THE MORPHOLOGY, MIGRATION AND PRESSURE DEVELOPMENT OF ORIENTED PLASMODIA OF THE SLIME MOLD

DONALD M. MILLER and JOHN D. ANDERSON
Southern Illinois University and University of Illinois, Urbana

ABSTRACT.—Starved plasmodia of the myxomycete, *Physarum polycephalum*, were unidirectionally oriented on non-nutrient agar and exhibited both a characteristic morphology and migration rate. The morphology described consists of five distinct areas: (1) the advancing front, (2) the flattened sheet area, (3) the tubule area, (4) the major tube area and (5) the residual flattened sheet area. Alteration of migrating plasmodia indicates that a unified advancing front behaves as the anterior end of a single organism and determines the polarity of a plasmodium. Analyses of migration rates of twenty plasmodia demonstrate that starving plasmodia migrate at approximately seven hour intervals which are separated by pauses. As the time of starvation is increased, the migration rate increases and the length of the pause period also increases. Pressure measurements indicate that only limited volumes of endoplasm are in the sol condition at any one time.

The endoplasm of myxomycetes streams to and fro in a regular periodic fashion termed shuttle flow. Associated with the alternating flow of protoplasm is the movement of the organism over a surface. The development of advancing areas and the movement of a plasmodium on a uniform substrate (such as agar) usually takes place in an apparently random fashion. Therefore, early investigations of movement, potentials, and the effects of external factors on myxomycetous plasmodia are made suspect by the failure of the investigator to define adequately the physiological state of the organism and to put the organism in such a condition that one could predict, with some certainty, its future behavior under the same conditions. The introduction of the double chamber technique (Kamiya, 1940) provided considerable impetus to research on slime mold plasmodia, since Kamiya had put the organism into an experimental situation which could be repeated.

The discovery by Anderson (1961) that the organism would not migrate any appreciable distance over parafilm led to his development of a method for orienting plasmodia. This technique is more advantageous than the double chamber method for the following reasons: (1) the organism is in a starved condition, migrating at a measurable rate, which constitutes a semidefined physiological state; (2) this state is readily reproduced; (3) the direction of migration can be controlled; (4) the future behavior of the organism can be predicted; and (5) a much larger organism is obtained; this lends itself to certain experiments.

MATERIALS AND METHODS

The organism, *Physarum polycephalum*, was cultured in the dark on rolled oats using a modification of the method described by Camp (1936). A continuous succession of subcultures were maintained during the three year period of these experiments. The plasmodia were migrated on agar filled trays after the method described by Miller, Anderson and Peterjohn (1964).

MORPHOLOGY

A unidirectionally oriented migrating plasmodium exhibits a characteristic morphology which can be designated as having five distinct areas (Fig. 1): (1) the advancing front area, (2) the secondary front or the flattened sheet area, (3) the tubule area, (4) the major tube or tubes, and (5) the residual flattened sheet area. Each of these areas is subject to change with changes in the ex-

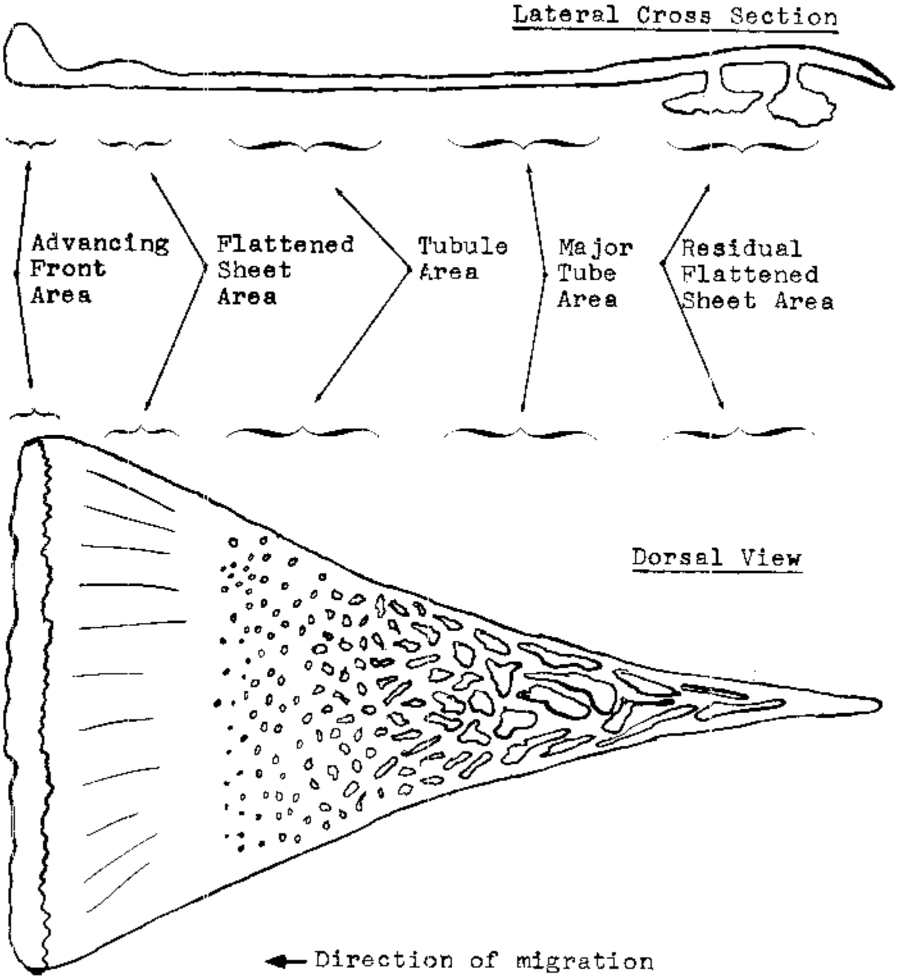

FIGURE 1.—Diagramatic Morphology of an Oriented Plasmodium. Illustrated are the Five Distinctive Morphological Areas Seen in a Unidirectionally Oriented Plasmodium.

ternal environment and time, but their general forms can be seen in the average oriented plasmodium.

Advancing Front Area.—The advancing front area is the part of the plasmodium which determines the direction of movement of the mass of the plasmodium. If viewed from above, this advancing front area is usually lobulated and thicker than the remainder of the frontal area. This lobular area is preceded by the hyaline layer of its foremost portion. If the advancing edge is viewed under a coverslip, the hyaline layer is then clearly seen. In non-oriented migrating plasmodia, this advancing area proceeds in one direction for a period of time, after which it is retracted, and a new advancing area develops in another part of the organism.

Flattened Sheet Area.—The area immediately behind the advancing front (i.e., the secondary front) is much flatter and usually has a shiny appearance. Under the microscope small tubules can be seen internally.

Tubule Area.—This is the part of the plasmodium where the tubes increase in size and become visible macroscopically. Unlike the tubules of the flattened sheet area, these are more permanent structurally and are not so readily changed. It is also in this area that the intertubular protoplasm begins to thin out. Thus, the flattened sheet area is transformed into a reticular area with residual flattened sheet areas in the reticulum. This in turn is transformed into a reticulated network of tubes with no protoplasm in between.

Major Tube Area.—The tubes finally coalesce near the rear into the major tube or tubes. A major tube is usually elevated above the substrate by smaller projecting tubules which lead laterally outward to residual flattened sheet areas. Lewis (1942) also noticed this condition in non-oriented plasmodia. The space between each projecting tubule and the substratum is usually filled with slime. In some plasmodia, the portions of the tubes between anchoring sites may curl into knots. The major tubes eventually trail off into slime tracks in the posterior.

Residual Flattened Sheet Area.—The part of the plasmodium interspersed between the reticular tubules is referred to as the residual flattened sheet area. It represents islands of protoplasm which have not yet completed the transformation to the tubule but are in some intermediate stage.

MIGRATION

Horizontally Migrated Plasmodia.—Plasmodia were allowed to migrate horizontally on agar (2% in tap water), and the distance migrated was recorded as a function of time. A plot of time versus distance for three such experiments is shown in Figure 2. These figures indicate that the migration rate of a starving plasmodium is not constant but increases with time. In addition, pauses in migration were apparent. Analyses of twenty horizontally-oriented plasmodia show that the same pattern occurred in each one (that is, an increase of migration with time). On close inspection of migration rate plots and time lapse films, it was noted that migration was interrupted by pauses at regular intervals, but that the migration rate was approximately uniform during each of the seven to ten hour activity periods between pauses (Fig. 3). The longer a plasmodium migrated the longer became each succeeding pause. Guttes and Guttes (1963) demonstrated that in non-oriented plasmodia motility ceases when nuclear division occurs. Phase contrast microscopical observation of two different plasmodia during pause in migration showed division figures. Although each successive pause

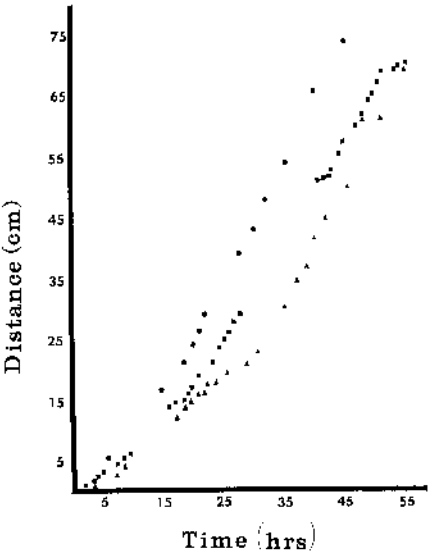

FIGURE 2.—Migration Rates of Three Oriented Plasmodia.

was of longer duration than the previous one, the periods of migration were of approximately the same length. However, the rate of migration increased after each successive pause. Plasmodia with the morphology and activity described above have been kept migrating on non-nutrient agar in the starved condition for over five days.

Relationship of Morphology to Migration. — Because oriented plasmodia display these different morphological regions, experiments were undertaken to remove or alter portions of these regions to determine their role in the motile phenomenon. If the advancing front were destroyed or removed, translocation over the substratum stopped until a new advancing front area developed. Interference with the advancing front area, such as mutilation, usually resulted in a change in polarity (i.e., direction of migration). In contrast to this, the removal of one third of the major tube area of a plasmodium did not affect its direction or rate of movement.

Vertically Migrated Plasmodia. — The shuttle flow of protoplasm does not vary its rate of flow or period even though the migration rate increases (Kamiya, 1959; Anderson, 1949). Kamiya indicated that the two largest factors determining the migration rate of a plasmodium were the net volume of protoplasm transported and the motive force developed. In terms of the measurements obtained from his double chamber, Kamiya reported a pressure difference of fifteen cm of water. Assuming that the endoplasm is a continuous sol from the anterior to the posterior end of a plasmodium, we thought it seemed likely that if a large plasmodium were forced to migrate vertically, then large hydrostatic pressures should develop in the lower (rear) tubes of the plasmodium. It was assumed that the maximum height a plasmodium could then migrate upwards would be approximately fifteen cm. This was predicated on the basis of the motive force measurements of Kamiya. Because we had found that the presence of nutrient material in an area prolonged the maintenance of tubes to that area, we supplied large vertically-oriented plasmodia with a very small amount of food (three oat grains or more) at the initial transplant site. Under these circumstances, the plasmodia migrated vertically in the same manner as horizontally except that there was not so pronounced an increase in the rate of movement and their tubes were maintained over extended distances.

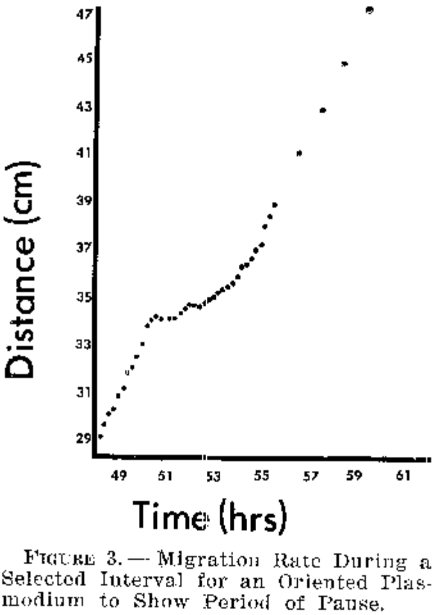

FIGURE 3. — Migration Rate During a Selected Interval for an Oriented Plasmodium to Show Period of Pause.

In one of eight experiments conducted with vertically-oriented plasmodia, a plasmodium migrated 66 cm up on an agar surface and was continuous from the bottom to the top. The migration rate of a vertically-oriented plasmodium followed the same general pattern as one which migrated horizontally. Since these results were not in agreement with what had been predicted on the basis of pressures determined by Kamiya's double chamber experiments, an additional experiment was designed to measure the hydrostatic pressure at the base of a vertically-oriented plasmodium.

Pressure Measurements. — To measure the pressure in the lower tubes, a vertically migrating culture was oriented through a one inch inflatable rubber cuff connected to a water manometer. After a large tube had formed inside, the cuff was slowly inflated and the pressure changes recorded. The maximum static pressure (i.e., pressure which was maintained over several minutes) was five to six cm of water. Changes in pressure of only two to three cm of water were detected. Two supplemental experiments gave similar results except that the values were lower. A similar experiment was conducted in which a plasmodium was horizontally-oriented with a tube within the rubber cuff. After the tube was formed, the entire tray was erected so that the plasmodium was in a vertical position, and the pressure measurements then made. The results were the same as for the originally vertically-oriented plasmodium. In addition, no macroscopic change in the appearance of the plasmodium was detected that would have indicated a large change in hydrostatic pressure.

Many authors have described the exudation of protoplasmic drops after the puncture of a plasmodial tube (Belar, 1930; Camp, 1937; Cohen, 1942; Wohlfarth-Bottermann, 1962). A lower tube (approximately 3 mm in diameter) of a vertically-oriented plasmodium was punctured. The resultant drop which flowed out was approximately four mm in diameter and has the same volume as one from a similarly punctured tube of the same size in a horizontally-oriented plasmodium.

DISCUSSION

These studies show that oriented plasmodia develop and maintain a characteristic morphology. Unpublished microscopical studies on oriented plasmodia by Sheen, Miller and Anderson, (1965) have confirmed this morphology. These plasmodia are in a starved condition, and the energy for the protoplasmic movement, and hence the translocation over the agar surface, must result from some autophagous process. The protoplasm of the organism undergoes a sequential change from an advancing front, to a flattened sheet, and to a posterior tubular morphology at a rate which is related to the rate of locomotion of the plasmodium over a surface. These processes appear to be accelerated during starvation of the plasmodium. Conversely, the presence of food materials results in the maintenance of tubes from the plasmodium to the food, and a retardation of migration rates. These observations suggest that the availability of food inhibits the rate of movement and the sequential change in morphology of the organism which is characteristic of locomotion. The results of altering the morphology of a plasmodium indicate that a unified front behaves as the anterior end of a single organism and determines the polarity of a plasmodium.

At intervals the plasmodia interrupted their migration. These results, in agreement with Guttes and Guttes (1963), indicated that plasmodial migration is suspended during the synchronous nuclear divisions which occur in this organism. In addition, the increase in the length of each pause period suggests that the longer a plasmodium is starved, the more the processes required for nuclear division are prolonged, or that perhaps nuclear divisions are becoming less and less synchronous, thereby extending the pause period until all nuclear divisions are completed.

The use of oriented plasmodia to study the pressure developed in vertical versus horizontal situations indicates that in both cases the measurement of hydrostatic pressure is approximately the same and the changes in pressure measured are also the same. These results are in apparent contradiction to both the conclusions of Kamiya (1959) and the concept of a pressure flow mechanism for plasmodial movement. It has occurred to the authors, as it has to others (for example Stewart, 1964), that perhaps the incongruity lies in the previous tacit assumption that the endoplasmic sol is continuous over long distances at the same instant. These experimental results suggest to us that only very limited volumes of plasmodial endoplasm may be in the sol condition at any one time. If this be the case, then, to some degree, the amount of protoplasm which exudes from a punctured tube could rationally be independent of the height of the tube and dependent upon the limits of solation of the endoplasm in that particular area.

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LITERATURE CITED

ANDERSON, J. D. 1949. Effects of direct current on *Physarum polycephalum*. PhD Thesis. Stanford University.

———, B. T. STEWART, and P. A. STEWART. A biochemical correlate of galvanotaxis in slime mold plasmodium. The Physiologist 4:3.

BELAR, K. 1930. Über reversible Entmischung des lebendigen Protoplasmas. Protoplasma 9: 209-244.

CAMP, W. G. 1936. A method of cultivating myxomycete plasmodia. Bull. Torrey Botanical Club 63: 205-210.

———. 1937. The structure and activities of myxomycete plasmodia. Bull. Torrey Botanical Club 64: 307-335.

COHEN, A. L. 1942. The organization of protoplasm: a possible experimental approach. Growth 6: 259-272.

GUTTES, E., and S. GUTTES. 1963. Arrest of plasmodial motility during mitosis in *Physarum polycephalum*. Experimental Cell Research 30: 242-244.

KAMIYA, N. 1940. The control of protoplasmic streaming. Science 92: 462-463.

———. 1959. Protoplasmic streaming. Protoplasmalogia 8: 1-199.

LEWIS, W. H. 1942. The relation of the viscosity changes of protoplasm to ameboid locomotion and cell division in Structure of Protoplasm (ed. W. Seifriz) Iowa St. Press.

MILLER, D. M., J. D. ANDERSON, and G. W. PETERJOHN. 1964. Protoplasmic distribution and plasmodial fusion in a myxomycete, *Physarum polycephalum*. Trans. Illinois St. Acad. Sci. 57: 94-97.

STEWART, P. A. 1964. The organization of movement in slime mold plasmodia in Primitive Motile Systems in Cell Biology (ed. R. Allen and N. Kamiya) Academic Press N. Y.

WOHLFARTH-BOTTERMANN, K. E. 1962. Weitreichende, fibrilläre Protoplasmadifferenzierungen und ihre Bedeutung für die Protoplasmaströmung. Protoplasma 54: 514-539.

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