

# Light and Dark Adaptation in *Phycomyces* Light-Growth Response

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**ABSTRACT** Sporangiohores of the fungus *Phycomyces* exhibit adaptation to light stimuli over a dynamic range of  $10^{10}$ . This range applies to both phototropism and the closely related light-growth response; in the latter response, the elongation rate is modulated transiently by changes in the light intensity. We have performed light- and dark-adaptation experiments on growing sporangiohores using an automated tracking machine that allows a continuous measurement of growth velocity under controlled conditions. The results are examined in terms of the adaptation model of Delbrück and Reichardt (1956, Cellular Mechanisms in Differentiation and Growth, 3-44). The "level of adaptation,"  $A$ , was inferred from responses to test pulses of light by means of a series of intensity-response curves. For dark adaptation to steps down in the normal intensity range ( $10^{-6}$ - $10^{-2}$  W/m<sup>2</sup>),  $A$  decays exponentially with a time constant  $b = 6.1 \pm 0.3$  min. This result is in agreement with the model. Higher-order kinetics are indicated, however, for dark adaptation in the high-intensity range ( $10^{-2}$ -1 W/m<sup>2</sup>). Adaptation in this range is compared with predictions of a model relating changes in  $A$  to the inactivation and recovery of a receptor pigment. In response to steps up in intensity in the normal range,  $A$  was found to increase rapidly, overshoot the applied intensity level, and then relax to that level within 40 min. These results are incompatible with the Delbrück-Reichardt model or any simple generalizations of it. The asymmetry and overshoot are similar to adaptation phenomena observed in systems as diverse as bacterial chemotaxis and human vision. It appears likely that light and dark adaptation in *Phycomyces* are mediated by altogether different processes.

## INTRODUCTION

Lower organisms have evolved highly complex behavioral responses to environmental stimuli (Haupt and Feinleib, 1979; Lenci and Colombetti, 1980). Many of these sensory responses are adaptive. A remarkable example is found in the fungus *Phycomyces blakesleeanus*: the light-induced growth modulation of its sporangiohore, or fruiting body, exhibits adaptation over a dynamic range of  $10^{10}$  in light intensity, a range similar to that of vision in higher organisms.

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We have performed light- and dark-adaptation experiments on growing sporangiophores of *Phycomyces* at both normal and high intensities, and have used the results to test a model for adaptation originally proposed by Delbrück and Reichardt (1956).

*Phycomyces* has long been studied as a model system for sensory transduction (Bergman et al., 1969; Russo and Galland, 1980; Lipson, 1980). The sporangiophore can be considered a naturally isolated photoreceptor cell. Responses take the form of changes in the growth rate, rather than electrical potential. The enormous cell size, rapid growth rate, short generation time, and the availability of sensory mutants make *Phycomyces* an attractive organism for sensory physiology.

The aerial sporangiophores are produced during the growth of a highly branched filamentous mycelium, produced from a single spore. Each sporangiophore consists of a thin, cylindrical stalk 0.1 mm in diameter bearing a spherical sporangium 0.5 mm in diameter at the top. When mature, the sporangium contains some  $10^5$  multinucleate spores. The elongation rate of the sporangiophore is quite rapid, typically 3 mm/h during the stationary growth phase, which lasts many hours. The unicellular sporangiophores can thus grow to heights in excess of 10 cm. The elongation, and associated axial twist, are confined to a growing zone that extends some 2.5 mm below the sporangium.

Sporangiophores have evolved a repertoire of responses that promote the efficient distribution of spores. The direction and rate of growth may be altered by a number of environmental stimuli, including light, gravity, stretch, wind, chemicals, and the presence of nearby objects (Bergman et al., 1969). When stimuli are applied from one side or another, sporangiophores bend after a latent period of a few minutes. In response to unilateral blue light, sporangiophores exhibit positive phototropism, bending towards the source at a maximal rate of several degrees per minute. If, instead, the light stimulus is applied symmetrically from both sides, but with a temporal variation in intensity, the elongation rate of the sporangiophore is modulated. In particular, pulses or steps up in light intensity induce a transient increase in growth rate referred to as the light-growth response. Phototropism arises from differential light-growth responses around the growing zones, induced by the focusing of light in the cylindrical lens formed by the sporangiophore (Bergman et al., 1969; Dennison and Foster, 1977; Medina and Cerdá-Olmedo, 1977). The light-growth response and phototropism therefore share a common blue-light action spectrum (Presti and Delbrück, 1978), as well as the same dynamic range. Study of the dynamic light-growth response thus provides a closer look at the basis of phototropism. By means of an automated tracking machine, the light-growth response has been studied by traditional methods (Foster and Lipson, 1973) and by modern system-identification techniques (Lipson, 1975a, b, c).

The photoreceptor(s) for the *Phycomyces* light responses have not been positively identified, although current evidence points strongly towards a flavin-based chromophore (Otto et al., 1981; Galland, 1983). It is not known

to what extent, if any, the adaptation processes are associated with the photochemical cycle of the photoreceptor. Given that the organism is able to adapt over a prodigious intensity range ( $10^{-9}$ – $10$  W/m<sup>2</sup>), it is reasonable to assume that adaptation begins quite early in the transduction pathway, most likely at the photoreceptor level. In this paper, we consider a simple model that identifies the adaptation kinetics with the photochemical kinetics of the receptor pigment.

There are two aspects to adaptation in the light-growth response. The first is that, under constant conditions, the growth rate is independent of the background light level (except at very high light intensity; Foster and Lipson, 1973). After a stepwise change in light intensity, the growth rate recovers to its prestimulus level of  $\sim 3$  mm/h after an interval of  $\sim 40$  min. Given that the growth rate is modulated only by changes in intensity, the light-growth response system behaves like a high-pass filter or differentiator. We will refer to this feature of adaptation as the recovery of growth rate. The second feature is that the magnitude of the light-growth response depends primarily on the relative change in intensity made with respect to the adapted level (i.e., on the ratio of the new level to the old), with a weak dependence on the initial intensity level. This range adjustment of sensitivity is a common attribute of adaptation phenomena; it involves a shift in the “operating point” of the sensory system. Such shifts are often associated with logarithmic transduction.

A model for the kinetics of adaptation in *Phycomyces* was proposed some time ago by Delbrück and Reichardt (1956); it has remained the foundation for the interpretation of physiological results in this organism. The sporangiophore is assumed to maintain an internal variable,  $A$ , the “level of adaptation,” which can be defined as “either the actual intensity to which the specimen has been adapted or as the virtual intensity to which it would have to be adapted to give a corresponding response to a standard stimulus” (Bergman et al., 1969). It is further proposed that  $A$  follows the light intensity,  $I$ , with first-order kinetics, according to

$$dA/dt = (I - A)/b, \quad (1)$$

where  $b$  is the time constant for adaptation. The growth rate is assumed to be a function only of the ratio ( $I/A$ ), so that when  $I = A$ , the cell is adapted and no extra growth occurs. During light responses, the departure of  $I$  from  $A$  before recovery results in a modulation of the growth rate. Delbrück and Reichardt found that, after a step down to total darkness,  $A$  fell with a time constant of 3.8 min during the first 10 min of the response. For later times, the decay appeared to be somewhat slower.

The recovery of the growth rate has been modeled in terms of a high-pass-filter component in the transduction chain (Lipson, 1975a, b). This sort of filtering is essentially a linear process. However, the process that mediates range adjustment (see above) is inherently nonlinear. The “white-noise” method of system identification has been employed to examine these nonlinearities (Lipson, 1975a, b). Unfortunately, the large-range aspects of adaptation are rendered inaccessible by this approach because of masking by other

nonlinearities, notably rectification and saturation, both of which limit the range of the response to large sudden changes in stimulus intensity.

To study range adjustment in terms of the Delbrück-Reichardt (1956) model, we have returned to traditional techniques, similar to those used in the original work. The development of the *Phycomyces* tracking machine, which incorporates a temperature-regulated chamber, has made possible long-term measurements of the growth of a single sporangiophore under controlled conditions. We have found that the model of Delbrück and Reichardt describes adaptation to darkness rather well, but fails to account for the adaptation kinetics observed for increases in light intensity. The model also breaks down in the high-intensity range. For this region, we consider an alternative kinetic model (Lipson, 1975*b*) that associates the adaptation process with receptor pigment inactivation and regeneration.

#### MATERIALS AND METHODS

##### *Strains*

The albino strain C2 [genotype *carA5(-)*], derived by nitrosoguanidine mutagenesis from the wild-type strain NRRL1555, was used in all the experiments. It has photoresponses indistinguishable from those of the wild type (Lipson, 1975*c*). Sporangiophores were grown on potato dextrose agar in shell vials, as described previously (Foster and Lipson, 1973).

##### *Tracking Machine*

Sporangiophores were examined individually on a machine designed to track their movement (Foster and Lipson, 1973). A vial with a single sporangiophore rested upon an *x-y-z* stage that was driven by means of servos so as to keep the sporangium fixed in space; the stage displacement was then equal and opposite to that of the sporangium. Vertical growth velocity was recorded continuously on a strip-chart recorder; measurements of the instantaneous growth were accurate to better than 1  $\mu\text{m}/\text{min}$ . Movement in the *x* and *y* directions was also monitored; excessive bending in any one sporangiophore led to its elimination from the data base. Specimens were tracked in a temperature-regulated chamber maintained at  $20.5 \pm 0.5^\circ\text{C}$ . The chamber was enclosed to suppress wind currents.

##### *Illumination*

The light source was an argon-ion laser operated in an intensity-regulation mode at a wavelength of 488 nm (model 52G; Coherent Radiation, Palo Alto, CA). The beam was expanded to 10 mm diam by means of lenses. Beam intensity was varied by means of a pair of inconel-coated, circular neutral density wedges, as described in Lipson (1975*a, b*). Bilateral oblique illumination, which stabilizes vertical growth, was used for all experiments. After attenuation by the wedges, the beam was split and directed bilaterally, at  $30^\circ$  below the horizontal, towards the growing zone of the specimen. Filters and light intensities were calibrated as described previously (Lipson, 1975*a*).

##### *Stimulus Programs and Response Definition*

An electronic instrument was constructed to produce light-stimulus programs in a cyclic fashion. The instrument can produce up to six distinct patterns in succession;

experiments were generally concluded before completion of the final cycle. High and low resting-intensity levels, which we designate  $I_H$  and  $I_L$ , respectively, were preset for each experiment. Up to six different values for the test-pulse intensity could be specified in advance. In addition, the electronic programmer could be set to generate a fourth intensity level,  $I_2$ , just before a transition between  $I_H$  and  $I_L$ ; this "conditioning pulse" was used for the light-adaptation experiments. Times between transitions among the light intensity levels were set in advance. The three types of stimulus program used are shown in Fig. 1.

Intensity-response curves for adapted sporangiophores were determined by administering a series of 12-s test pulses at 50-min intervals to sporangiophores adapted to  $I_L$ . Different values for  $I_L$  were used for each curve. Test pulses of various magnitudes were applied above this level in a random order; a typical result is shown in Fig. 1*a*.

For the dark-adaptation experiments, sporangiophores were adapted to  $I_H$  for 50 min, and then the light level was dropped to  $I_L$  (Fig. 1*b*). The level of adaptation was inferred from responses to test pulses (again, of 12 s duration) applied at various delay times,  $\Delta t_d$ , after the transition. After a recovery time (generally, at least 30 min), the cell was readapted to  $I_H$  and the process was repeated.

In the light-adaptation experiments (Fig. 1*c*), sporangiophores were adapted to  $I_H$  for 50 min. Then the light level was raised briefly to  $I_2$ , the conditioning level, for a variable time,  $\Delta t_2$  (30 s in the sample shown), during which the cell would begin to adapt to the higher level. The intensity was then dropped to  $I_L$ , as in the dark-adaptation experiments, and 12-s test pulses were applied after a fixed delay time,  $\Delta t_d$  (20 min), to determine the level of adaptation.

Different schemes for measuring the light-growth response have been used previously (Foster and Lipson, 1973). For the present work, we adopted the peak-to-peak height of the growth response after test pulses. These were measured from the strip-chart records of the experiments.

### *Error Analysis*

To calculate the level of adaptation from the response to a test pulse, we first had to determine a value for the subjective stimulus from the response. Since the intensity-response curves (Fig. 2) were determined with the stimulus (rather than the response) as the independent variable, we had to calculate the error involved in inverting the relationship. These errors were obtained (by standard error-propagation methods) from the parameter errors obtained in fitting the intensity-response data to Eq. 3 via a nonlinear least-squares approach (Hamilton, 1964). These fits gave estimates of the covariance of the parameters  $R_0$  and  $S_0$  as well as their individual variances. The variance-covariance matrix was used to fix the standard error bars for  $A$  shown in Figs. 3 and 4. In the high-intensity range (see Results), the error analysis was generalized to include interpolation errors in fixing  $R_0$  and  $S_0$ .

## RESULTS

### *Intensity-Response Curves*

The relation between the test-pulse size and the magnitude of the light-growth response is essential for the determination of the level of adaptation under dynamic conditions. In the intensity-response experiments, the specimen was exposed to a 12-s pulse of light at various relative amplitudes over the background illumination, to which it was adapted (Fig. 1*a*). The specimen was permitted to readapt to this level during the 50 min between pulses.

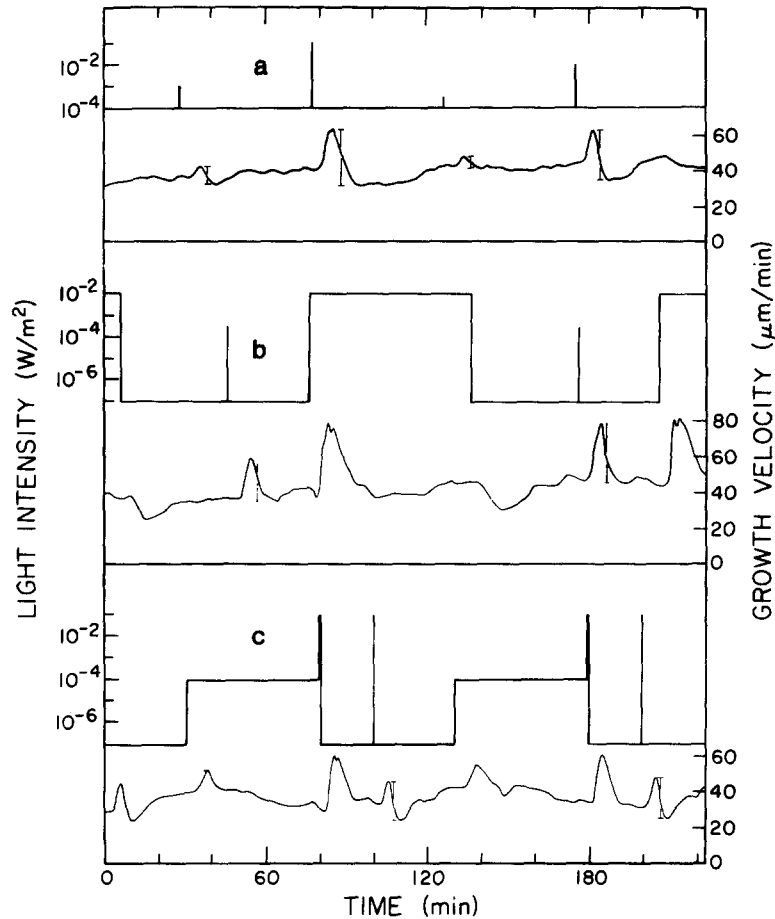


FIGURE 1. Portions of typical stimulus programs and responses. (a) Upper trace: light level representing a series of test pulses of 12 s duration delivered at 50-min intervals; the baseline intensity in this case was  $A = 10^{-4} \text{ W/m}^2$ . Pulses of various amplitudes above baseline were applied in a random order. Lower trace: a strip-chart record of the instantaneous growth rate measured with the tracking machine. The bars indicate the peak-to-peak amplitude of light growth response, used in these experiments as a measure of the response. (b) Upper trace: light levels during a dark-adaptation experiment. Sporangiophores were adapted to  $I_H = 10^{-2} \text{ W/m}^2$  for 60 min, after which the intensity was dropped to  $I_L = 10^{-7} \text{ W/m}^2$ . Test pulses of 12 s duration were given after a variable decay,  $t_d$  (here 40 min). Lower trace: the light-growth responses to the test pulses, marked by bars, as in a. Responses to the transitions between  $I_H$  and  $I_L$  can also be seen. (c) Upper trace: light levels during a light-adaptation experiment. Sporangiophores were adapted to  $I_H = 10^{-4} \text{ W/m}^2$  for 50 min, after which a conditioning stimulus of duration  $\Delta t_2 = 30 \text{ s}$  was given up to the level  $I_2 = 10^{-1} \text{ W/m}^2$ . The intensity was then stepped down to  $I_L = 10^{-7} \text{ W/m}^2$  for 20 min, after which a test pulse of 12 s duration was given (in the example, coincidentally equal to  $I_2$ ). Lower trace: the light growth responses to the test pulses, marked by bars, as in a. Responses to other light-level transitions can be seen.

The subjective stimulus (Foster and Lipson, 1973) is defined as

$$S = \frac{(I - A)}{A} \Delta t, \quad (2)$$

where  $I$  is the light intensity,  $A$  is the level of adaptation, and  $\Delta t$  is the duration of the light impulse. Assuming the specimen to be fully adapted prior to the pulse, we have  $A = I_L$ . When the cell is adapted, we have  $A = I$  and  $S = 0$ . For a stimulus of fixed  $\Delta t$ ,  $S$  is a function only of the ratio  $I/A$ , which can be taken to be the subjective intensity.

Multiple experiments of the type shown in Fig. 1a were performed for the following background light intensities:  $A = 10^{-4}$ ,  $10^{-2}$ ,  $10^{-1}$ , and  $10^0$  W/m<sup>2</sup>; the results are shown in Fig. 2. Data from each series were fit to the hyperbolic saturating function (Foster and Lipson, 1973)

$$R = R_0 S / (S + S_0), \quad (3)$$

which exhibits a sigmoidal shape (a hyperbolic tangent) when  $R$  is plotted against the logarithm of  $S$ . In the "normal range" (Delbrück and Reichardt, 1956), the curves for  $10^{-4}$  and  $10^{-2}$  W/m<sup>2</sup> are virtually identical. This feature is a reflection of the range-adjustment aspect of adaptation: the response depends primarily on the subjective intensity and not upon the absolute intensity. Curves for different adapted levels in the normal range are therefore

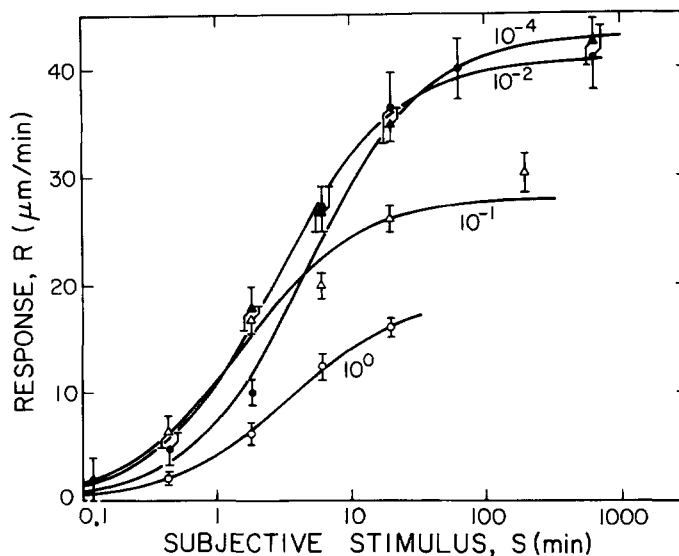


FIGURE 2. Intensity-response curves. The light-growth response amplitudes,  $R$ , to test pulses of the type shown in Fig. 1a are plotted as a function of the subjective stimulus,  $S$  (see text, Eq. 2). Series for baseline (adapted) light levels  $A = 1$  W/m<sup>2</sup> (open circles),  $10^{-1}$  W/m<sup>2</sup> (open triangles),  $10^{-2}$  W/m<sup>2</sup> (filled triangles), and  $10^{-4}$  W/m<sup>2</sup> (filled circles) are displayed. Data points represent the average of at least 10 responses obtained with several different sporangio-phores; the error bars represent standard errors. The solid curves are nonlinear least-squares fits to Eq. 3.

superposable. At higher intensities ( $A = 10^{-1}$  and  $10^0$  W/m<sup>2</sup>), the response saturates at a lower level; this diminution of the response to bright light is well established (Bergman et al., 1969; Lipson, 1975*b*) and is roughly analogous to the progressive loss of vision at high light intensities, associated with pigment bleaching (Rushton, 1965).

#### *Dark Adaptation in the Normal Range*

Dark-adaptation experiments determine the behavior of  $A$  as a function of time after a step down to darkness. In these experiments (Fig. 1*a*), specimens were adapted for 50 min to  $I_H = 10^{-2}$  W/m<sup>2</sup>; then the intensity was attenuated by five orders of magnitude to  $I_L = 10^{-7}$  W/m<sup>2</sup>. The level of adaptation was inferred from the responses to test pulses at delay times  $t_d = 10, 20, 30, 40,$  and 50 min after the step. The values of  $R$  were averaged from many individual responses; the subjective stimulus was then calculated from the inverted form of Eq. 3,

$$S = S_0/(R/R_0 - 1), \quad (4)$$

where  $R_0$  and  $S_0$  are constants obtained from the fit to intensity-response data at  $10^{-4}$  W/m<sup>-2</sup> (Fig. 2). Values of  $A$  were determined from  $S$  by means of the inverted form of Eq. 2:

$$A = \frac{I}{(1 + S/\Delta t)}, \quad (5)$$

where  $I$  is the intensity of the test pulse, and  $\Delta t = 12$  s. Eq. 1 implies that  $A$  decays exponentially after a step to darkness according to

$$A = I_H \exp(-t/b), \quad (6)$$

where  $I_H$  is the initial adapting level. The data were fit to the linearized form

$$\log(A/I_H) = -2.303(at), \quad (7)$$

where  $a = 1/b$ . A least-squares fit gave  $a = 0.164 \pm 0.008$  min<sup>-1</sup>, corresponding to  $b = 6.1 \pm 0.3$  min.

#### *Dark Adaptation in the High-Intensity Range*

Dark-adaptation experiments were performed as in the normal range, but with  $I_H = 10$  W/m<sup>2</sup> and  $I_L = 10^{-4}$  W/m<sup>2</sup>. Because of the substantial dependence of the intensity-response curves upon absolute intensity in this range (Fig. 2), the analysis and interpretation became more involved.

In the normal range, a single intensity-response curve was sufficient to analyze all the test pulse data. In the high-intensity region, we had to interpolate among a family of such curves, one for each adapted intensity. The procedure used to deduce  $A$  from the response,  $R$ , was generalized as follows. The theoretical intensity-response curve appropriate to a particular value of  $A$  was derived by an iterative procedure involving interpolation of the parameters  $R_0$  and  $S_0$  for the various experimental curves at  $10^{-2}$ ,  $10^{-1}$ , and  $10^0$  W/m<sup>2</sup>. In effect, the level of adaptation was calculated from the intensity-response curve that would have been obtained from pulse experi-



ments based on that level. In the upper portion of Fig. 3,  $A$  is plotted as a function of time after a step down. The straight-line fit is based on Eq. 7. The slope corresponds to  $b = 6.2 \pm 0.6$  min. The fit is not as good as in the normal intensity range (lower curve, Fig. 3).

A model associating adaptation kinetics with those of a receptor pigment (Lipson, 1975*b*) gives

$$A = I_c(1 - p)/p, \quad (8)$$

where  $p$  is the fraction of photoreceptor molecules in the active state and the critical intensity is given by  $I_c = k/\bar{\sigma}$ , with  $k$  is the regeneration rate of the

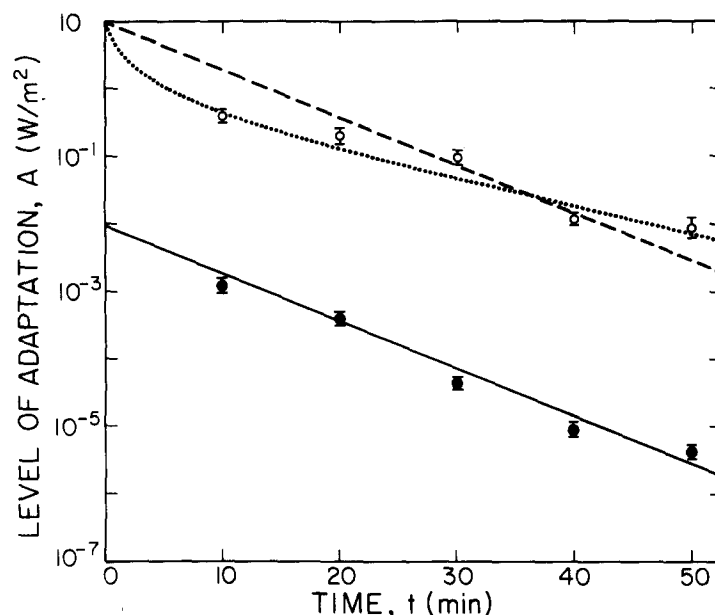


FIGURE 3. Time course of dark adaptation. The inferred behavior of the level of adaptation,  $A$ , as a function of time after a step down to darkness in the normal range (filled circles) and the high-intensity range (open circles). The solid and dashed lines are least-squares fits to Eq. 7 (derived from Delbrück-Reichardt model). The dotted curve is a nonlinear least-squares fit to Eq. 10 (photochemical model). The bars represent standard errors derived by error-propagation methods.

pigment and  $\bar{\sigma}$  is the partial cross section for inactivation (the product of the total absorption cross section and the quantum efficiency; Lipson and Presti, 1980). Assuming  $p$  follows a first-order, monomolecular rate equation, one can derive (Lipson, 1975*b*)

$$dA/dt = k(I - A)/(1 + A/I_c). \quad (9)$$

In the normal range ( $A \ll I_c$ ), this reproduces Eq. 1 with  $k = 1/b$ . For high intensities, neglecting  $I_L$  in comparison with  $I_H$ ,  $I_c$ , and  $A$ , the approximate solution is

$$A = I_c / [(I_c / I_H + 1)e^{kt} - 1]. \quad (10)$$

The dotted line in Fig. 3 is a fit to this equation. The parameter values are deduced from the fit

$$\begin{aligned} I_c &= (8 \pm 8) \times 10^{-1} \text{ W/m}^2; \\ k &= (9.2 \pm 2.7) \times 10^{-2} \text{ min}^{-1}; \\ \tau &= 1/k = 10.8 \pm 3.1 \text{ min.} \end{aligned}$$

The quality of this fit is much better than that for the straight line (Fig. 3). The large statistical error in  $I_c$  reflects the relative insensitivity of the fit to the value of  $I_c$ ; the determination is consistent with the value  $I_c = (6 \pm 1) \times 10^{-1} \text{ W/m}^2$ , determined previously from the diminution of response at high intensity (Lipson, 1975*b*). However, the value for  $\tau$  differs considerably from the corresponding value,  $2.7 \pm 0.6 \text{ min}$ , obtained earlier, as well as from the present value of  $b = 6.1 \pm 0.3 \text{ min}$ .

#### *Light Adaptation*

By analogy with the dark-adaptation experiments, one would hope to measure light adaptation using the reciprocal treatment, i.e., by administering test pulses at various times after a step up in light intensity. However, the large saturated response to steps up (see Fig. 1*b*) would obscure the responses to the test pulses, especially those with short delay times. The greater sensitivity of *Phycomyces* to increases in light (a rectification phenomenon), and the faster kinetics of light adaptation (see below), render this approach unfeasible. Indeed, the dark-adaptation experiments were possible only because the small transient response to the step down had passed by the time the earliest test pulses were given.

Accordingly, an indirect method was used to measure light adaptation. In this method, we assumed the kinetics of dark adaptation (Fig. 3, lower curve). A conditioning stimulus,  $I_1 = 10 \text{ W/m}^2$ , of variable width was applied above the adapted level,  $I_H = 10^{-4} \text{ W/m}^2$ . Pulses of the usual 12 s duration were applied at  $t_a = 20 \text{ min}$  to infer the level of adaptation. The effect of the conditioning stimulus is to raise the level of adaptation above  $I_H$  before the step down; the response to test pulses a fixed time later is therefore diminished. We obtained the level of adaptation at the end of the conditioning stimulus by extrapolating back from the inferred level,  $A_d$ , according to

$$A_o = A_d \exp(t_a/b), \quad (11)$$

with  $b = 6.1 \text{ min}$ . Combining the results for several values of the conditioning interval, we reconstructed of the time course for  $A$  after a step up (Fig. 4). The solid curve is the prediction of the Delbrück-Reichardt model,

$$A = I_1 [1 - \exp(-t/b)]. \quad (12)$$

The data are incompatible with this prediction. Within the first minute,  $A$  overshoots the conditioning stimulus level, peaks, and eventually relaxes to that level. Two features of the model are incompatible: (a) the observed overshoot and relaxation cannot be accounted for by any first-order relaxation

equation, such as Eq. 1, and (b) the linear dependence of  $A$  upon  $I$ , implicit in Eq. 1, is contradicted by the data. Thus, light adaptation appears fundamentally different from dark adaptation.

#### DISCUSSION

Our experiments were performed within the framework of the adaptation model developed by Delbrück and Reichardt (1956) for the light-growth response of *Phycomyces*. The model incorporates the following key assumptions: (a) that the state of adaptation of the organism can be described by a single variable  $A(t)$ , the "level of adaptation"; (b) that  $A$  follows the ambient light intensity with first-order kinetics given by Eq. 1; and (c) that the growth response depends functionally upon a subjective intensity, given by the ratio  $I/A$ .

In general, the concept of a level of adaptation presumes that organisms are able to maintain an internal parameter (such as the level of a biochemical intermediate, or a membrane potential), which reflects information about their environment in the recent past. Although biochemical correlates of  $A$  have not yet been identified in *Phycomyces*, such correlates have already been found in other systems (cf. Springer et al., 1979). The dependence of growth upon a subjective—rather than absolute—intensity incorporates the phenomenon of range adjustment in a system that has a logarithmic (Weber-Fechner) input-output relationship: the response depends upon a ratio, as opposed to a difference, of quantities. The characteristic times associated with the kinetics by which  $A$  follows  $I$  indicate the "memory" the system maintains of past stimuli.

For dark adaptation, where Eq. 1 reduces to  $dA/dt = -A/b$ , the level of adaptation should fall exponentially in the dark with time constant  $b$ , according to Eq. 6. Our data on dark adaptation in the normal range were fit very well by an exponential with  $b = 6.1 \pm 0.3$  min. Indeed, our data fit an exponential process much better than the original data of Delbrück and Reichardt (1956). The time constant of  $\sim 6$  min found here for dark adaptation is comparable to the 7-min time constant associated with the recovery of sensitivity and regeneration of visual pigment in human rods (Rushton, 1965). However, in *Phycomyces*, the level of adaptation (analogous to visual threshold)<sup>1</sup> falls exponentially in the dark, whereas it is the logarithm of the threshold that falls exponentially in the case of scotopic vision. Such threshold shifts associated with adaptation are generally among the slowest processes associated with sensory transduction.

Dark adaptation in the high-intensity range ( $\sim 10$  W/m<sup>2</sup>) displays different kinetics from those in the normal intensity range ( $\sim 10^{-2}$  W/m<sup>2</sup>). An exponen-

<sup>1</sup> The level of adaptation may be related to a threshold measure as follows: in connection with intensity-response data (Fig. 2), define a threshold stimulus,  $S_T$ , and threshold intensity,  $I_T$ , such that the response  $R$  is 10% of the saturation value  $R_0$ . Then, by Eq. 3,  $S_T = S_0/9$ . Let us adopt the value  $S_0 = 4.8$  min for the  $A = 10^{-4}$  W/m<sup>2</sup> curve. Then, since  $\Delta t = 0.2$  min, we have from Eq. 5 that  $I_T/A = 1 + S_T/\Delta t = 3.7$ . Thus, within the range of validity of a particular intensity-response curve, the threshold is proportional to the level of adaptation.

tial fit to the data gave a time constant in reasonable agreement with the corresponding value for the normal range, but the fit was less satisfactory (Fig. 3). Moreover, there appears to be a systematic curvature to the semilogarithmic plot not predicted by the linear model of Eq. 1.

A better fit was obtained from the photochemical model implicit in Eq. 9. Two parameters were obtained from this fit: the critical intensity,  $I_c$ , and the time constant,  $\tau$  (see Results). Since the photochemical model must reduce to the linear model of Delbrück and Reichardt in the limit of  $A \ll I_c$ , we should identify  $\tau$  with the time constant  $b$  in Eq. 1. However, the value obtained from the fit,  $\tau = 10.8 \pm 3.1$  min, is higher than expected. A previous estimate, derived from white-noise analysis of *Phycomyces* in the high-intensity range (Lipson, 1975*b*), gave  $\tau = 2.7 \pm 0.6$  min. These two estimates of  $\tau$  bracket the value for  $b$  inferred from our data, but the differences are too large to be explained by statistical error. Therefore, despite the improved fit of Eq. 9 over Eq. 1, the specific model relating adaptation kinetics to a first-order set of photochemical reactions is inadequate. Nevertheless, the substantial improvement of the curvilinear fit (Fig. 3, upper portion) over the straight-line fit encourages future refinement of this photochemical model.

Our results for light adaptation are incompatible with the Delbrück-Reichardt model. After a step up in light intensity, the adaptation level rises very rapidly, overshooting the mark before relaxing to the final intensity level. There was already an indication of asymmetry in the kinetics of light and dark adaptation in the original work of Delbrück and Reichardt (1956). They suggested that Eq. 1 might still hold for light adaptation, but with a shorter time constant,  $b$ . Our results clearly do not support this notion. Although light adaptation proceeds rapidly during the first few minutes of the response, the peaking and subsequent slow relaxation of  $A$  cannot be accommodated by any first-order, linear differential equation such as Eq. 1.

We have considered alternative mathematical models up to second order with nonlinear dependence of  $A$  upon  $I$ , subject to the constraint that they reduce to Eq. 1 when  $I = 0$ . However, these generalized models were unable to fit the data well. This outcome is not surprising, because it is common for organisms to exhibit asymmetries that arise, for example, when forward and backward reactions proceed along entirely different pathways (Milsum, 1966). Thus, it may be inappropriate to generalize Eq. 1 to include both light and dark adaptation. Analogous asymmetries (and overshoots) have long been known in human vision (Crawford, 1947; Baker, 1963) and appear also in the adaptation kinetics for bacterial chemotaxis (Berg and Tedesco, 1975). In the light-growth response itself, there is a substantial asymmetry in the response to positive and negative stimuli: the response to a positive step or pulse of light is generally much stronger than to a corresponding negative stimulus (Foster and Lipson, 1973; Lipson 1975*a, b*; and see Fig. 1*b*). This rectification property may itself be based upon asymmetries in adaptation processes.

It should be emphasized that the kinetics of light adaptation in Fig. 4 were obtained under the assumption that the adaptation to darkness after the conditioning pulse remained in accordance with the Delbrück-Reichardt

model, i.e., fell exponentially. (An indirect protocol for light adaptation was undertaken for reasons given in Results.) In particular, we assumed that the dark phase of adaptation occurred with the same time constant of 6.1 min, as measured in our earlier experiments. If, however, dark-adaptation kinetics were altered in some way by the prior conditioning stimulus, then the apparent time course for  $A$  would differ.

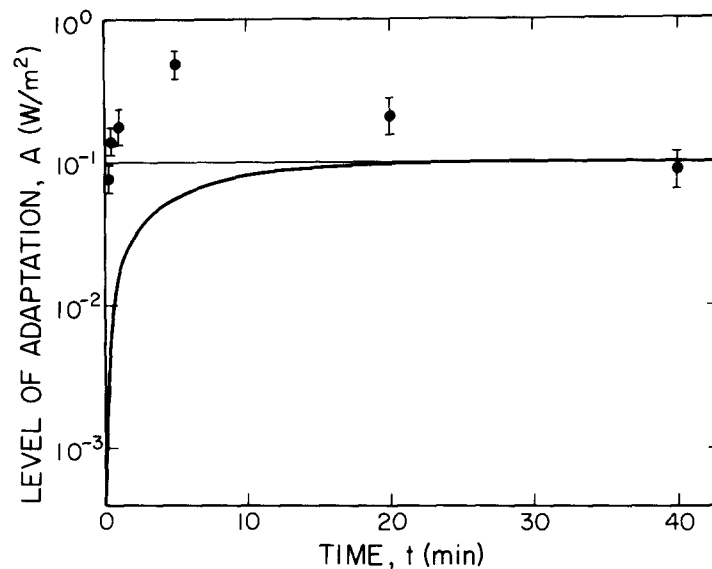


FIGURE 4. Time course of light adaptation. The behavior of the level of adaptation,  $A$ , was inferred as a function of the time,  $\Delta t$ , after a step upwards from  $I_H = 10^{-4} \text{ W/m}^2$  to  $I_L = 10^{-1} \text{ W/m}^2$ . The time on the abscissa represents the duration of conditioning pulses from  $I_H$  to  $I_L$ . As shown in Fig. 1c, these pulses were followed by 20 min of dark adaptation and then a test pulse. The adaptation level shown here on the ordinate was obtained by extrapolation (Eq. 11) backwards from the adaptation level at the time of the test pulse (see text). The solid curve is the prediction of Eq. 12 (Delbrück-Reichardt model), assuming the value  $b = 6.1 \text{ min}$  for the dark-adaptation time constant. The bars represent standard errors. These were computed by error-propagation methods carried through the equations used to derive  $A$ .

We have examined our data with this possibility in mind and have found that the dark decay would have to be slowed enormously to eliminate the observed overshoot; a dark time constant of some 30 min is required to bias the early points sufficiently to bring them into line with the solid curve of Fig. 4. We view this relatively drastic interference of light- and dark-adaptation kinetics to be unlikely; in any case, such an effect would represent a further contradiction of the model (Eq. 1).

Another type of departure from the Delbrück-Reichardt model, which could account for the overshoot deduced by our procedure, would arise if the conditioning stimulus (Fig. 1c) introduced a refractory period whose influence

persisted out to the time of the test pulse; if so, then the stronger test pulse needed to produce a criterion (half-maximal) response would lead us to infer a higher level of adaptation at the time of the test pulse and in turn at the end of the conditioning pulse. Such refractoriness might arise if there were two elements in the sensory transduction pathway that were responsible for adaptation, for example, one associated with the receptor pigment that managed range adjustment of sensitivity and another associated with the growth control output that might become refractory after a strong conditioning stimulus. In other words, the range adjustment and growth rate aspects of adaptation may be separately controlled.

The nonexponential decay of dark adaptation in the high-intensity range (Fig. 3, upper part) also suggests that there may be two components for dark adaptation. Studies of dark adaptation by a phototropic delay method (Russo and Galland, 1980; E. D. Lipson and S. M. Block, unpublished data) provide evidence for biphasic decay of dark adaptation from initial levels at high intensity and low intensity; in the normal range, the decay appeared describable by a single exponential as we find here (Fig. 3, lower part). In particular, Russo and Galland (1980) found that for an initial adapting level of  $6 \text{ W/m}^2$ , the adaptation level fell with time constants of 3 and 9 min. A phototropism mutant affected in gene *madB* lacks the fast component. The biphasic decay found in phototropic adaptation at high intensity is similar kinetically to what we find in Fig. 3. There the decay from the initial adapted level to the first (10 min) point corresponds to a time constant of  $\sim 3$  min; the asymptotic slope of the dotted curve at later times corresponds to a time constant of 10 min. Therefore, similar dark-adaptation kinetics seem to apply for both the light-growth response and phototropism in the high-intensity range.

In summary, although the Delbrück-Reichardt adaptation model has provided a very useful framework for analysis of adaptation phenomena in *Phycomyces*, the specific kinetics of Eq. 1 are valid only for dark adaptation in the normal range. At high intensity, the kinetics are higher order—probably biphasic and perhaps associated with the photochemical kinetics of the receptor pigment. As in vision, light adaptation appears to proceed much faster than dark adaptation; the two phenomena may be mediated by very different processes. This asymmetry may underlie the rectification property of the light-growth response. The development of a comprehensive kinetic theory of adaptation in *Phycomyces* will have to await the outcome of more experiments, particularly on light adaptation.

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