Phototropism in Conidiobolus, Some Preliminary Observations

Abstract. The action spectrum for phototropism of *Conidiobolus* conidiophores was determined crudely with glass filters and by projecting a spectrum on cultures of the fungus. The active wavelengths (about 400 to 650 mμ) corresponded in general with those absorbed by extracts containing a pigment with an absorption spectrum characteristic of a porphyrin.

It is well known that conidiophores of *Conidiobolus*, a member of the phycomycetous order Entomophthorales, grow toward a source of light. It is the purpose of this paper to report the results of a preliminary investigation of phototropism in this fungus.

The strain of *Conidiobolus* used for these trials was isolated by the canopy plate technique of Drechsler (*1*) from decaying leaves of *Sequoia sempervirens* Endl. The fungus is similar to one studied by Morrow (*2*) and resembles *Conidiobolus villosus* Martin in that it produces villose conidia under some conditions. It was grown on potato-dextrose agar or on a medium which contained 0.5 percent asparagine, 2.0 percent glucose, and salts. When cultures were illuminated unilaterally by an incandescent lamp, positive phototropism was indicated by an accumulation of conidia and young mycelia at the side of the culture nearest the light.

In order to determine what wavelengths of light are capable of eliciting a phototropic response, Corning glass filters were interposed between the lamp and the cultures. Phototropism was induced not only by light passed through a blue filter (5543), but also by light passed through sharp cut filters which transmit less than 0.5 percent of wavelengths shorter than 471 and 599 mμ. The fact that light from a red photographic safelight was effective even when passed through a red filter (2408) is additional evidence that this species of *Conidiobolus* is capable of responding to orange or red light as well as to blue light. That the response is not to heat or to far-red light is indicated by the fact that no response was shown by cultures which were illuminated by light from a photographic safelight passed through a far-red filter (5840).

In an attempt to obtain further information on the effective wavelengths of light, a spectrum was projected on a culture of *Conidiobolus*. Light from an incandescent lamp was focused through a slit on a collimating lens, dispersed by a 60° prism, and directed on the fungus by a second lens. The apparatus was calibrated by placing a fluorescent lamp in front of the slit and marking the emission lines of mercury. The fungus was grown in a transparent plastic box with a glass plate in the bottom and an agar slant partially covering the plate. The slant was inoculated by streaking conidia near its edge in a line at right angles to the direction of the light, so that conidia discharged toward the light collected on the glass plate. After 4 or 5 days, the results were recorded by placing the plate in a photographic enlarger and projecting the image on sensitized paper.

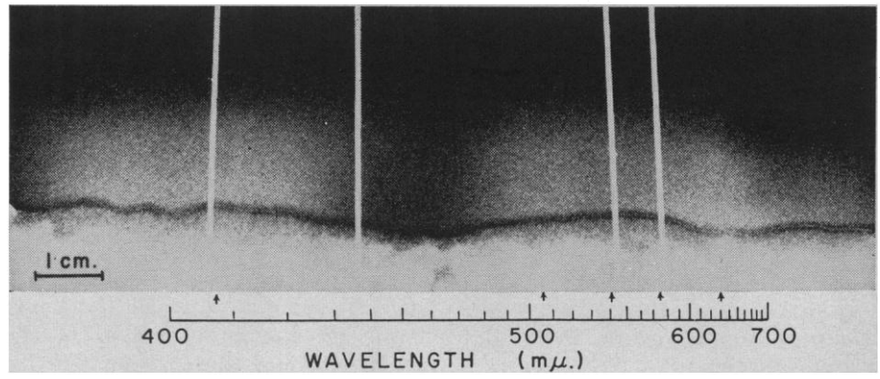

Fig. 1. Action spectrum for phototropism of *Conidiobolus* obtained by projecting spectrum on culture. Degree of positive response is indicated by density of conidia (white) on glass. Lines show approximate positions of emission lines of mercury. Arrows show positions of peaks of absorption spectrum shown in Fig. 2.

The results indicated that the fungus responds to wavelengths as long as about 650 mμ (Fig. 1). Conidia accumulated most densely on the glass plate opposite those portions of the growth illuminated by violet (about 400 mμ) and green-red light (about 490 to 650 mμ).

In order to discover whether *Conidiobolus* contains a pigment which absorbs light of wavelengths similar to those effective in inducing phototropism, absorption spectra of extracts were determined. The fungus was grown in Roux bottles containing 100 ml of liquid asparagine-glucose medium. Mats of mycelium from 12-day-old cultures were ground with acetone. The acetone was extracted with petroleum ether, and after partition, the petroleum ether layer was discarded. The fact that the petroleum ether extract was colorless indicates that this species of *Conidiobolus* does not contain carotinoids in appreciable quantities. The yellowish acetone layer was clarified by centrifugation, and its absorption spectrum was determined with a Bausch and Lomb recording spectrophotometer.

As shown in Fig. 2, the absorption spectrum is of a type characteristic of certain porphyrins (*3*) with a strong absorption peak in the violet and four peaks in the green, yellow, and orange portions of the spectrum. Dioxane extracts showed peaks at 412, 506, 543, 580, and 630 mμ.

The results of these preliminary experiments indicate that *Conidiobolus*—unlike higher plants and such fungi as *Phycomyces* and *Pilobolus* (*4*)—is capable of responding phototropically to green, yellow, and orange light as well as to violet and blue light. Further, this fungus contains a porphyrin pigment which absorbs light in these same general portions of the visible spec-

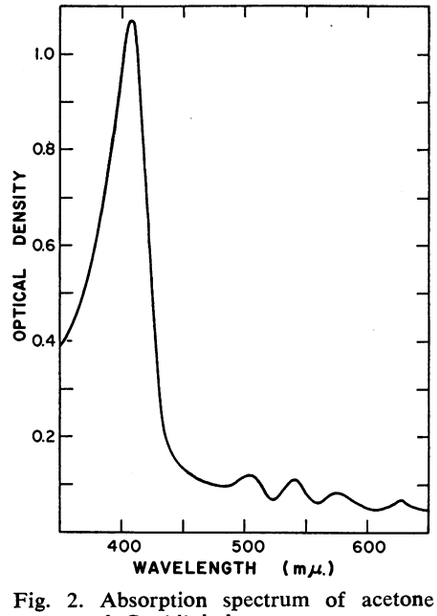

Fig. 2. Absorption spectrum of acetone extract of *Conidiobolus*.

trum. Since the wavelengths of light absorbed by the pigment are similar to those effective in phototropism, it is possible that the porphyrin is the photosensitive pigment; however, further investigation will be required to determine whether this interpretation is correct (*5*).

ROBERT M. PAGE
JOANNE BRUNGARD
Department of Biological Sciences, Stanford University, Stanford, California

References and Notes

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5. The authors are grateful to W. R. Briggs for the use of equipment.

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Antigen Disappearance in Hibernating Ground Squirrels

Abstract. The rate of antigen disappearance was studied in hibernating ground squirrels (*Citellus tridecemlineatus*) injected with I^{131}-labeled bovine serum albumin. There was no detectable disappearance of antigen during 14 days of hibernation. The induction period, however, ended 5 days after arousal as compared to a 7-day induction period in nonhibernating ground squirrels.

The resistance of hibernating animals to infection has been studied by a number of workers (*1*). It would appear that resistance to infection is increased with entry into hibernation. There is no evidence to suggest that increased resistance to infection is other than a nonspecific effect associated with physiological changes accompanying this process. To our knowledge, the immune response of mammals in hibernation has not been studied. The present study is concerned with antigen disappearance in the hibernating ground squirrel, *Citellus tridecemlineatus* (*2*).

The ground squirrels were collected in northern Illinois during September 1960 and were maintained in a room at 23°C for 6 weeks before use. During this period they were individually caged and allowed free access to Rockland Guinea Pig Diet with supplements of carrots twice weekly. Hibernation was induced by placing the animals, individually caged in a deep bed of wood shavings without food or water, in a room at 5°C and 50-percent relative humidity. They were checked twice daily to determine their state of hibernation.

We followed the disappearance of I^{131}-labeled bovine serum albumin from the circulation of hibernating and nonhibernating ground squirrels. All squirrels received a single intraperitoneal injection of 10 mg of bovine serum albumin labeled with I^{131} by the method of Talmage *et al.* (*3*). Serum from ground squirrels was iodinated and injected by the same procedure.

Blood samples from the tail were collected on tared filter paper, weighed, and counted in a well-type scintillation counter. Counts were corrected for background, disintegration, and weight of sample, converted to percentage of the activity of the sample taken 1 day after antigen injection, and plotted on a semilogarithmic scale. The rate of antigen disappearance was in three phases, as indicated by marked changes in the slope of the plot. These three phases were, in the terminology of Dixon *et al.* (*4*), (i) the equilibration phase, (ii) the nonimmune elimination phase, and (iii) the immune disappearance phase which marks the appearance of antibody.

In Fig. 1 the mean slopes are plotted for each phase of antigen disappearance in four treatment groups. The mean slope for the period of equilibration is an average of the slopes of that phase for the individuals in the group. The mean slopes for the nonimmune elimination and the immune phases were obtained by means of the same method.

Group A was composed of 14 squirrels that remained at room temperature after antigen was injected. The first blood sample was taken 1 day after the injection, four during the next 6 days, and three in the course of the next 5 days. The period of equilibration was less than 1 day in ten animals and as long as 3.5 days in four animals where the mean half-disappearance time was 2.3 days. During the nonimmune elimination phase that followed, the rate of antigen disappearance slowed so that the antigen level decreased by half in 5.4 days. On the seventh day after antigen injection the half-disappearance time decreased to 1.5 days. This increase in rate of disappearance is considered to mark the end of the induction period and the beginning of the immune phase wherein the appearance of antibody in the circulation is followed by rapid removal of the circulating antigen-antibody complexes. This sequence is qualitatively similar to that observed by Dixon *et al.* (*4*) in rabbits.

Group B was composed of 11 animals that were placed in the cold room immediately after antigen injection. They all entered hibernation within a day. During the 2-week hibernation period only four samples were taken; after arousal the sampling schedule was the same as for group A. There was little or no disappearance of antigen during the period of hibernation. After arousal, the half-disappearance time became 4.4 days, which was not statistically different

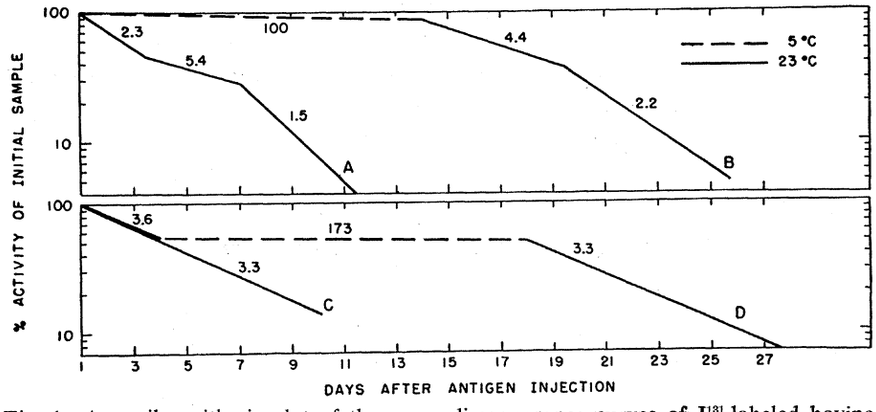

Fig. 1. A semilogarithmic plot of the mean disappearance curves of I^{131}-labeled bovine serum albumin in groups A and B, and of I^{131}-labeled ground squirrel serum in groups C and D. After injection of labeled material on day 0: (*A*) 14 animals maintained at 23°C; (*B*) 11 animals maintained at 5°C for 14 days in hibernation followed by a return to 23°C; (*C*) 4 animals maintained at 23°C; (*D*) 5 animals at 23°C for 4 days, then in hibernation at 5°C for 14 days followed by a return to 23°C. The numbers associated with each phase of the plot show the half-disappearance time in days.