T H E S I S

THE GERMINATION OF SLIME MOULDS

Submitted by

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TABLE OF CONTENTS

THE GERMINATION OF SLIME MOULDS

E. C. Smith

INTRODUCTION

The germination of slime moulds is a subject of intrinsic interest. The life cycle of these peculiar organisms presents a sequence of forms not paralleled in that of any other organism, plant or animal. Moreover, being near the dividing line between plants and animals, yet not in any direct line of ascent, a study of their behavior may well bring one close to the secret of life itself. Despite the large amount of study which has been devoted to this group, even a beginner, if he comes with a new focus of attention, may add somewhat to the knowledge of their behavior. Only a few species, and these chiefly European, have been studied intensively by other investigators and the generalizations made are clearly based upon too limited observation.

It is the purpose of this discussion to consider the germination of several American species figuring photographically for the first time their morphologic peculiarities.

A study of this group may well expect to obtain results which will afford a basis for comparison with the

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process of germination so thoroughly worked out for fungi. This study is limited to the consideration of the endosporic group of Myxomycetes, to such species as were readily obtainable and to so much of the life cycle of these as is comprised between the ripe spore and the formation of the plasmodium. The points on which attention is centered are,— the method of emergence of the swarm spores, their shapes and sizes at different stages of their career, their characteristic movements at each stage of development, the function and the occurrence of the biflagellate forms, the divisions and fusions observable and the formation of cysts and plasmodia. It is hoped to demonstrate more clearly defined stages, correlated with definite forms and movements, than have been hitherto recognized and to furnish data for some ~~for some~~ species which have not hitherto been the subject of careful study.

LITERATURE

Up to the middle of the 19th century the interesting life cycle of the Myxomycetes was unknown and unsuspected. From the early part of the 18th century certain species were known in the fruiting stage and described and named by their discoverers. Dillenius (21) and Buxbaum (18) were among the first to describe various species. Micheli (32) figured some

forms so exactly that his types are still recognizable today. The great names in this field of description, however, came somewhat later; Persoon (36), Schrader (39), and Fries (22). All of these writers assumed that what we now call "Myxomycetes" were Fungi and that they belonged to the order Gasteromycetes. Fries grouped them in a suborder which he called Myxogastres, Link (7) in 1833, perceiving the independence of the group, suggested as a substitute for the sub-order Myxogastres, the order Myxomycetes, slime-moulds.

The literature fails to make clear who was the first to observe and record the fact that in this group of organisms the spores do not produce hyphae, but ciliate swarm-cells or zoospores which, in turn, coalesce to form plasmodia, from which the fruiting bodies develop. It is probable that this discovery was one result of a rather general application of new and more thorough methods of research to all of the more minute forms of both animal and vegetable life which spread rather rapidly in the middle decade of the 19th century. Certain it is that the modern period in which this cycle is assumed as a matter of course begins with the various publications by deBary and his pupils and contemporaries.

In consequence of this discovery, which indicated a relationship with the lower forms of animal life, deBary (1) in

in 1858 introduced the name Mycetozoa. This name was accepted by his pupil Rostafinski (38), who in 1875 brought out his Sluzowce Monographia, which is the basis on which all subsequent systematic work in this group rests. Lister (8) in his Mycetozoa, which describes all species in the collection of the British Museum, also accepts the name, while Massee (30) goes back to the name given by Fries, and Macbride (9) prefers the name suggested by Link, Myxomycetes. Thus does knowledge of life processes affect ideas of relationship and classification.

The literature of description and classification is voluminous, but some of the systematic writers, notably Arthur Lister (8) in England, Rostafinski (38) in Poland and Schroeter (13) in Germany, have contributed much to our knowledge of the physiology of these organisms and many workers in many lands have devoted their efforts for years at a time to tracing out the life processes of the group or single species of the group and to a study of the conditions which affect those processes, while cytologists have investigated various aspects of cell behavior in single species with special reference to the behavior of the chromosomes. For all these investigations the work of deBary has been the starting point. Much information has been added as the years have gone by,

minor corrections have been made, new methods and appliances used, but in the main the account given by this pioneer holds good today.

DeBary's "Die Mycetozoen", (The Mycetozoa) was first published in 1859 in the Zeitschrift für wissenschaftliche Zoologie, pp. 88-176, and reissued in book form in 1864. This was followed in 1886 by his "Morphologie der Pilze, Mycetozoa und Bacterien" (Morphology of Fungi, Mycetozoa and Bacteria) which in the following year appeared in an authorized English edition, the translation being made by Henry E. F. Garnsey, Fellow of Magdalen College, Oxford, revised by Isaac Bayley Balfour, Sherardian Professor of Botany at Oxford.

One other name associated with the decade of the 60's should be mentioned, that of L. Cienkowski (2) who was a fellow pioneer with deBary and who is frequently quoted and referred to by deBary in his later book. He it was who discovered the cysts and described their function. Some of his figures, as well as those of deBary, still appear in comparatively recent works.

DeBary's (1) brief statement concerning germination conditions seems sounder than some more recent and more elaborate statements: "The requisite conditions for germination in most known forms are the usual spring and summer temperatures of our temperate climate and a sufficient supply of water. The majority germinate readily when placed in pure water, well-

developed fresh material often in a few hours. Nutrient substances dissolved in the water do not hinder germination; this at least was found to be the case in Fuligo and Chondrioderma."

He excepts from this statement the Ceratieae and Acrasieae. Famintsin and Woronin (16) working at St. Petersburg had announced in 1873 that "The spores of Ceratium germinate only when they have been thoroughly dried, and then only when they are not cultivated in pure water. To bring on germination under favorable circumstances in 30 hours it is necessary to bring the thoroughly dried spores into a drop of water to which has been added a little piece of coniferous wood which has begun to decay."

A great number of different media were successfully used by different workers, usually a few species only being used in each test. Ward (41) used water with small amounts of mineral salts (Ca, Mg., K., Na.) and with roots of hyacinth; Strasburger (40) cultivated Chondrioderma difforme in an extract of dry stalks of Vicia faba. Didymium effusum also developed well in this medium in the experiments of Klebs (26). Miller (33) used hay extract and water with one percent of milk for cultures of Physarum cinereum, Stemonitis, Chondrioderma difforme and Didymium microcarpum. Lendner (29) used extract of Vicia faba for several species; Potts (37) cultivated Dictyostelium mucoroides in extract of kernels of Zea Mays.

Solid media in great variety were also tried out. DeBary (11), Cienkowsky (2), Rostafinsky (38) and van Tieghem (41) turned to the natural substratum on which the species experimented with grew. Lister (8) used bits of Stereum hirsutum for the culture of Badhamia utricularis; Cienkowsky (2) cultivated Licea pannorum on bits of carrot. Other experimentors used agar agar with extracts of organic substances. Nadson (34) cultivated Dictyostelium in the following nutrient solution: distilled water 100 cc., glucose 5 gr., peptone 1 gr., potassium phosphate 0.1 gr., magnesium sulphate 0.1 gr., calcium phosphate and iron phosphate.

The most extended work on the conditions of germination of slime-moulds was done by J. C. Constantineanu (3) at Halle in the Laboratory of Professor Klebs. In his inaugural address at Halle, Constantineanu presented the results of three years work. In this he says : "My own experiments had for their goal first, to determine for several species the conditions under which germination of the spores takes place. I was further concerned with the question by what means plasmodia might be attained in the culture and under what conditions the formation of plasmodia and of fruiting bodies takes place."

In the first instance he takes issue with Vuillemin (14) and Pinoy (12) . The former had stated that the spores

of Dictyostelium mucoroides never germinate alone, but only from the moment when one adds to them a suitable kind of bacteria. Pinoy went much further. He wrote (12) :
"In a series of researches directed to endosporic myxomycetes I have been able to show that if, taking all necessary precautions, one sows the pure spores of either Chondrioderma difforme or Didymium effusum, even upon a maceration of decayed wood, one observes no development; if, on the other hand, one adds bacteria, one observes successively the germination of the spores, the formation of amoebae, of the plasmodium and of the spore-bearing apparatus. One of these bacteria (Bacillus luteus) has shown itself the most favorable."

Constantineanu contends that in the case of quickly germinating species introduced bacteria could not possibly develop rapidly enough to have a direct influence on germination and that even in the case of slowly germinating kinds the influence of bacteria was not on germination, but on later development. This influence on later development had already been made clear by experiments of Lister, Nadson, Potts and Celakovsky.

Jahn (6) in the preceding year had said: "Whoever busies himself with the spores of very common species (Aethalium septicum, Lycogala epidendron, Stemonitis fusca,

Trichia varia) has the unpleasant experience that they generally do not germinate. Almost certainly capable of germination in distilled water are only the spores of *Reticularia* and *Amaurochaete*; to be depended on to some degree are those of *Dictydium difforme*, *Stemonitis fusca* and *Badhamia macrocarpa*. The species of *Stemonitis* do not germinate." That this is a common experience is shown by the statement of Durand (4) that out of a large number of recently gathered slime-moulds only one, *Enteridium rozeanum*, germinated, though many cultures of each species were made.

Constantineanu was successful in germinating the spores of fourteen species, among them those of a *Cribraria*, a genus where no germination had previously been obtained. His table showing the elapsed time between the sowing in distilled water and the beginning of germination is as follows:

Reticularia Lycoperdon	30 minutes to 1 hour
Aethalium septicum	30 minutes to 1 and 1/2 hours
Stemonitis splendens var. *flaccida*	5 to 6 hours
Stemonitis fusca	5 to 6 hours
Perichaena depressa	5 to 6 hours
Amaurochaete atra	6 to 10 hours
Didymium effusum	12 hours
Badhamia macrocarpa	12 to 18 hours
Lycogala miniatum	60 hours

Leocarpus vernicosus	24 hours to 6 days
Physarum didermoides	24 hours to 10 days
Dictydium umbilicatum	3 to 20 days
Cribraria aurantiaca	20 days.

His cultures were in hanging drops in van Tieghem cells. The percentage of germination ran from 55 percent to 100 percent. He concluded that the spores of most endosporic species could be germinated in distilled water. He further found that tap water did just as well as distilled water and with some species gave more regular results. He also secured the germination of various species in the different nutrient media which had been used by others. He found that free mineral and organic acids exercise a harmful influence; that carbohydrates, especially certain sugars, are a help in the germination of certain species, but affect others very slightly. He secured slight germination of some species at a temperature of 2 to 4°C., and found that the maximum temperature endurable for some species to be 30°, for others 35, and for two, 40°. High temperature for a limited time followed by room temperature hastened germination in most species.

This last statement had been proved by Jahn in the preceding year. He recognized several stages in germination, the first stage having a much higher optimum temperature

than succeeding stages. He gives as an instance: "Spores of Reticularia which have lain dry for eight months germinate at 21° after 30 minutes. When sowed in water at 37° and allowed to remain in the thermostat at this temperature they usually did not germinate. When the temperature of 37° was endured for only five minutes and then lowered to 21° they germinated in 11 minutes from the first sowing.

All these statements are mere refinements upon the general statements of deBary and the end result appears to be that germination generally takes place in water at a temperature varying from 21° C. to 30°C., at times varying from 30 minutes to 20 days, the time being fairly constant for each species under similar conditions, but that not all the spores germinate and that sometimes none do.

What now follows the rupture of the spore envelope? Again let the pioneer lead off. DeBary(1)(p. 422) tells the simple story: "The germinating spore swells first of all by absorption of water, and one or two small vacuoles, which disappear and reappear alternately, are seen near the upper surface of the protoplasm in which rotating movements are often observed; at length the membrane bursts and the protoplasm oozes or creeps slowly out of the opening. The protoplasmic body then either at once, as is the rule, or after a

transitory period of rest, during which it assumes a spherical form, commences amoeboid movements, undulating changes of outline and protrusions and withdrawls of pointed processes, and in this way becomes an elongated body which moves about in the water like a swarm spore and is known by the name of swarm-cell." ---------- "The swarm cell has no firm membrane but careful observation shows that it is surrounded by a tolerably broad, pellucid and indistinctly defined envelope of the consistence of mucilage."

"The movements of swarm cells are of two kinds: a hopping and an amoeboid creeping movement. In the first the cell floats freely about the water with its anterior extremity usually turned upwards. This extremity is finely pointed, the point being drawn out into a long cilium or flagellum with an undulating and swinging movement; in exceptional cases only are there two cilia. -- The body thus constituted rotates round its longitudinal axis in the circumference of a cone the apex of which is formed by the posterior extremity. The cilium swings with an undulating motion from side to side, making the swarm cell move in a similar manner and advance in one direction; sometimes there is no rotation. The body at the same time exhibits constantly varying undulatory movements of its surface, with bending and contraction and recurrent expansion of parts."

"In the creeping movement the swarm cell lies on the firm substratum , and either advances in one direction with a vermicular movement and with the cilium stretched out in front; or it assumes a roundish form and thrusts out pseudopodia in every direction and then draws them in again. Swarm cells with purely amoeboid motion have been unnecessarily distinguished by the name myxamoebae."

"The swarm cells multiply by bipartition. Before division the swarm cell contracts into a spherical form and the cilium and vacuoles disappear. This is followed by the appearance of an annular constriction in the middle which speedily becomes deeper and in a few minutes divides the body into two spherical halves which at once resume the characters of motile swarm cells."

"In a few days most of the swarm cells have the creeping form in which they are without cilia, and many have increased in size and contain single large strongly refringent granules. Then they approach close to one another and again separate, till at length two or three are seen to come into close contact with one another and to become fused into a single body, the young plasmodium."

Between the years 1899 and 1912 hardly a year passed without some publication on Myxomycetes by E. Jahn of the University of Berlin . A few of these publications dealt with

geographical distribution, but most of them were devoted to intensive studies of different portions of the life cycle of single species. In 1905 one communication was devoted to "The Germination of the Spores"(6). In this paper he divides the whole group into sections on the basis of the method of germination. He says: "Two entirely different types of germination are to be distinguished, that of Ceratiomyxa and that of other myxomycetes." In Ceratiomyxa the spores are borne on stipes and there is no envelope enclosing them. When mature, the spore nucleus divides in karyokinetic fashion and then these divide again, so that the spore has four nuclei. It then passes into a state of rest. On germination an amoeba appears which divides into four little balls which divide again, passing into eight swarm spores, each with its own single nucleus. The flagellum is then produced and the swarm spores conduct themselves like those of other species."

His statement regarding the endosporic species seems contradictory. He first makes the general statement that in all other species besides Ceratiomyxa normal spores have only one nucleus. He then distinguishes in these two subordinate groups according to the course of germination. I give his exact wording so far as a translation may give it:

" a. The Reticularia Type. In Reticularia Lycoperdon Bull. an amoeba first comes forth from the spore, remaining quiet for a short time after germination. Then, with vigorous

streamings of the plasm, it acquires a nose-shaped projection and begins, with peculiar twistings, to push the flagellum out of the projection. One can easily follow all the stages of the growth of the flagellum. The swarm spore then stretches itself and takes the form of an interrogation point or an "S". The flagellum may be complete in 15 minutes. During the whole time a pulsating vacuole at the rear end is in action.".

" b. Didymium Type. DeBary studied the germination of Didymium. He discovered that a division of the amoeba takes place within the closed spore membrane. He avoided more exact statements concerning the process of flagellum building."

"It is easy to establish the fact that the swarm spores which are killed and stained during the germination come forth from the spore envelope with shorter and longer flagella. The first appearance of the flagellum therefore probably takes place within the closed membrane. After germination the swarm spores at first remain quiet, then they stretch themselves and complete the formation of the flagellum, but without the characteristic twistings of the foregoing type. In many of the species which germinate in this fashion balls of slime, as described by deBary and Cienkowski come forth and after germination are pushed aside. These slime balls are lacking in type 2a. (The Reticularia Type)."

" I have as yet become familiar with only one kind which behaves in the fashion of Reticularia, Enteridium rozeanum, an American form. The Physareae and the Trichieae germinate after the fashion of 2b. (Didymium Type). Transition forms between 2 a and 2b. appear among the Stemoniteae."

Clearly he makes his types depend on whether the flagellum is formed before or after emergence from the spore envelope. The implication is that only a few species are of the Reticularia type and that all Physareae and Trichieae belong to the Didymium type. But what about the division of the amoebae within the closed spore membrane? Jahn does not again refer to this, nor does he apparently see that this contradicts his statement that "in all other species besides Ceratiomyxa normal spores have only one nucleus ?"

In 1899 Henrique Plenge (11) discovered and described a connecting structure between the flagellum and the nucleus in the swarm spores of myxomycetes and Flagellates. In the same paper he dealt with the relationship between the cilia and the protoplasm and nuclei of Metazoans, later turning his attention exclusively to these latter forms. It remained for Jahn (5) to exploit and carry further this discovery, discriminating between the flagellum base and the bell-shaped body which connects it with the nucelus, the movement of the latter pulling the lower part of the flagellum with it.

Plenge worked with Didymium, Jahn with Stemonitis flaccida. Jahn connected his treatment of the formation of the flagellum with a discussion of the division of the nucleus. He says: "I have sought out the nuclear divisions and followed the formation of the flagellum in seven species. In five of these species the flagellum sprouts after the completed division and reconstitution of the nucleus. The division itself is a more or less normal karyokinesis, the course of which was already given by Arthur Lister in 1893."

"In two other species , on the other hand, Stemonitis flaccida Lister,and Reticularia Lycoperdon Bull., the flagellum takes place during the last stages of the karyokinetic nuclear division. In the first of these species the division generally follows immediately upon the germination." This article is elaborately illustrated by figures enlarged to a scale of four thousand diameters, showing each stage in a complete mitosis.

Olive (35) and Harper (23) in this country also contributed much to our knowledge of the ordinary and reduction divisions of the nuclei, Olive working especially with Ceratiomyxa, Harper with Fuligo varians and Didymium. Both these workers as well as Jahn agree in the belief that the evidence indicates a sexual reproduction.

Lister describes other motions of the swarm spores besides those mentioned by deBary, a dancing motion while

while floating freely in the drop of water and a snail-like creeping movement on a flat surface, this movement being associated with a linear form, the flagellum extended in advance. Lister implies, though he does not explicitly state that the flagellum is invariably formed after emergence from the spore envelope.

Cienkowski described and named the cysts . DeBary gives a very clear statement based on Cienkowski's researches, Lister adds an interesting and significant note, while Macbride fails to mention them in his introduction.

DeBary correlates the two kinds of cysts with the sclerotia, regarding all three as Transitory Resting States. Into these states all stages having the power of motion may pass, returning again under favorable conditions to the state of movement. To quote deBary once more: "It appears ------ that these transitory resting states are not necessary members of the course of development. Their formation would seem to take place only when the development of the swarm-cells into plasmodia or of plasmodia into sporangia is interrupted by insufficiency of food, by slow desiccation, or by slow cooling to below a certain minimum. But there are a number of observations which also point to other at present unknown causes. The state of movement is restored when the bodies after desiccation are again placed in water at the proper temperature."

Lister's observation is " In all cultivations of germinating spores, a number of swarm-cells, after a short period of activity, withdraw the flagellum and become encysted in a globular form (the microcysts of Cienkowski) After being dried and rewetted, the contents burst the membranous cyst-wall, which remains as an empty hyaline sac, and emerge to resume their activity."

The microcysts are resting-stages of swarm cells. They are spherical, surrounded by a hyaline wall or a firm marginal layer and are usually slightly smaller than the spores. The marcocysts are resting-states of very young plasmodia. They, are also spherical, but have a double wall much thicker and less permeable than that of the microcysts. Just how long these cysts maintain their vitality is not known. Microcysts have been revived after a duration of two months. Owing to the thinner envelope the microcysts revive much more quickly than the macrocysts. Sclerotia are resting-states of full-grown plasmodia. The shape depends somewhat on the species, but in all cases masses or strings become brittle and horny. This state has been observed to endure for eight months. Placed in water such sclerotia return to the ordinary plasmodial form in a period varying from six hours to several days.

European writers generally make a distinction between the truly amoeboid, non-flagellate forms appearing at a late stage of development and the plasmodia which result from the coalescence of two or more of these forms, calling the former "amoebae" or "Myxamoebae", although some writers call the form taken on emergence "Amoeba." English and American writers generally do not make such a distinction, but include both stages under the term "Young Plasmodium" or "small Plasmodium."

The plasmodium has received attention ever since the time of deBary and is now included in the descriptions of species in systematic works. Besides the treatment in all general works of recent date special articles are available by Cienkowski , Strasburger (40), Lister (8), Ayres (19), Clifford (20), Harshbarger (24) and Hilton (25).

For our purpose one citation is sufficient, calling attention to two distinct types of movement exhibited by myxamoebae. We quote from Schroeter's (13) introduction to "Myxogastres " in Engler and Prantl's "Die Natürlichen Pflanzenfamilien."

"They now move by creeping, a hyaline projection pushing out in one or more parts of the border and the granulated portion moving into these. Through the pushing forth of new projections, the drawing in of some, the pulling up of the mass, they constantly change their form and move

forwards. Certain differences may be observed in the kinds of movements of the separate species. One distinguishes the Limax-form, in which the amoebae creep about slowly with a pushing forward of the front end and a dragging after of the hinder end, and the Proteus-form, where the blunt or pointed small processes (pseudopodia) are pushed out on different sides and the form thereby rapidly changed. "

Directions for laboratory treatment, based on the extended studies of many investigators and on personal experience are given in Ernst Küster's "Kultur der Mikroorganismen" (27) . American laboratory manuals (notably that of Chamberlain) base the section on Myxomycetes largely on an article in the Journal of applied Microscopy by Howard Ayres (17).

Lister in the introduction to his "Mycetozoa" adds an item regarding the relation of bacteria to swarm-cells. He says: "If bacteria are introduced into a cultivation of swarm-cells on the stage of the microscope, they are seen to be laid hold of by the pseudopodia and drawn into the interior of the swarm-cells, where they are enclosed in a digestive vacuole. Several bacteria are brought in turn to the same chamber, or fresh captures are conveyed into one or more additional vacuoles. The protrusion of pseudopodia usually ceases for a time after such ingestion, and the hinder end of the swarm-cell takes a rounded form. In the course of an hour

or two the bacteria are assimilated, and the digestive vacuoles disappear."

This directly contradicts the statement of deBary (p. 452): "The facts recorded above show that the food is taken in during the swarm-cell condition only in the fluid state or state of solution, and this is the case, at least in most instances, with the plasmodia."

In the literature reviewed one finds broad general agreements, diversities of opinion on specific points, a tendency to generalize from observations on a few species, a focus of attention on certain points to such an exclusion of other easily observed phenomena that the reader often gets a blurred impression of the sequence of stages in the life cycle. Durand (4) in the Botanical Gazette of March, 1894, gives the story of the germination of Enteridium rozeanum, stage by stage, correlating the movements observed with the stage of development and describing the features taken for granted in the reports of older and more experienced men.

The present study does not concern itself with nuclear divisions, but it does attempt to see and describe what happens in succession in the life cycle of a number of Myxomycetes and to correlate the size and movements of a number of species with the stage of development reached.

MATERIALS AND METHODS.

The material available for study consisted of 23 species of Myxomycetes, some gathered by the author in Estes Park, Colorado, during week-ends in October and November, some collected by Professor C. D. Learn in eastern Iowa in August, 1925 and some secured from Mr. Frank A. Gilbert of the Farlow Herbarium at Harvard University, these last having been collected in eastern Massachusetts during the summer of 1926.

The species are as follows, comprising representatives of four out of five of the grand divisions of the groups set forth in Macbride's North American Slime-Moulds: Fuligo septica (Linn.) Gmel., Badhamia lilacina (Fries) Rost., Badhamia rubiginosa (Chev.) Rost., Physarum sinosum (Bull.) Weinm., Physarum notabile Macbride, Cienkowski reticulata (Alb. and Schwein.) Rost., Leocarpus fragilis (Dickson) Rost., Mucilago spongiosa (Leyss.) Morgan, Didymium melanospermum (Pers.) Macbride, Didymium xanthopus (Detmar) Fr., Diderma testaceum (Schrad.) Pers., Diderma radiatum (Linn.) Morgan, Stemonitis fusca (Roth.) Rost., Diachaea leucopodia (Bull.) Rost., Enteridium splendens Morg. (Enteridium rozeanum (Rost.) Dictydium cancellatum Macbride, Arcyria incarnata Pers., Hemitrichia serpula (Scop.) Rost., Hemitrichia vesparium (Batsch.) Macbride, Hemitrichia vlavata (Pers.) Rost, Trichia varia (Pers.) Rost., Trichia decipiens (Pers.) Macbride.

Determinations were made by the use of Lister's "Mycetozoa", Macbride's North American Slime-Moulds" and Sturgis' "The Myxomycetes of Colorado."(48) Duplicate specimens were sent to Professor Macbride at Iowa City and to the Farlow Herbarium at Harvard University where the determinations were confirmed and in one case corrected.

The method of study employed was that of continued observation of cultures of the spores of the different species in hanging drops of water. The customary van Tieghem cell was discarded in favor of a support designed by Dr. L. W. Durrell.(31) This support and the usual slide from which the drop of water depended was set in a Petri dish lined with filter paper and wet with water from the same receptacle from which the drop had been taken.

It was realized that "pure water" or even "distilled water" is not the same thing at all times and in all places and that some of the conflicting results in attempts at germination might be due to differences in the water. According to Kusano (28) acids bring on germination and alkalies retard it. Webb (15) showed that the optimum concentration varied for different species and that in most species there was considerable latitude on either side of the optimum in which germination took place in almost equal degree. The object of this study not being the conditions under which germination takes place, but the successive stages in the development, only so much attention was given to this matter

as was necessary to secure germination in reasonable amount. Experiments were made with solutions of tap water at different pH values from 4 to 8. It was found that the tap water as it came from the tap varied between pH 5.5 and pH 6.8 and gave rather better results as it came from the tap than when acidified with HCl. Distilled water with a pH value of 5 was tried in a series of cultures, but was found to work not quite so well as the tap water. That results might be comparable tap water with pH value of 6.8 was used in the experiments which were finally tabulated.

Each cultivation consisted of six plates, the spores for all six drops being taken from one sporangium whenever that provided sufficient material. While the experiments with regard to optimum ddgree of acidity were being carried on six plates of the same species were carried under similar conditions as to temperature at each degree of acidity used. For a considerable time parallel cultures were carried in distilled water with a value of pH 5 and in tap water with a known pH value of 6.8

Experiments with regard to optimum temperature were merely incidental to securing germination. For a time parallel cultures were carried in a water-jacketed chamber kept at a temperature of 20° C., and on a table in a room where the temperature varied from 22° to 26° C. There was a

slight advantage shown in the higher temperature and this was used for purposes of comparative record. On a few occasions where it was desired to hasten germination the cultures were given five minutes in a thermostat at 37°, 30 minutes in an oven at 30°, or moistened with water at 40° which was allowed to cool to room temperature.

As experience was gained cultures were observed under the microscope immediately after wetting, with the surprising result that occasionally swarm-cells were observed in these perfectly fresh cultures, not active, indeed, and possibly cysts, rather than swarm-cells. Moreover, it was advisable to know precisely what else besides spores of the given species was present in the culture. Knowing this at the outset enabled one to discriminate more clearly later between swarm-cells and other objects. Bits of the capillitium and of the outer envelope of the sporangium sometimes closely resemble swarm-cells. No attempt was made to secure so-called "pure cultures", except to see that only one species of Myxomycete spores was present. Bacteria are considered indispensable to the development, if not to the germination of all species of the group. It was not necessary to introduce them into the cultures as Pinoy contended; they were always present, introduced with the spores. It was noted that

the bacteria in some cultures were quite different from those in others.

After the time of germination was approximately known the cultures were set at such times that the very beginning of the process might be observed. Observation of the living cultures was checked by study of stained slides, these in turn being interpreted by the living forms. It was found advisable to scrutinize the slides after the cultures were killed and fixed before staining, as occasionally certain details were plainer here than in the stained slides. Again, the stained slide was examined before the film of water from the washing had dried, experience proving that frequently there was a greater clarity in the slide at this time.

The stain constantly used was an acid fuchsin in alcoholic solution. Various other stains were experimented with, safranin, gentian violet, methylene-blue, orange G and Haidenhain's iron-alum-haematoxylin, but none gave as good results as the fuchsin. If the slides required clearing, they were slightly over-stained and then, when dry, were cleared and the stain thinned by placing for a moment in alcohol. The preparations were fixed and killed in one operation, the slide bearing the culture drop being inverted for a minute or so over a wide-mouthed bottle containing osmic acid, covered meanwhile by the inverted glass stopper of the bottle. After killing the slide was allowed to dry

and then stained. The stain was applied directly by a glass rod and after 30 seconds the excess was washed off under the tap.

In sufficient material or difficulties in the germination made the results less satisfying with some species than with others, complete observations only being possible when sets of cultures were carried through many times. However, in all cases the cultures were carried through to the point designated by Macbride as "small plasmodia" and by German writers as "myxamoebae" and in most cases true plasmodia were obtained, in a few cases being kept alive for 10 days or more without addition of nutrient media.

EXPERIMENTAL DATA

Introductory.

Germination was secured in all the species worked with and carried through to the plasmodial stage with all but one, the experiment ending at the myxamoeba stage in this case because there were no further spores obtainable. In only a limited number of species was complete germination obtained, the percentage of germinating spores varying from 5 to 90 percent.

While scrutinizing the cultures for evidences of emerging swarm-cells certain features of the spores which might have an influence upon germination were forced upon the

attention. In the first place, the dry spores differed in shape from the wetted spores quite frequently, being of varied shapes and exhibiting certain sharp angles which looked as if they might become lines of rupture when the swarm spores issued. Whether this was the case was not definitely determined, though it was observed later that some of the spores did rupture in a straight, smooth line. The wetted spores in all cases became spherical or nearly so. In the second place, there was a striking difference in the size of the spores of the same species, even in spores taken from the same sporangium. Mere size, perhaps would have no effect upon germination, but the striking difference in this particular suggested a possible difference in thickness of envelope which would affect its permeability. Even more important was the clear indication in some cases of immaturity which would unquestionably affect the germination, perhaps in more than one particular. This initial intimation of difference in power of germination was fully borne out by the results obtained in the cultures.

Another interesting feature which one needs to be on his guard against is the apparent appearance of swarm-cells in freshly made cultures. In not a few instances when cultures were examined immediately after the wetting of the spores several objects indistinguishable from non-flagellate swarm-cells were clearly visible. The natural inference is that some germination had taken place before the sporangia

were gathered and in a gradual drying out the swarm-cells had become encysted. If the culture had not been examined until some time had elapsed these would naturally have been taken as evidences of recent germination.

Another phenomenon observed at this pre-germination stage occurred in a single species only, but was suggestive of the changes going on within the spore at this time. While watching a culture of Enteridium rozeanum a single spore, in a field containing many spores, was seen to revolve on its axis at a moderate rate, turning three to six times in one direction, checking its movement, revolving a few times in the opposite direction, then stopping or revolving in the first direction, then coming to a rest. Changing the field, this same procedure was noted in other spores. There was no sign of a projecting flagellum, (this was verified by fixing, killing and staining the preparation); the movement was due to some interior force, probably correlated with movements of the swarm-cells as it lay within the spore.

The Emergence of the Swarm-Cell.

The emergence of the swarm-cell is dismissed in a few words by deBary, Lister and Macbride, but is a fascinating process to watch, with certain suggestions of mystery surrounding it. The statement usually made is that the spore-wall is

ruptured by the swelling of its contents, "which slowly emerge and lie as a nearly pellucid globule by the side of the empty spore-case." The spore-case is ruptured, indeed, but in most cases there is no visible sign of rupture while the swarm-cell is emerging or after it has emerged. First there appears a tiny bead upon the curving surface of the spore. This gradually increases in size until it nearly equals the spore. As seen under the lens it is, indeed, nearly pellucid, of a beautiful light green color, but with visible flecks or granules which show as the focus is changed. Later, when it has moved away from the spore, the empty case can not, in most species, be distinguished from the ungerminated spore. Apparently the swarm-cell passes through the spore-wall without leaving any sign of its passage.

While this is true of most species, it is not true of all those worked with. In the case of Badhamia lilacina, Badhamia rubiginosa and occasionally Didymium melanocarpum and Physarum notabile a definite line of rupture appears through which the swarm-cell on account of its color is plainly discerned. This line of rupture in these species is straight and extends over half or nearly half the circumference of the spore. As the pressure increases the slit becomes a v-shaped opening filled by the swarm-cell. At last the swarm-cell slips out much as the pulp of a grape slips out of its covering when pressure is applied. Some writers, having observed this method of visible emergence, conclude that this

is the usual procedure. Even in this case the valves, if we may so name the separate portions of the spore-case, usually come together after the escape of the contents and present an appearance hardly to be distinguished from that of ungerminated spores. While the turgid spore-cases are gay deceivers, the shrunken cases when dried and stained reveal a V-shaped openings or smooth lines of rupture which indicates a possible V-shaped openings at some stage. Sometimes the line of rupture is less regular, but in general a large class of species conform to this type of emergence.

Still another variation in the manner of emergence is presented by a few species in which the swarm-cell is decidedly larger than the spore from which it issures. Here the swarm cell apparently fills a circular opening while emerging, an opening almost as large as the total circumference of the spore. There is no suggestion of a slit or a V. Usually after emergence of the swarm-cell the wall seems to be miraculously renewed, but the empty cases can usually be distinguished by their color. In old cultures an occasional spore-case appears as a hemisphere with an irregular or ragged edge as if one half the spore had been torn away by the cell in its emergence. Illustrations of this type are Fuligo septica and Enteridium rozeanum.

Swarm-cells on emergence vary in size from 6 μ in Badhamia rubiginosa to 10μ. in Physarum notabile, averaging

8μ in diameter. In some cases the swarm-cells are distinctly smaller than the spores, in others distinctly larger, those of Enteridium rozeanum sometimes being one and one-half times the size of the spore.

Along with the swarm-cells, sometimes in advance of them, slime-balls issue from the spore-envelope. These were first noted and described by Cienkowski. They are little transparent globules with a slightly denser center. When abundant they cbud the drop of water and make exact observation of minute details difficult, if not impossible. Slime-balls were observed in cultures of the following species: Badhamia lilacina, Badhamia rubiginosa, Physarum sinuosum, Didymium melanospermum, Diderma testaceum, Diderma radiatum, Stemonitis fusca, Hemitrichia vesparium, Trichia decipiens and Trichia varia.

Jahn's Didymium type is based upon Didymium difforme, a species not accessible to the writer. Jahn states that the swarm-cells issue with the flagellum fully or partly formed and that the Physareae and Trichieae are of this type. Of the species worked with, only one, Diderma radiatum, showed the flagellum even partly formed as the swarm-cell emerged. Lister, in his "Introduction" makes no exception to the rule that the flagellum is formed after the swarm-cell has emerged from the spore-case. Certainly in the species of Physareae and Tricieae observed by the writer no swarm-cells emerged with the flagellum formed.

Jahn states that deBary studied the germination of **Didymium** and discovered that a division of the amoeba takes place within the closed spore membrane. This division takes place in other species and seems a sounder basis for types than Jahn's presence or absence of flagella at emergence. In six species the emergence of two swarm-cells from one spore envelope was clearly observed, in two species in several different cultures of different ages. The most striking cases were in Badhamia lilacina and Badhamia rubiginosa. In the former a bluish bead first appeared on the rim of the spore. This had a reddish point at its center. This bead grew until it reached a diameter of 6 μ then detached itself from the spore. Itwas followed almost immediately by a second bead which started with a diameter of 2 μ or less, swelled until its diameter was 6 μ to 7 μ and then detached itself from the spore. These two swarm-cells had no suggestion of a flagellum. This process was observed in three different cultures. There was one variation, the two swarm-cells in one case issuing, not one after the other, tandem-fashion, but at the same time from a distinct cleft in the spore a short distance from each other. In early cultures of Badhamia rubiginosa it was noticed that recently issued swarm-cells were always in pairs and very small in comparison to the size of the spores. Later two nuclei were seen in several ungerminated spores. Later still the actual emergence of two swarm-cells from the same spore was witnessed. In one case the division was not complete and the two swarm-cells were still united after

emergence, presenting an appearance similar to that of a peanut with a rather deep constriction. The division was completed soon after the spore envelope was left. The other species in which two swarm-cells were observed to come from the same spore were Physarum sinuosum, Physarum notabile, Diderma radiatum, Hemitrichia serpula and Trichia decipiens. Two species of Didymium, D. melanospermum and D. nigripes var. xanthopus were observed in many cultures without arriving at a satisfactory conclusion on this point.

The elapsed time between the wetting of the spores and the observed emergence of the first swarm-cells varied from a minimum of 25 minutes with Fuligo septica to a maximum of 20 hours with Mucilago spongiosa. The time for each of the species observed will be given in Table 1. While there are differences in the average time for different species it seems probable that in certain cases the times shown in the table are likely to need revision. Some species were carried through many sets of cultures, up to 10 and even in one case to 15 sets of six cultures each, while in a few cases, owing to extremely limited material, only one set of six cultures was made.

A very noticeable phenomenon is the long period over which emergence of swarm-cells continues. In the case of one set of cultures of Physarum notabile, Macbride, the first escape of a swarm-cell from its envelope was noted at six and one half hours from the wetting of the spores. Germination was

meagre, but fairly continuous up to the ninth day, when suddenly the culture was filled with recently issued swarm-cells Even on the 13th day some germination was observed. This was doubtless unusual and an extreme case, but the rule was to find in cultures three days old or older every stage from the emerging cell to the myxamoeba. This was true even when the spores were taken from one sporangium.

Formation of the Flagellum.

The swarm-cell emerges in the form of a sphere or near-sphere. It lies near the spore in a resting state, at least quiescent, for a period varying from a few minutes to several hours. No distinct amoeboid stage with characteristic amoeboid movements apart from the formation of the flagellum was observed except possibly in the case of Leocarpus fragilis and even here there was room for doubt. Observation of a given culture could not be continuous and such movements may possibly have taken place without being noted. Other movements were occasionally observed. Both at this stage and later non-flagellate swarm-cells were seen to advance by a spasmodic hitching or twitching movement which carried them forward about half their diameter with a recoil about half as great. In the cases where amoeboid movement was possibly connected with this stage the movement was never vigorous and no slender pseudopodia were projected.

The definite amoeboid movements seen were connected with the formation of the flagellum. This process from beginning to end was observed in several species. While alike in general features the process varied in certain particulars as seen in different species. Whether the differences were due to the species observed or to the limited number of observations can only be determined by further experimentation.

As seen in a culture of Fuligo septica four days old the process was as follows: Watching a quiescent spherical individual apparently recently issued from the spore the writer saw it begin to change its shape. First a slight protuberance appeared at one point on the sphere. After a little this was pushed out into a point. Then the whole body elongated until, except for the point, it resembled a sausage. After an interval of rest movement was resumed. While the movements involved change of outline they did not seem like true amoeboid movements, but rather due to violent activity of the body as a whole, properly described as twistings and jerkings of the whole organism. These movements brought about a partial projection of the flagellum which was plainly visible under the power used (magnification of 640 diameters). The flagellum, after being thus pushed out, was withdrawn.

The twisting and pumping was resumed. At this point a change was noticed. The base grew wider until the body was shaped like a wide-based pear. The wide base was seen to contract, pushing the body-content forward into the anterior portion and holding it there by a deep constriction, like that between the links of a chain of sausage or that between the thorax and abdomen of ants. The anterior portion, now swollen to nearly twice its normal size, contracted and forced the flagellum out further than the earlier attempt. This was accompanied and followed by convulsive jerks, twistings and corkscrew movements. The contortions continued for some time, becoming less violent, with restperiods between. When the flagellum was fully formed the spherical shape was resumed with the flagellum floating nearly upright. The whole performance consumed 12 minutes.

In Badhamia lilacina the beginning of the process was identical. A spherical swarm-cell slowly changed its shape but did not become nearly so elongated as in the case of Fuligo . There was no observable constriction. Instead, something of the same effect was produced secured by a pronounced bending to one side, the anterior portion pressing strongly against the wider base and thus pushing the body content forward. There were some corkscrew contortions and some spinning on the axis. Again, the spherical shape was resumed on the completion of the flagellum.

In Didymium melanospermum there was no visible constriction and the shape assumed was much like that of the swarm-cell of Badhamia. The commonest, most often repeated movement was one in which the free tip of the anterior portion traced a circle of some size, the base fixed and the body tracing the form of an inverted cone. The outstanding difference, however, was the fact that when the flagellum was completed the spherical shape was not resumed, but the sausage-shape or elongated pear-shape was retained.

In Enteridium rozeanum an extreme elongation of the body precedes the flagellum formation, so that it measures 12 μ by 2 to 3 μ. The contortions do not seem to be more violent than in the other species observed, though Jahn states that they are distinctly more violent than in Didymium difforme.

The First Flagellate Stage.

After the flagellum is formed the swarm-cells enter upon a stage which may endure a few hours only, but which in some cases lasts for a couple of days. It is practically impossible to determine the exact duration because the emergence of fresh swarm-cells and their transformation into flagellate forms goes on usually for at least two days and sometimes for more than a week.

A great variety of forms is assumed, some species being less stable in this particular than others. The term "pyriform" has been in general use ever since the swarm-cells were first observed and perhaps is a s descriptive of general

type as any one term may well be. In notes made at the time of the observation it occurs as frequently as any other. The writer's preference is for "fig-shaped." Other descriptive terms, sometimes accompanied by rough sketches in the notes, are "sausage-shaped", " ellipsoid", "oblong in sense used in descriptions of leaves," "ovoid," "triangular," "flatiron-shaped." In some species all these forms are assumed, in others only a part of them. These changes of shape are frequently spoken of as " amoeboid", but in the writer's experience there is rarely any exhibition of pseudopodia at this stage and it seems better to reserve that term for movements where pseudopodia are concerned. Apparently the spherical form is basic, even in the species where it is least often assumed, for from it all the other forms come and to it all the forms at last return. Badhamia rubiginosa, Badhamia lilacina, Hemitrichia vesparium for the most part maintain the spherical form; Diderma radiatum, Cienkowski reticulata, Arcyria incarnata and Trichia decipiens show a preponderance of oval, ovoid, oblong or triangular shapes; while the other species observed exhibit the pear or fig shape most frequently.

Biflagellate swarm-cells at this stage were noted in the following species:- Fuligo septica, Badhamia lilacina, Didymium melanospermum and Diderma radiatum. In every case the two flagella were on the same end of pointed or

elongate forms, but in one Diderma culture several spherical swarm-cells had flagella extending from opposite sides of the sphere, like wings. The cells were rather small and occurred in a culture 21 hours old, so there is a possibility that cell division had taken place, and that these forms belong to the second flagellate stage.(See Table 2.)

Certain movements, if not precisely limited to this stage of development, are at least characteristic of it. Chief among these is a continuation of some of the movements associated with flagellum formation long after that organ has reached its extreme extension. The broad base of the pear-shaped form rests upon the glass of the slide, the upper part of the body moving with the flagellum so that the surface of in inverted cone is traced, creating a little vortex and in some cases visibly drawing bacteria to a point where they are ingested by the swarm-cell. Sometimes, it is true, this movement is associated with progression, the hold on the slide being released, but in the great majority of cases observed there was no progression. Occasionally this movement is varied by a snapping and curling of the flagellum. This movement , minus the body participation is also exhibited by the spherical forms. In a few cases the movements irresistably suggested a fisherman "whipping" a troutbrook. Darting movements with the flagellum leading are also common. Sometimes

a circling movement is indulged in, the circles being of both large and small radius. In notes taken at the time this movement of the swarm-cells of Fuligo is likened to that of a dog chasing his tail. This note recurs in connection with movements of cells of Physarum notabile, Didymium melanospermum, Cienkowskia reticulata, Stemonitis fusca and Hemitrichia versparium. Spinning movements were also observed. There was a conspicuous absence of movement through the water by "the rapid lashing of the cilium."

Cell Division.

In Ceratiomyxa, an exosporic species, Jahn reported that the amoeba which issues from the spore divides into four separate swarm-cells which divide again before any flagellum is formed.

In six endosporic species (named in the section on Emergence of the Swarm-cell) a definite cell division takes place within the closed spore envelope. Here we seem to have a connecting link between the methods of the endosporic and exosporic species.

The occurrence in very young cultures, soon after swarm-cells have begun to emerge, of swarm-cells hardly more than half the normal size leads to the suspicion that a cell division sometimes occurs after the emergence and before the formation of the flagellum.

In most of the species observed, this division takes place only after a flagellate stage of uncertain duration has been followed by a resumption of the spherical form and the withdrawal of the flagellum. This last process is called by the German writers "rounding off" (runden sich ab).

The actual cell division was several times observed in this and succeeding stages. The spherical swarm-cell changes its shape, the outline becoming elliptical or oblong, a constriction a pears in the middle section, becomes deeper and deeper until only a thread unites the two parts; this finally gives way and the two daughter cells begin a separate existence, after a time producing flagella. The whole process occupies from thirty seconds to a little over a minute and so is easily missed. The daughter cells are at first decidedly smaller than the mother cells, but in time reach the size of the original issue from the spore.

Macbride (9) states that division beyond the first of course takes place only in suitable nutrient media. In the experience of the writer it has gone through to the fourth generation both in tap water and in distilled water in some cases. In a culture of Enteridium 12 days old, which had exhibited myxamoebae and plasmodia in their due season, a large number of flagellate swarm-cells, not over one-fourth the size of the original issue, were found. Occasionally the

formation of the flagellum in these daughter spores was observed.

Apparently this division did not take place in all cases, but the "rounding off" was followed immediately by the myxamoeba stage.

Second flagellate stage.

While this stage in some ways resembles the first flagellate stage there are other differences than that of initial difference in size. When the shape is not spherical it is less pointed than in the first stage and more regular. The elongated forms are best described as ellipsoidal. Frequently they are angular , presenting an outline almost that of a rectangle longer than broad, but somewhat narrower at the flagellate end. The movements are more frequent and more rapid. A spinning movement, continued for some time, and occasionally reversed was quite common. Notes taken at the time describe this as like the motion of a pinwheel. Dancing movements were also very common. Dartings to and fro with frequent change of direction were noted. In this darting motion the broad end was in the lead and the flagellum trailed behind like a trailing piece of string or the tail of a horseshoe crab. A less frequent movement, always associated with an elongated, worm-like shape, was that of creeping on the glass slide. This was never observed in the first flagellate stage, but only after division had taken place. Perhaps it is even more characteristic of still later stages, having been observed

in cultures five, six and seven days old and in one case in the swarm-cells germinated from cysts on the 23rd day. Notes taken at the time of observation describe the form in various terms. Now it is called slug-like, now larva-like and again caterpillar-like in form and movement. It progresses by "undulating movements, the flagellum upraised and advanced." In Stemonitis fusca the movement is described as "waddling like a duck" In cultures of Diderma testaceum and Enteridium rozeanum it was noted that the flagellum and anterior portion of the body were elevated and waved with a weaving motion like that frequently seen in caterpillars. In these cultures the creeping forms predominated. In both the last named species biflagellate forms were observed quite different from those previously mentioned. The second flagellum came from the posterior end of the body and trailed behind apparently as a dead load. It was about the length of the normal flagellum, but slightly thicker. Continued observation demonstrated the fact that this second flagellum was more easily retracted than the normal one. In one case it was distinctly forked at the end. In the stained preparation the body was shortened and the rear flagellum frequently disappeared. Finally, it was retracted almost completely while under observation, thus demonstrating its nature as a pseudopodium, even though of a peculiar type. (See Table 2 and Figure 8.)

Again the loss of the flagellum and the resumption of the spherical form are noted. Here one occasionally notes movements which may be connected with the withdrawal of the flagellum. Without any progression twitching or jerking motions becomes almost continuous. At times, when two cells are near each other it looks as though they were indulging in a jig; at other times two gamecocks sparring at each other are irresistibly suggested. It was not until it was realized that these particular movements bore no relation to the nearness of the other cells and that they characteristically occurred just before the loss of the flagellum that they were connected with that process.

Microcysts.

It is just at this time of loss of flagellum and "rounding off" that microcysts usually appear if at all. Earlier writers generally state that the occurrence of cysts depends on a gradual drying out of the culture. Lister (8) says: "In all cultivations of germinating spores, a number of swarm-cells , after a short period of activity, withdraw the flagellum and become encysted in globular form. After being dried and rewetted, the contents burst and the membranous wall, which remains an empty, hyaline sac, and emerge to resume their activity." In our experience no gradual drying out was necessary to their formation; nor did they occur in all cultures or with all species; nor was drying out and rewetting a necessary

preliminary to their germination. Some, kept continually in a waterdrop in a moist chamber, germinated after the lapse of several days; others were allowed to dry out and, after remaining dry for several days were rewetted; these usually germinated within 12 hours from the rewetting. The germination resembled that from a spore; some of the cells issuing were no different in appearance from those emerging from a spore, others assumed almost at once the worm-like form previously described.

The Myxamoeba stage.

While some swarm-cells become cysts, most of them, whether without dividing or after one or more divisions, come to the myxamoeba stage. Here a striking change is to be observed. While growth in size may be observed in cells of the second flagellate stage, these forms never surpass and rarely equal in size the original swarm-cells. Suddenly in place of forms 6 μ to 8 μ in diameter the culture teems with forms 16 μ to 20 μ in diameter. This surprising increase in size is accompanied, probably caused by a change either in the substance of the cell or in the arrangement of its various constituents. The granular and hyaloplastic elements are differently arranged and the proportion of the latter is greatly increased. Stained preparations show that this change, at least in its beginnings, is under way before the flagellum is lost, but is not complete until the flagellum has entirely

disappeared. Two distinct types of movement, both amoeboid in character, were observed. Certain relatively small forms, not exceeding 8 μ in length, displayed the movements called by Schroeter " Protean". These affected principally a rather elongated form associated with the projection of numerous rather slender pseudopodia, first in one direction and then in another. The form was constantly changing and streaming was frequently observed. Even when at rest the form was very irregular , quite like the figures presented by Cienkowski and deBary. The forms most frequently noted, however, were of a very different type, the one Schroeter calls the "Limax type". Here there was no projection of slender pseudopodia in many directions, but a slow steady advance along a relatively broad front of the hyaline ectoplasm which widened as it advanced until the shape resembled that of a fan. The tip suggested a photograph of the tip of a lava-flow as it reaches nearly level ground. The distinction between the granular and the hyaloplasm portion was very definite. Gradually the granular substance flowed into this expanded portion and usually after a moment of rest the movement was continued. There was a constant tendency to assume a circularoutline as seen under the lens and this was the characteristic resting form. In stained preparations this tendency was accentuated. Some of these myxamoebae were seen to be in contact with the glass slide, but for the most part they were observed at or near the surface of the drop. The general assumption seems to have

been that they creep about on the substratum, whether of glass or of some solid media. No mention of this movement at or near the surface has been met with, though Woronin calls attention to the fact that the zoöspores of Chromophyton rosanoffii are able to creep on the surface of the water and Pfeffer (10) says that this is not surprising, " since the surface tension film is capable of affording the required resistance."

The Plasmodial Stage.

While in a real sense this stage is distinct from the preceding one, the two overlap in such a way that the distinction is frequently blurred. If size is made the criterion we are confronted with the fact that some myxamoebae are larger than some plasmodia; if we make the number of nuclei the distinguisning characteristic we are often baffled by our inability to locate any nuclei in the plasmodia; if we make fusion the test, we are faced with the fact that in certain species the large flagellate swarm-cells which precede the myxamoeba stage are demonstrably the result of the fusion of smaller swarm-cells. Yet in general the distinction holds good and serves a useful purpose. Plasmodia are always the result of fusion of myxamoebae; they are usually larger; and, if the nuclei can be made out are seen to be multi-nuclear. Moreover, there are ill-defined, not easily described differences springing out of this combination of characters which,

after some experience, makes the discrimination easy and natural.

Myxamoebae approach each other, touch and draw back. This contact without fusion may be repeated a number of times, but at last a contact is established and maintained. Sometimes two, more often three were observed thus in contact, still maintaining separate identity. Gradually an envelope of ectoplasm surrounds and binds together the different individuals of the group, the outline as defined by the ectoplasm being circular. At this stage the original limiting membranes and the separate nuclei are clearly visible in stained preparations. ~~Gradually the membranes disappear and all signs of the original limiting membranes and the separate nuclei are clearly visible in stained preparations.~~ Gradually the membranes disappear and all signs of the original outlines are lost. During this fusion there are no visible movements, no extrusion of pseudopodia.

After a time the newly formed plasmodium begins to move about in characteristic amoeboid fashion, extruding pseudopodia and drawing them back, sometimes proceeding with Limax movements, sometimes extending a pseudopod and capturing bacteria. This last is frequent at this stage. After the ingestion of bacteria ~~and~~ the plasmodium usually remains quiescent for a time before resuming its activity. During this

quiescence a spherical or ellipsoid form is assumed which makes it difficult to distinguish from large myxamoebae.

The formation of plasmodia was made the "terminus ad quem" of the present study, but several cultures were carried far beyond this stage and several interesting facts were noted. Despite the ingestion of bacteria the plasmodia thrived better and lived longer if a nutrient, like dextrose, was added to the culture in weak concentrations. Occasionally macrocysts were formed, both when the culture was allowed to dry out and when the drop and the moisture in the chamber were maintained. These cysts have walls which enable them to live through a protracted drought. Sometimes fresh germination of spores continued long after plasmodia were formed, in one case up to the 20th day. Sometimes plasmodia were seen to ingest swarm-cells of different ages and sizes and even spores and empty spore-envelopes. Sometimes, after germination had ceased and no intermediate forms were present, the plasmodia were associated with numerous, flagellate, minute and exceedingly active swarm-cells.

The limitations of drop cultures force a separate treatment of the normal development of plasmodia and the formation of sporangia.

CONCLUSIONS

The experimental results are interpreted in the light of the reported results of previous investigations. Many of these reports concern European species and are accessible only in German or French. The present experiments are confined to species found in America, but a sufficient number are identical with those reported on in the European experiments to afford a useful check. In most cases the European investigators worked with a very small number of species and directed their attention to certain particulars - flagellum formation, nuclear division, conditions under which germination takes place. Constantineanu, indeed, carried on continuous observations ~~tion~~ of 14 species and determined the elapsed time from the wetting of the spores to the first emergence of the swarm-cells and the first appearance of plasmodia in the cultures, but he did not describe the appearance or the movements during any stage of the development. Jahn observed and described the flagellum formation in two species, but his attention was focused upon the accompanying mitosis.

Present experience enforces the conclusion that cultures of any species must be repeated again and again before any statement is justified. Cultures from the same gathering of fruiting forms and even cultures of spores from the same sporangium act differently under identical laboratory

conditions. Jahn's statement that certain species generally did not germinate and Constantineanu's statement that these same species did germinate readily in distilled and tap water are both exemplified in the experience of the present writer. It is well also to remember that water, and even distilled water, is not the same at all times and in all places. The discrepancies in results might possible be explained in some cases if the pH values of the water used were known.

Comparison of present observations with the general statement of Jahn concerning the emergence of swarm-cells with the flagellum already formed and Lister's observations, repeated by the present writer, on the ingestion of bacteria by swarm-cells, with the statement of deBary that food is taken only in a fluid state or in solution, suggests the need of adequate observation of many species before general statements are indulged in.

The experiments reported in the preceding pages show that there is far greater latitude in the conditions under which germination takes place than is stated by most authors and completely corroborates the statement of deBary. As Kuester (27) points out, in the case of myxomycetes there is no question of "pure cultures," since bacteria are indispensable to the normal development, if not to the germination of

the spores. Certain writers, notably Pinoy, insisted on the need of introducing bacteria. As a matter of present experience, these were always introduced with the spores, being present either upon the spores themselves or upon the spore-cases.

It was surprising and pleasing to find in the drop culture an almost complete absence of the fungi which so bedevel cultures on nutrient agar. In over eight hundred preparations these occurred less than a dozen times. It was thought necessary to familiarize one's self with the forms of zoospores of the Flagellatae and Chytridiaceae, but none of these occurred in the cultures. Planarians were usually present, but these were easily distinguished from the swarm-cells.

The latitude in temperature and acidity proved to be quite extensive. Germination of nearly all the species was secured in temperatures ranging from 18 to 28°C. Several species tested germinated in solution ranging from pH 4 to pH 7. Doubtless the optimum was often missed, but temperatures between 20° and 25°C. and pH values between 5.5 and 6.8 proved very good common denominators.

Under these average conditions the striking differences in germination and development observed were seen to be due primarily to differences within the spores themselves and

not to external causes. These differences, as suggested by the appearance and behavior of the spores, were due to differences in the maturity or differences in the nutrition content of the spores.

In most of the literature relating to the germination of spores of slime-moulds no distinction of stages is made in describing the forms assumed and the movements made by the swarm-cell. The present experiments show that even within the swarm-cell period there are distinctive shapes and movements associated with the first swarm-cell stage different from those exhibited after division has taken place.

The observations of elapsed time between the wetting of the spores and the first observed emergence of swarm-cells give results fairly parallel to those obtained by Constantineanu, but cover more species, and for the most part, different species.

The movements were seen to be far more varied than the literature prepared one for . In particular the movements of the flagellum not connected with locomotion impressed the observer.

The temporary character of the flagellum and its similarity to a pseudopodium were strongly impressed upon the observer. Instead of being the outstanding mark of a swarm-cell, it was present less than 50 percent of the swarm-cell

period and where cell division took place more than once was produced and withdrawn several times. When this fact is brought into relation with the not infrequent appearance of two flagella on oneindividual and the observed pseudopodial relation of one flagellum in cases where this second cilium appears at the opposite end of the body, a conviction grows that these are two alike innature if not entirely so in function and permanence, the shape assumed being due to the same properties in protoplasm exhibited under different degrees of surface and internal tension.

In the species studied, Jahn's statement concerning the emergence of swarm-cells in certain large groups with the flagellum already formed was not confirmed. It is true that he worked with different species from any observed by the writer and that we have no reason to question his statements concerning these species.

The emergence of two swarm-cells from one spore envelope observed in six species, does not seem to have found previous record in the literature.

The tendency of swarm-cells and plasmodia alike to assume the spherical form at various times and under various conditions of internal or external shock or stress is very pronounced. The resting stages before and after cell division,

as well as those before and after flagellum formation, come to be associated by the observer with those more protracted resting stages known as cysts. The changing form and the projection of flagella are dynamic phenomena; the spherical form is static and basic, due to the surface tension as in other liquids segregated in minute quantities. Here we find ourselves face to face not merely with the debated question whether these forms are animal or vegetable, but also near to the very line between the living and the non-living. This protoplasm, " the material basis of life," shows itself akin in its static phases to other liquids, but in its dynamic phases it seems to hold all the possibilities of all the various types of life which the universe displays.

The definitely limited work here reported on invites further study along two distinct lines: first, the securing of data parallel to those here presented for an increasing number of species of slime-moulds that a broader basis for generalization may be secured; second, the intensive study of selected species that the life processes and physiological reactions may be more clearly and intimately known.

SUMMARY

This study is limited to endosporic fungi and to that part of the life cycle included between the ripe spore and the young plasmodium.

The materials for study consist of 23 species illustrating four of Macbride's five sections of endosporic myxomycetes. The identification of the species was checked by sending duplicates for identification to the Farlow Herbarium of Harvard University and to Professor Macbride at Iowa University.

The method used was that of observation of living cultures in hanging drops of water taken from the tap, but of a known pH value (5.5 to 6.8), at a room temperature varying from 20 to 25°C., cultures were prepared in sets of six, usually all the spores being taken from one sporangium. These cultures were repeated, usually several times. Observations of living cultures was checked by study of stained preparations made at regular intervals.

Germination was secured in all species worked with and development to the plasmodial stage was observed in all but one.

In the emergence of swarm-cells as ordinarily observed the cells appear to issue from the spore without any visible opening through which it passes. In a few cases such

such an opening does appear; in other cases the rupture of the spore envelope is revealed only in old cultures and mostly in stained preparations.

In all species except Diderma radiatum the swarm-cells observed issued without flagella; in six species two swarm-cells emerged from each spore.

The elapsed time from the wetting of the spore to the first observed emergence of swarm-cells varied from 25 minutes to 23 hours. Swarm-cells continued to emerge for several days.

The production of the flagellum was observed in cultures of several different species. This was accompanied by a change from the spherical to an elongated form and was accomplished by a variety of violent contortions varying slightly in the different species observed.

These flagellate forms do not preserve a constant shape, but different species do affect a more or less prevailing form. The forms most characteristic of this stage exhibit also some movements which are characteristic, particularly those of the flagellum in securing food. In this stage biflagellate forms occur.

Cell division in certain species takes place before the swarm-cells leave the spore envelope; in a few cases the division probably takes place soon after the swarm-cell emerges and before flagellum-formation takes place; in most cases it comes after the flagellum has been formed, used and withdrawn.

After division has occurred the daughter cells extrude flagella and take on shapes and exhibit movements different in some respects from those exhibited by the original swarm-cells. For a time they are also of a smaller size. Biflagellate forms with the two flagella on opposite ends of the body are fairly common at this stage. Occasional fusions of these cells to form larger swarm-cells are observed.

These swarm-cells eventually withdraw their flagella and take on the spherical shape, sometimes maintaining this for a number of hours and forming a distinctive feature of cultures.

A remarkable change then occurs. The size increases greatly and the general appearance undergoes a marked change. Not only the size, but also the consistency and arrangement of elements in the body is strikingly different. True amoeboid movements are displayed and bacteria are ingested more markedly than in previous stages. This myxamoeba stage lasts from two to four days. At this time cysts are frequently formed.

These myxamoebae fuse to form plasmodia characterized by larger size and plural nuclei, but exhibiting much the same appearance and movements as the myxamoebae. Macrocysts are formed at this stage, with double walls.

Present results warrant the statement that there is a great latitude in the conditions under which slime-moulds will germinate. There is no need for nutrient solutions, no need for the introduction of bacteria, no need for great care in excluding other organisms or in controlling acidity or temperature.

Under the conditions of observation it seems clear that the striking differences in germination and development are primarily due to causes within the spores and not to external conditions.

The time of emergence of swarm-cells averaged less than in the experiments of Constantineanu and the extremes were much closer together.

The temporary character of the flagellum and its behavior in certain biflagellate forms leads to the conclusion that it is more akin to the pseudopodia than has been commonly thought.

Jahn's generalization upon the emergence of the swarm-cells in large classes of slime-moulds with the flagellum already formed is challenged on the ground that he presents no proof to cover his statement and that slides at hand disprove the truth of the general statement.

LITERATURE CITED.

(1) Bary H. A. de
1887. Comparative Morphology and Biology of the Fungi, Mycetozoa and Bacteria. Eng. Trans. of "Vergleichende Morphologie und Biologie der Pilze, Mycetozoen und Bacterien. Leipzig. 1884.

(2) Cienkowski, L.
1863. Zur Entwickelungsgeschichte der Myxomyceten, in Pringsheim's Jahrbuecher fuer wissenscaftlicher Botanik III.

(3) Constantineanu, J. C.
1906. Ueber die Entwickelungsbedingungen der Myxomyceten , in Annales Mycologici IV: pp. 495-540.

(4) Durand, E. J.
1894. Notes on the Germination of Enteridium rozeanum. Bot. Gaz. XIX:89-

(5) Jahn, E.
1904. Myxomycetenstudien III: Kernteilung und Geisselbildung bei den Schwaermern von Stemonitis flaccida Lister, Berichte der deutschen botanischen Gesellscaft XXII: 84-92.

(6) ———
1905. Myxomycetenstudien IV: Die Keimung der Sporen Ber , d.d. bot. Gesell. XXIII:489-497.

(7) Link, H. F.
1833. Handbuch zur Erkennung der nutzbarsten und am haufigsten verkommenden Gewaechse. III.

(8) Lister, Arthur
1911. Mycetozoa . 2nd ed.

(9) Macbride, Thomas H.
1922. North American Slime-Moulds.

(10) Pfeffer,
Physiology of Plants. Eng. Trans.

(11) Plenge, Henrique
1899. Ueber die Verbindungen zwischen Geissel und Kern bei den Schwaermerzellen der Mycetozoen und bei den Flagellaten und ueber die an Metazoen aufgefundenen Beziehungen der Flimmerapparate zum Protoplasma und Kern. Verhandlungen Naturhist. -- Med. Vereins, Heidelberg.N.F.VI:217-275.

(12) Pinoy, E.
1902. Necessite de la presence d'une Bacterie pour obtenir la culture de certain Myxomycetes. Bull. Soc. Mycol. France. XVIII:288-289.

(13) Schroeter, J.
1889. Myxomycetes, in Engler und Prantl, Natuerlichen Pflanzenfamilien.

(14) Vuillemin, P.
1910. The Myxomycetes. Rev. Gen. Sciences Pure Et Appl. XXI:432-443, 473-484.

(15) Webb,
Germination of the Spores of Certain Fungi in Relation to Hydrogen-ion Concentration. Ann. Mo. Bot. Gard. VIII:283-341.

(16) Woronin, M. S. and Famintsin, A. S.
1873. Ueber zwei neue Formen von Scleimpilzin in Memoires de L'Academie Imperiale des Sciences de St. Petersbourg.

LITERATURE REFERRED TO.

(17) Ayres, H.
1898. Methods of Study of the myxamoebae and plasmodia of the Mycetozoa. Journ.Ap. Micros. I:1-3, 15-17.

(18) Buxbaum, J. C.
1721. Enumeratio Plantarum

(19) Celakovsky, L.
1877 . Ueber den dreifachen Generationswechsel der Pflanzen. Sitzungsber. K. Boehm. Gesell, Wissensch . Prag. die Plasmodien der Myxomyceten. Flora 76: 182-244.

(20) Clifford, J. B.
1897. Notes on some physiologicalproperties of a myxomycete plasmodium.Ann. Bot. 11:179.

(21) Dillenius, J. J.
1719. Catalogus Plantarum circa Cissam nascentium.

(22) Fries, Flias M.
1817. Symbolae Gasteromycetum
1818. Observationes Mycologicae
1820. Systema Mycologicum

(23) Harper, R. A.
1900. Cell and nuclear division in Fuligo varians. Bota. Gaz. 30:217-251.
1907. Figures produced by ptotoplasmic streamings in fungi and slime-moulds. Sci. N.S.25: 282.
1908. Progressive cleavage in Didymium. Sci. N.S. 27:341

(24) Harshbarger, J. W.
1901. Observations upon the feeding plasmodia of Fuligo septica. Bot. Gaz. 31: 1.
1902. Distribution of nuclei in the feeding plasmodia of Fuligo septica. Journ. Mycol. 8: 158-160.

(25) Hilton, A. F.
1910. On the study of Mycetozoa. Journ. Quekett. Micros. Club. 2: 55-60

(26) Klebs, G.
1900. Zur Physiologie der Fortpflanzung, einiger Pilze. Pringsheim's Jahrb. Wissensch. Bot. 35: 80-203.

(27). Kuester, Ernst
1913. Die Kulture der Microorganismen. B.G.Teubner. Leipzig- Berlin. 2nd ed. 1913.

(28) Kusano, S.
1909. Studies of the Chemotactic and other reactions of the swarm-spores of Myxomycetes. Journ. Col. of Agr. Imp. University of Tokyo. 2: 1-83. (Bot. Centralbl 114: 383-385.

(29) Lendner, A.
1902. Essai de Cultures de Myxomycetes. Bul. Herb. Boisier LI. 2:336.

(30) Massee, G.
1892. A monograph of the Myxomycetes. London (Methuen) 1892.

(31) Melhus, I. E. and Durrell, L. W.
1919. Studies on the Crown Rust of Oats. Iowa Agr. Exp. Sta. ResearchBul. 49:113-144.

(32) Micheli, P. A.
1729. Nova Plantarum Genera

(33) Miller, C. C.
1898. The aseptic cultivation of mycetozoa. Quart. Journ. Micros. Soc. 41:43-71.

(34) Nadson, A.
1899. Des Cultures du Dictyostelium mucoroides. Scripta Botanica. fasc. XV. St. Petersbourg.

(35) Olive, E. W.
1898. Affinities of the Mycetozoa. Proc. Ind. Acad. Sci. 1898.:169-174.
1907. Evidences of Sexual reproduction in the slime-moulds Sci. N.S. 25: 266.
Cytological studies on Ceratiomyxa. Trans.Wis. Acad. Sci. 15:753-754.

(36) Persoon, C. H.
1795. Observationes Mycologicae Pars. prima
1796. " " " secunda
1801 Synopsis Methodica Fungorum

(37) Potts, G.
1902. Zur Physiologie d. Dictyostelium mucoroides Flora B. 91.

(38) Rostafinski, J.
1873. Versuch eines Systems der Mycetozoen.
1875 Sluzowce Monografia 432 pp. 13 pls. Paris. 1875. Supplement. 43 pp 1 pl. 1876.

(39) Schrader,H. A.
1797. Nova Genera Plantarum.

(40) Strasburger, E.
1878. Wirkung des Lichtes und der Waerme auf Schwaermsporen. 75 pp. Jena (Fischer) 1878.

(41) Tieghem, P. Van.
1876. Neurelles Observationes sunle Devellopement du Fruit et sun.

(42) Ward, H. M.
1884. The morphology and physiology of an Aquatic Myxomycete. Quart. Journ. Micros. Sci. 24: 64-86.

(43) Sturgis, Wm. C.
1907. The Myxomycetes of Colorado. Col. Col. Pub. Sci. Ser. Vol. XII. No. 1, pp. 1-43
1913. The Myxomycetes of Colorado. II. Col. Col. Pub. Sci. Ser. Vol. XII, No. 12. pp. 435-454.

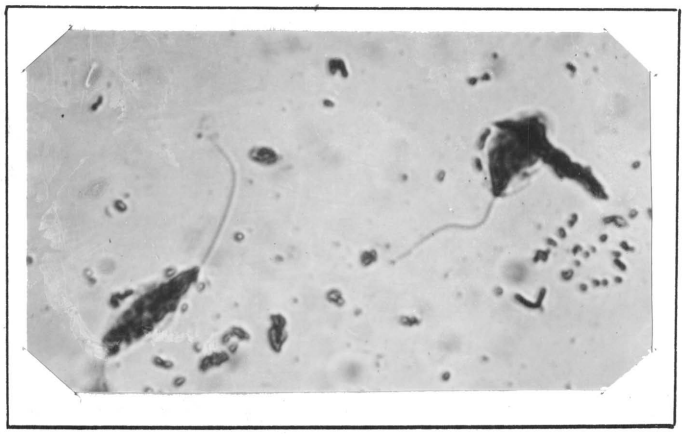

Fig. 1. Typical swarm-cells of Didymium melanocarpum of the first flagellate stage. The "indispensable" bacteria appear in this and all succeeding figures. X 1000

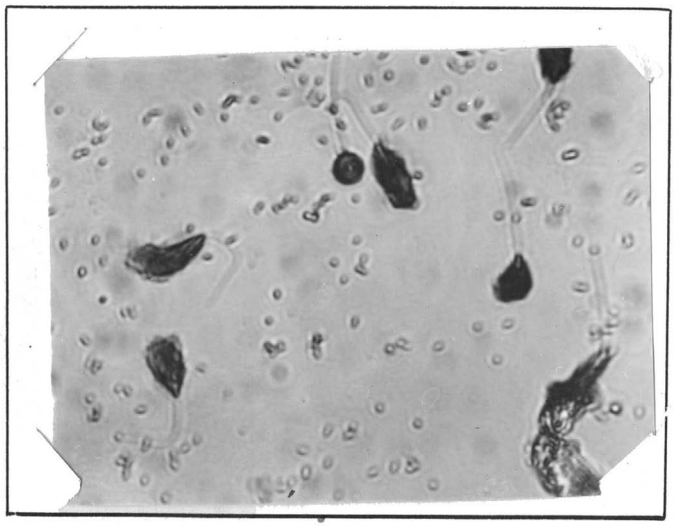

Fig. 2. Swarm-cells of Didymium melanocarpum of the first flagellate stage showing spherical cells and variations of the pyriform type. X 800

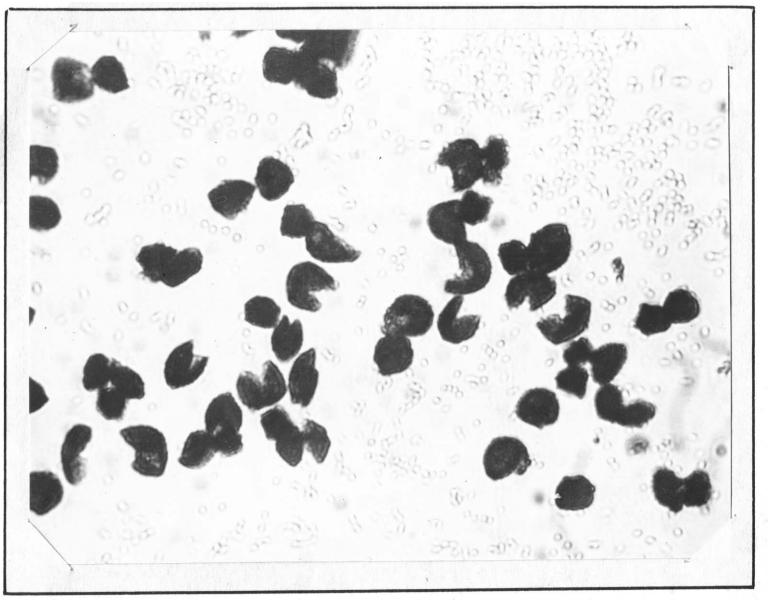

Fig. 3. Empty spore envelopes showing characteristic rupture of the envelope in Didymium melanocarpum. X 450

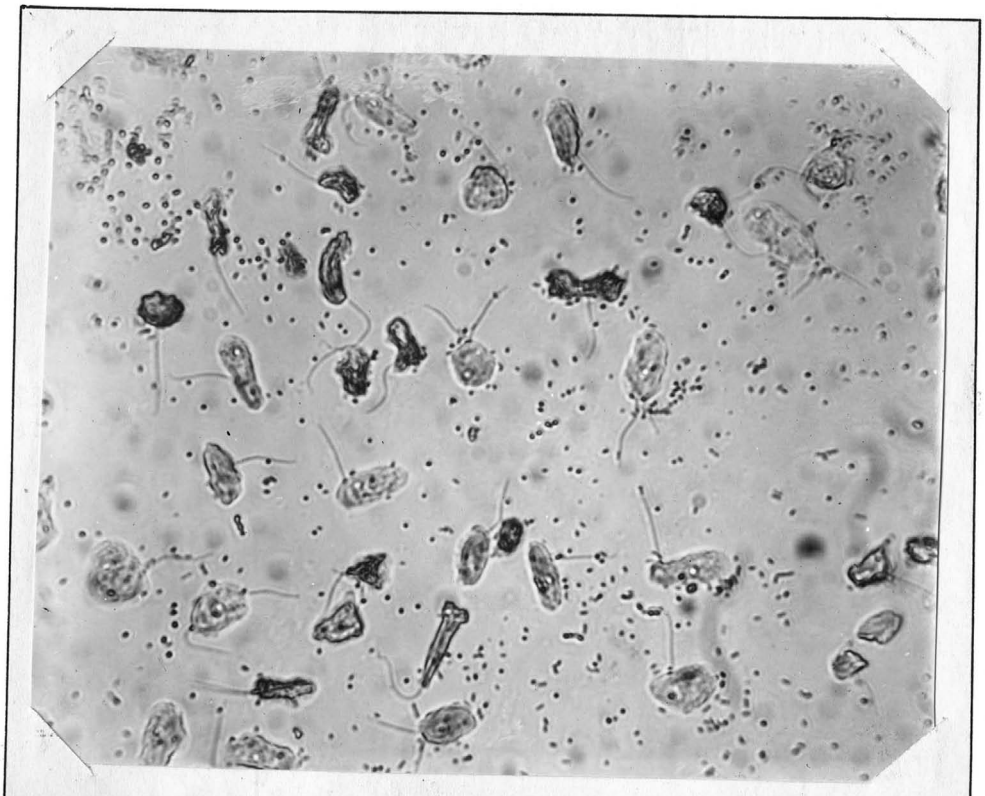

Fig. 4. Colony of swarm-cells of Didymium melanocarpum in which germination is nearly complete, showing variety in size, in shape, in location of flagella and several bi-flagellate individuals . X 640

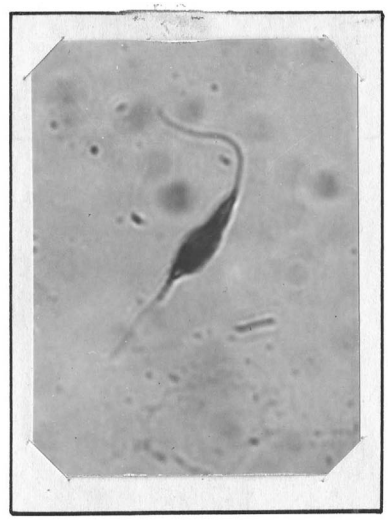

Fig. 5. Bi-flagellate swarm-cell of second flagellate stage with flagella at opposite ends. X 1000

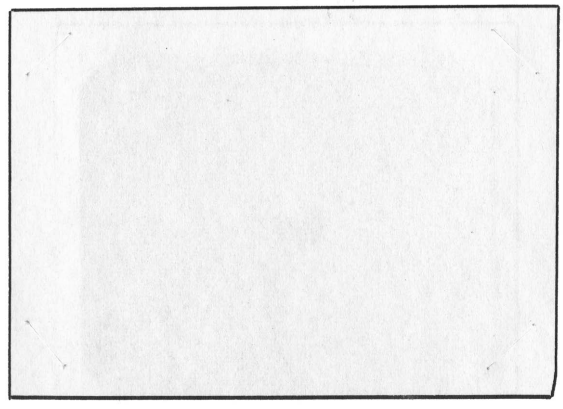

Fig. 6. Uniflagellate swarm-cells in old culture, but belonging to first flagellate stage. X 1000

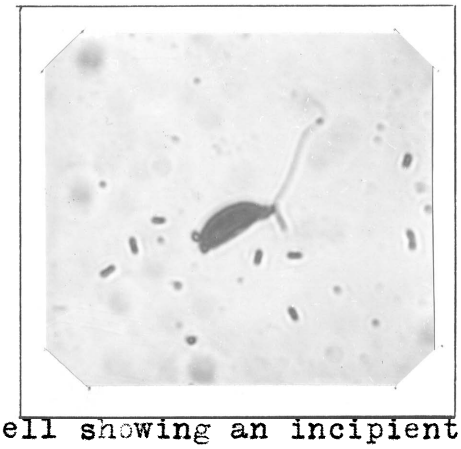

Fig. 7. Swarm-cell showing an incipient second flagellum originating at same point as normal flagellum. X 700

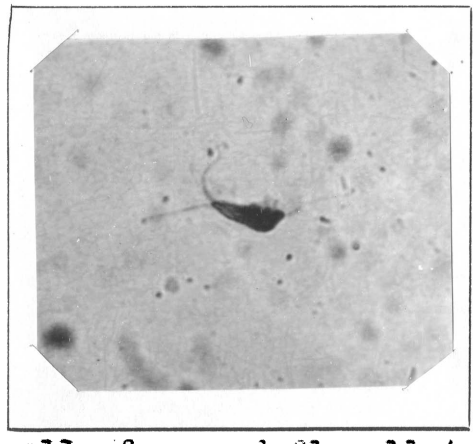

Fig. 8. Swarm-cell of second flagellate stage showing two flagella originating at same point and (rather faintly) a third flagellum trailing from posterior end. The pseudopodial character of this posterior flagellum is further indicated by the fact that in killing and staining it always shrinks and sometimes disappears. X 700

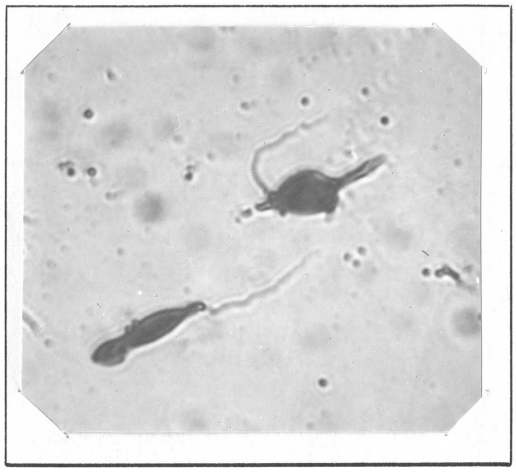

Fig. 9. Swarm-cells which exhibit amoeboid movements of two kinds, constriction of the cell and protrusion of pseudopodia. X 1000

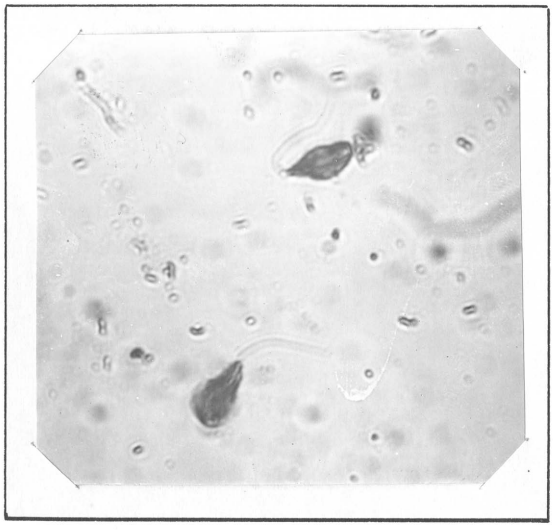

Fig. 10. Swarm-cells of very common occurrence in cultures from 16 to 60 hours old. X 1000

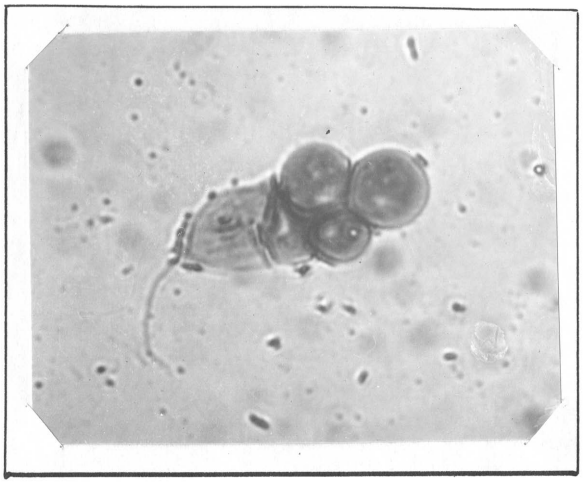

Fig. 11. Flagellate and non-flagellate swarm-cells fusing (apparently). The flagellate form is unusually large, the non-flagellate forms show the spherical shape usually assumed between the second flagellate and the myxamoeba stage. X 1000

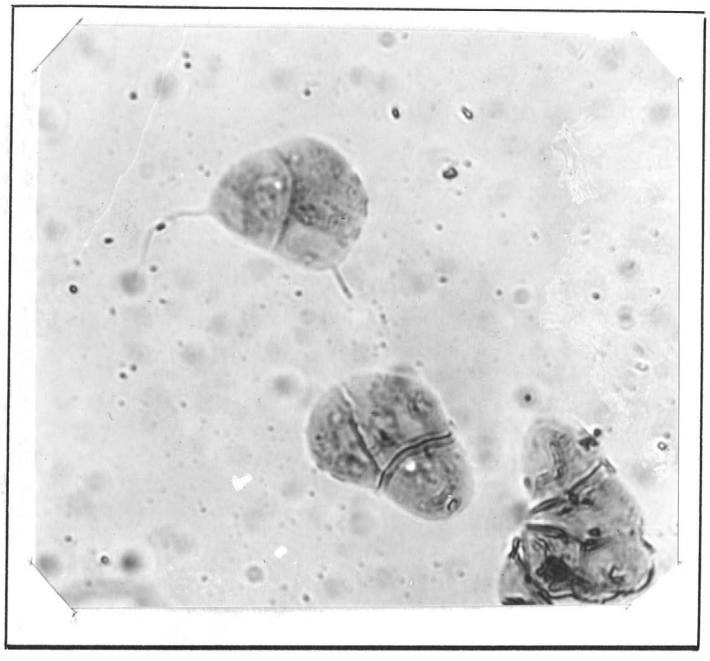

Fig. 12. Myxamoebae, some still retaining vestiges of their flagella, fusing to form plasmodia, individual cells still distinct. X 1000

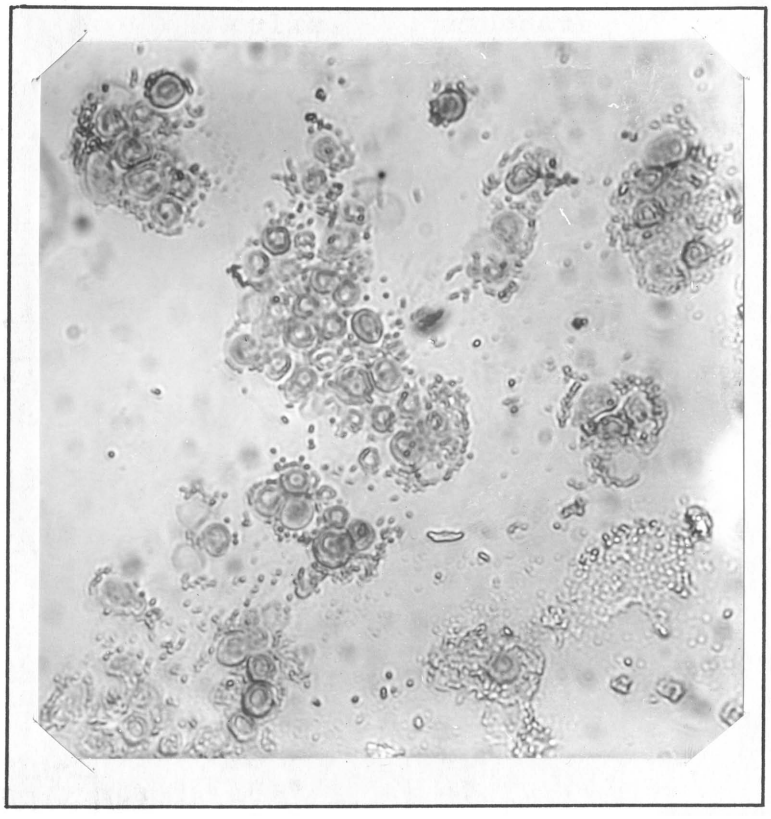

Fig. 13. Plasmodia in which the original cells have lost their identity. The outline of the ectoplasm does not show; the vacuoles, some containing ingested bacteria, are the most prominent feature, appearing as circles or ellipses. X 1000

Table 1.

Showing elapsed time between wetting of spores and first observed emergence of swarm-cells, myxamoebae, plasmodia and cysts.

Name of Species	Emergence of swarm-cells.	Appearance of myx-amoebae	Appearance of cysts	Appear-ance of plasmodia
Fuligo septica	25 min.	30 hrs.	68 hrs.	3 days
Stemonitis fusca	50 "	72 "		5 days
Badhamia lilacina	2 hrs.	48 "		4 "
Didymium melanosper-mum	2 "	48 "		4 "
Didymium nigripes var. xanthopus	2 "	25 "		3 "
Leocarpus fragilis	2 "	50 "		3½ "
Hemitrichia serpula	2½ "	48 "	48 hrs.	5 "
Hemitrichia ves-parium	2½ "	43 "		3 "
Dictydium cancella-tum	2½ "	53 "		3 "
Hemitrichia clavata	3½ "	50 "		3 "
Enteridium rozeanum	3½ "	40 "		3 "
Trichia decipiens	4½ "	40 "	6 days	3½ "
Physarum sinuosum	5 "	29 "	4 days	3½ "
Physarum notabile	6½ "	19 "	6 "	4 "
Badhamia rubininosa	10 "	19 "		4 "
Diderma radiatum	10 "	50 "	4 Days	4½ "
Cienkowskia reticulata	10 "	62 "		
Diachaea leucopodia	18 "	46 "		6 days
Trichia varia	18 "	40 "		3 days
Arcyria incarnata	16 "	47 "		4 "
Diderma testaceum	20 "	48 "		3½ "
Mucilago spongiosum	20 "	46 "		5 "
Arcyria nutans	23 "	48 "		4 "

Table 2. Showing occurrence of biflagellate Swarm-cells.

Both flagella on one end of swarm-cell.	Flagella on opposite ends of swarm-cell.	Flagella on opposite sides of spherical swarm-cells.
Fuligo septica	Physarum sinuosum	Diderma radiatum.
Badhamia lilacina	Physarum notabile	
Physarum notabile	Didymium melanospermum	
Didymium melanospermum	Diderma radiatum	
Diderma radiatum	Diderma testaceum	
Dictydium cancellatum	Enteridium rozeanum	
Mucilago spongiosa	Trichia decipiens.	