Plasmodium berghei infection (*11*). In each case, females or castrates are more susceptible than normal males. The possibility exists that the increased susceptibility of these animals is related to a deficiency of at least two late-acting components of complement. These observations suggest that mice lacking more than one component of complement may be useful for studies on the in vivo functions of complement.

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References and Notes

1. L. T. Rosenberg and D. K. Tachibana, *J. Immunol.* **89**, 861 (1962); R. M. Weintraub, W. H. Churchill, Jr., C. Crisler, *Fed. Proc.*, in press (1966); R. M. Weintraub, H. J. Rapp, T. Borsos, in "Complement workshop," *Immunochemistry*, in press.
2. W. D. Terry, T. Borsos, H. J. Rapp, *J. Immunol.* **92**, 576 (1964).
3. L. D. Caren and L. T. Rosenberg, *Science*, this issue.
4. H. J. Rapp, *ibid.* **127**, 234 (1958); K. Inoue and R. A. Nelson, Jr., *J. Immunol.* **95**, 355 (1965).
5. Supplied by the Schering Corporation, Bloomfield, N.J., as Oreton (aqueous testosterone, given in the dosage of 2.5 mg per mouse) and as Progynon (estradiol benzoate in oil, given in the dosage of 0.1 mg per mouse). Controls were given Oreton vehicle (supplied by Schering) or sesame oil. The C'^{m}-EDTA titers of the serums from animals receiving vehicle or oil were the same as those from untreated controls.
6. L. Herzenberg, D. K. Tachibana, L. T. Rosenberg, *Genetics* **48**, 711 (1963); B. Cinader, S. Dubiski, A. C. Wardlaw, *J. Exp. Med.* **120**, 897 (1964).
7. U. R. Nilsson and H. J. Muller-Eberhard, *Fed. Proc.* **24**, 620 (1965).
8. C. Crisler and M. M. Frank, *ibid.* **24**, 620 (1965); L. D. Caren and L. T. Rosenberg, *Immunology* **9**, 359 (1965); Z. Ovary, *Int. Arch. Allergy Appl. Immunol.* **3**, 162 (1952); S. Ben-Efraim and B. Cinader, *J. Exp. Med.* **120**, 925 (1964); C. Stiffel, G. Biozzi, D. Mouton, Y. Bouthillier, C. Decreusefond, *J. Immunol.* **93**, 246 (1964); L. D. Caren, J. F. MacKnight, L. T. Rosenberg, *Fed. Proc.* **24**, 698 (1965); L. T. Rosenberg, in "Complement workshop," *Immunochemistry*, in press.
9. A. Kirschbaum, A. G. Liebelt, N. G. Falls, *Cancer Res.* **15**, 685 (1955).
10. B. A. Rubin, *Progr. Exp. Tumor Res.* **5**, 217 (1964).
11. J. Greenberg, E. Nadel, G. R. Coatney, *J. Infect. Dis.* **93**, 96 (1953).
12. We thank Dr. E. P. Vollmer for suggestions and advice, and M. O. Harris for able technical assistance.
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Antigenic Differences in the Surfaces of Hyphae and Rhizoids in Allomyces

Abstract. *Immunofluorescent techniques have demonstrated a difference in surface components of hyphae and rhizoids of* Allomyces macrogynus. *An antigenic component detected on the rhizoidal surface may be present, but masked, in the hyphal-wall matrix material. The system also allows visualization of the hyphal wall during aging, when changes from a smooth to a fissured surface are noted, and differences in adsorptive properties occur.*

Many Phycomycetes produce rhizoids as outgrowths of a thallus or of hyphal filaments. These rhizoids are thought to serve for support and, particularly in the case of some of the chyridiaceous fungi where they penetrate the host cells, as absorptive organs. Despite their wide occurrence little is known of their composition or structure. Aronson and Preston (*1*) have published electron micrographs of rhizoids of *Allomyces macrogynus* prepared by chemical treatment, which show microfibrils that are continuous with those in the wall of the parent hyphal segment. Analysis of purified cell wall preparations of this organism has disclosed the presence of components other than the *N*-acetylglucosamine resulting from hydrolysis of chitin microfibrils (*2, 3*). There has been no evidence to indicate that the composition of rhizoid walls differs from that of hyphal ones. When mention has been made of walls of rhizoids they have been analyzed together with hyphal walls and assumed to have the same composition. However, using immunofluorescent techniques, we have shown that differences in the composition of the walls of those two structures do exist in *A. macrogynus*.

Cell wall extracts containing one or two antigens were used to absorb portions of an antiserum which had been produced against whole mycelium. Young plants of *A. macrogynus* were incubated in absorbed antiserum, washed several times, and treated with the same antiserum, unabsorbed but labeled with fluorescein isothiocyanate. The results of such a procedure can be seen in Fig. 1 where only the rhizoids show attachment of the fluorescein-labeled antiserum.

The rabbit antiserum was prepared by injecting rabbits beneath the scapulae with ground lyophilized vegetative mycelium mixed with Freund's adjuvant. We used serum from a single bleeding; it showed at least seven precipitin lines when tested by the Ouchterlony technique with fresh mycelial extracts. Labeled antiserum was prepared from a globulin fraction of the specific serum by the method of Riggs *et al.* (*4*). Fluorescein-labeled goat antiserum to rabbit globulin (*5*) was used in some tests.

Table 1. Fluorescent antibody labeling patterns of 2-day-old mycelium of *Allomyces macrogynus*.

Antiserum treatment of mycelium		Fluorescence	
First	Second	Hyphae	Rhizoids
Absorbed	Labeled	−	+
Labeled	None	+	+
Unabsorbed	Labeled goat*	+	+
Labeled goat	None	−	−
Unabsorbed	Labeled	−	+†
Absorbed	Labeled goat*	+	+

* Goat antiserum to rabbit globulin. † Weak positive.

Various procedures have been employed to release antigens from the cell walls, including enzymatic digestion and treatment with sodium deoxycholate. As many as three antigens were detected in such extracts and each can be obtained by more than one extraction procedure. Preparation of the cell walls by mechanical means and a preliminary chemical analysis of antigen-containing fractions have been described (*2, 6*).

Two different absorbed antiserum preparations were used in these experiments: One (I) was absorbed with a heated enzymatic wall digest which contained one antigen, and the other (II) was absorbed with a digest containing two antigens, one being common to both extracts. Both preparations gave identical results in the labeling patterns obtained. Absorptions were carried out by mixing equal volumes of the antiserum and the antigen solution and incubating the mixture at 4°C for 48 hours (I) or 1 week (II). Precipitates were removed by centrifugation, and the supernatants were tested for excess antigen or antibody. Preparation I contained excess antigen; preparation II gave no detectable precipitin lines in the agar diffusion tests with either antiserum or antigen. Figure 4 shows a double diffusion test prepared with one of the wall digests and several of the serum samples used.

Two- or three-day-old plants grown from zoospores in Emerson broth were used for most experiments. Plants grown

in a defined medium gave similar results. The plants were washed twice in 0.02*M* phosphate-buffered saline, *p*H 7.2, placed in the appropriate serum for 30 minutes, washed three times in buffered saline, and observed or incubated in the second reagent, as required. For observation, the mycelium was placed in buffered glycerol and examined with a Zeiss fluorescence microscope, operated with an Osram HBO 200 mercury lamp in combination with a UG 5 exciter filter (Table 1).

The results with the "reverse" staining procedure (line 6, Table 1) can be explained if the rhizoids contain antigen or antigens in addition to those present in wall digests used for absorption. Caution is always necessary in interpreting results with immunofluorescent techniques and our findings will be checked with antiserum to be produced against purified wall antigen. However, regardless of the mechanism invoked for the staining patterns obtained, it seems clear that a difference in surface does exist between rhizoids and hyphae.

It appears unlikely that the component or components responsible for attachment of labeled antibody is an integral part of the microfibrils since wall fractions in which the antigen occurs contain no glucosamine. Although the antigen, common to both of the digests used for absorbing the antiserum, can be removed from the walls by treatment with the proteolytic enzymes Pronase and trypsin, it contains none of the released amino acids. From precipitin inhibition studies, galactose is a part of the determinant site (*7*).

Rhizoids of all lengths were fluorescent. Immature meiosporangia and zoosporangia were not labeled. Hyphal tips not uncommonly were fluorescent, as were "bumps" along the hyphae, which apparently were developing rhizoids and branches (see Fig. 3*a* and arrows in Fig. 1). This suggests that the components on the rhizoids are not unique, but are masked in the hyphal wall by other constituents which have not yet covered the actively growing regions. In addition, a zone, rarely two, close behind the tip, occasionally fluoresced (Fig. 3*a*). No cytological differences were noted in tips which fluoresced, and examination of the same microscopic fields with phase contrast optics showed protoplasm of normal appearance in them (compare Figs. 3*a* and 3*b*). Although further observations must be made on cultures during growth to clarify the relationship of the fluorescing tips to growth of the hypha, our results indicate that all of the cell wall components may not be formed simultaneously during new wall synthesis. This is not surprising in light of information available on higher plant cell walls, but contrasts with recent suggestions concerning streptococcal wall growth (*8*).

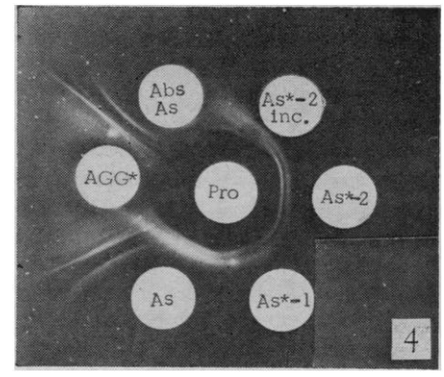

Fig. 4. Agar double diffusion test after 4 days at 15°C. Center well: Heated Pronase digest of purified *A. macrogynus* cell walls. Peripheral wells, beginning at lower left: As, rabbit antiserum prepared against whole mycelium; As*-1 and As*-2, two-column fractions of the same antiserum after fluorescein isothiocyanate conjugation (As*-2 was the preparation used in tests reported herein); As*-2, inc., a portion of As*-2 after mycelium had been incubated in it; Abs As, specific antiserum after absorption with the wall digest; AGG*, fluorescein-labeled goat antiserum to rabbit globulin (distance between wells, 4 mm).

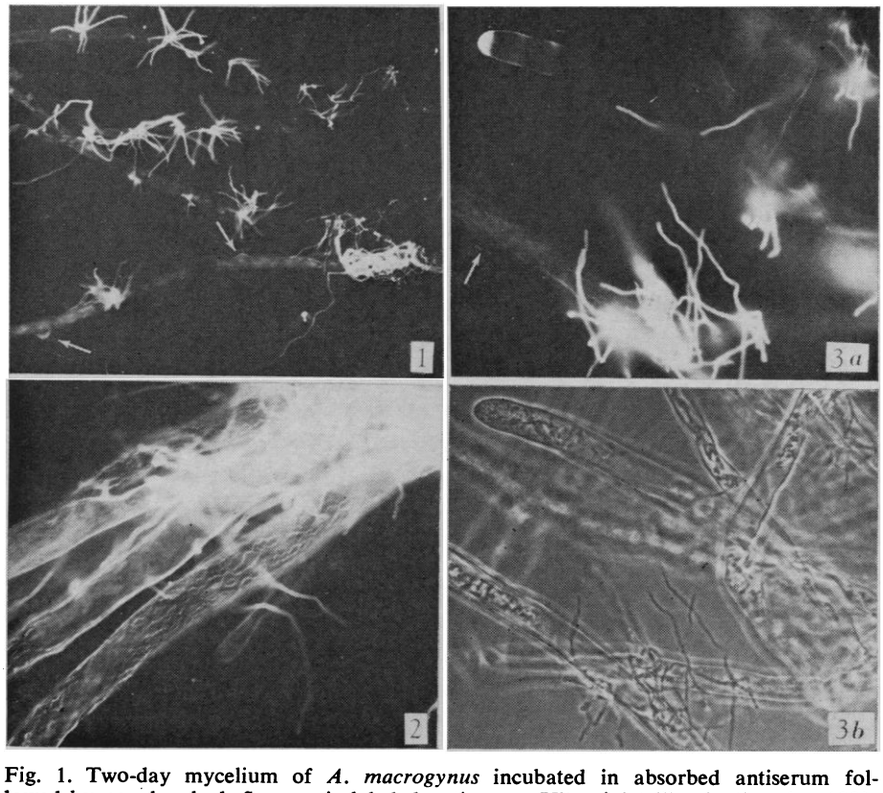

Fig. 1. Two-day mycelium of *A. macrogynus* incubated in absorbed antiserum followed by nonabsorbed, fluorescein-labeled antiserum. Ultraviolet illumination (× 100). Fig. 2. Mycelium harvested at 4 days, washed in distilled water, and stored at 4°C for 24 hours. Treatment as in Fig. 2 (× 250). Fig. 3. Three-day mycelium treated as described for Fig. 1. *a*, Ultraviolet illumination. *b*, Same field, viewed with phase contrast optics (× 250).

The specific labeling patterns described in our results could only be obtained with 3-day-old or younger mycelium. Beginning at 3 to 4 days, areas of hyphae appear which show uptake of labeled serum, either specific or nonspecific. Such areas increase with age. Observations of these preparations provided further evidence for the difference in hyphal and rhizoidal surfaces and information on changes, both in appearance and adsorption properties, which occur in the hyphal surface with age.

The uptake of label is first seen as faint elongated markings parallel to the long axis of the hypha (arrow in Fig. 3*a*). These surface patterns become more obvious in older cultures where it appears as though an outer layer of wall develops longitudinal cracks whose exposed edges contain the component which takes up the label (Fig. 2).

Careful examination of a number of preparations led to this interpretation of the fluorescent surface markings as rents. In some cases the entire outer surface, as well as the exposed edges, appears somewhat fluorescent with only the exposed under layer, the "holes," being dark. These rents in the hyphal surface are more frequently seen on older and larger hyphae and may arise during the increase in the diameter of the filament. Most tip regions remain free of the fluorescent patterns; however, it is not unusual to find lengths of a single hypha with a fissured area intercalated between two nonfluorescent areas, and with no obvious differences in diameter. The torn appearance is marked in mycelia which have been placed in water for the induction of zoosporangia and in mycelia which have been washed and stored at 4°C. It should be emphasized that in all older cultures where the hyphae show an adsorption of nonspecific labeled serum, the rhizoids never fluoresce, again a demonstration of a difference in surface properties between the two structures.

It thus appears that there are changes in surface properties of hyphae with age, apparently due to exposure of underlying constituents, and that at least some of the hyphal components differ from those present on the rhizoidal surface. Possibly these dissimilarities are a reflection of functional differences between the two structures, and they might be expected if the supposition is true that rhizoids effect nutrient uptake for the organism. The observations herein not only raise the question of the nature of the unique hyphal component or components but the more intriguing possibility that different parts of a wall with a continuous substructure can synthesize different superficial compounds.

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References and Notes

1. J. M. Aronson and R. D. Preston, *J. Biophys. Biochem. Cytology* **8**, 247 (1960).
2. J. M. Aronson and L. Machlis, *Am. J. Bot.* **46**, 292 (1959).
3. S. A. Fultz, thesis, University of Michigan, 1965.
4. J. L. Riggs, P. C. Loh, W. C. Eveland, *Proc. Soc. Exp. Biol. Med.* **105**, 655 (1960).
5. Serum obtained from Difco Laboratories, Detroit, Mich.
6. S. A. Fultz and A. S. Sussman, *Bacteriol. Proc.* **1964**, 20 (1964).
7. ———, in preparation.
8. R. M. Cole, *Bact. Rev.* **29**, 326 (1965).
9. Supported by a Horace Rackham postdoctoral fellowship to S.A.F.
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Regeneration in Spinal Neurons: Proteosynthesis Following Nerve Growth Factor Administration

Abstract. *Incorporation of H^3-leucine into dorsal root ganglion cells in rats was markedly increased over that of controls following section of sciatic and femoral nerves. Crush lesion of dorsal roots did not increase the H^3-leucine uptake of these cells except in animals which had received nerve growth factor after the operation.*

Regeneration of nerve cells involves a high rate of nucleoprotein production in the nerve cell related to increased functional demands (*1*), and increased uptake of S^{35}-methionine (*2*) and C^{14}-orotic acid (*3*) has been found during regeneration. Section of motor nerves close to the spinal cord has also resulted in early increase in incorporation of S^{35}-methionine into motor horn cells (*4*), which suggests a close relation between the early intrinsic changes in the neuron and the regenerative process.

Section of the peripheral process of the sensory neuron is followed by active regeneration, but this is not true after interruption of the central process (*5*). A comparable inequality in chromatolytic response of the dorsal root ganglion cells has also been observed (*6*). In our study we sought a basis for this differential regenerative response between the peripheral and central processes of sensory neurons through measurement of protein synthesis.

Marked enhancement of the regenerative response of sensory neurons results from application of nerve growth factor (NGF)(*7*) both in the living chick embryo and in tissue culture, but this result was confined largely to the medio-dorsal cell group of the dorsal root ganglion (*8*). Although this effect of NGF is most pronounced in developing neurons, a regenerative enhancement has been observed in the dorsal funicular axons of kittens 6 to 8 months old (*9*). Augmented growth of sympathetic neurons in the chick embryo has been closely correlated with protein synthesis (*10*). Such initial proliferative growth in the embryo can be contrasted with regenerative growth in the adult by comparing relative rates of protein synthesis. In the second part of this study we examined the effect of NGF on protein uptake by dorsal root ganglion cells 6 days after crush of their central or peripheral processes.

Rats, 1 month old and weighing approximately 100 g each, were used in all experiments, and each experimental procedure and its control employed animals from the same litter. One group of four animals sustained unilateral high section of the sciatic and femoral nerves, the unoperated side serving as control. For comparison, section of dorsal roots L2 to S1 close to the spinal cord was performed on one side in a separate group of four animals.

The second procedure employed unilateral crush lesions (using watchmakers' forceps) of either the central or peripheral process of dorsal root ganglion cells in groups of four animals each. Location and extent of the lesions were similar to those in the first procedure, the normal side serving as control. To examine the effect of NGF, one group submitting to each procedure received this substance for 6 days, while a similar group remained untreated. Each animal received 1 ml per day of a buffered solution of NGF in saline that contained 2.3×10^5 biological units of activity per milliliter. Six days after the operative procedure, H^3-leucine (specific activity, 576 mc/mmole) was injected intraperitoneally at a dosage of 6 mc/100 g of animal weight. Three hours after injection, the dorsal root ganglia of L4 and L5 were removed and fixed in a solution containing 6 percent formol and 0.5 percent trichloroacetic acid. Quantitative evaluation of the autoradiograms was carried out by visual grain counting. In each ganglion the number of grains in 10 large cells at the level of the nucleus was counted and was calculated per unit surface area of the cell (*4*).

Results of these experiments are summarized in Table 1. Each value expresses the average grain count determined in 80 cells. A significant increase (32 ± 5 percent; $P < .001$) in the number of grains per unit area