PROTOPLASMIC DISTRIBUTION AND PLASMODIAL FUSION IN A MYXOMYCETE *PHYSARUM POLYCEPHALUM*

D. M. MILLER, J. D. ANDERSON, and G. W. PETERJOHN
University of Illinois, Urbana, and Baldwin-Wallace College, Berea, Ohio

ABSTRACT—A small plasmodium of the myxomycete, *Physarum polycephalum*, which had incorporated the isotope, P^{32}, was fused with a large migrating plasmodium of the same species. Distribution of the radioactive phosphorus at varying time intervals after fusion of the plasmodia indicated that streaming in this organism is an effective circulatory system, even during migration. The demonstration that fusable plasmodia intermix completely indicates that the lack of ability to distribute material to an area is not the direct cause of the migration phenomenon.

The primary function of rhythmic protoplasmic streaming in the myxomycete, *Physarum polycephalum*, has been assumed to be the movement of the plasmodium over substrate. While the streaming undoubtedly has other functions, a search of the literature reveals only research which has as its primary concern the relation of the protoplasmic streaming (or motive force) to plasmodial movement (Kamiya, 1959). Normally, migration does not occur when *P. polycephalum* is grown upon an adequate medium, such as after the method of Daniel and Rusch (1961). However, as food material becomes exhausted, the plasmodium begins migration. It has also been observed that when the plasmodium grows off of oat flakes upon which it has been cultured, it begins to migrate. Could it be that the failure of materials to be distributed from the food source to the periphera of the plasmodium is a factor in initiation of migration? A second possible function would be that of oxygen transport in the organism. Oxygen needs in laboratory cultures are probably met by simple diffusion, but in natural conditions parts of the plasmodium which are entrenched in decaying wood are undoubtedly subjected to very low partial pressures of oxygen. While the organism is known to tolerate tensions of oxygen as low as 3mm Hg (Kitching, 1940), trace amounts of oxygen are essential for continued survival (Locwy, 1950). All of these considerations suggested the following question: How effective is the shuttle type streaming in the distribution of components throughout the plasmodium of the acellular slime molds?

The plasmodia of *P. polycephalum* are capable of rapid fusion with one another. This peculiar property prompted another question. To what extent is one plasmodium itself an individual from another? When a small plasmodium is fused to a larger one, does it remain together as a unit within the larger plasmodium or is it distributed by the larger one literally as part of itself?

Finally, what is the effectiveness of distribution of a particular component by protoplasmic streaming during movement of the plasmodia? It is easy enough to assume that dis-

tribution of materials may occur while the organism is not migrating, but what happens when the organism is advancing at a rate of 3 to 4 cm/hr, as has been previously reported by Anderson (1963)?

To obtain answers to some of the above questions, a labeled plasmodium (one which has incorporated the isotope P^{32}) and a non-labeled plasmodium were allowed to fuse and the distribution of the isotope in the unlabeled plasmodium was followed as a function of time.

Materials and Methods

The organism, *P. polycephalum*, was grown after the method of Camp (1936) on rolled oats. The isotope P^{32} was incorporated into a growing culture by mixing with oats fed to the myxomycete. Both the labeled and unlabeled plasmodia were subcultured from plasmodia obtained from Dr. D. P. Rogers, of the University of Illinois. Previous work (Anderson, 1963) has shown that unidirectional migration of plasmodia on an agar surface can be obtained by outlining the projected path with Parafilm, a product of the Marathon Co. The larger unlabeled plasmodium was placed at one end of a lucite tray (30 cm long, 30 cm wide, and 5 cm deep) which was filled with 3% agar to a depth of one cm. A two inch perimeter of the agar surface was overlain with Parafilm. Since the plasmodium will not migrate onto the Parafilm, it migrated unidirectionally down the tray. After the large unlabeled plasmodium had migrated unidirectionally a distance of 19 cm, 71.4 mg of a plasmodium, which had incorporated P^{32} to the extent of 50 counts per minute per milligram of wet weight, was put in apposition to a large tube of the migrating plasmodium (Figure 1). Counting was performed with a General Scaler Ratemeter, Nucleonic Corporation of America, Model RCR2, with an end window tube. For all counts the tube was held at a fixed distance of 1.5 cm from the agar surface. Counts were taken at various distances along the left side of the organism on which the tagged explant had been placed. In one instance (at 3 hrs and 50 min), the arm holding the counting tube was extended to perform a series of counts on the right side of the plasmodium.

Figure 1. — Diagram of experimental set up. Explant of a small labeled plasmodium which had incorporated P^{32} was put in apposition to the tube of a large unidirectionally migrating unlabeled plasmodium nineteen centimeters behind the advancing front.

Results and Discussion

Within an hour after fusion of the P^{32} labeled transplant with the larger non-labeled migrating plasmodium, distribution of P^{32} could be detected throughout the organism (reading 2 of Table 1). Distribution of material in an anterior-posterior direction would be expected since this is the axis of migration and the direction of orientation of the large tubes. Detection of the isotope along the edge of the plasmodium opposite to the site of the explant indicates that

TABLE 1.—Distribution of P^{32} in a Migrating Plasmodium as a Function of Time and Distance Posterior to Advancing Front

Reading No.	Elapsed time (hrs:min)	Side of explant reading taken	Distance leading edge had migrated (cm)	Counts per minute corrected for background[a]				
				at leading edge	10 cm back from leading edge	20 cm back from leading edge	30 cm back from leading edge	40 cm back from leading edge
			10.0	(Tagged explant put in place)				
1.....	0:20	Left	22.0	0.0[b]	4.0[b]	.....	.....	.....
2.....	0:50	Left	23.0	5.0	33.0	.....	.....	.....
3.....	1:10	Left	24.0	1.0	15.0	.....	.....	.....
4.....	2:15	Left	27.0	4.0	12.0	42.0	.....	.....
5.....	3:00	Left	29.0	20.0[b]	33.0	141.0	.....	.....
6.....	3:20	Right	30.0	53.0[c]	65.0	157.0	.....	.....
7.....	3:50	Left	31.0	40.0	102.0	87.0	135.0	.....
8.....	10:40	Left	32.0	84.0	86.0	77.0[c]	71.0	77.0

[a] Unless otherwise indicated experimental counts were for two minutes.
[b] Counting period was one minute.
[c] Counting period was three minutes.

there is considerable distribution laterally (reading 6 of Table 1). These findings demonstrate that the shuttle type streaming of *P. polycephalum* might very well serve to transport oxygen or some other nutrient even while the organism is moving over a substrate or in the interstices of decaying wood.

It should be noted that during the experiment the large plasmodium was migrating at the rate of 3 cm/hr. Since the agar surface on which it was migrating was 20 cm wide, this meant that the plasmodium was increasing its surface area at the rate of 60 cm^2/hr. A fixed area was counted. The effect of the migration, therefore, is to dilute the number of counts by increasing the overall area and volume.

The results indicate that the distribution of the isotope to the advancing front is essentially the same as to other areas. This implies that the lack of ability to distribute material to an area (that is, the advancing front) could not be involved in the migration phenomena.

It is assumed in this experiment that some of the isotope in the explant had been incorporated into nuclei and other cellular components. Therefore, the distribution of isotope in the migrating plasmodium may be taken as an index of the distribution of nuclei and cytoplasm from the smaller plasmodium throughout the matrix of the larger. Thus the experiment shows there is a complete intermixing of fusable plasmodia.

ACKNOWLEDGMENTS

Discussions with Drs. John W. Daniel and H. P. Rusch of the McArdle Memorial Laboratories, Madison, Wisconsin, and Dr. W. Ross

Ashby of Departments of Electrical Engineering and Biophysics, University of Illinois, led the authors to formulate this experiment.

Literature Cited

Anderson, John D. 1963. Regional differences in ion concentration in migrating plasmodia. pp. 125-134. *In* Primitive Motile Systems. R. D. Allen and N. Kamiya [eds.]. Academic Press Inc. New York.

Camp, W. G. 1936. A method of cultivating Myxomycete plasmodia. Bull. Torrey Bot. Club 63:206-210.

Daniel, J. W., and H. P. Rusch. 1961. The pure culture of *Physarum polycephalum* on a partially defined soluble medium. J. Gen. Microbiol. 25:47-59.

Kamiya, N. 1952. The motive force responsible for protoplasmic streaming in the Myxomycete plasmodium. Annual Report of Scientific Works from Faculty of Science, Osaka University 1:53-58.

Kitching, J. A., and M. H. Pirenne. 1940. The influence of low tensions of oxygen on the protoplasmic streaming of Myxomycetes. J. Cell. Comp. Physiol. 16:131-133.

Loewy, A. G. 1950. Protoplasmic streaming under anaerobic conditions in a Myxomycete. J. Cell. Comp. Physiol. 35:151-153.

Manuscript received November 9, 1963.