A TAXONOMIC AND CULTURAL STUDY
OF THE
WHITE-SPORED POLYSPHONDYLIA

BY

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A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE
(Bacteriology)

at the

UNIVERSITY OF WISCONSIN

1959
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INTRODUCTION

In 1901 Olive described two species of white-spored Polysphondylium, *Polysphondylium pallidum* and *Polysphondylium album*. In his "Monograph of the Acrasieae" (1902) he reported using dung decoction and peptone agar as media and his "pure cultures" contained unidentified bacteria. However, he had no idea of the relationship of the bacterial associate to the slime mold, although he was aware that various kinds of bacteria were usually present and that bacteria-free cultures were difficult to obtain.

Olive apparently had three isolates of *P. pallidum* and one isolate of *P. album* available for study. Since that time hundreds of white-spored Polysphondylium have been isolated by Raper and co-workers and there are nearly 200 isolates in his collection alone. Raper noted that there was much variation among the isolates but came to no decision regarding their separation into the two species described by Olive. Notwithstanding the frequency with which the white-spored Polysphondylium are isolated, these organisms have not been studied carefully since Olive worked with them. Raper (1951) referred to the difficulty of culturing *P. pallidum* and noted that media suitable for the cultivation of the larger Dictyosteliaceae are not suitable for *P. pallidum*. He observed that even under the best conditions of cultivation optimal fruiting often occurred only at the point of inoculation. He included *P. pallidum* in his generalized considerations of the whole group of the
Dictyosteliaceae, and other workers (Blaskovics and Raper, 1957, Bonner and Shaw, 1957, and Shaffer, 1957) mentioned it in studies primarily concerned with other species; but there has been no detailed study of the Polysphondylium pallidum-Polysphondylium album complex as such.

This investigation involved the study of numerous isolates of white-spored Polysphondylium for the purpose of determining the validity of the claim for two species, demonstrating the influence of various environmental factors on the morphology of the sorocarps, and determining optimal conditions for growth and fructification of these slime molds.
LITERATURE REVIEW

While most of the studies on the Acrasieae have been carried out in the last twenty-five years, our knowledge of these organisms dates back to Brefeld's discovery and description of Dictyostelium mucoroides in a culture of Mucor mucedo in 1869. His observations provided a highly accurate picture of the life cycle, differing from that we know today only in that he believed that a transient plasmodium was formed at the time of aggregation. A brief description of their life cycle reveals the singularity of this group of organisms. Myxamoebae, which at one stage are independent of one another and indistinguishable from many soil amoebae, aggregate and build a communal fruiting structure in which some cells are utilized in the formation of a stalk and the rest become spores that will eventually disperse and give rise to a new generation of myxamoebae.

Brefeld realized he was not the first to see this organism. De Bary had observed it; Coemans had previously studied and regarded it as a form of one of the Mucorales; and Cienkowski had described Amoeba limax cysts found on rotting substances which Brefeld thought were probably D. mucoroides. He believed D. mucoroides differed from the known Myxomycetes in the absence of swarm cells and the short duration of the plasmodium. Taxonomically, he placed Dictyostelium next to and on the same level as the Myxomycetes, bridging the gap between the Myxomycetes and true fungi through the Mucoraceae. Van Tieghem (1880) divided the
Myxomycetes into two groups: those with fused plasmodium and those with aggregated plasmodium. The latter, whose cells remained distinct and individual throughout the life cycle, were named the Acrasieae. Here he included *Dictyostelium* since his culture did not exhibit fusion of myxamoebae. Brefeld (1884) retracted his claim of a true plasmodium and named the organization of myxamoebae following aggregation the pseudo-plasmodium.

In 1884 Brefeld described a new and more complex relative of *Dictyostelium*. This organism resembled *Dictyostelium* in all stages of its life cycle except the final fructification. The completed fruiting structure consisted of a main stalk and a terminal spore mass, but bore at regular intervals along the main axis verticillate arrangements of side branches, each terminated by a spore mass. These branches were perpendicular to the main axis or inclined slightly upward. Each side branch in a whorl was structurally a miniature of the main axis and its terminal spore mass and was loosely attached to the exterior of the central axis by secreted slime. The size of the branches and the number of branches per whorl decreased distally.* The distance between whorls was approximately equal; the distance from the most distal whorl to the terminal spore mass was approximately three to four times the interval between whorls; and the distance from the substrate to the most proximal whorl was even longer. The completed fruiting structures were a deep violet color so that they blended in with the dark substrate. Brefeld suggested that this natural camouflage delayed their discovery. The

*The terms distal and proximal are used in relation to distance from the base of the stalk.*
entire structure was weak and collapsed easily when the culture dish lid was removed. He named the organism *Polysphondylium violaceum* because of its many branches and its violet color.

Brefeld also described in great detail the formation of the fruiting structure. The life cycle of *P. violaceum* paralleled that of *Dictyostelium mucoroides* to the point where the structure consisted of a long drawn out mass of amoebae at the upper end of the stalk. Then, beginning at the proximal end of the mass, individual segments of amoebae separated themselves from the mass. These individual segments which constrict off and contract to a spherical form were destined to compose a single whorl of side branches. This constriction and separation resulted from the formation of a new center of organization within the segment. Each spherical mass divided radially into a number of sections, each of which ultimately developed into a side branch bearing a small sorus. Stalk and sorus formation in each of these sections duplicated that of the main axis and terminal spore mass. Development was completed from the bottom up, so that a single developing fruiting structure might have a completed whorl of branches near the base and yet undifferentiated masses of amoebae at the top. The number of whorls and the number of branches per whorl increased and decreased with the size of the main axis.

Olive (1902) in his "Monograph of the Acrasieae" stated that the terminal spore mass of *Polysphondylium* was normally about twice the diameter of those of the lower whorls. He considered this a constant characteristic. He also believed negative hydrotaxis caused the fruiting structure to be directed away from the substrate and at right angles
to it. As further evidence he noted the upward inclination of the branches of *Polysphondylium*, believing this to reflect direction away from the moist substrate as well as the main stalk.

Olive (1901) described two new species isolated from dung: *Polysphondylium pallidum* and *Polysphondylium album*. Both species were strikingly different from *P. violaceum* in that their spore masses and stalks were white. Spore masses of both species were somewhat smaller than the largest spore masses of *P. violaceum*, and the spores were slightly less elongate. He differentiated between the two white-spored species by stating that *P. album* had larger spore masses, 100 to 200 μ in diameter instead of 50 to 80 μ in diameter as in *P. pallidum*. Additionally, the spore masses of *P. album* were usually more numerous in a whorl, making the fructifications more conspicuous, and its stalks were rather constantly weak at the base so that the fructifications lay close to the substratum in a characteristic fashion.

Harper (1926, 1929) introduced the system of terminology which is most commonly used today. The spore mass of the fruiting structure was termed the sorus. The stalk was called the sorophore. He termed the entire spore-bearing structure the sorocarp. He suggested that, for clarity, the amoebae should be called myxamoebae; but for convenience, the term might occasionally be abbreviated. The pseudoplasmodial mass was called the sorogen after it left the substratum and began building the stalk and branches.

In Harper's studies of *Polysphondylium violaceum* (1929) the relative dimensions of parts were measured and described. His observations agreed quite well with Olive. Both workers stated that the terminal
sorus is always larger than any of those on the lateral branches. However, Harper noted variation in size, number of branches, and general proportions comparable to that in higher fungi, algae, and in seed plants and indicated that environmental factors had a very definite effect on the structure of the sorocarps. Harper noted that sometimes Polysphondylium is unbranched and occasionally Dictyostelium is branched. He believed that the branch of Dictyostelium did not arise from a segment of the apical sorogen but from a pseudoplasmodium that crept up the stalk after the sorocarp was completed and built its own sorocarp using the side of the stalk as a support.

Harper set up a series of experiments to determine the effect of light on the growth and morphogenesis of Polysphondylium violaceum (1932). He found that alternation of day and night or continuous darkness made no difference in the amount of cell growth and division. However, alternation of day and night resulted in more and smaller plants and more unbranched plants, while "continuous darkness" resulted in larger and fewer plants with more branching. The condition of continuous darkness was not one of total darkness. The bell jar in which the cultures were kept was covered with a black cloth that did not totally exclude light, and the cloth was removed for a few minutes daily to allow the examination of the cultures. Harper stated that he was trying to duplicate conditions that might possibly occur in nature, such as development of plants in shaded locations.

Nadson (1889) considered the possibility that the accompanying bacteria changed the culture medium. He cultivated D. mucoroides in association with Bacillus fluorescens liquefaciens and concluded that the
bacteria favored the growth of *Dictyostelium* by creating an alkaline medium through the production of ammonia. Potts (1902) cultivated *D. mucoroides* in association with bacteria on a synthetic medium containing both a nitrogen and a carbon source. He realized that *Dictyostelium* was dependent on bacteria for nutriment. Like Nadson, he recognized the importance of acidity and alkalinity and found that *D. mucoroides* could grow in slightly acid to strongly alkaline media but grew best in media of a mild alkaline reaction. Pinoy (1907) noted that agar containing bouillon or peptone would not support the growth of *D. mucoroides* even though abundant bacteria were present unless lactose, maltose, or glucose was added. By the elimination of non-essential components from Endo's medium which supported normal growth and development of *D. discoideum* Raper, Raper (1939) devised a medium containing only lactose and peptone which likewise supported normal growth and development of this species. He concluded that *D. discoideum* required a medium that the bacterial associate could utilize and that contained both utilizable nitrogen and carbon sources which were broken down simultaneously to yield alkaline and acid products. He found pH 5 to 7 was most favorable for growth and development of *D. discoideum*, with pH 6 optimal. He noted that *D. discoideum* grew and developed normally in some cultures contaminated by fungi while it failed to grow in similar uncontaminated cultures. He showed that the favorable influence of the fungus could be attributed to the creation of an acid medium. He also demonstrated that if culture plates with *Escherichia coli* were incubated at 35 C for three days prior to inoculation with *Dictyostelium*, the slime mold grew poorly and its development was abnormal. He attributed this to the
strongly acid reaction of the medium. He concluded that the manner of attaining and maintaining a favorable hydrogen ion concentration was unimportant. The balanced utilization of carbon and nitrogen sources by the bacterial associate, the addition of free acid to cultures of alkaline reaction, the presence of a fungal associate, or the incorporation of buffers in the medium, all favored the growth and development of *D. discoideum*. He also found that the physical characteristics of the bacterial associate affected the growth and development of the slime mold. Extremely gummy cultures made it difficult for the myxamoebae to engulf the bacteria, while spores of Gram positive bacilli were ingested but not digested (Raper, 1937).

An understanding of the influence of environmental factors on the growth and development of slime molds was increased by the work of Whittingham and Raper (1957) on *Dictyostelium polycephalum* Raper. When *D. polycephalum* was first cultured in the laboratory, it required the presence of both *Aerobacter aerogenes* and a yeast-like fungus, *Dematium nigrum*, in order to fruit optimally (Raper, 1956). Whittingham and Raper found that the primary beneficial function of *Dematium* was the reduction of humidity in the culture vessel, and a second function could have been the removal or neutralization of acidic or basic end-products resulting from the metabolism of the bacterial associate. In the course of their investigation they demonstrated that fruiting of *D. polycephalum* could be inhibited by volatile acidic metabolics products of *E. coli* and that this inhibition could be attributed to the production of an adverse pH. Optimum pH for fruiting was 6.5.

Potts (1902) first demonstrated the importance of humidity in
the development of *D. mucoroides*. Raper (1940b) noted that cultures of
*D. discoideum* fruited 2 to 4 hours earlier if the lids of the culture
dishes were raised slightly and attributed this to a decrease in rela-
tive humidity within the culture dish. Bonner and Shaw (1957) were able
to prolong the migration period in *D. mucoroides*, *P. violaceum* and *P.
pallidum* in the presence of high relative humidity and low solute agar.
In *P. pallidum*, stalks four centimeters in length were produced with
small whorls or individual branches occurring over their entire length.
The authors pointed out the effectiveness of spore dispersal under such
circumstances. Raper (1940b) reported that an increase in temperature
would stimulate sorocarp formation. Bonner and Shaw's demonstration
that prolonged normal migration of *D. discoideum* and *D. mucoroides* could
be obtained at high temperatures as long as a high constant humidity was
maintained suggests that increased temperature stimulates sorocarp for-
mation by temporarily lowering the relative humidity. Whittingham and
Raper's photographs of the degree of differentiation achieved by *D.
polycephalum* under specified percentages of relative humidity indicate
that ninety-eight per cent relative humidity is probably optimal for
that species and illustrate the importance of relative humidity in ob-
taining optimum fruiting. Both Whittingham and Raper and Bonner and
Shaw suggest that decreased relative humidity is an important factor in
stimulating sorocarp formation.

Raper (1939) noted that the accumulation of ammonia from the
breakdown of peptone resulted in an increased alkalinity of the medium
and inhibition of the growth of *Dictyostelium*. Other nitrogen sources
such as ammonium chloride, asparagine, ammonium phosphate, and ammonium
nitrate had a similar effect. *D. discoideum* grew poorly or not at all in cultures more alkaline than pH 8.0 and produced normal sorocarps in cultures from pH 4.8 to 7.0. Cohen (1953) sought to establish the role of free ammonia as the morphogenetic suppressor. He stated that ammonia could inhibit growth entirely, allow the development of vegetative amoebae only, or if more dilute allow the formation of simple fruiting structures resembling *Guttulina* or *Guttulinopsis*, some of the simplest genera of slime molds. Normal development occurred when ammonia was present in traces or undetectable. As confirmation of the role of free ammonia rather than the ammonium ion, he cultured and obtained normal fruits of *P. pallidum* and *Dictyostelium purpureum* Olive on *E. coli* plates containing ammonium chloride at M/33 concentration over M/20 sulfuric acid to absorb any free ammonia present. He stated that this represented a concentration of ammonium ion many times greater than that of free ammonia necessary to produce inhibition of morphogenesis.

Previous discussion has centered around environmental factors which may inhibit fruiting in the slime molds. A more pointed question is what causes the initiation of fruiting in the slime molds. Potts (1902), Oehler (1922), and Schuckmann (1924) believed fruiting was a result of the exhaustion of the food supply. Raper (1940b) noted that pseudoplasmodia normally do not begin development until the available bacteria have been consumed and that pseudoplasmodium formation can be considerably delayed by the addition of fresh bacteria to the vegetative culture (Raper, 1940a). Increased temperature, decreased humidity, and light are other factors known to hasten pseudoplasmodium formation (Raper, 1940b). None of them, however, explains the aggregation of
amoebae at particular points. Schuckman (1925) and Harper (1926) suggested that it represented a negative hygrotropic response. The failure of D. discoideum to fruit at its point of aggregation and the separation of interspecific mixtures of amoebae into two separate fruiting structures (Raper and Thom, 1941) indicate that negative hygrotropism is not the answer. Olive (1902) suggested a chemotactic influence and noted that it apparently differed for white and dark spored Dictyostelia. Potts (1902) also suggested a chemotactic influence. Arndt (1937) considered exhaustion of the food supply as a preliminary condition indirectly stimulating fruiting. He postulated an attracting substance arising from the amoebae themselves and noted that variation in the strength of the stimulus gave rise to waves in the aggregating mass. Raper and Thom (1941) demonstrated the specificity of the attracting substance when they showed the sorting out of interspecific mixtures of myxamoebae at the aggregation stage. Runyon (1942) proved that the attracting substance was diffusible in water and could exert its effect through a dialyzing membrane. Bonner (1947) verified the fact that the attracting substance was diffusible in water by its ability to attract amoebae around corners or with no connecting interface when the amoebae were cultivated under water. He also observed that amoebae downstream from a previously established center oriented to the center while those upstream from it did not. He suggested that the substance was produced continuously or intermittently at the center of an aggregation and formed a chemical gradient outward from the center. He suggested the term acrasin for this substance. Further study by Bonner (1949) revealed acrasin production by the migrating pseudoplasmodium of D. dis-
coideum as well.

Shaffer (1953) was able to induce aggregation by rapid and continuous transfer of washings from previously established centers and concluded that acrasin was very unstable at room temperature. He noted that acrasin was genus specific in young centers and streams. However, older Polysphondylium centers would attract both Polysphondylium and Dictyostelium amoebae, while older Dictyostelium centers attracted only Dictyostelium amoebae. He suggested that Polysphondylium had the power to produce Dictyostelium acrasin at a later stage and that this may have some as yet unknown function other than in the attraction of amoebae. He pointed out that this ability to produce both acrasins suggested that Polysphondylium evolved from Dictyostelium and that this is consistent with morphological evidence. Further work by Shaffer (1956a, 1956b) revealed that an acrasin solution passed through a dialysis membrane was stable at room temperature and he concluded that the original instability was due to reaction with larger molecules, such as an extracellular enzyme. He devised a method for extracting acrasin chemically with cold methanol and vacuum drying at -10 C. The acrasin acts to orient sensitive amoebae and also causes them to secrete acrasin. This extends the area over which acrasin can act by forming a chemical relay system. The destruction of acrasin by an enzyme helps in the maintenance of a chemical gradient and reduces the background against which a gradient must be detected. Sussman, Kerr and Lee (1956) also found a method for isolating and stabilizing acrasin by extracting with cold dilute hydrochloric acid at pH 3.5. They came to the same conclusions as Shaffer regarding the inactivation of acrasin. They were able to separate acrasin into
two fractions, A and B, neither active separately. They found that the enzyme destroyed the B fraction, apparently by converting it into the A fraction. Later investigations by Sussman and workers (Sussman, 1958) revealed a third component in the acrassin complex, and additional purification yielded an A fraction which was active alone. Mention was made that fractionation of Polysphondylium acrassin revealed the presence of three functionally identical components. Again we see that investigators must be cautious about drawing general conclusions on the basis of study of a single species or strain. Shaffer (1957) states that acrassin not only orients an amoeba but induces it to become sticky and secrete acrassin. The intercellular adhesiveness thus achieved appears to be species-specific and plays an important role in aggregation along with the secretion of acrassin.

Gregg (1956) assumed on the basis of cross-agglutination tests that certain surface antigens of 24 to 26 hour D. discoideum, D. purpureum, and P. violaceum amoebae are species-specific and persist through later stages of development. He also assumed on the same basis that non-species-specific surface antigens arise at a later stage (30 to 48 hour cultures). He suggested that these surface antigens might have been a factor in the results obtained by Raper and Thom (1941) in their experiments with interspecific mixtures. Bonner and Adams (1958) also demonstrated this differing amount of cell compatibility or adhesion in their experiments with interspecific mixtures. Later work by Gregg and Trygstad (1958) suggested that the failure of certain aggregateless variants to complete development was associated with the alteration or elimination of certain surface antigens in the amoebae.
Bonner (1947) stated that agglutination, unlike aggregation, is irreversible and that cells come together by chance collision. However, aggregation is not normally achieved by agglutination. While antigen-antibody reactions may be factors in development at a later stage, some other factor must initiate the normal aggregation of the *Dictyostellium* amoebae. Ennis and Sussman (1958) state that the loci of the aggregations are determined by the presence of initiator cells which, as their name implies, initiate aggregation. Furthermore, these initiator cells are present in a constant proportion in a given species of slime mold and are morphologically distinguishable. Their mechanism of action is unknown. Their uniqueness possibly lies in their ability to secrete acrasin without the stimulus of previously secreted acrasin.

A number of factors are thus shown to be influential in the initiation of the fruiting process of the Acrasiæae: first a set of favorable environmental conditions—exhaustion of food supply, adequate number and density of amoebae, a decrease in humidity, and a favorable pH; then certain physiological conditions of the amoebae themselves—possible presence of initiator cells, ability to secrete acrasin and maintain a chemical gradient by the enzymatic destruction of acrasin, stickiness of the amoebae, and specificity of the surface stickiness and/or the acrasin produced. The once believed simplicity of a large number of cells coming together to form a communal fruiting structure reveals itself as a quite complex process indeed.
MATERIALS AND METHODS

All cultures of white-spored *Polysphondylium*, *Polysphondylium violaceum*, *Dictyostelium discoideum*, and bacterial cultures used in this study were obtained from the culture collection of Dr. K. B. Raper at the University of Wisconsin. Raper's isolate WS 320 was arbitrarily selected as reference culture for the *Polysphondylium pallidum*-P. album group of slime molds because of its erect, regularly branched fruiting structures. It was collected in the Horticultural Society Gardens of the Madras University Botanical Experiment Station in September, 1955. Strain number 281 of *Escherichia coli* was regularly used as a bacterial associate unless another is designated.

Stock cultures were maintained in Petri dishes on modified hay* or dilute hay infusion agar (Raper, 1937) by inoculating spores onto the center of a cross-streak of bacterial suspension previously placed upon the medium. Stocks were transferred monthly by touching a sterile needle to the deliquescent sori of several sorocarps and subsequently touching the needle to the fresh medium, thus transferring quantities of spores to the new culture. Where the amount of inoculum was not critical, entire sorocarps were transferred with the needle. In order to facilitate the preparation of spore suspensions, stock cultures for use

*Modification of original hay infusion agar formula currently used: 60 g of partially decomposed hay infused in a liter of tap water for one-half hour in an autoclave at 15 pounds pressure; infusion filtered through cheesecloth folded around a layer of cotton; filtrate made up to one liter; 0.2 per cent K₂HPO₄ and 2 per cent agar added; adjusted to pH 6.0 to 6.5 and sterilized at 15 pounds pressure for 20 minutes.
in the serological experiments were maintained on agar slants of a medium containing 0.1 per cent lactose, 0.1 per cent peptone and 1.5 per cent agar designated 0.1 L-P agar. Thus heavy spore suspensions could be prepared simply by adding sterile distilled water and vigorously shaking the tubes. Since the presence of bacteria in these suspensions was desirable, and bacteria were being added as well, those which had not been consumed by the slime mold were not detrimental to the experiments. Neither did the presence of sorophores and stalk cells in the suspension have any harmful effect. Transfer of these stocks was accomplished in the same manner as described for those in Petri plates. It was necessary to transfer them more frequently, however, since the cotton-plugged tubes dried out more rapidly than the Petri plates. Cultures were maintained at room temperature (24 to 26 C) or in an incubator at 25 C.

Many kinds of media used commonly or uncommonly in the laboratory were tested to select a medium most suitable for the maintenance of stock cultures. Many of them supported abundant vegetative growth but no fruiting. Others allowed abundant fruiting which, however, was considered atypical. If the medium were rich in nutrients the heavy bacterial growth seemed to impair the development of the slime mold. If the medium were very low in nutrient, the fruiting might be typical but very sparse. No medium was found to be superior to hay infusion agar or dilute hay infusion agar for stock maintenance. Cross-streaked plates usually yielded better fruiting than plates spread with a uniform suspension of slime mold spores and E. coli.

Measurements of pH were taken with a Beckman model G portable pH
meter. For the experiments with bacterial associates, the agar from a single plate was finely chopped in distilled water. For more critical measurements in later experiments, a 0.5 centimeter square block of agar was removed aseptically from the Petri dish with a sterile blade, the agar was finely chopped, 0.5 milliliters of distilled water was added, and the measurement was taken in an adapter-beaker for small samples.

Many different strains of white-spored Polysphondylium from widely scattered natural sources were included in this comparative study, some of these believed to be representative of possible sub-groups being investigated more intensively than others. Hereafter, these are referred to by the numbers they bear in Raper's culture collection. Detailed discussion of techniques used in the individual experiments will be considered in connection with experimental results.
EXPERIMENTAL RESULTS

Characterization of cultures

The white-spored members of the genus *Polysphondylium* seem to vary quite markedly as these are isolated from soil and other natural sources, and over the past two decades Raper and his associates have accumulated more than two hundred slime molds of this type. Time did not permit a detailed comparative study of all of these, and from them seven isolates which appeared morphologically different under similar cultural conditions were selected for intensive investigation. Following a period of growth and subsequent fructification on hay infusion agar plates cross-streaked with *Escherichia coli*, these strains could be characterized as follows:

Ill 7

Sorocarps confined to bacterial streaks, commonly up to 10 mm in length, somewhat tangled; numerous sorocarps arising from a single pseudoplasmodium; stalks relatively thick, fairly erect to the point of the first whorl of side branches, with approximately the basal one-third of the stalk devoid of branches and sori; beyond this point, stalks weak and tangled; spacing of whorls somewhat irregular, often quite close together; stems of lateral branches very long, sori mostly of medium to small diameter, with rarely more than eight sori per whorl; terminal sorus similar to lateral ones in size and not far removed from the most distal whorl; spores elliptical, 4.7 to 8.2 μ by 2.4 to 4.7 μ.
Sorocarps largely confined to bacterial streaks and fruiting often patchy and discontinuous; stalks up to 15 mm in length, relatively weak and trailing the substrate for some distance, then rising and bearing three or four whorls of side branches with large, milky white sori, often attaining a diameter of 110 μ, on relatively long branches that are frequently rebranched; side branches are commonly 300 to 400 μ long; rarely more than five sori per whorl; terminal segment of stalk very long, often equal to the remainder of the sorophore and devoid of whorls, often quite tangled and bearing a very small terminal sorus that is commonly only one third the diameter of the lateral sori (figure 1); spores 4.7 to 10.6 μ by 3.5 to 4.7 μ.

WS 222-1

Sorocarps extending out from bacterial streak; stalks long, weak, trailing, frequently interrupted; fructifications so tangled it is difficult to follow an individual sorophore; stalks rise and alternately touch the underlying substrate; whorls irregularly spaced, rather far apart, often atypical; seldom more than five sori per whorl; lateral branches medium to long bearing sori of medium to small dimensions; terminal sorus difficult to identify because stalk is so weak and growth is so tangled; spores 4.7 to 7.1 μ by 2.4 to 3.5 μ.

WS 225-2

Fructifications confined to bacterial streak; appearance of culture somewhat like Ill 7, but sorocarps more delicate; numerous sorocarps arising from a single pseudoplasmodium; stalks relatively thick,
often irregular near the base, fairly erect; whorls irregularly spaced, approximately five sori per whorl; side branches of medium length; sori small and terminal sorus not far from the most distal whorl; spores 5.9 to 7.1 μ by 3.0 to 5.9 μ.

WS 320

Sorocarps confined to bacterial streak; individual structures distinct, erect, fir-like, usually arise from individual pseudoplasmodia; lower one-third to one-half of sorophore frequently devoid of sori, sometimes trailing on substrate; whorls abundant, regularly spaced; side branches usually 200 to 300 μ long; up to ten sori per whorl; sori commonly 50 to 80 μ in diameter; terminal sorus typically within this size range and not far from the last branch (figure 1); spores 5.9 to 9.4 μ by 2.4 to 4.7 μ.

WS 406

Fructifications confined to bacterial streak; sorocarps arise from individual pseudoplasmodia; stalk (basal portion) frequently trails substrate; side branches with sori borne shortly after sorophore becomes aerial; few typical whorls with lateral branches short or absent; sori opaque, commonly 30 to 50 μ in diameter, closely spaced and often borne directly against the stalk; large lateral sori frequently formed by the fusion of completed neighboring sori in the whorl; terminal sorus typically larger than lateral sori, ranging from 100 to 125 μ in diameter, milky white (figure 1); spores 3.8 to 6.3 μ by 2.5 to 3.8 μ.
Figure 1. Sorocarps of three strains of white-spored Polysphondyliia cultured on dilute hay infusion agar, x16. a. WS 406. Note the very short lateral branches and the large terminal sorî of three of the sorocarps visible in the picture. b. Fr 47. Note the tiny terminal sorus (arrow) borne on an extremely long internode and the very long stemmed side branches. c. WS 320. The side branches which appear to bear no sorî are in contact with the agar surface and the spores have dispersed onto the agar.
Fructifications mostly confined to bacterial streak; stalks very weak, trailing, becoming aerial then frequently retouching substrate; sorophores quite tangled, bearing few typical whorls; lateral sori more abundant than on WS 222-1, ranging in size from small to large (large milky white ones common), irregularly spaced; side branches varying in length; terminal sorus usually medium to large, and not far from last side branch; spores 5.0 to 7.5 μ by 3.2 to 5.0 μ.

The degree to which sorocarps are confined to the immediate area of the bacterial streak depends on the relative strength of the stalks and the extent of migratory activity of the sorogen when this retouches the substrate -- whether it immediately rises again, or whether the mass of cells migrates for some distance along the surface. A break in stalk formation at this point may enhance the migratory activity of the slug thus formed.

Ill 7 and WS 225-2 are similar in appearance, both presenting a deep, tangled aspect. WS 225-2 possibly has fewer sori per whorl, shorter side branches, and smaller sori. WS 222-1 and WS 425-2 are somewhat similar in appearance; however, WS 222-1 is more delicate, and the colony tends to spread farther from the original bacterial streak. Fr 47 is quite distinctive: it has few whorls, large sori, and a long expanse of thin stalk between the last side branch and the very small terminal sorus. All other isolates examined varied morphologically only slightly from one or the other of the seven strains described above.
Bacterial associates

Each of the seven isolates was cultivated in combination with a number of eubacteria in order to determine the influence of the bacterial associate upon growth and development, as had been done by Raper (1937) for D. discoideum. Hay agar plates were cross-streaked with suspensions of the following bacteria: *Escherichia coli* 281, *Serratia marcescens* 175, *Pseudomonas fluorescens* 112, *Aerobacter aerogenes* 900, *Sarcina lutea* 1018, *Bacillus megaterium* 160, and *Bacillus subtilis* WBS. This group includes both Gram negative and Gram positive bacteria, non-sporeformers and sporeformers. Each of the previously described slime molds was inoculated onto duplicate plates with the seven bacterial species, incubated at room temperature (24 to 26 C) and examined over a period of twelve days.

Growth with the Gram positive organisms, *B. megaterium*, *B. subtilis*, and *S. lutea* produced typical but rather few fructifications, and clearing of the bacterial streaks was neither as extensive nor as complete as with the Gram negative species. There was no pigmentation of any of the mature fruiting structures with *S. marcescens* as occurs in *Dictyostelium discoideum* and certain other species (Raper and Thom, 1941); however, some pigmentation was evident in the aggregations of Fr 47. Fr 47 also appeared to consume the bacteria and to fruit more slowly with all associates. The pH of the hay medium before inoculation with either the slime molds or the bacterial associates was 7.15. Following the twelve day period of slime mold growth the reaction was somewhat more alkaline, pH 7.2 to 7.9.
By substituting 1.5 per cent agar with 0.1 per cent glucose and 0.1 per cent yeast extract in the above experiment, a much more luxuriant bacterial growth was obtained. However, consumption of the bacteria was less complete and fruiting of the slime mold was less satisfactory than on hay agar. The initial pH of the medium was 6.95, and after slime mold growth had occurred, the pH ranged from 7.1 to 8.3. The bacterial associates grew less abundantly when 0.1 per cent peptone was substituted for yeast extract. *S. marcescens* grew poorly and with no pigmentation, and subsequent growth of the slime molds with this associate was poor. As on glucose-yeast extract agar, clearing of the plates and fruiting of the slime mold was frequently not as good as on hay agar. Fruiting occurred with all associates, except for Fr 47 which failed to fruit with *S. marcescens*. The initial pH of the medium was 6.95. After the growth period, the pH of cultures with *S. marcescens* ranged from 4.5 to 4.8 and with *S. lutea* from 4.9 to 5.5. With the other bacterial associates the medium became consistently alkaline, ranging from pH 7.1 to 8.2. It is possible that the pH of the cultures of *S. marcescens* and *S. lutea* had been somewhat higher at an earlier period when fructification occurred.

**Effect of temperature on growth and development**

It was noted in preliminary experiments that clearing of the bacterial associate and subsequent fruiting of the slime mold occurred more slowly at lower temperatures, and that Fr 47 seemed to have a slower growth rate than the other isolates. Hay agar and 0.1 per cent glucose-0.1 per cent peptone agar (0.1 G-P) plates were cross-streaked
with *E. coli* and inoculated with spores from a single sorus of each of the selected isolates. The plates were then incubated at 10, 15, 20, 25, and 30 °C respectively. Results of the experiment appear in figures 2, 3, 4, and 5, which are based on averages of the diameters of areas cleared of bacterial growth in duplicate plates. Eight and five-tenths centimeters is considered to be complete clearance. Comparative rates of growth and reproduction of the amoebae of WS 320 and Fr 47 at varying temperatures is demonstrated. WS 320 is typical of the remaining five isolates. No attempt was made to determine quantity, quality, or rapidity of fructification.

The graphs indicate that over this temperature range an increase in temperature is generally accompanied by an increase in rate of growth. This is particularly true within the range from 10 to 25 °C. The difference in growth rate between 25 and 30 °C is less clear cut. There is little or no growth of Fr 47 at 30 °C, indicating that this temperature is very near the maximum temperature for this isolate. Also, Fr.47 grows more slowly at all temperatures except 10 °C on hay infusion agar.

At 20 to 30 °C, the rate of growth is fairly constant after the initial two day interval. This apparent slower growth in the period immediately following inoculation is a result of the necessary population buildup, whereas the actual rate of growth and cell division is probably the same from the time of spore germination to the point when the amoebae have cleared most of the bacteria and have ceased feeding. At 10 to 15 °C this lag may extend to the fifth and occasionally the seventh day. Delayed or incomplete germination at these lower temperatures may be a factor also. After the initial lag period, growth is limited only by the
Figure 2. Rate of growth of Fr 47 inoculated onto hay infusion agar cross-streaked with *Escherichia coli*.

Figure 3. Rate of growth of Fr 47 inoculated onto 0.1 G-P agar cross-streaked with *Escherichia coli*. 
Figure 2

FR 47 on Hay Infusion Agar

Diameter of Area Cleared in CM

= 30°
= 25°
= 20°
= 15°
= 10°

Time in Days

Figure 3

FR 47 on 0.1 GP Agar

Diameter of Area Cleared in CM

= 30°
= 25°
= 20°
= 15°
= 10°

Time in Days
Figure 4. Rate of growth of WS 320 inoculated onto hay infusion agar cross-streaked with *Escherichia coli*. Note that the bacterial streaks are completely cleared in five days at 25 and 30°C.

Figure 5. Rate of growth of WS 320 inoculated onto 0.1 G-P agar cross-streaked with *Escherichia coli*. Note that complete clearance of the bacterial streak is not accomplished at any temperature within the fourteen day observation period.
availability of bacteria for food. Since the food supply is constant as the myxamoebae advance through the pre-grown streak, the rate of clearing of the bacterial colony is also relatively constant. As the clearing of the plate reaches completion in some cases -- particularly at the higher temperatures -- the graphs show an apparent decrease in the growth rate. This is an artifact, however. The plates were examined at two or three day intervals so that the exact time within that interval when complete clearance was reached was not determined.

The seven isolates were inoculated on hay agar cross-streaked with *Escherichia coli* and incubated at various temperatures in order to determine the range of temperatures at which each will grow. Plates were examined periodically for multiplication of amoebae as exhibited by the clearing of the bacterial streak and for fruiting. None of the seven isolates grew at 5 C or at 37 C. All of them grew from 10 C to 30 C. WS 225-2, WS 406, and WS 425-2 exhibited growth and fruiting at 34 C, while Fr 47 showed only very slight vegetative growth at 30 C. A strain designated "Salvador," that had been isolated originally by Miss Dorothy Fennell at the Northern Regional Research Laboratory (Peoria, Illinois) and was reported to tolerate higher temperatures, was investigated. Her observation was verified by growing it on hay agar cross-streaked with *E. coli* at 37 C where growth and fruiting was adequate and normal. There was no growth at 44 C. Subsequent to determining the temperature range within which each of the selected strains would grow and fruit, 91 additional cultures of white-spored Polysphondylia obtained from Raper's Collection were tested for growth at 30, 34, and 37 C to ascertain whether any of them possessed the
Salvador strain's capability to grow at high temperatures. None was found; but four isolates, namely Africa 6, Peoria 12A, WS 426 and 3-1, grew and fruited normally at 34 C. These cultures included isolates collected in the Eastern United States, the Midwest, Africa, and Panama.

Response to light

In several cultures the sorocarps appeared to be oriented toward a source of light. An experiment was set up to determine which of the isolates responded to light and to obtain, if possible, some measure of their response. A single streak of *E. coli* was spread across the center of each hay agar plate. Spores of each of the test slime molds were inoculated in the very center of the streak, and the plates were incubated at room temperature in a box with a slit on one side to admit the light. A fluorescent lamp served as a light source at night. The plates were oriented so the bacterial streaks were perpendicular to the light source. Eleven days later the plates were removed and examined. This experiment was carried out twice. Response was evidenced by the leaning of the sorocarps toward the light source. Ill 7 and Fr 47 showed no response. WS 222-1 was inconsistent -- one time no response, the next time a strong response. WS 406 exhibited a slight response, while WS 225-2 responded moderately. WS 320, WS 425-2, and Campbell (an eighth isolate added in this experiment) exhibited a strong response with the sorocarps often constructed nearly parallel with the substrate. The next logical question was whether light is required for growth and fructification. To determine this, inoculated
plates similar to those in the above experiment were placed in a dark box -- a closed metal canister which was placed in a cabinet in the laboratory -- and remained there, away from all light, for a week. Examination at this time revealed that absence of light had little or no effect on the vegetative stage, growth having proceeded at the normal rate. However, light did seem to influence somewhat the quantity of fruiting. Sorocarps were less abundant, and in some isolates very few in the absence of light, but were not noticeably larger when developed in darkness. These results, therefore, are not in complete agreement with Harper's findings that continuous darkness yields larger and fewer plants with more branching. However, since there is a difference in the definition of continuous darkness in the two experiments, as noted in the Literature Review, direct comparison of results is difficult.

**Effect of humidity**

Whittingham and Raper (1957) suggested that a decrease in relative humidity stimulates sorocarp formation in *Dictyostelium polycephalum*. Raper. It was noted that cotton plugged tubes of WS 320 on 0.1 L-P agar slants frequently fruitied much more abundantly than corresponding cultures in glass topped Petri dishes. If this were an effect of decreasing humidity, plate cultures covered with glazed clay tops should fruit better than those covered with glass lids (figure 6). Four 0.1 L-P plates were cross-streaked with *E. coli* and inoculated with WS 320 spores. Two plates were covered with glass tops and two with glazed clay tops and all four were incubated at 25 C. At 48 hours after inoculation, there was abundant fruiting in the center of the
Figure 6. Comparison of sorocarps when cultured in dishes covered with glass lids and with glazed clay lids. Plates containing modified Sussman-Bradley medium were spread with a suspension of *Escherichia coli* cells and WS 320 spores and incubated at 25 C. a. Area of fruiting in dish covered with glass lid, x6.3. Dense fruiting occurs, but the stalks of the sorocarps are weak, tangled, and the side branching is quite irregular. b. Area of fruiting in dish covered with glazed clay lid, x6.3. Note the very dense fruiting and the increased sorus formation. Although it is not distinguishable in the photograph, the sorocarps were much more erect.
clay-topped dishes. The glass-topped cultures had only one or two sorocarps at the point of inoculation. At the end of one week both types of cultures had cleared an equal amount of the bacterial streak, indicating that there was no differential effect on the vegetative stage. The glass-topped cultures had numerous sorocarps with weak stalks in contact with the substrate for great distances before becoming upright and branching. The clay-topped cultures had definitely more abundant fruiting, and the sorocarps were more erect and thus taller. The slime mold may build the same length stalk under both sets of conditions, but in the latter case less of it is in contact with the substrate.

Increasing the percentage of agar in the medium has an effect similar to using clay-topped dishes, but the effects are not quite as marked. Duplicate cross-streaked plates of dilute hay infusion agar and of 0.1 L-P were prepared with 1.0, 1.5, 2.0, 2.5, and 3.0 per cent agar. The plates were cross-streaked with *E. coli*, inoculated with WS 320 spores, incubated at 25 C and examined periodically for one week. It was found that the percentage of agar in the medium did not affect the quantity of fruiting. However, a higher concentration of agar in the medium yielded more continuous fruiting to the ends of the streaks and more erect fruiting structures. Fruiting on dilute hay agar is in all cases superior to that on 0.1 L-P agar. The amoebae seem to travel farther from the bacterial streak on the firmer agar, and on 0.1 L-P with 3.0 per cent agar the vegetative amoebae showed a greater tendency to encyst.
Effect of pH

In the experiments with various bacterial associates the pH of the cultures was measured after the growing period was completed. Considering that pH may play a very important role in the fructification of the slime mold, a closer study seemed necessary. Hay infusion agar plates were cross-streaked with a suspension of *E. coli* and inoculated with spores of each of the slime molds. Sixteen plates with each of the slime molds was prepared so that two plates could be utilized for pH measurement at six 48 hour intervals. The four extra plates allowed for the discard of contaminated plates. Sixteen plates were cross-streaked with bacteria but not inoculated with slime molds, and sixteen plates were left unstreaked and uninoculated to serve as controls. A similar series of plates was prepared using *Aerobacter aerogenes* as the bacterial associate, and all the plates were incubated at 25°C. The initial pH of the medium prior to incubation was 6.5.

There was no apparent difference in the effect of the different strains of the slime mold, so results of all strains can be considered as one. The pH of the cultures gradually became slightly more basic over the 10 day growing period, resulting in a final pH of 6.7 to 7.0. The pH of the plates cross-streaked with bacteria but not inoculated with slime mold spores paralleled those in which slime mold growth had occurred. The plates with no slime mold or bacterial inoculation remained at pH 6.5. These results indicate that the bacterial associate rather than the slime mold itself alters the pH of the medium.
Since the hay agar is buffered with $\text{K}_2\text{HPO}_4$, an unbuffered medium, 0.1 L-P, was used to set up an experiment parallel to the one above. The initial pH of the medium prior to incubation was 7.3. The final pH of the slime mold-bacterial associate cultures after 10 days growth was 7.8 to 8.2. The plates with bacterial streaks but no slime mold had a pH of 7.8 to 7.9, while the control plates containing only medium had a pH of 7.4 to 7.5, very close to the original. As expected, there was a greater pH change with the unbuffered medium. The plates which contained bacteria but no slime mold were not quite as basic as those which had been inoculated with slime mold also. Although the differences in final pH are not great enough to be conclusive, they suggest that the slime mold also contributes to the alkalinity of the medium.

Gregg, Hackney, and Krivanek (1954) demonstrated the excretion of ammonia by the slime mold during the transition from migrating pseudoplasmodium to mature sorocarp.

It was realized that measurement of the pH of the entire plate content might not provide definitive information about the relationship of pH to the development of the slime mold itself since this was in all cases localized. Therefore, attempts were made to measure the pH of the medium at the exact sites of vegetative amoebae, aggregating amoebae, and sorocarps. This was accomplished by aseptically removing a 0.5 cm square of agar at locations supporting successively vegetative amoebae, aggregating amoebae, or mature sorocarps; crushing the agar with distilled water in an adapter-beaker; and measuring the pH with a Beckman portable model G pH meter. However, the results were too inconsistent to be of substantial value. The complexities of the two-membered system
obscured any changes in pH which might have been effected by the slime mold itself.

Cultures of slime molds on plates cross-streaked with bacteria frequently failed to show a uniform development of fruiting structures throughout the streak. Typically the sorocarps were well formed in the central one-half of the cross-streak. As fruiting progressed outward, however, the sorocarps became increasingly abnormal, twisted and tangled, with less sorus formation; and on some plates no true fruiting occurred, the massed myxamoebae culminating in lumpy aggregations or encysted cells. Five to seven days are required to completely clear the bacterial streaks when slime mold spores are inoculated onto the center of a cross-streak. Therefore, the myxamoebae at the center of the cross-streak feed on a bacterial colony that is one day old, while the advancing myxamoebae at the outer ends of the streaks are consuming a culture five to seven days old. If the age of the bacterial culture has any influence on the development of the slime mold, uniform fruiting throughout the streak would not be expected.

In order to provide bacterial cultures of approximately the same age to a growing slime mold culture, a loopful of *E. coli* suspension was streaked across one side of a hay agar plate and spores of WS 320 were inoculated onto the center of it. The following day a second streak of *E. coli* suspension was placed parallel to and in contact with the first streak. Two more streaks were added in a similar manner on consecutive days. Thus, as the myxamoebae cleared the first streak, a second streak of young bacterial culture was provided. In this manner, day old bacterial cultures were presented to the slime mold culture consecutively
over a period of four days. At five days after the first inoculation, all four streaks were cleared of bacteria. Normal sorocarps were present within the first streak and only at the interfaces between the subsequent streaks. The pH of the agar beneath fruiting and non-fruiting areas was 7.0 to 7.2, while the pH of the agar on the opposite side of the plate from the streaks was 6.65. It seems that the slime mold prefers a young bacterial colony to an old one and that the accumulation of diffusible waste products from existing older bacterial cultures may have had an inhibitory effect on fruiting of the slime mold. Unfavorable pH may partially explain why slime molds fruit well in the center of a cross-streak but not farther out. However, unfavorable pH does not explain the failure of the slime mold to fruit uniformly when fresh bacterial streaks were provided daily since the pH of fruiting and non-fruiting areas was very nearly the same and was always within a pH range thought to be suitable for fruiting. If the slime mold were acutely sensitive to pH, perhaps there was a significant difference in pH at the instant when aggregation was initiated. Perhaps more metabolic products of the bacteria accumulated in the center of the streak, or possibly fruiting in the central area was inhibited by metabolic products diffusing through the agar from the previous bacterial streak. Possibly volatile waste products of the bacteria are inhibitory.

On cross-streak plates of richer media such as 2 per cent agar containing 0.25 per cent lactose and 0.25 per cent peptone (0.25 L-P), or 0.5 per cent lactose and 0.5 per cent peptone (0.5 L-P), a more dense fruiting of the slime mold occurs in the immediate area of the inoculation but only at this site. At this locus the richer medium provides a
greater food supply and there is a faster buildup of the myxamoeba popula-
lation which takes place before enough "inhibitor" is produced and
favorable conditions for fruiting of the slime mold cease to exist.

Whittingham and Raper (1957) showed that certain volatile organic
acids inhibit the fruiting of *Dictyostelium polycephalum*. A concentric
plate system was used to demonstrate this. The bottom half of a 50 by
15 mm Petri dish was placed inside a 90 by 20 mm Petri dish. The height
of the smaller dish was low enough to permit free diffusion of gases be-
tween the two cultures. Liquid diffusion between the cultures was pre-
vented by coating the lip of the smaller dish with silicone grease. In
the central dish was placed an agar medium containing 1.0 per cent tryp-
tone, 1.0 per cent glucose, and 1.0 per cent yeast extract (1.0 TGY) and
a medium favorable for the fruiting of *D. polycephalum* was placed in the
surrounding vessel. A suspension of *E. coli* was inoculated onto the
medium in the central dish and a suspension of *E. coli* and spores of *D.
polycephalum* was inoculated onto the medium in the surrounding vessel.
During the period of incubation at 30 °C with glazed clay covers, the pH
of the medium in both chambers decreased steadily, attaining a final
value of about pH 5.0. Under these circumstances the slime mold failed
to fruit in the outer chamber, while control plates which had not been
inoculated with *E. coli* in the center dish exhibited normal sorocarp
formation on the fruiting medium surrounding this.

An attempt was made to duplicate the above experiment. The cen-
ter dish contained 1.0 TGY agar inoculated with *E. coli* while the area
surrounding this contained either dilute hay infusion agar or 0.1 L-P
agar inoculated with a suspension of *E. coli* and WS 320 spores. The
plates were covered with glass lids and incubated at 25 C. The pH of the medium in the center dish dropped to 4.8 but that outside decreased only to pH 6.6 to 6.8 and supported good growth and fructification of Polysphondylium. Control plates similarly inoculated with E. coli and slime mold spores in the outer chamber, but with 1.0 TGY uninoculated in the central dish, remained at pH 7.0 and produced normal sorocarps. Lowering the incubation temperature from 30 C as used by Whittingham and Raper to 25 C apparently reduced the rate at which the volatile acid was produced by the bacteria from 1.0 TGY agar and pH outside remained within a range suitable for normal fruiting.

None of the combinations of white-spored Polysphondylium, bacteria, and media previously tried had produced a strongly acid medium. Numerous cultures had become strongly alkaline and failed to fruit normally. This suggested that a basic end product of the metabolism of E. coli might be the "inhibitor" of normal fruiting. Therefore, a modification of 1.0 TGY medium designated as NTGY*, which becomes strongly alkaline when E. coli is grown on it, was substituted in the central dish. On the fourth day of incubation at 25 C, concentric systems with E. coli in the central dish showed abundant amoebae in the outer dish but no fruiting. In a few instances there were scattered twisted, abnormal fruits. The pH in the central dish was 8.4 and the pH in the outer dish was 7.9 to 8.2. Concentric systems with uninoculated NTGY in the center showed excellent fruiting in the outer dish where the pH remained at 7.1 to 7.2. Obviously some volatile basic end product of

*Composition of NTGY: 5 gm tryptone, 5 gm yeast extract, 1 gm glucose, 1 gm K₂HPO₄, 20 gm agar, 1 liter tap water; adjust pH to 7.0.
the *E. coli* metabolism was inhibiting the aggregation and fruiting of the slime mold in the outer chamber of the test system. This inhibition was not, however, sufficiently severe to affect the proliferation of vegetative amoebae.

If the volatile substance produced by the *E. coli* does not destroy the aggregative mechanism of the slime mold but merely inhibits it, removal of the inhibitor and return of the pH of the medium to a range favorable for sorocarp formation should allow the culture to fruit. However, once the inhibited amoebae encyst the reaction would not be reversible. Therefore, removal of the inhibitor and reversal of the pH must occur when the control plates are fruiting and the vegetative amoebae in the inhibited plates are healthy and not yet encysted. Attempts were made to provide these conditions by removing the *E. coli* and the medium in the central dish and replacing it with various concentrations of acetic acid. Phenol red at 0.002 per cent had been added to the medium so that pH changes could be observed without opening the dish and removing agar. However, concentrations of acetic acid which were volatile enough to change the pH in the time interval required proved to be toxic to the amoebae. Therefore, attempts to renew the fruiting ability of the culture failed.

Cohen (1953) postulated that free ammonia rather than the ammonium ion was responsible for the morphogenetic suppression of the slime mold. If this is true, a highly buffered medium in the outer dish of a concentric system with NTGY medium inoculated with *E. coli* in the
center should produce just as good fruiting as a system without *E. coli* in the center. A modification of Sussman and Bradley's medium* (1954) gives very luxuriant fruiting when inoculated with a suspension of *E. coli* and WS 320 spores. The sorocarps, however, are neither as erect nor as regular as those on cross-streaked hay plates. Since the test medium also supports a heavy growth of bacteria, it is assumed that the buffering capacity of the medium permits the fruiting of the slime mold. This medium was therefore substituted in the outer dish of the concentric plate systems. After four days incubation the system lacking *E. coli* in the center yielded excellent fruits in the chamber surrounding this where the pH of the medium was 7.1. The system containing *E. coli* in the center yielded only twisted abortive fruits in the outer dish where the pH of the medium was 7.7 (figure 7). The medium was not highly enough buffered to yield any conclusions. Substitution of $10^{-1}M \text{KH}_2\text{PO}_4$ for the normal amount of potassium salt in the medium provided enough buffering capacity but was toxic to the slime mold and prevented normal growth and development. Substitution of $0.5 \times 10^{-1}M \text{KH}_2\text{PO}_4$ yielded very light fruiting with scattered small, regular sorocarps.

An attempt was made to determine whether a basic pH alone could inhibit fruiting. Four concentric plates were prepared with NTGY in the center and the modification of Sussman-Bradley's medium in the outer dish. *E. coli* was inoculated onto the central dish of two plates but

*Composition of modified Sussman-Bradley medium: 15 gm agar, 2.5 gm yeast extract, 2.0 gm beef extract, 4.0 gm glucose, 2.0 gm \text{KH}_2\text{PO}_4, 0.5 gm \text{MgSO}_4 \cdot 7\text{H}_2\text{O}, 0.1 gm cholesterol, 20 ml 0.1 per cent phenol red, and 1 liter $H_2O$; adjust to pH 6.0 to 6.4.*
Figure 7. Comparison of cultures of WS 320 on modified Sussman-Bradley medium in the presence and absence of deleterious Escherichia coli in the central dish of concentric plates.  
a. Area of fruiting in outer dish when central dish contains uninoculated NTGY medium, x6.3.
b. Suboptimal morphogenetic expression achieved in outer dish when central dish contains NTGY medium inoculated with E. coli, x6.3. Note the complete absence of normal sorocarps with regular branching.
not the other two, and all four plates were incubated at 25 C for ten days until the two containing E. coli were strongly basic as revealed by the indicator in the medium. The agar was then removed from all four of the central dishes, the outer dishes were inoculated with a suspension of E. coli and slime mold spores, the dishes were covered with glazed clay tops to allow the diffusion of gases, and the plates were again incubated at 25 C. Six days later the plates which had been exposed to the volatile E. coli products showed no fruiting or very abnormal sori-gens, while those which had contained uninoculated NTGY in the central dish were fruiting abundantly. The pH of all four plates was essentially the same seven days after inoculation. In a duplicate experiment, however, four days after inoculation, nearer the actual time of fruiting, plates with many sorocarps had a pH of 7.1, while non-fruiting plates had a pH of 7.6. At the time of inoculation with the slime mold, plates exposed to the by-products of E. coli were at pH 7.1 while those not so exposed were at pH 6.2.

The above experiments show that fruiting of WS 320 can be inhibited by basic volatile end products of E. coli metabolism. Medium that has been exposed to such volatile products for a number of days will not support the growth of WS 320, even though the deleterious E. coli is no longer present and the plates are covered with glazed clay tops so that gases may diffuse through the lid. Control medium not exposed to volatile products of E. coli will support luxuriant, dense fruiting. The sorocarps, however, exhibit few regular whorls.

In order to ascertain the effect of pH alone, a series of plate cultures uniformly inoculated with spores of WS 320 and E. coli in sus-
pension was set up on the modified Sussman-Bradley medium ranging in pH from 5.3 to 6.9. The initial pH was attained by adding NaOH or HCl to the medium. After one week of incubation at 25 C, cultures with an initial pH of 5.3 and 5.8 showed normal fruiting and a final pH of 6.8 and 7.1. The culture with an initial pH of 6.4 contained only encysted amoebae and had a final pH of 7.3. Cultures with an initial pH of 6.6 to 6.9 showed no growth of the slime mold at all and had a final pH of from 7.9 to 8.3.

To avoid the change in pH of the medium caused by the growth of the bacterial associate, WS 320 spores and E. coli suspension were inoculated onto 0.1 L-P medium and allowed to grow at 25 C for 48 hours to obtain a maximum number of amoebae in the pre-aggregative stage. The amoebae were washed off the plates with distilled water, centrifuged once at 2700 rpm for four minutes, and the supernatant poured off. The amoebae were then spread with a loop over a 2 cm diameter area of non-nutrient plates with 1.5 per cent agar containing 0.002 per cent phenol red and ranging in pH from 5.8 to 6.6 and again incubated at 25 C. Twenty-four hours later fruiting was accomplished on all plates. However, the final pH at the point of fruiting ranged from 6.4 to 6.7. The slight shift toward alkalinity could be attributed to growth of E. coli carried over with the slime mold amoebae. Therefore, the experiment was repeated using amoebae that had been centrifuged and washed three times; and also, as a control, a very light suspension of E. coli was spread on the opposite side of the water agar plate from the slime mold amoebae. There was some increase in alkalinity of the medium in the immediate area of the added bacteria. There was no bacterial growth and no fruit-
ing of the slime mold on the plate at pH 5.8. All other plates showed
normal sorocarp formation. An occasional plate with particularly heavy
fruiting showed a slight increase in alkalinity as revealed by a change
in the indicator in the immediate area. Perhaps this can be attributed
to the consumption of the bacteria and the release of ammonia by the
slime mold itself during morphogenesis (Gregg, Hackney, and Krivanek,
1954).

If other environmental conditions are favorable, normal sorocarp
formation occurs from about pH 6.1 to pH 7.2.

Serology

Classification of the Dictyosteliales at the present time is
based entirely on morphological characteristics. On this basis there
seems to be no valid reason for separating the white-spored Polysphondylia
into two species. Any further separation, therefore, will require
new means of discrimination. If the species-specificity of surface anti-
gens of the slime mold amoebae demonstrated by Gregg (1956) can be veri-
fied and extended to P. pallidum, serology may prove to be a very valu-
able tool in the classification of the white-spored Polysphondylia.

In order to explore this possibility, an experiment was set up
to determine the specificity of the surface antigens of two isolates of
white-spored Polysphondylia (WS 320 and Fr 47) and one isolate of Polys-
phondylium violaceum (V 6).

Slants of 0.1 L-P agar were inoculated with E. coli and spores
of the three slime molds. When fructification appeared complete, several
milliliters of distilled water was added to the tubes and they were
shaken thoroughly to obtain a heavy suspension of spores. An approximately equal volume of a heavy *E. coli* suspension was added to the tubes and thoroughly mixed. Three drops of this suspension was then pipetted onto each of five 0.1 L-P agar plates and spread with a sterile glass rod. V 6, which grew more rapidly than the other two cultures, was incubated at 20 C; WS 320 and Fr 47 were incubated at 25 C.

At 44 to 48 hours when the amoebae had nearly cleared the plates of bacteria the amoebae were washed from the plates with sterile distilled water. They were centrifuged at approximately 2700 rpm for three to four minutes to separate the bacteria from the amoebae. The supernatant liquid containing the bacteria was decanted off, the remaining amoebae were resuspended in sterile distilled water and again centrifuged. This process was carried out three to five times until the supernatant liquid appeared to be relatively free from bacteria, at which time it was replaced with sterile 0.85 per cent NaCl solution. Saline was used as a suspending medium instead of distilled water because it was thought to be less of an irritant to the rabbit. Suspension in saline did not appear to decrease the viability of the amoebae. The suspensions of amoebae were adjusted to a certain optical density each time in order to standardize the number of amoebae injected. Kjeldahl determinations were made prior to the injection series in order to approximate the number of amoebae injected by Gregg (1956).

Preinoculation serum for controls was taken from the rabbits by heart puncture before any injections were made. The blood was placed at 37 C for an hour to hasten coagulation and then refrigerated overnight. The sera were obtained the following day by centrifugation for 30 min-
utes at approximately 5300 rpm and dispensed in 2 ml quantities into sterile 15 ml screwcapped tubes. The sera were heated in a water bath at 56 C for thirty minutes to destroy complement, then stored at -20 C.

Ten intravenous injections of the amoebae suspensions of the three slime molds were given at alternating three and four day intervals in the marginal ear vein of each of three mature rabbits. The initial injection consisted of 1.0 ml. Each subsequent injection was increased by 0.5 ml until the ninth and tenth injections, which consisted of 5.0 ml each.

The procedure used for determination of titer follows closely that described by Zaczyński (1951). The amoebae were prepared in the same manner as those for injection. However, a much heavier suspension was used. For each of the three slime molds and also for E. coli a series of 14 1.0 cm OD tubes was set up with 0.5 ml of 0.85 per cent NaCl in each tube. To the first tube 0.5 ml of antiserum was added, mixed thoroughly, and 0.5 ml of this dilution was transferred to the second tube. This process was repeated until each of the 13 tubes contained a total of 0.5 ml of saline and antiserum in an increasingly higher dilution. The fourteenth tube is a control tube containing only 0.85 per cent NaCl. To each of the fourteen tubes 0.1 ml antigen was added and the tubes were incubated at 37 C for one hour, then removed to 10 C for two hours. Titer was established by determining the highest dilution of the antiserum which would agglutinate 48 hour homologous amoebae.

The rabbits were test bled and the titer determined two days after the ninth injection at which time the titer was shown to be too low for use in the testing program. Therefore, the rabbits were again
test bled nine days after the last V 6 injection and twelve days after the last Fr 47 and WS 320 injections and the titer was determined. Antisera of V 6 showed a titer of 1:8192, and WS 320 showed a titer of 1:2048. Fr 47 could not be tested because of growth failure on the plates inoculated to provide amoebae for the test. Antisera tested against *E. coli* showed a titer of 1:32.

These titers appeared more than adequate for further testing purposes. Therefore, 14 days after the last injection of Fr 47 and WS 320 and 11 days after the last injection of V 6 the antisera were obtained by heart puncture, prepared and stored in the same manner as the preinoculation sera.

### TABLE I

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>V 6</th>
<th>Fr 47</th>
<th>WS 320</th>
<th><em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>V 6</td>
<td>1:8192</td>
<td>*</td>
<td>*</td>
<td>1:16</td>
</tr>
<tr>
<td>Fr 47</td>
<td>1:32</td>
<td>1:2048</td>
<td>1:256</td>
<td>*</td>
</tr>
<tr>
<td>WS 320</td>
<td>1:16</td>
<td>*</td>
<td>1:256</td>
<td>1:16</td>
</tr>
</tbody>
</table>

*indicates combinations not tested.

Titers of the antisera thus obtained are recorded in table 1. Note that the highest titers are between homologous antisera and antigens, while WS 320 and V 6, the *P. violaceum*, and Fr 47 and V 6 show only a very low titer. WS 320 and Fr 47 show a closer antigenic
relationship to each other than to V 6. These results parallel the
degree of morphological similarity among the cultures. E. coli had
a negligible titer with any of the antisera.

The antisera were tested against 12 strains of white-spored
Polysphondylium, one strain of P. violaceum, (V 6) and one strain of
Dictyostelium discoideum (V 12) by drop agglutination tests similar to
those performed by Gregg (1956). Amoebae were harvested at 24 hours,
centrifuged and washed with distilled water until relatively free of
bacteria, and adjusted to a density of approximately 0.75 x 10^8/cc.
A drop of amoebae suspension and a drop of antiserum was mixed thoroughly
on a clean glass slide with a fine glass rod and examined under the 10x
objective of a compound microscope. Positive (+) agglutination was
indicated by clumping of the amoebae. None of the myxamoebae tested
were agglutinated by either saline solution or pre-inoculation serum.
Results are indicated in table 2. Note that none of the Polysphondy-
lium antisera was able to agglutinate D. discoideum amoebae, and the
Fr 47 antiserum was unable to agglutinate the P. violaceum amoebae.
WS 452B, a rangy culture with very little Polysphondylium-like branching,
can be antigenically classified with the white-spored Polysphondylium.
Forty-four hour amoebae tested in a similar manner showed no speci-
ficity whatsoever.
TABLE 2

Results of agglutination tests between antisera and 14 strains of slime mold amoebae

<table>
<thead>
<tr>
<th>Antigens</th>
<th>V 6</th>
<th>Fr 47</th>
<th>WS 320</th>
</tr>
</thead>
<tbody>
<tr>
<td>V 6</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>V 12</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WS 320</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Campbell</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>II 7</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>WS 406</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pan 12</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Salvador</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fr 47</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>WS 450A</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>WS 452B</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>WS 446C</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>WS 446D</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>WS 425-2</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ indicates agglutination of amoebae

- indicates no agglutination of amoebae
No species-specificity of 24 hour amoebae could be demonstrated in *Polysphondylium* using this technique, although the inability of Fr 47 antiserum to agglutinate V 6 amoebae is some indication of this. Instead, there appears to be a genus-specificity. However, table 1 indicates that the antigenicities of the white-spored *Polysphondylias* are more closely linked to each other than to *P. violaceum*. Probably absorption techniques would more clearly delineate the antigenic relationships of members of the genus *Polysphondylium*. 
DISCUSSION

A primary objective of this study was to determine whether the white-spored Polysphondylium could be separated satisfactorily into the two species described by Olive, (1901, 1902) P. pallidum and P. album, on the basis of characteristics described by him. Or failing in this, could other criteria be found which might provide a basis for species separation among these abundant but variable, Polysphondylium. Additional objectives were to determine optimal conditions for growth and fructification of these slime mold, as had been done for the larger species of Dictyostelium, such as D. discoideum, D. mucoroides and D. purpureum Olive, and for Polysphondylium violaceum (Raper 1940a, 1951).

It was found that the isolates examined could not be clearly divided into two species according to Olive's classification. Rather, there seemed to be a continuous spectrum of characteristics among the isolates, each isolate varying from the others to a degree, but none sufficiently different from all the other isolates to warrant its being considered as a focal point for a separate species. Dimensions of spores in one strain, for example, might cover a wider range than that described by Olive in either species. The weakness of the base of the stalk was a matter of degree. Olive's differentiation was based largely on gross morphology, which has been shown to be very sensitive to changes in the environment. In his discussion of P. violaceum (Olive, 1901,
1902), he mentions that the spores of the Maine and Florida forms are somewhat smaller, and the fructifications are different in that they are more delicate and less luxuriant and the sori have less diameter than do those of typical strains. He considers these differences, however, as varietal. Yet, using differences in the same criteria as a basis, he describes two species of white-spored Polysphondylium. Olive undoubtedly had more isolates of P. violaceum than of P. pallidum available for study and was thus able to see the range of characteristics of the dark-spored Polysphondylium. Since he lists only three isolates of P. pallidum and one of P. album, the latter may have been sufficiently distinctive to cause him to consider it a separate species. Additionally, culture conditions were not emphasized in his study of more than a half-century ago, and his slime molds were grown upon dung or dung agar with unknown bacteria and without knowledge of their role in slime mold nutrition, or of their influence on morphogenesis.

Since Olive's division of the white-spored Polysphondylium into two species was unsubstantiated by sorocarp morphology in this study, another means of differentiation was explored. Gregg's serological study of Dictyostelium purpureum, D. discoideum, and P. violaceum showing species-specificity of 24 to 26 hour amoebae suggested that serology might be a basis for differentiating the white-spored Polysphondylium. However, 24 to 26 hour amoebae of Polysphondylium proved to be genus-specific rather than species-specific. Since Gregg used only one species of Polysphondylium his study could not reveal that the antigenic structure of P. violaceum and the white-spored Polysphondylium would be so similar. More intensive study will probably show antigenic
differences between white and dark-spored Polysphondylium as is indicated by the failure of Fr 47 antiserum to agglutinate *P. violaceum* (V 6) amoebae. Even differences between the isolates of the white-spored Polysphondylium may be demonstrated. Such differences, however, will hardly be sufficient to separate species.

The question next arises as to which species name should be retained for the white-spored Polysphondylium. The two species were named at the same time, and the names are of Latin origin and are equally descriptive. Since neither name has priority, it is suggested that the name *Polysphondylium pallidum* be retained since it has been most commonly used in the literature.

The influence of the nutritional and physical environment was investigated. The utilization of a number of different bacterial associates revealed several requirements for a suitable food source. The bacteria must display adequate growth on the substrate provided; there must be no great accumulation of waste products toxic or inhibitory to the slime mold; and the bacteria must be suitable for ingestion and utilization as food by the slime mold. *E. coli* has proved to be generally adequate as an associate for *P. pallidum*. Production of harmful wastes must be prevented by control of the substrate and the temperature of incubation. Nearly simultaneous inoculation of bacteria and slime mold is preferred. This prevents an accumulation of waste products of the bacteria preceding the growth of the slime mold, as would occur if slime mold inoculation were delayed a day or two.

Rate of growth and reproduction of the slime mold amoebae increases with an increase in temperature to the point where growth fails
to occur entirely. An increase in temperature normally increases the food supply available to the slime mold amoebae and probably increases their activity and rate of cell division. Fr 47 has a slower rate of growth than the other isolates examined. A number of factors might be responsible for this. Perhaps the Fr 47 amoebae have less motility and cannot move as rapidly. Perhaps it is more difficult for them to ingest or digest and assimilate the bacterial food supply. Possibly the rate of cell division is slower. And, since their delayed development seems to carry over into the aggregative stage, possibly they are slower to respond to acrasin or produce acrasin at a slower rate or lower level than the other isolates. A temperature of 25 C is most suitable for maintenance of the majority of strains of P. pallidum. This temperature is optimum for a great number of strains, and all isolates examined fruited at this temperature.

Response to light seems to be quite variable among the isolates, and even inconsistent in a single isolate, although the latter possibility must be explored further.

Extremes of pH were inhibitory to fruiting of the slime mold. In the usual culture methods employed and with E. coli as an associate, excessive acidity was never a problem. However, cultures frequently became highly alkaline with subsequent inhibition of normal fruiting, particularly the suppression of branching and normal sorus formation. In some cases aggregation was prevented where there was no apparent inhibition of growth and reproduction of the amoebae, as had been shown earlier for D. discoideum and other species (Raper, 1939). It was demonstrated that the inhibitor was a volatile basic waste product of
E. coli metabolism. The precise nature and the mechanism of this inhibition is unknown. Possibly it interferes with the normal intercellular stickiness of the surfaces of the myxamoebae. It may suppress acrasin secretion, or it may prevent the production of the enzyme which normally destroys acrasin, thereby allowing the accumulation of excess acrasin and upsetting the chemical gradient.

Hay infusion agar and dilute hay infusion agar continue to be superior for maximum expression of morphogenetic capabilities. While the Sussman-Bradley medium gives increased growth and the heaviest fruiting of any medium in plates spread with a suspension of bacteria and slime mold spores, the sorocarps are definitely atypical and fail to show the erectness of the stalk and regularity of branching that is possible with the two hay infusion media. The value of the hay media perhaps lies in their buffering capacity and in their low nutrient content which supports light but adequate growth of E. coli. The hay media are usually made with a two per cent agar content instead of the usual 1.5 per cent agar contained in most media. Higher agar content and the consequent increase in hardness of the surface seemed to encourage continuous and more erect fruiting. Clay-topped dishes, which allowed for a gaseous exchange and for a continued decrease in humidity, definitely encouraged more continuous and erect fruiting but had no effect on the growth and reproduction of the amoebae.

Many experiments in slime mold research are complicated by the presence of bacteria. Inquiries into the physiology of the slime mold frequently must take into account the physiology of the bacterial associate as well. While bacteria-free spores are relatively easy to
procure, it is difficult to obtain a preparation of amoebae that is free of bacteria, especially in the first 30 hours after inoculation when the amoebae are still actively feeding on the bacteria and have not yet cleared the plate of the majority of bacterial cells. Sussman and Bradley (1954) have taken a step in the right direction with their preparation of a synthetic medium requiring only the addition of a paste of a protein growth factor of bacterial origin. However, growth on this medium represents only 10 to 20 per cent of the growth attained with live bacteria (Bradley and Sussman, 1952). The accomplishment that would give the greatest impetus to the field of slime mold research would be the discovery of a defined medium upon which the slime mold would grow as abundantly and fruit as characteristically as it does in the presence of a suitable bacterial associate.
SUMMARY

Seven strains of white-spored Polysphondylia, believed to encompass the limits of natural variability among these slime molds, were selected for intensive study and were characterized under a stated set of cultural conditions. A number of other isolates were examined and were found to vary morphologically only slightly from one or the other of the selected strains.

Escherichia coli was found to be the most favorable bacterial associate for these slime molds. Production of harmful amounts of basic volatile waste products of E. coli metabolism which were inhibitory to the fruiting of the slime mold could be prevented by controlling the substrate and the temperature of incubation. The range from pH 6.1 to 7.2 was optimal for sorocarp formation, and germination of spores and proliferation of the vegetative amoebae occurred from somewhat below pH 6 up to about pH 8.2. Hay or dilute hay infusion agar allowed the highest morphogenetic expression of the slime molds. A gradual decrease in humidity stimulated sorocarp formation. The optimal temperature for growth and fructification of most of the isolates examined was 25 C, and growth occurred in each of the selected strains from 10 to 30 C. Within this range, with the exception of Fr 47, an increase in temperature was accompanied by an increase in growth. Fr 47 showed slight growth but no fruiting at 30 C. The strain designated as Salvador grew and produced normal sorocarps at 37 C. Response
to light was variable among the isolates examined. In limited tests, the surface antigens of 24 to 26 hour amoebae of *Polysphondylium* were shown to be genus-specific, hence probably of little or no value for separating strains or species within the genus.

The isolates examined could not be divided satisfactorily into the two species described by Olive (1901) but composed a continuous spectrum of morphological types. It was suggested, therefore, that the name *Polysphondylium pallidum* be retained for the entire group of white-spored *Polysphondylium* since this name has been most used in the limited literature relative to these slime molds.
REFERENCES


Brefeld, O. 1884 Polysphondylium violaceum und Dictyostelium mucoroides nebst Bemerkungen zur Systemstik der Schleimpilze. His Untersuchungen aus dem Gesamtgebiete der Mykologie, Heft 6, pp. 1-34.


Potts, G. 1902 Zur Physiologie des *Dictyostelium mucoroides*. Flora (Jena), 91, 281-347.


Runyon, E. H. 1942 Aggregation of separate cells of Dictyostelium to form a multicellular body. Collecting Net, 17, 88.


APPROVED

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Aug. 12, 1959