

Absorption and Screening in *Phycomyces*

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ABSTRACT In vivo absorption measurements were made through the photosensitive zones of *Phycomyces* sporangiophores and absorption spectra are presented for various growth media and for wavelengths between 400 and 580 m μ . As in mycelia, β -carotene was the major pigment ordinarily found. The addition of diphenylamine to the growth media caused a decrease in β -carotene and an increase in certain other carotenoids. Growth in the dark substantially reduced the amount of β -carotene in the photosensitive zone; however, growth on a lactate medium failed to suppress β -carotene in the growing zone although the mycelia appeared almost colorless. Also when diphenylamine was added to the medium the absorption in the growing zone at 460 m μ was not diminished although the colored carotenoids in the bulk of the sporangiophore were drastically reduced. Absorption which is characteristic of the action spectra was not found. Sporangiophores immersed in fluids with a critical refractive index show neither positive nor negative tropism. Measurements were made of the critical refractive indices for light at 495 and 510 m μ . The critical indices differed only slightly. Assuming primary photoreceptors at the cell wall, the change in screening due to absorption appears too large to be counterbalanced solely by a simple effect of the focusing change. The possibility is therefore advanced that the receptors are internal to most of the cytoplasm; i.e., near the vacuole.

INTRODUCTION

Research on the photoresponses of *Phycomyces* has been reviewed by Banbury (1959), Reinert (1959), Thimann and Curry (1960), Delbrück (1962), and Shropshire (1963). Interpretation of this research is hampered by our incomplete knowledge of the optical properties of the photosensitive zones of sporangiophores. These properties, and in particular the absorption spectra through sporangiophores in the visible region, are investigated here in more detail. These properties are discussed in terms of their relationship to the nature and radial location of the primary photoreceptors.

Absorption spectra have been reported previously by Delbrück and Shropshire (1960) and by Abbot and Grove (1959). We have extended this work by making more detailed measurements of sporangiophores grown under a variety of conditions. Absorption spectra were taken through the

photosensitive zones of single sporangiophores. Since most of the recent research used sporangiophores grown either on potato dextrose agar or on a glucose asparagine medium, spectra for specimens grown on these media are presented.

A direct *in vivo* observation on the absorption of the photoreceptor pigment would provide information for the isolation of this pigment, since one could determine the optimum conditions for isolation and perhaps establish an *in vivo* assay procedure. A comparison of the absorption spectra with the action spectra for growth and tropic responses is made in order to relate one to the other and to consider what role the observable pigments might have as primary photoreceptors. For this purpose it was found convenient to vary the carotenoid composition by adding diphenylamine to the media and to present the absorption spectra of the resulting sporangiophores. Absorption spectra of specimens grown on a lactate medium are also presented, since *Phycomyces* sporangiophores grown on such a medium were reported (Schopfer and Grob, 1950) to contain very little β -carotene. β -carotene has previously been suggested as a candidate for the primary photoreceptor pigment. Sporangiophores were also grown in the dark, to see whether the β -carotene content in the photosensitive zone decreases, as has been reported for mycelia (Garton, Goodwin, and Lijinsky, 1951).

Precise knowledge of the location of the primary photoreceptors would be helpful to cytologists attempting to identify structures associated with the photoreceptors and to spectroscopists attempting to observe *in vivo* absorption of the photopigment. The effects of screening on phototropism were investigated in order to gain more information concerning the radial position of the primary photoreceptors. Phototropism in *Phycomyces* is caused by a concentration of light on the side distal to the incident light, this concentration being produced by the lens action of the sporangiophore. Buder (1918, 1920) showed that when the sporangiophores are immersed in paraffin oil this lens effect is reversed and negative phototropism occurs. Shropshire (1962) immersed sporangiophores in inert transparent media of appropriate refractive index, such that he could observe the rate and direction of tropism as a function of the refractive index. He found that for a critical refractive index, slightly lower than that of the cell contents, the effects of light reaching the proximal and distal sides of the sporangiophore balance, and (on the average) no phototropism occurs. In this situation, the small remaining lens effect is presumably counterbalanced by the attenuation of light on its path from photopigments on the proximal side to that on the distal side. An understanding of the components of this balance would lead to a determination of the location of the primary photoreceptors, i.e. whether the receptors are uniformly distributed throughout the cytoplasm, are near the tonoplast (membrane separating vacuole from cytoplasm), or are near the plasmalemma (membrane just inside the cell wall). In an attempt to segre-

gate the effects of absorption and refraction from the complicating effects of scattering, reflection, etc., we have determined the critical refractive indices at two closely spaced wavelengths in a wavelength range for which the absorption change is large.

List of abbreviations and terms used:

PDA, potato dextrose agar medium; GA, glucose asparagine medium; LAC, lactate medium; DPA, diphenylamine; T, cytoplasm thickness, i.e. sporangiophore radius minus vacuole radius; D, sporangiophore diameter; plasmalemma, membrane just inside cell wall; tonoplast, membrane separating vacuole from cytoplasm; I, effective intensity in units of \log_2 with zero equivalent to 5μ watts/cm² at the peak of the action spectra.

CULTURE PROCEDURES

Sporangiophores of *Phycomyces blakesleeanus* were grown under diffuse incandescent light on agar in 12 x 35 mm shell vials, or in 100 X 20 mm Petri dishes. A list of the media used follows:

Potato dextrose agar (PDA):

40 g Difco potato dextrose agar; 1 ml of 0.05 % thiamine HCl; 1 liter H₂O.

Glucose asparagine (GA): 10 g agar; 30 g glucose; 2 g L-asparagine·H₂O; 0.5 g MgSO₄·7 H₂O; 1.5 g KH₂PO₄; 0.5 ml of 0.05 % thiamine HCl; 1 liter H₂O; (Agar and glucose were autoclaved separately in solutions.)

Lactate (LAC):

10 g agar; 2.5 g 80 % ammonium lactate; 2 g magnesium lactate; 1.5 g KH₂PO₄; 0.5 g MgSO₄·7 H₂O; 0.1 g MnSO₄·H₂O; 0.1 ml 0.05 % thiamine HCl; 1 liter H₂O; (The agar was autoclaved separately in solution.)

Diphenylamine (DPA) when used was added to the media from a stock solution of 1.25 g DPA, dissolved in 25 ml Tween 80, which was then diluted to 1 liter with H₂O. This solution was autoclaved separately.

The above media were autoclaved at 125°C for 15–25 min.

The sporangiophores were grown at 60–80 % relative humidity at about 22°C. The light intensities during growth were not varied. These intensities will be given in terms of biological effectiveness as deduced from the action spectra of Delbrück and Shropshire and will be quoted in terms of the intensity scale described by Delbrück and Reichardt (1956). The scale is in units of \log_2 , with zero equivalent to about 5μ w/cm² at the peak of the action spectra.

MICROSPECTROPHOTOMETRY

Apparatus and Procedures

Absorption spectra through photosensitive zones of single stage IV sporangiophores were obtained using a Cary Model 15 recording spectrophotometer of the Applied Physics Corporation, Monrovia, Calif., and a microscope attachment on loan from this company. The microscope attachment replaced the usual sample compartment of the spectrophotometer.

The sample beam of the spectrophotometer was focused with a 20X, 0.5 NA

microscope objective used as a condenser to form a $5 \mu \times 100 \mu$ image at the axis of the sporangiophore. The transmitted light was collected by a 90X, 1.30 NA, oil immersion objective. The high numerical aperture objective used was found to be satisfactory for collecting much of the scattered light, but limited the size of the image that could be viewed to 100μ or less. Viewing was accomplished by means of a removable mirror and auxiliary ocular. The specimens could be positioned on a rotating microscope stage.

Metal discs with holes were used in the reference beam as neutral density filters to balance losses in the microscope optics. Further balancing was done with the balancing potentiometers of the spectrophotometer. It was not possible to completely balance the sample and the reference beams. A base line was obtained by moving the sporangiophore slightly out of the beam and sending the beam through a section of the slide adjacent to the specimen. The base line was then recorded, the sample moved back into the beam, and another line recorded. The absorbance was then taken as the difference between these lines. Since this difference was measured at every $5 \text{ m}\mu$, all the data presented are for smooth curves drawn through averages of points spaced $5 \text{ m}\mu$ apart.

The photomultiplier compartment and electronics of the spectrophotometer were not altered. The monochromator slits were held fixed at 0.3 mm, resulting in a spectral band width of 2.5 to $8.5 \text{ m}\mu$ in the wavelength range from 400 to $580 \text{ m}\mu$. The apparatus was capable of measuring absorbance to 0.003. Measurements below $400 \text{ m}\mu$ were not possible with the glass lenses used.

Sporangiophores were plucked from the mycelia, mounted in microscope oil on microscope slides, and covered with cover slips which were spaced from the slides with dabs of stopcock grease. Under these conditions the protoplasm continued to stream during the measurements. Control sporangiophores immersed in microscope oil continued to grow and to exhibit tropism.

Unless otherwise indicated, measurements were made at about 1.3 mm below the base of the sporangium since Delbrück and Varjú (1961) showed this to be near the center of the photosensitive zone. Most of the sporangiophores used were 4–5 cm long, and all were between 3.5 and 5.5 cm. At times the sporangia were gently removed, without tearing the columella, to facilitate mounting. No differences were observed in the spectra of specimens with and without sporangia. For most measurements no care was taken to shield sporangiophores from light, since a search for light-induced absorption changes in the photosensitive zone had given negative results.

It was found that for any given set of growth conditions the absorbance sometimes varied more than a factor of two. For sporangiophores grown on PDA, the variation was much less; for those grown on GA there seemed to be a correlation between absorbance and cytoplasm thickness; in the other cases growth was very erratic. Shifting the point of measurement several tenths of a millimeter from the 1.3 mm resulted in differences less than those seen between specimens. The average cytoplasm thicknesses (T), i.e. cell radius minus vacuole radius, and the average cell diameter (D) at the point of measurement, are given in the figure legends.

Sporangiophores grown on GA matured about a day later than those grown on PDA. The addition of diphenylamine slowed maturation: $15 \mu\text{g}/\text{ml}$ of DPA added to PDA slowed maturation 1 day; $15 \mu\text{g}/\text{ml}$ added to GA slowed maturation 3 days.

The specimens grown on GA in the dark came up about a day later than those grown at $I = -1$.

MICROSPECTROPHOTOMETRY

Experimental Results

GLUCOSE ASPARAGINE, INTENSITY 2^{-1} TIMES STANDARD

Sporangiophores were grown on GA at an intensity of -1 . Fig. 1 *a* contains a plot of the average absorbance through the photosensitive zones of seven sporangiophores vs. wavelength. The peaks at about 462 and 490 $m\mu$ are undoubtedly due to β -carotene, which predominates in cell extracts. Absorption spectra of extruded cell contents had similar peaks at about the same wavelengths. These latter spectra were obtained using opal glass (Shibata et al., 1954) to lessen the influence of sample scattering.

GLUCOSE ASPARAGINE, DARK GROWN

Sporangiophores were grown on GA in total darkness, except for short periods of observation with red light. They were plucked and mounted in room light ($I \cong -2$), since this procedure did not appear to result in gross changes in the absorption. Fig. 1 *b* is a plot of the average absorbance of five such sporangiophores.

POTATO DEXTROSE AGAR, INTENSITIES 2^{-1} AND 2^{-5} TIMES STANDARD

Sporangiophores grown on PDA at intensities of -1 and -5 gave average absorbances as shown in Figs. 1 *c* and 1 *d*. The β -carotene peaks appear slightly shifted from those seen for specimens grown on GA (Fig. 1 *a*). These shifts are most likely caused by the increase of scattering relative to absorption for those grown on PDA, but may be influenced by absorption by other pigments.

LACTATE, INTENSITY 2^{-1} TIMES STANDARD

Phycomyces grown on a lactate medium had mycelia colorless to the eye and very thin sporangiophores. The cytoplasm below the top centimeter contained few colored particles. These particles streamed in what appeared to be thin, filamentous lines. The growing zone had a large number of particles and the spectrum (Fig. 1 *e*) through this zone is very similar in shape to that of specimens grown on GA which leads us to conclude that the major pigment absorbing in the blue is very probably β -carotene. The specimens selected for measurement were thicker than most, since they were easier to handle without crushing.

POTATO DEXTROSE AGAR AND GLUCOSE ASPARAGINE, WITH ADDED DIPHENYLAMINE, INTENSITY 2^{-1} TIMES STANDARD

Sporangiophores were grown on PDA with 15 $\mu\text{g/ml}$ diphenylamine added. The average absorbance of six such sporangiophores is plotted vs. wavelength in Fig. 1 *f*. The specimens used in the average were selected for high absorption in order to more clearly show the positions of the absorption peaks. The spectrum for four sporangio-

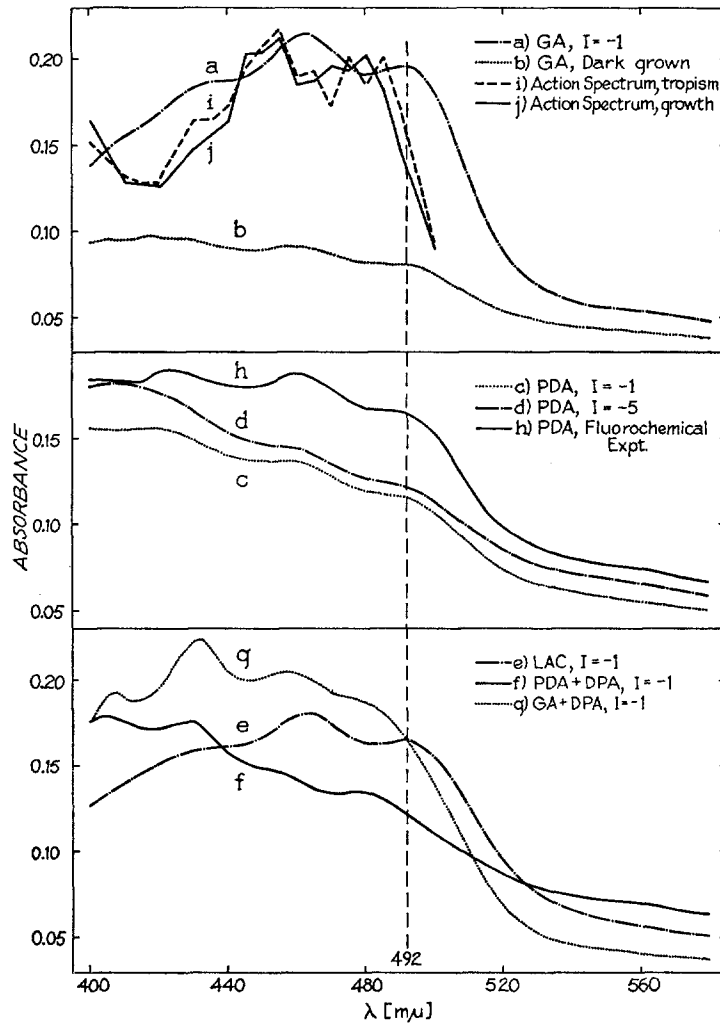


FIGURE 1. Absorbance of the photosensitive zone of sporangiophores vs. wavelength; averages of 4 to 10 specimens in each case.

Medium I	GA -1	GA dark	PDA -1	PDA -5	LAC -1	PDA + DPA -1	GA + DPA -1	PDA in fluoro- chemicals -7
<i>D</i> , diameter, μ	100	67	100	110	64	80	78	102
<i>T</i> , cytoplasm thickness, μ	26	19	27	28	29	22	19	30

phores grown on GA with 15 $\mu\text{g}/\text{ml}$ diphenylamine is plotted in Fig. 1 *g*. At a lower concentration of DPA (5 $\mu\text{g}/\text{ml}$) the spectra were similar to those without DPA.

Diphenylamine, when added to the media, results in a reduction of β -carotene in the mycelia and an increase of certain other carotenoids (Goodwin, 1952) which are β -carotene precursors. Using growth conditions similar to ours, G. Meissner¹ has

¹ Personal communication by G. Meissner and Charles David, California Institute of Technology.

found that hexane extracts of sporangiophores grown on PDA with 15 $\mu\text{g}/\text{ml}$ di-phenylamine show changes in carotenoid content similar to those found in the mycelia by Goodwin. The shifts of absorption peaks in the transmission curves of the growing zone (Figs. 1 *f* and 1 *g*) also indicate changes in carotenoid content. (Similar shifts were seen in spectra of extruded cell contents.) Because of the overlapping absorption, the specific positions of the peaks for the individual carotenoids cannot accurately be assigned. It is very notable, however, that although the hexane extracts showed a drastic reduction in the total amount of carotenoids absorbing at 460 $\text{m}\mu$ (β -carotene and its colored precursors), the spectra through the growing zones show no such drastic decrease, probably indicating that these pigments are accumulated here.

DETERMINATION OF THE CRITICAL REFRACTIVE INDEX FOR TWO WAVELENGTHS

Procedure

The method for investigating the phototropic behavior in immersion fluids of various refractive indices was similar to that used by Shropshire (1962). Sporangiophores were grown in vials on PDA at an intensity of about -1 . These were then inverted and placed vertically (about five at a time) in a flat-walled glass container containing an appropriate mixture of the fluorochemicals. The mycelia were not immersed. Care was taken to avoid light screening of one specimen by another. The sporangiophores were allowed to exhibit tropism for 4 hr under the influence of a horizontal monochromatic light beam. The light source was a 2 mm image of the exit slit of a Bausch and Lomb high intensity monochromator. This image was 80 cm from the sporangiophores. The desired indices of refraction for the immersion liquid were obtained by mixing FC-75 and KEL-F grade 3 manufactured by the Minnesota Mining and Manufacturing Co. (St. Paul, Minn.). Measurements of the angle of tropism after 4 hr were made for various indices of refraction, using for stimulation the wavelengths $\lambda = 495 \text{ m}\mu$ and $\lambda = 510 \text{ m}\mu$. The half-band width of the monochromator was 5 $\text{m}\mu$. Higher order diffraction was eliminated by using a Corning (Corning Glass Works, Corning, N.Y.) 0-54 filter at 495 $\text{m}\mu$ and a Schott GG-14 filter (Fish-Schurman Corp., New Rochelle, N.Y.) at 510 $\text{m}\mu$. The latter was a sharp cutoff filter having a rapidly increasing absorption with decreasing wavelength at about 495 $\text{m}\mu$ and therefore serving to lessen stray light at the lower wavelengths. The effective intensity of the beam was about -7 , assuming the effectiveness at 495 $\text{m}\mu$ given by the action spectra of Delbrück and Shropshire, and assuming that light at 510 $\text{m}\mu$ is one-tenth as effective as that at 495 $\text{m}\mu$.

DETERMINATION OF THE CRITICAL REFRACTIVE INDICES FOR TWO WAVELENGTHS

Experimental Results

A plot of the average angle of tropism after 4 hr vs. the index of refraction of the immersion medium is shown in Fig. 2. Each point represents an average of at least 15

specimens. Fig. 1 *h* is a plot of the average absorbance of five of these specimens (at about 1.3 mm. below the base of the sporangia) vs. wavelength. The absorbance measurements are for typical specimens at the end of the tropism experiments. The average specimen diameter was 102 μ , the average vacuole diameter 42 μ . Note that these experimental conditions apparently had the effect of increasing the absorbance and cytoplasm thickness. (Compare Figs. 1 *c* and 1 *h*.) The reason for this consistent increase is not known.

It is quite clear from the data presented that at 510 $m\mu$ the sporangiophores tend to a more positive tropism than at 495 $m\mu$. Assuming straight lines through the two sets of points, $n_o = 1.312$ for $\lambda = 510 m\mu$; and $n_o = 1.306$ for $\lambda = 495 m\mu$.

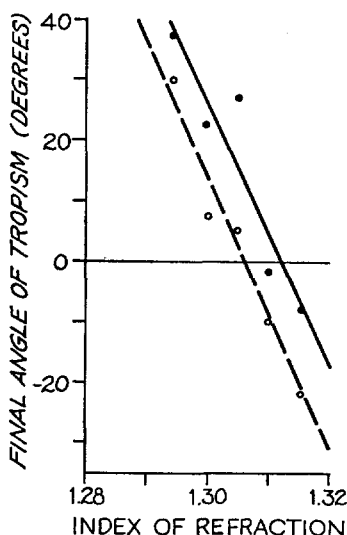


FIGURE 2. Angle of tropism after 4 hr vs. index of refraction of immersion fluid at (a) open circles and dashed line, 495 $m\mu$; (b) filled circles and solid line, 510 $m\mu$. Each point is an average for more than 15 sporangiophores. The standard error of the mean for each point was between 5 and 8.5 degrees.

DISCUSSION AND CONCLUSIONS

Comparison of Absorption and Action Spectra

The similarity, for wavelengths above 420 $m\mu$, of the action spectra for light responses of *Phycomyces* to the absorption spectrum of β -carotene have led to the speculation that, in this range, β -carotene may be the primary photoreceptor pigment even though the presence of an action spectra maximum at about 380 was thus left unexplained. The visible portions of the action spectra of Delbrück and Shropshire (1960) are compared to the absorption spectra for sporangiophores grown on GA at $I = -1$ in Figs. 1 *i*, 1 *j*, and 1 *a*. (Comparison is made with GA-grown sporangiophores, since the β -carotene peaks are better defined than for PDA-grown sporangiophores.) It is seen that absorption and action spectra differ significantly. The absorption peaks of the sporangiophores come about 10 $m\mu$ higher than those of the action spectra and the sharp

drop-off of the action spectrum at the long wavelength side comes at a β -carotene absorption peak. Thus, if it is assumed that the absorption spectrum follows the action spectra, this bulk β -carotene cannot be the primary photoreceptor pigment. One comes to the same conclusion if the absorption spectrum is compared to the action spectrum obtained by Curry and Gruen (1959). It must be emphasized that this argument does not rule out some other form of β -carotene as the primary photoreceptor, nor does it rule out that β -carotene is in some other respect necessary for photoresponses.

The assumption that the absorption spectrum follows the action spectrum appears to be a good one. Screening effects are small since for PDA the variation in absorption between 420 and 500 $m\mu$ is less than 10% of the incident light. Unless there is some other reason why the quantum yield is strongly dependent on wavelength (such as a localized screening pigment around the receptor), it must be concluded that the bulk β -carotene is not the primary receptor pigment.

Attempts to eliminate β -carotene, by using diphenylamine and by growing specimens on a lactate medium, were not successful. For a lactate medium, even though almost colorless mycelia were obtained, the β -carotene content in the photosensitive zone (which is roughly also the growing zone) was, in fact, normal (at least as far as could be determined from the *in vivo* absorption measurements). For diphenylamine, the β -carotene was reduced but perhaps by not more than 50%. Growth in the dark reduced the amount of β -carotene in the light-sensitive zone, but did not eliminate it.

In sum, a component of absorption corresponding to the action spectra was not found, probably because the photoreceptor pigments are present in quantities that are too small to be observable in the presence of scattering and of absorption by the bulk pigments. Perhaps detailed threshold measurements under a variety of growth conditions and for various stages of development might uncover a system with larger amounts of photopigment, thus enabling its observation. Also, off-axis measurements of absorption, at places thought to have high concentrations of photopigment, may prove useful.

The Location of Receptor Pigment

Sporangiophores can be immersed in inert media having an index of refraction such that the sporangiophores are balanced phototropically (Buder, 1918, 1920; Shropshire, 1962). Positive phototropism occurs because the sporangiophore acts as a lens and focuses light on the distal side. Shropshire (1962) found that n_c , the critical index of refraction of the immersing medium needed for balance, is slightly less than that of the cell contents. It appears, then, that at n_c there is a residual focusing which counterbalances some other effects. It has been assumed (Shropshire, 1962; Castle, 1965) that the balance is between

the positive effect of refraction and the negative effect of absorption of light on its path between the receptors on the proximal and distal sides. Thus, a detailed understanding of the balance should give information concerning the radial location of the receptors since only pigments lying between the receptors on the two sides would influence this balance.

Since the absorption and focusing effects are small, reflections and scattering defeat out attempts to understand this balance quantitatively. The reflections are influenced by interference phenomena, while the nature of the scatterers and the detailed behavior of the scattering are unknown. To reduce

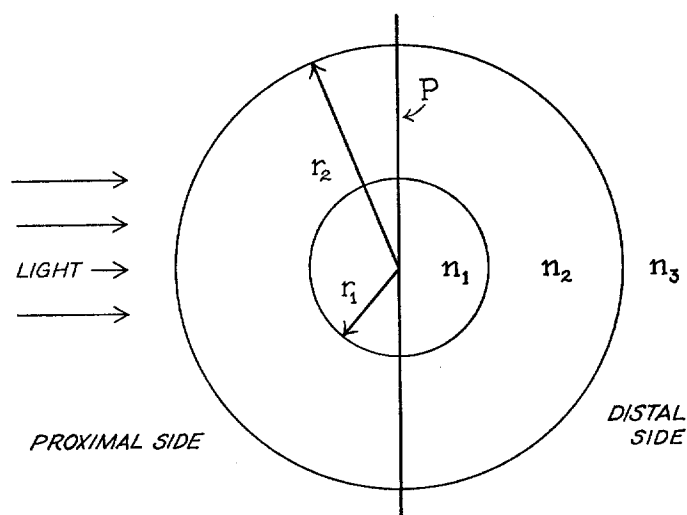


FIGURE 3. Diagram of the model sporangiophore consisting of a vacuole with radius r_1 and refractive index n_1 , cytoplasm with inner and outer radii r_1 and r_2 , and refractive index n_2 and a surrounding medium with refractive index n_3 . The plane P through the axis of the cylinder has its normal in the direction of the incident light rays.

these uncertainties the critical refractive indices were measured at two closely spaced wavelengths, $495 \text{ m}\mu$ and $510 \text{ m}\mu$, where absorption changes considerably. In this situation, hopefully, only changes in refraction and changes in screening need to be considered. It was found that the critical indices definitely differed: $n_o = 1.306$ for $\lambda = 495 \text{ m}\mu$ and $n_o = 1.312$ for $\lambda = 510 \text{ m}\mu$. The corresponding change in optical density was about 0.035; i.e., a shift from 510 to $495 \text{ m}\mu$ results in a reduction of light on the distal side of approximately 8%.

To estimate the reduction in focusing advantage due to the change in critical index we considered a model sporangiophore consisting of concentric cylinders (see Fig. 3) with radii $r_1 = 20 \mu$ and $r_2 = 50 \mu$; with refractive indices n_1 for $r < r_1$, n_2 for $r_1 < r < r_2$, and n_3 for $r_2 < r$, corresponding to an

inner vacuole, surrounded by cytoplasm, which, in turn, is surrounded by the external medium. The cell wall was neglected.

Computer calculations of the influences of refraction were made for a range of refractive indices bracketing our uncertainty of these indices. Paths of 20 rays going through half of such a model sporangiophore, with $n_1 = 1.335$, $n_2 = 1.350$, and $n_3 = 1.306$, are shown in Fig. 4. These values of the refractive indices are the estimated and measured parameters for balance at 495 m μ .

We assumed that, for each side of the sporangiophore (proximal and distal), the effectiveness of absorbed light is proportional to the distance of the intercepting photoreceptor from the midplane (P in Fig. 3); i.e., that the action is proportional to the length of the lever arm of the receptor (mechanical ad-

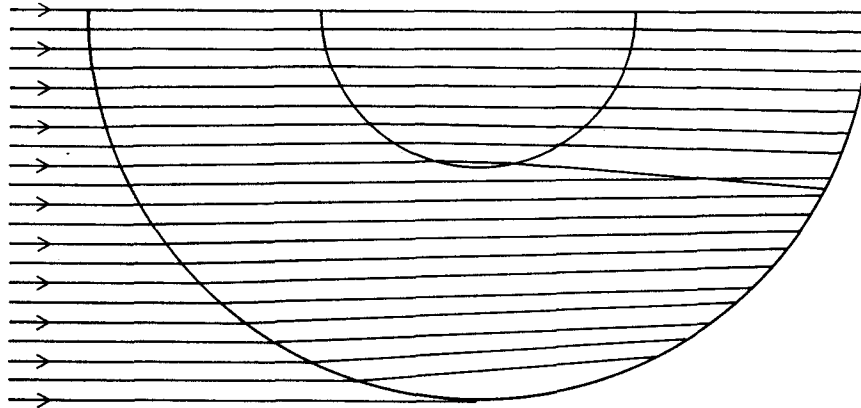


FIGURE 4. 20 ray paths through half of a sporangiophore with $r_2/r_1 = 2.5$, $n_1 = 1.335$, $n_2 = 1.350$, and $n_3 = 1.306$. The incident rays are parallel to each other and perpendicular to the sporangiophore axis.

vantage theory, Buder,² and Jaffe, 1960). This assumption is made because some method is needed to transform ray patterns into effectiveness, in order to compare the influences of refraction and screening. Focusing advantages were computed using 200 rays per sporangiophore, with totally reflected rays eliminated by the computer program at the start.

Over a wide range of the parameters, n_1 and n_2 , the calculated change in focusing advantage at the *plasmalemma* was about an order of magnitude less than the measured change in screening between proximal and distal sides. Attempts to account for this discrepancy as being due to changes in the influence of reflections and scattering led to the conclusion that each of these corrections is either too small or in the wrong direction. However, the optics is sufficiently complex to leave a residue of doubt at this point.

² Buder, J. 1946. Uebersicht über Ergebnisse einiger noch ungedruckter Arbeiten aus den botanischen Anstalten der Universität Breslau. Mimeographed private communication.

It appears then, that the assumption of receptors on the plasmalemma leads to the difficulty of reconciling the large changes in screening when the wavelength is shifted from 495 to 510 $m\mu$ with the minute changes in dioptrics when n_o is changed from 1.306 to 1.312. The arguments presented are just as valid and almost as gross for receptors assumed to be distributed throughout the cytoplasm, although they depend on the assumption that the β -carotene is not strongly radially concentrated near the plasmalemma.

If we assume that the receptors are near the tonoplast, the calculated change in focusing advantage is even less than for the plasmalemma. We cannot be certain that this assumption would lessen the difficulties. It is true that the differential screening at 495 and 510 $m\mu$ would be much less since the screening pigments now lie external to the receptor pigment. It is also true that the differential focusing effect is also small. However, both of these effects are so small that scattered and internally reflected light may come to play decisive roles, and these are impossible to evaluate with sufficient accuracy.

Perhaps it would appear more natural to assume that the receptors are at the plasmalemma, since responses to light are observed as growth responses at the cell wall. The assumption of receptors at the tonoplast raises the difficulty of transmitting information concerning small changes in light distribution across the cytoplasm to the cell wall. We pose against this the difficulty of explaining how, in our experiments, the small change of critical index can offset the change of screening between receptors at the proximal and distal sides, unless the receptors are, in fact, near the tonoplast. Only further experiments can resolve this dilemma. In any event, it appears that the tropic balance in fluorochemicals cannot be explained solely in terms of a screening effect balanced by a simple focusing effect, if the receptors are either at the plasmalemma or uniformly distributed in the cytoplasm.

SUMMARY

1. The β -carotene absorption that can be seen in the photosensitive zones of single sporangiophores of *Phycomyces* differs by a red shift of about 10 $m\mu$ from the action spectra. This implies that the bulk β -carotene is not the primary photoreceptor pigment. The bulk β -carotene absorption in vivo differs from that in hexane also by a red shift of about 10 $m\mu$.

2. For growth in the presence of light, the absorption in the growing zone at 460 $m\mu$ could not be greatly diminished, even under conditions which drastically reduced the colored carotenoids in the mycelia and in the bulk of the sporangiophores. Growth in the dark reduces β -carotene in the growing zone and in the mycelia.

3. Absorption characteristic of the action spectra could not be found in the absorption spectra. The absorption of photopigment is masked by the "bulk" absorption of carotenoids and by scattering.

4. The critical indices of refraction for phototropic balance at 495 and 510 $m\mu$ differed only slightly in spite of a very considerable difference in the total absorption. If the primary photoreceptors lie anywhere but near the vacuole, the small changes in dioptrics seem to be inadequate to compensate for the changes in absorption of light on its path between the receptors on the proximal and distal sides. The possibility is therefore discussed that the photoreceptors lie near the vacuole. Further experiments are needed to decide this issue.

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