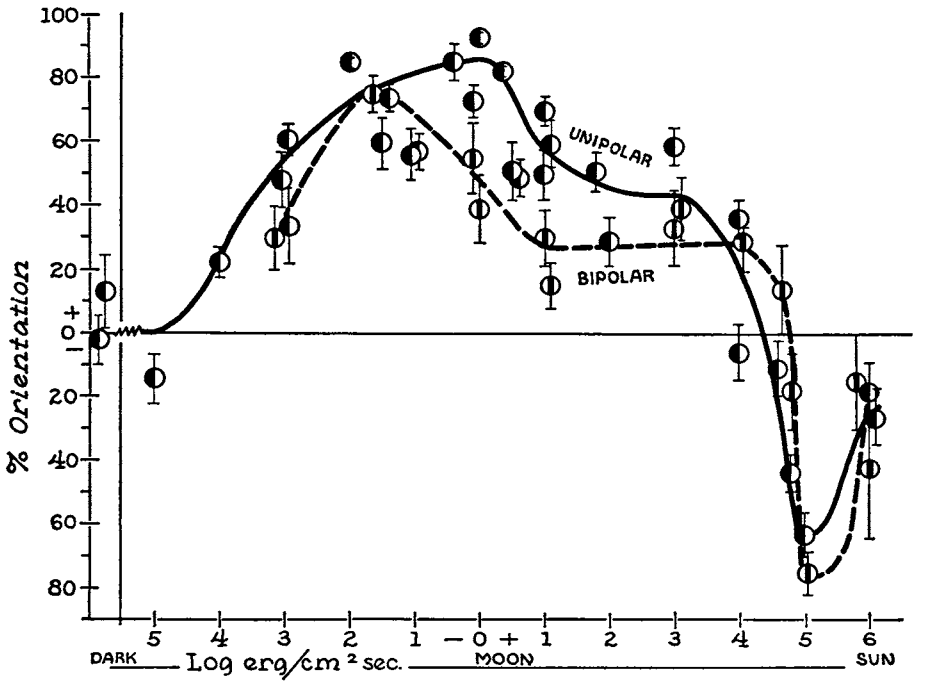

FIGURE 4*a* Orientation of chloronemal origins by partial illumination as a function of intensity. Plus and minus orientation values indicate tendencies to grow from the bright and dark parts of the cell respectively. Each point characterizes one experimental distribution; the bar through each is the theoretical standard deviation arising from a finite sample size.

Since the plane of preferred growth is perpendicular to the plane of polarization, and since it is practically necessary to restrict observations to the horizontal plane, we explored the projection of the response *within* the plane of preference through the use of horizontally directed and *vertically* polarized light. In determining these responses, we measured 2097 chloronemal outgrowth angles to obtain 15 distributions. We have grouped all of these distributions into six intensity ranges and plotted the pooled distributions of each group in Fig. 7.

They are of three types. (*a*) In the low intensity range from 10^{-3} to 10^{-1} erg/cm²second, the distribution is *uniform* except for a decline beyond 115°, in the rear parts of the cells. (*b*) In the medium intensity range, from $10^{-0.2}$ to $10^{0.5}$ erg/cm²second, there is a broad low maximum at the cells' *front poles.* (*c*) In the high intensity range from $10^{5.2}$ to $10^{5.8}$ erg/cm²second there is a sharp *"preequatorial"* peak at about 85° from the light. The three distributions obtained between the medium and high ranges may all be considered combinations of these last two types mixed in various proportions, this being particularly clear at $10^{1.5}$ erg/cm²second.

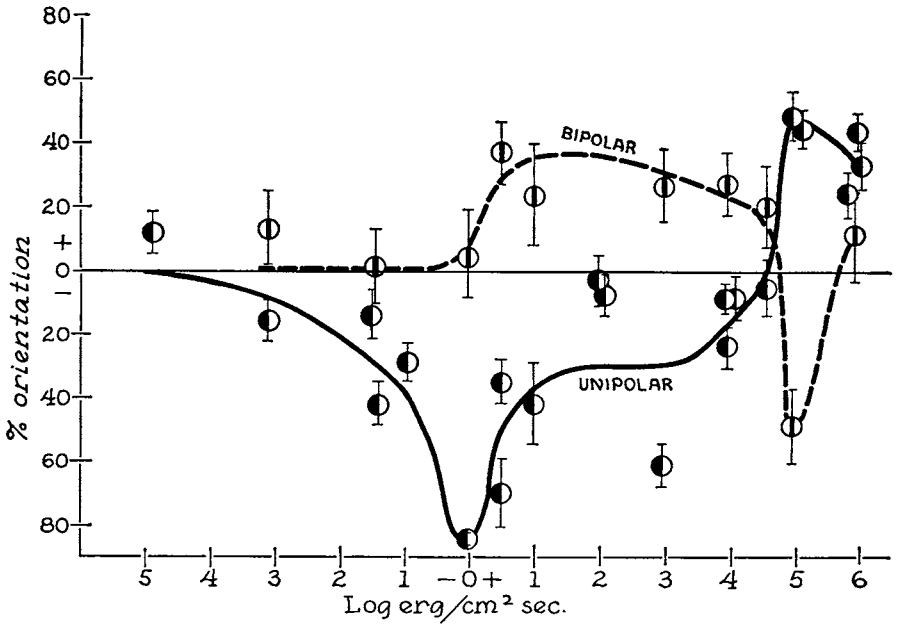

FIGURE 4*b* The same for rhizoidal origins.

In order to quantitatively characterize these responses, we use the parameter:

$$V_2^F = \sum_{0\text{-}90^\circ} p \cos 2\theta$$

where p is the per cent among those outgrowths developing from the cells' front hemispheres. That is, we calculate the per cent orientation in the front half of the distribution only. Hence if all these outgrowths point forward, $V_2^F = +100$ per cent; if all of these point croswise, $V_2^F = -100$ per cent; while if the distribution is uniform in the front hemisphere, $V_2^F = 0$ per cent. We chose this parameter for two reasons: first, by ignoring the rear half of the distribution, one minimizes the interpretive difficulties produced by light transmission losses through the cell; secondly, V_2^F makes the three types of responses roughly commensurable in a simple manner.

Using V_2^F, then, all the responses to horizontally directed and vertically polarized light are plotted (and joined by the curve labeled "horizontal") in Fig. 6. The uniform response at low intensities is reflected in the near zero values of V_2^F there; the front pole tendency shows in the somewhat positive V_2^F values at medium intensities; the increasing preequatorial response at high intensities shows in the increasingly negative values there. Note, however, that the near zero values at the transitional intensity of $10^{1.5}$ erg/cm²second arises from a weakly bimodal distribution rather than a uniform one.

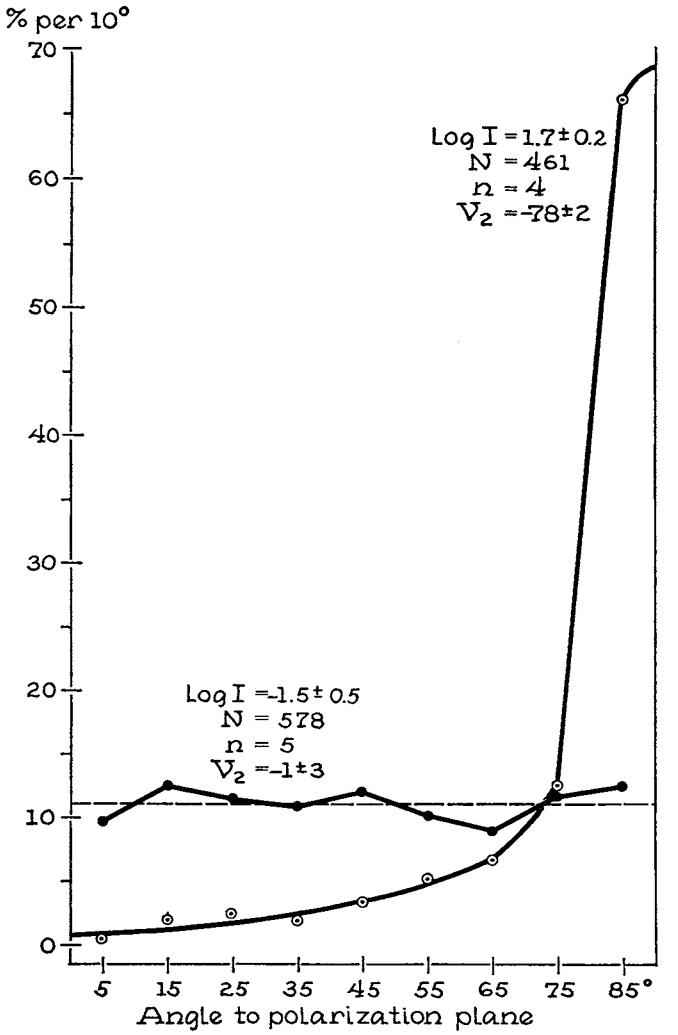

FIGURE 5 Representative distributions of chloronemal origins in response to vertically directed plane polarized light. Dashed horizontal line represents a uniform distribution. Log I = logarithm of intensity in ergs/cm²second; N = number of angles measured; n = number of experiments pooled; V_2 = per cent orientation.

The reader will recall that we have only been able to measure the horizontal component of each outgrowth's direction. Hence we have only determined outgrowth frequency as a function of the horizontal component of outgrowth direction. Such response curves were obtained for vertically directed polarized light (Fig. 5) and for horizontally directed but vertically polarized light (Fig. 7). Now it is instructive to compare these so as to imagine the surface which plots outgrowth

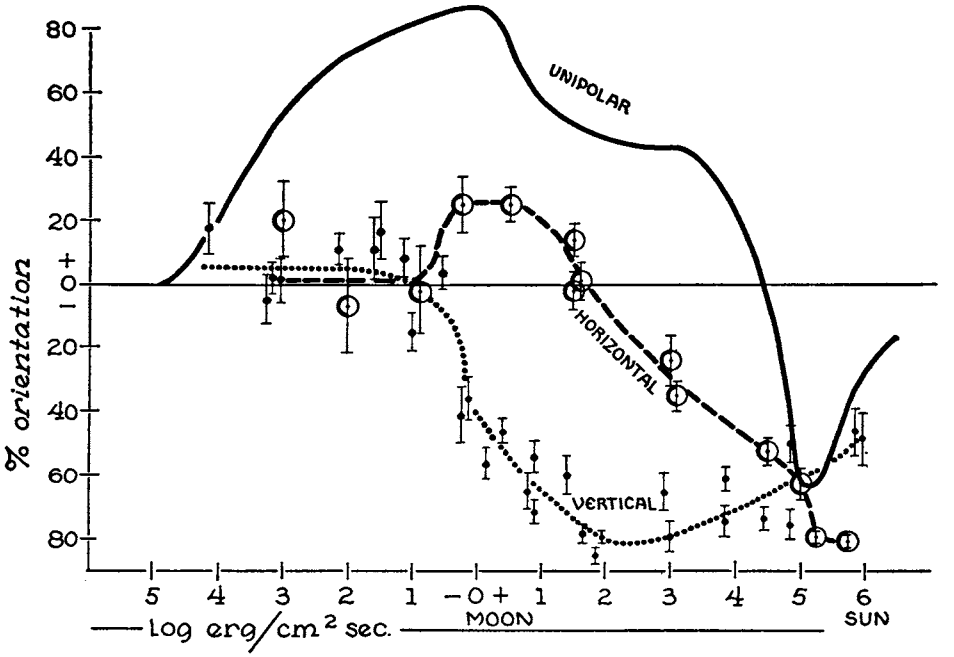

FIGURE 6 Orientation of chloronemal origins by polarized light as a function of intensity. "Vertical" refers to vertically directed plane polarized light; "horizontal" to horizontally directed and vertically polarized light. "Unipolar" curve (for comparison) is response to unipolar illumination with unpolarized light and is taken from Fig. 4*a*.

frequency (represented by distance from the origin) *versus* direction in space. That is, in the imagined absence of a substratum, gravity or other secondary orienting influences, let us visualize a plot of outgrowth frequency *versus* direction in spherical coordinates. It will be seen, that at high intensities, this surface is shaped like a wing or maple seed; the plane of the wings indicates the plane normal to the electric vector and their slight tilt, the preequatorial position of the modes. At medium and low intensities, the response surface resembles that of a fan or apple respectively; in both cases, the depression at the point of attachment indicates the fall in outgrowth likelihood away from the light.

Polarotropic Control of Rhizoidal Origin. Like the chloronemas, the rhizoids tend to start normal to the polarization plane. The degree of this orientation is displayed in Fig. 8. Again, like the chloronemas, at high intensities (10^3 to 10^6 erg/cm²second) the rhizoids tend to start at 85° to the propagation direction. Pooled distributions representing all observed rhizoidal responses to horizontally directed and vertically polarized light are shown in Fig. 9 (we have no such data below 10^3 erg/cm² second). The degree of this preequatorial orientation is shown in Fig. 8.

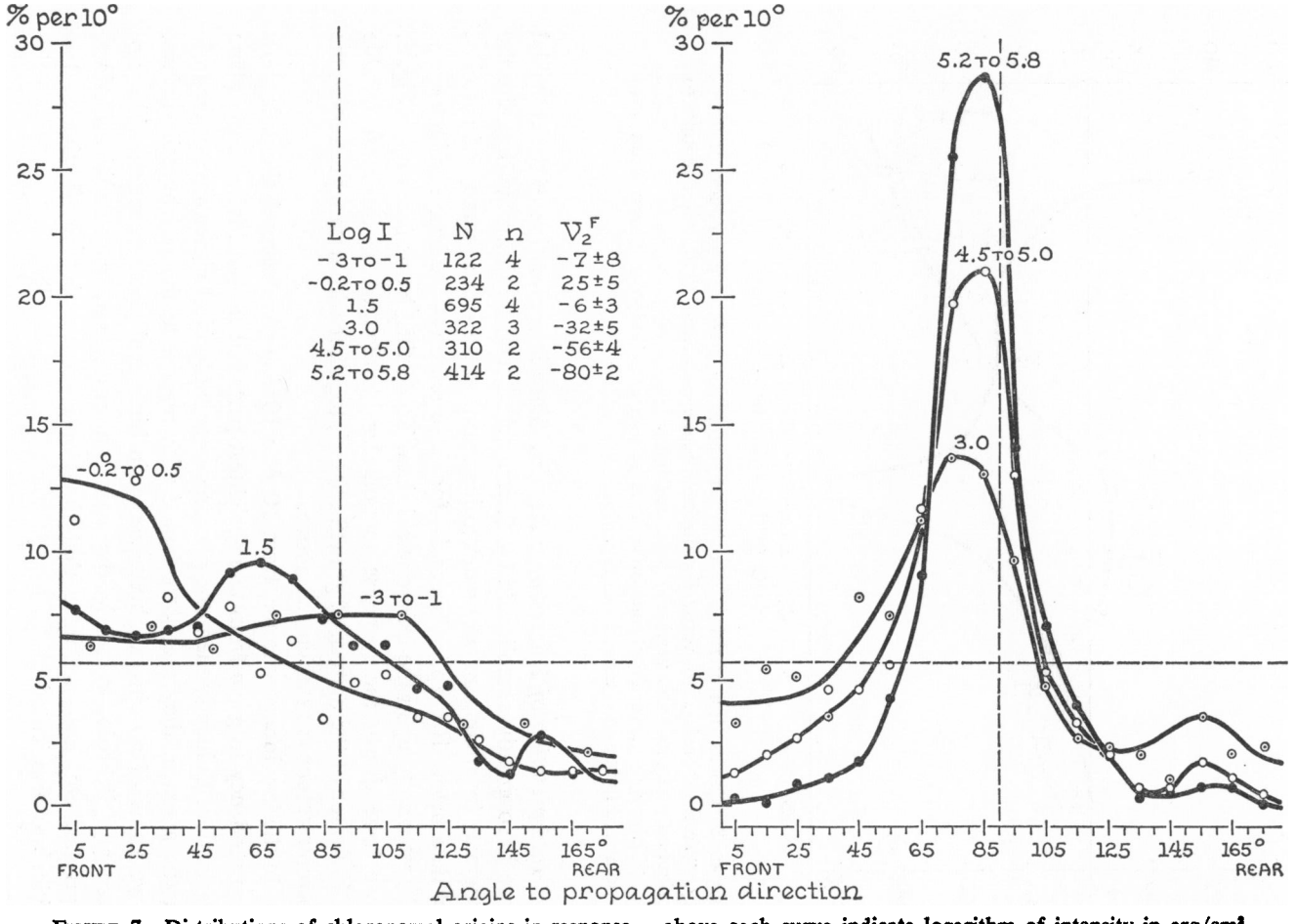

FIGURE 7 Distributions of chloronemal origins in response to horizontally directed and vertically polarized light. Dashed horizontal line represents a uniform distribution. Values above each curve indicate logarithm of intensity in erg/cm² second.

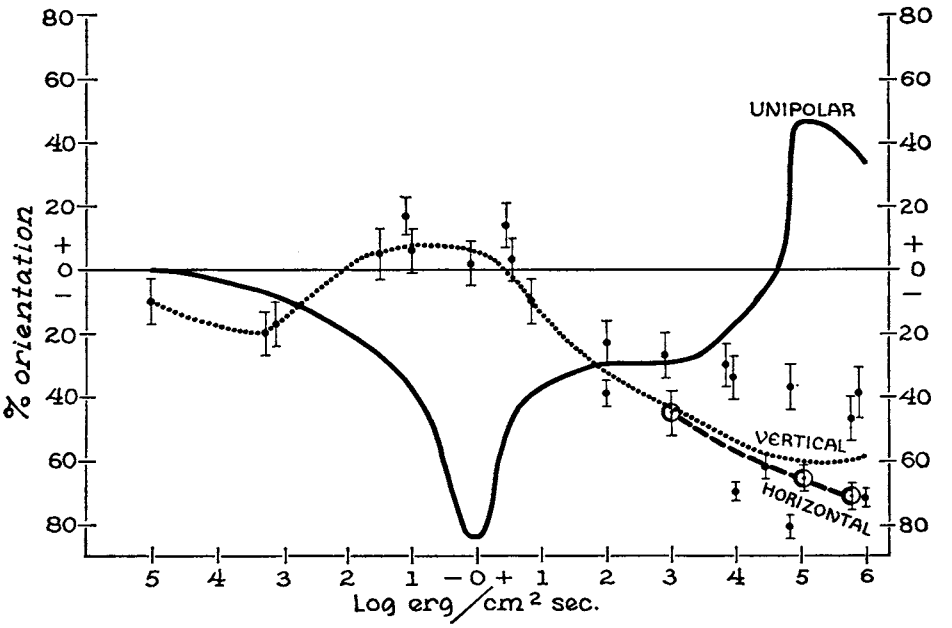

FIGURE 8 Orientation of rhizoidal origins by polarized light as a function of intensity. "Vertical" refers to vertically directed plane polarized light; "horizontal" to horizontally directed and vertically polarized light.

"Unipolar" curve (for comparison) is response to unipolar illumination with unpolarized light and is taken from Fig. 4*b*.

Interpretation of Polarotropic Results. Since interpretation of the rhizoidal response is so greatly complicated by their marked tendency to grow opposite the chloronemas, by their generally weak orientation, and by the scarcity of rhizoids, thus of data below 10^3 erg/cm^{2}second—for these reasons—we base our interpretation almost wholly upon the chloronemal data. To do this, let us focus upon Fig. 6. Here we have drawn the response to unipolar illumination as the most reliable simple indicator of the pattern of growth control, together with data representing the polarotropic response as discussed above.

(*a*) In the low intensity range, from 10^{-5} to 10^{-1} erg/cm^{2}second, the cells show a strong tendency to grow from their brightest parts but no sensitivity to the plane of polarization. Evidently then, the photoreceptor molecules[8] either are not dichroic or, on the average, they are not oriented with respect to the nearby cell surface. Since we know of no chromophores active in biological photoreception which are *not* strongly dichroic—certainly the common ones, *e.g.*, carotenoids, flavines, tetrapyrroles, etc. *are*—we infer that the tropic photoreceptors effective at low intensities are disoriented.

[8] Or, to be more precise, the photoreceptor chromophores.

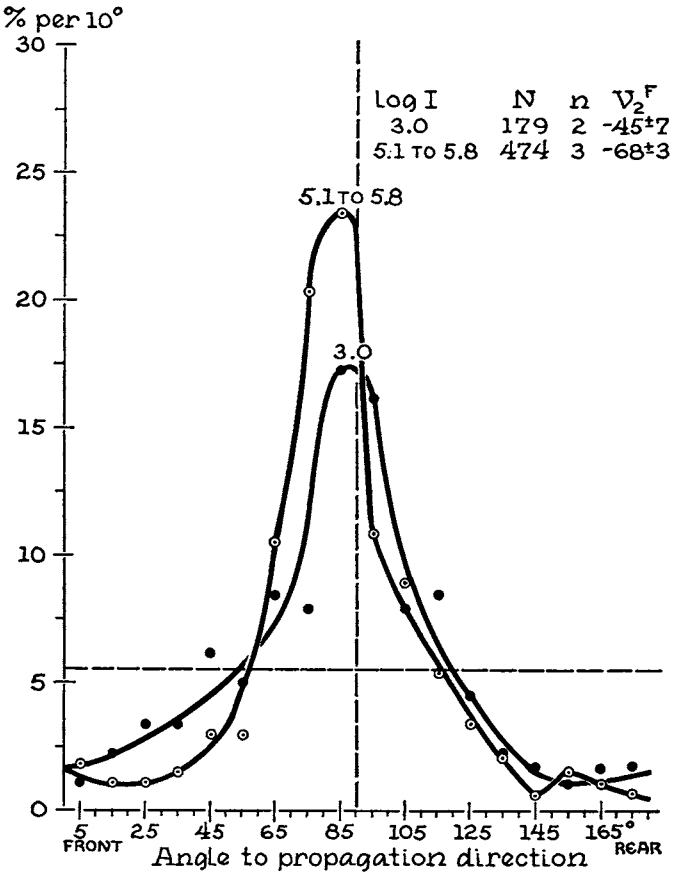

FIGURE 9 Distributions of rhizoidal origins in response to horizontally directed and vertically polarized light. Dashed horizontal line represents a uniform distribution. Values above each curve indicate logarithm of intensity in erg/cm²second.

In the absence of orientation, a symmetry argument cannot be used to find the mode of excitation (4); however, the effective intensities are so low—down to 10^{-4} erg/cm²second which is the limit of rod vision (8)—as to show that an electric transition is involved.

Illumination not only orients the spores' germination but also markedly increases the per cent which germinate at all. We ran one experiment designed to compare the intensity dependence of germination promotion and that of low intensity orientation. The results, shown in Fig. 10 seem to indicate a similar dependence. Since germination promotion is known to be controlled by a reversible red-far-red system (9), this suggests that the low intensity photoreceptor involved in orientation in phytochrome. This conclusion is further buttressed by the fact that the red-far-red system

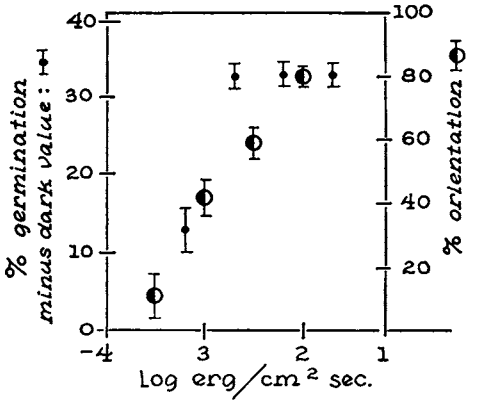

FIGURE 10 Stimulation of germination by red light compared to orientation by unipolar illumination with unpolarized light. In this experiment, 59 per cent of the spores grown in darkness germinated.

involves the only plant photoreceptor pigment yet known to be active at these extremely low intensities.

Finally, the relatively uniform distribution obtained in response to weak unilateral light suggests that the photoreceptor molecules are rather diffusely distributed within the cell. For if they were restricted to any shell near the cell's surface, then reflection and dioptric effects would probably create rather large inhomogeneities in intensity, even in the front part of this shell. Fig. 8 in reference (2) illustrates this phenomenon. Then, if there were such inhomogeneities, one would not expect the relatively uniform outgrowth distribution actually found in response to unilateral illumination in the low intensity range (Fig. 7).

(*b*) We reason that the medium intensity system, which is most clearly evident from $10^{-0.2}$ to $10^{1.5}$ erg/cm^{2}second, must have photoreceptor molecules which are tangentially oriented and electrically excited. The reasoning proceeds as follows.

First we assume that the intensity difference which is equivalent in effect to polarized light is approximately 100 per cent. Hence, as summarized in the introduction and discussed in a previous publication (2), the general mechanism of this polarotropic response must involve highly oriented and dichroic photoreceptors.

Why do we assume that the equivalent difference is close to 100 per cent? The method of partial illumination used imposes nearly 100 per cent differences upon the cells. In the medium intensity range, the degrees of orientation elicited by unilateral polarized light and by partial illumination are about the same. Hence it follows directly that polarized light corresponds in its orienting power to an approximately 100 per cent difference. Really then, we are only *assuming* that it corresponds uniquely to such a difference; thus we are only assuming that differences much smaller than 100 per cent do not produce a similar high degree of orientation. Even this loophole was experimentally closed in two simpler systems studied closely before (2), so we judge our assumption to be safe.

Secondly, consider the specific consequences of the combination of tangential receptor orientation and electric excitation illustrated in Fig. 11*a*. The resultant

pattern of light absorption is illustrated in Fig. 11*b*. As in *Fucus* (3) and in *Osmunda* (2), absorption is maximal along the equator which is in the plane normal to the vibration direction, and is minimal at this equator's poles. However, unlike these forms, *Funaria* (in this medium intensity range) tends to grow from its brightest part(s). Hence if receptor orientation were the only factor affecting the absorption pattern, then outgrowths should be most frequent in the plane normal to the vibration direction and equally frequent in all directions within this plane.

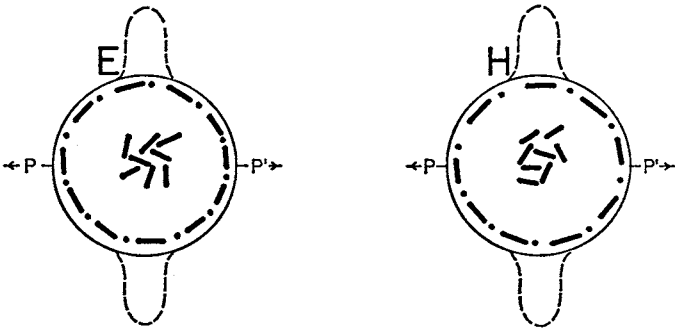

FIGURE 11*a* Diagrams of inferred receptor orientation. A dash in a cell represents a photoreceptor molecule's axis of highest absorption; a dot shows this axis end on. Symbols in the centers of the circles represent molecules at those cell poles which face toward and away from the reader. The arrows, marked PP^1, indicate the direction of the electric vector of polarized light propagated normal to the page. The dashed humps represent the projection in the plane of the page of the direction of most frequent germination. In the medium intensity model, as indicated by the *E*, absorption is excited by the electric vector. However, as indicated by the *H*, in the high intensity one; it is excited by the magnetic vector, which is perpendicular to the electric one.

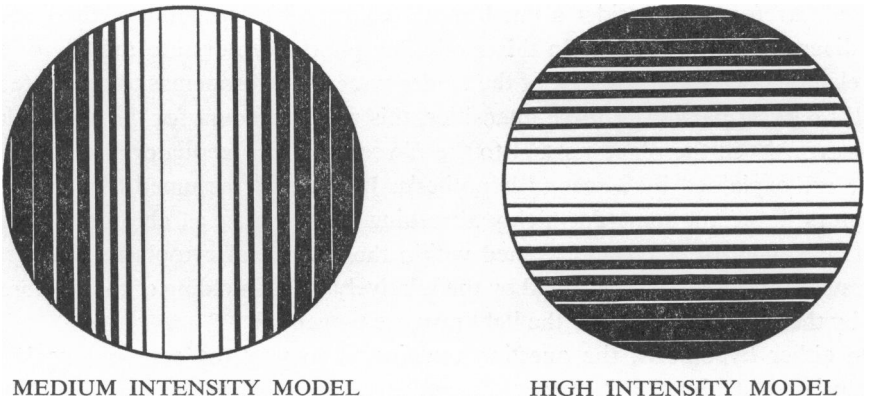

FIGURE 11 *b* Patterns of the inferred relative rates of light absorption by the photoreceptors. In the blackest regions, they absorb least light.

In fact, as already described, they are highly concentrated in this plane (Fig. 5) but deviate considerably from a uniform distribution within it (Fig. 7). However, these deviations are relatively small in the cell's front half and can be readily attributed to imperfect transmission of light through the cell.

The critical point is that this is the first case in which the oriented receptor theory predicts a uniform distribution in the plane of preference, and proves to show a far more uniform distribution within this plane, particularly its front half, than any other polarotropically responsive cell. It does *not* show the very sharp peaks nearly perpendicular to the light's propagation direction which have been found with all other systems (2, 3).

(*c*) The high intensity system, most clearly evident at about 10^5 erg/cm^{2}second presents a quiet surprising character. In terms of the oriented receptor theory—one so far found satisfactory in explaining polarotropic phenomena—the only explanation of the data is that shown in Fig. 11. The chloronema tends to start from the cell's darkest part. The receptors are still tangential but are *magnetically* excited (4).

Since the magnetic vector is perpendicular to both the electric vector and the propagation direction, this model yields the absorption pattern shown, one with minima in the directions of the magnetic vector and maxima in the equator defined by these poles. The assumption of magnetic excitation is required by the oriented receptor theory only in this case of the *Funaria* spore in the high intensity range because it is the only one known in which the unique directions of the growth frequency response are (approximately) in the directions of the magnetic vector. Conversely, only an assumption of magnetic excitation can yield an absorption pattern with unique direction, parallel to those of the magnetic vector (4).

However, according to an authority on excitation mechanisms (10), a degree of symmetry in the vicinity of the photoreceptor molecules which is so high as to be very unlikely *in vivo* is necessary for magnetic excitation to be dominant. We are driven, therefore, to consider a much more complex interpretation of these results, one diagrammed in Fig. 12. In this model the photoreceptors are radially oriented and electrically excited. In view of the tendency of the chloronemas to originate from a cell's darkest part(s) at these intensities, this would account for the accumulation of outgrowths in the plane normal to the *E*-vector. The preequatorial peaks in this plane are explained by a second hypothesis. It is further assumed that the photoreceptors lie *within* some flat highly absorbing organelles, *e.g.*, chloroplasts, which are tangentially oriented and located within the peripheral cytoplasm. The growth peak near 90° would be explained by the relatively great shielding of the photoreceptors by these organelles where the light rays are tangential.

On either hypothesis, the question remains as to why the response peaks preequatorially, at about 85°, rather than at 90°. To answer this, it should be considered that what both actually imply is that the response should peak where the light rays are parallel to the nearby surface while traversing the shell bearing the

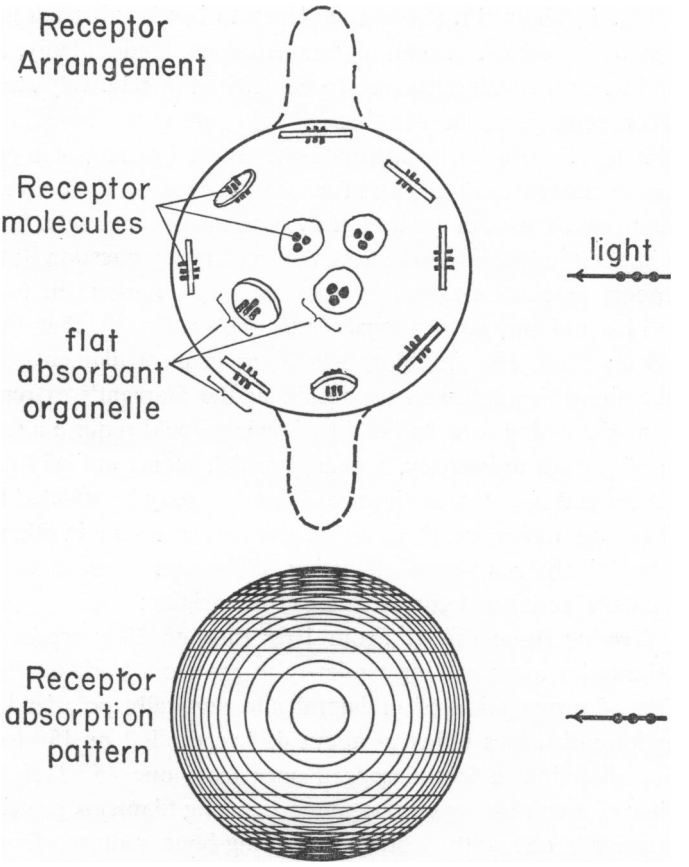

FIGURE 12 An alternative high intensity model. Light propagates from the right with the electric vector normal to the page. Symbols in the center of the upper cell represent organelles at those cell poles which face toward and away from the reader. As in Fig. 11, in the most heavily shaded parts of the lower diagram, the receptors absorb least light.

photoreceptors. Although we have not yet analyzed the dioptrics of the *Funaria* spore, an analysis of the *Botrytis* spore (2, Fig. 8) indicates that in this cell the rays are indeed tangential at about 85°, within the extreme periphery of the cytoplasm and only there. This argument, then, suggests a cortical locus for the photoreceptors of the high intensity system.

Phototropic Response of the Filaments. At most intensities, the relation to light of the *continued* growth of the filaments appeared similar to that of their *origins.*

For example, from 10^{-1} to 10^{1} erg/cm^{2}second (where the chloronemas showed a strong tendency to start from a spore's bright part but did not then tend strongly upwards as at lower intensities)—at these intensities, chloronemas growing upon a

40μ striping (Fig. 1) showed a striking tendency to bend or branch upon entering a shadow, so as to grow back into an illuminated zone. Undoubtedly, this behavior represents a tendency for wall extension to be favored in relatively strongly lighted parts of the chloronema just as it is in those of the spore at these medium intensities.

In the past it has been usual to attribute the tropic bending of a growing plant filament to *bowing* away from a region of greatest growth on the convex side rather than to *bulging* from a region of greatest growth on the concave side. Since a bulging mechanism is implicit in our interpretation, the reader may question it; the following arguments support bulging: (*a*) Observation of small markers in two cases, one chloronemal (11) and the other fungal (12) shows directly that bulging rather than bowing is involved. (*b*) In chloromemas, as in most filaments, elongation is mainly brought about through wall expansion at the filament's extreme tip (13). Now for such a tip-growing form to bend by bowing would require a radical change in the pattern of growth anisotropy, a change which seems unlikely on theoretical grounds. (*c*) As stated above, growth toward the light may be affected by branching as well as by bending; moreover, these alternatives are also seen in other cases (12).

However, we did observe some interesting differences between the relations to light of the filaments' continued growth and of their origins:

(1*a*) *Herring Bone Effect.* From $10^{0.5}$ through $10^{2.8}$ erg/cm^{2}second, the chloronemas showed a most striking tendency to grow out horizontally and *at 45°* to the direction of propagation of unilateral and vertically polarized light. While most grew for long distances either at about 45° to the left *or* 45° to the right of the propagation direction, a few zigzagged once from one 45° tack to the other. An over-all view of a culture containing numerous long filaments growing at plus or minus 45° impresses one with a striking herring-bone pattern. Distributions of measurements of the directions from a spore to its chloronemal tip are shown in Fig. 13.[9] The restriction to about 45° is sharper at $10^{1.2}$ to $10^{1.9}$ erg/cm^{2}second than at either $10^{0.5}$ erg/cm^{2}second or $10^{2.8}$ erg/cm^{2}second.

(1*b*) *Sinuous Growth.* Another curious phenomenon, illustrated in Fig. 14, is seen frequently in response to *un*polarized, vertically directed light of 10^2 erg/cm^{2}second (which is about the intensity inducing the clearest herring bone effect) and only rarely under any other conditions. The chloronemas, but not the rhizoids, tend to grow in a loosely horizontal and sinuous path, clockwise and counterclockwise arcs being of roughly the same frequency, and the radius of arc curvature averaging about nine times the filament radius. The meaning of this phenomenon is quite obscure.

(2) *Tight Helices.* An extraordinary, tight helical form, illustrated in Fig. 15, is frequently assumed by the chloronemas but not the rhizoids in response to vertically directed polarized light at 10^6 erg/cm^{2}second (which was the highest

[9] If a filament had obviously zigzagged, the measurement was taken from the elbow, instead of the spore, to the tip.

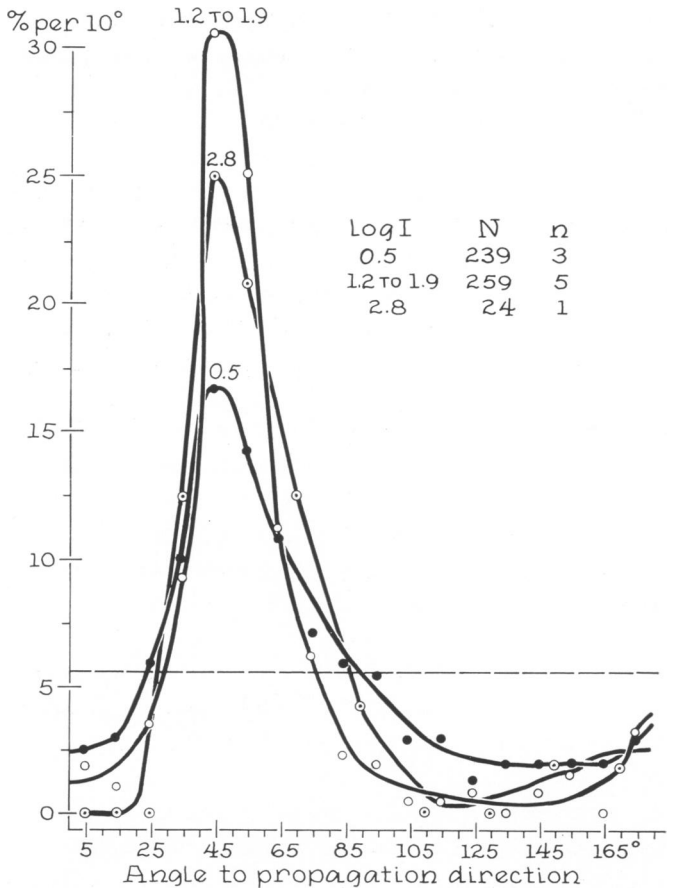

FIGURE 13 Distribution of chloronemal directions in response to horizontally directed and vertically polarized light.

intensity used). Specifically, in both of two experiments, about a third of all the sporelings had such corkscrew chloronemas when killed two days after the spores were wet. The only other condition in which we have seen these forms is in one but not the other of the above pair of experiments, in response to *un*polarized though vertically directed light of $10^{6.3}$ erg/cm^{2}second, where about one tenth of the sporelings developed corkscrews.

These helices spiral about as frequently clockwise as counterclockwise and are very tight: the helix diameter measures only about 2.2 times the diameter of the filament making it up, so its lumen would seem to have only about 1/10 the diameter of the helix. Moreover, the pitch angle, while varying rather widely, averages about 12°, little larger than the 9° calculated for a solid helix.

The screw axes of these forms show a strong tendency to lie horizontally and

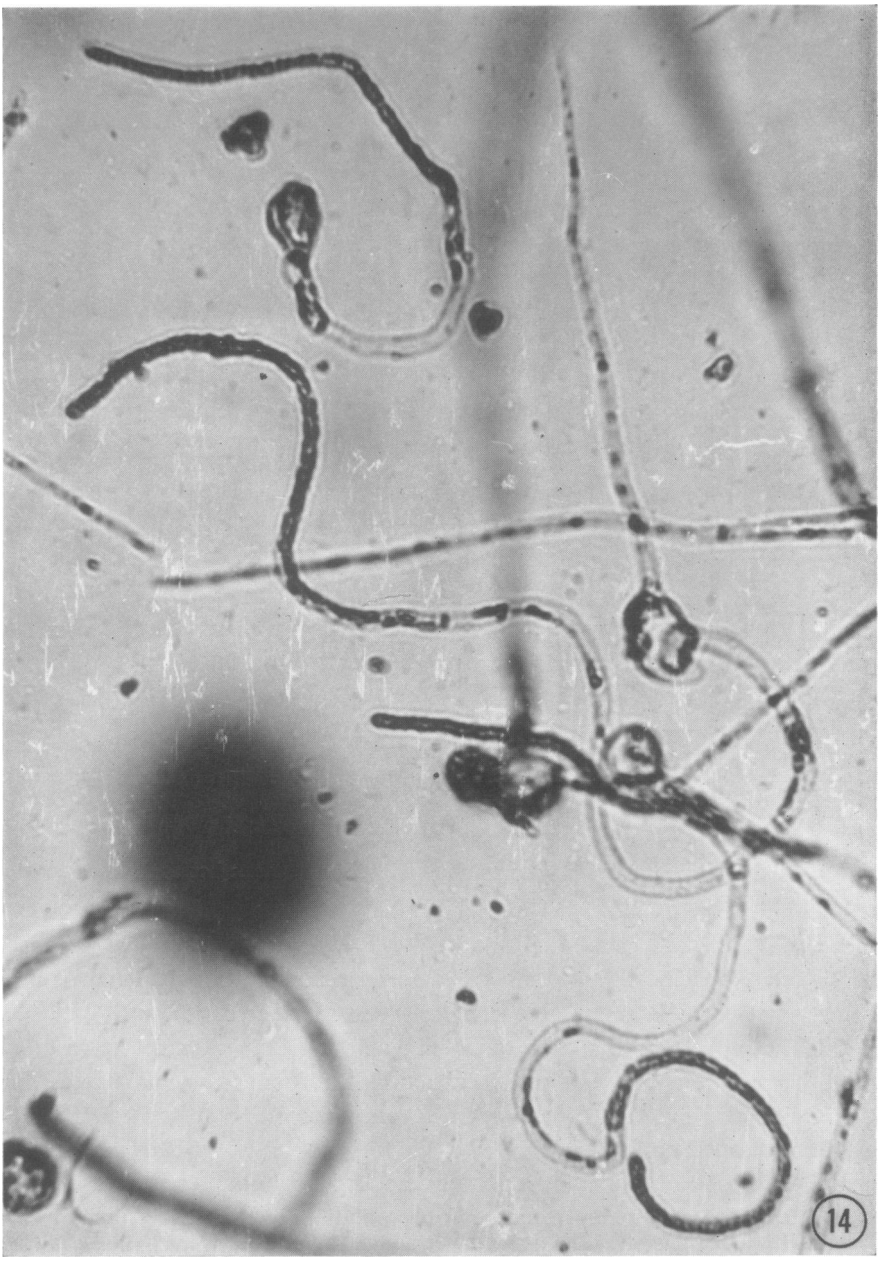

FIGURE 14 Sinuous forms of chloronemas. Cells grown for 48 hours (after being wet) under 100 erg/cm²second of unpolarized red light coming from below. Magnified 160 x.

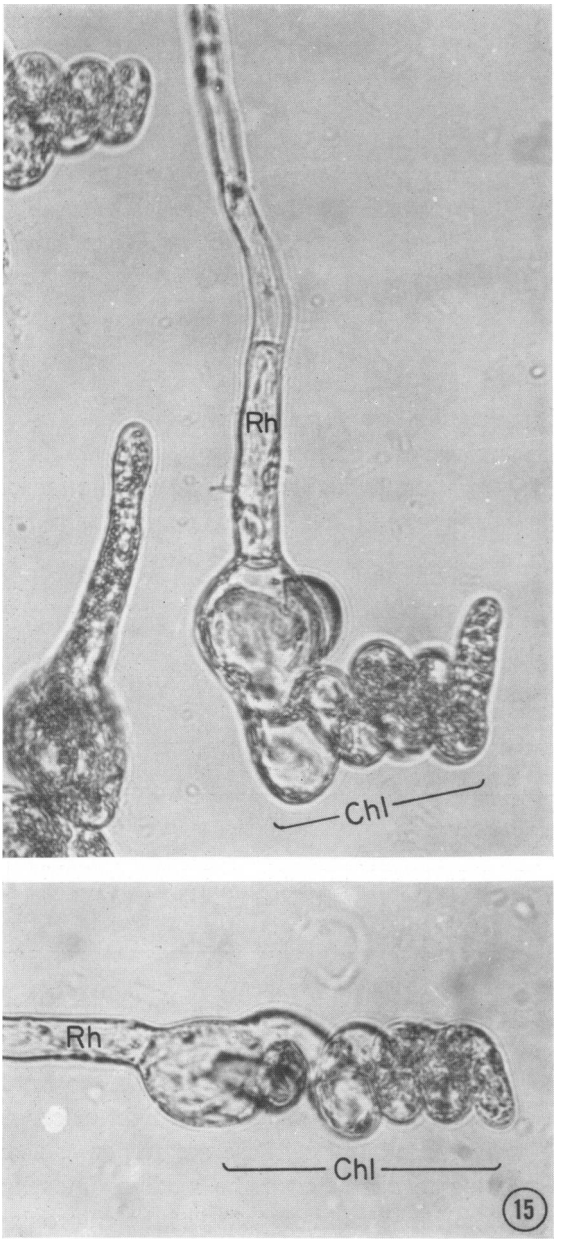

FIGURE 15 Tight helical forms of chloronemas. Cells grown for 52 hours (after being wet) under $10^{6.1}$ erg/cm^{2}second of plane polarized red light coming from below. Polarization axis shown by brackets. *Chl* = chloronema; *Rh* = Rhizoid. Magnified 610.

parallel to the plane of polarization. Thus in a representative sample of 32 such forms, the per cent orientation (V_2) was 93 ± 4 per cent. Hence the chloronemas continue to grow nearly normal to the polarization direction and are not really oriented too differently from their origins. Perhaps, then, this behavior arises not from a change in the phototropic system but from an extreme tendency of the chloronemas to grow toward themselves.

(3) Another, and perhaps related phenomenon, which is only seen among some cells in response to $10^{4.5}$ erg/cm^{2}second of unilateral, vertically polarized light, is a growth of chloronemas, which start out at about 90° as usual and then make a U-turn by bending forward and then right around the spore, while remaining in contact with it.

DISCUSSION

(*a*) Theoretically, the phototropic responses of a spherically symmetrical cell may be divided into twelve main cases depending upon whether growth occurs at the brightest or darkest part, and whether the receptors are tangential, or radial, or disoriented, and whether they are electrically or magnetically excited.[10] As Table I

TABLE I

POSSIBLE CLASSES OF THE PHOTOTROPIC RESPONSES OF SPHERICALLY SYMMETRICAL CELLS

Light is blue at all effective intensities unless otherwise noted. Cells are spores or zygotes.

Growth from	Electric excitation			Magnetic excitation	
Receptors...	Tangential (T)	Radial (R)	Disoriented (D)	T	R D
Brightest part	*Funaria* (medium, red)	*Botrytis* (2)	*Funaria* (low, red)		
Darkest part	*Fucus* (3, 15) *Equisetum* (15, 16) *Osmunda* (2)	[*Funaria* (high, red)] ?	[*Codium* (17)] ?	[*Funaria* (high, red)] ?	

shows, this study provides examples of several unfilled cases. Of the twelve, clear examples of four, and possible examples of three others have now been found.

(*b*) While no comparable studies on tropic responses to red light are found in the literature, Haupt's work on the so-called weak light chloroplast movements in *Mougeotia* seems closely related (14). These are responses to red light at doses similar to those appearing to characterize the medium intensity tropic effects in *Funaria* spores. Thus a half maximum response of the chloroplast occurs at about 10^4 erg/cm^2. The medium intensity response of *Funaria* reaches half maximal values

[10] Note that this classification is quite independent of the *mechanism* whereby local photon absorption controls growth localization.

at about $10^{-0.5}$ erg/cm^{2}second (Fig. 6). Assuming the effective period under continuous illumination to be about one day or the order of 10^5 seconds, then the product yields a dose of the order of 10^4 or 10^5 ergs/cm^2. Moreover, the pertinent *Mougeotia* receptors lie tangentially and the chloroplast comes to face the brightest part of the cell. Since these *Mougeotia* receptors are apparently phytochrome, this suggests that the medium intensity *Funaria* red receptor, like the low intensity one, will likewise prove to be phytochrome.

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