STUDIES IN THE ACRASIEAE FROM KANSAS SOILS

by

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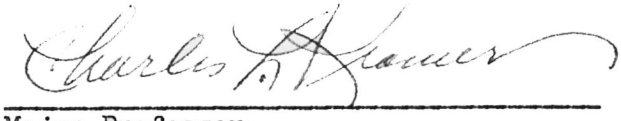

Major Professor

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INTRODUCTION

The class Acrasieae, which is often referred to as the cellular slime molds, is a small group of organisms found commonly in soils. The members of this group have two phases to their life history: a vegetative phase which is myxamoeboid, and feeds on soil bacteria and decaying organic material; and a fructifying stage in which a sorocarp containing the spores is formed. The sorocarp is usually composed of a cellular stalk which may be branched or unbranched bearing a terminal sorus composed of a mass of spores held together by a mucus-like substance.

Relatively little was known about the Acrasieae prior to the publication of E. W. Olive's monograph (Olive 1902). In this publication, Olive listed the species of cellular slime molds which were known at that time and also described the morphological characteristics of various phases in the life history of the species that he isolated. He also considered the phylogeny of the group.

In 1926 Harper (1926) studied the morphogenesis of Dictyostelium mucoroides and later (1929) he did a similar study of Polysphondylium violaceum. In 1935, Raper described a new species of Dictyostelium. This species, D. discoideum was unique in that it had a stalkless migrating pseudoplasmodium. Because of this fact, this species has been used to study the various aspects of the physiology of the cellular slime molds. Since 1935, eight new species and two varieties of Acrasieae have been added to the class (Raper 1941, 1956a, 1956b; Olive and Stoianovitch 1960; Olive 1962). One species, D. lacteum van Tieghem, which was known only from the original description was re-discovered by Raper in 1951 (Raper 1951). In 1960, Olive and Stoianovitch isolated a new species in the re-discovered genus Acrasis van Tieghem. Until

this time, this genus also was known only from the original description of the type species.

The Acrasieae are known to occur in many countries of the world and have been isolated from many different substrates; but they were generally considered to be coprophilous organisms prior to Raper and Thom's studies in 1932. They found that Dictyostelium mucoroides and Polysphondylium violaceum could be isolated eaily from forest soils. In 1947, Singh found D. mucoroides to be common in cultivated soils in England. In 1951, Raper found that cellular slime molds could be isolated from a number of different habitats although forest soils proved to be the most productive.

Because the Acrasieae are reported to be common soil inhabitants and easily cultured, this study was initiated to determine the number and kinds of cellular slime molds present in Kansas soils. In addition, the morphology of these organisms was studied, especially those in which detailed studies of the development of the organisms have not been made.

LITERATURE REVIEW

In 1902, E. W. Olive published a monograph on the Acrasieae. In this paper he included seven genera. Of these, six are now considered valid by most students of these organisms, particularily L. S. Olive (1960) and Raper (1960).

The genus Dictyostelium Brefeld (1869) is characterized as having stalked sori, the stalks simple or occasionally branched, the sori globose or subglobose and the sorocarps frequently gregarious. Seven species were included in Olive's monograph (1902). Since then four others have been described: Three by Raper (1935, 1941, 1956a, 1956b) and one by Singh (1947) constituting a total of eleven species.

The genus Polysphondylium Brefeld (1884) was described by Olive (1902) as having spherical sori born terminally on primary and secondary stalks, the latter branching in whorls from the main axis, although the fructification is sometimes simple. Three species have been described, but one of these is considered doubtful by Raper (1951).

The genus Guttulina Cienkowsky (1873) was described in Olive's monograph (1902) as having limax-shaped myxamoebae, without pseudopodia. Sori were irregular or spherical in shape, sessile or stalked, and composed of spores that had a definite protective wall. Four species were recognized by Olive (1902). Kessler and Raper (1960) discussed a fifth species, but no valid description exists as yet.

The genus Acrasis van Tieghem (1880) was described by Olive (1902) as having a fruiting structure with a single row of stalk cells on which a concatenate chain of spores was born. There has been only one species described since van Tieghem's description of the type species and this was by L. S. Olive (1960).

The genus Coenonia van Tieghem (1884) was described by Olive (1902) as having globose yellowish sori, terminating a stalk which was dilated into a sort of cupule in which the sorus rested. Only one species has been described and this is known only from the original description.

The genus Sappinia Dangeard (1896) was cited by Olive (1902) with some misgivings and is today no longer considered to be a valid genus of the cellular slime molds because it is a binucleated form. All cellular slime molds have a single nucleus (Olive 1960; Raper 1960).

The genus Guttulinopsis Olive (1901) is described as having myxamoebae with lobose pseudopodia. The sori are sessile or stalked and are composed of

pseudospores. A cell wall is not secreted about the resting myxamoebae, thus true spores are not formed (Olive 1901, 1902).

Since the publication of Olive's monograph no other genera were described until 1956 when Raper (1956a) described the genus Acytostelium. This genus is characterized by having terminal sori on acellular stalks. Only the type species has been described.

In 1960, L. S. Olive and Stoianovitch described the genus Protostelium. This genus is characterized by myxamoebae with filose pseudopodia, the presence of spherical microcysts, the absence of pseudoplasmodia, and spores borne singly at the apices of non-cellular stalks. Three species and two varieties have been described (Olive and Stoianovitch 1960; Olive 1962).

Morphology and Life History

The life history of the Acrasiales consists of two phases: a vegetative phase in which the myxamoebae feed and divide mitotically; and a fruiting stage in which the sorocarp containing the spores is formed. This latter phase is accomplished first by the aggregation of myxamoebae into a pseudoplasmodium which in turn culminates or develops into the mature fructification.

Germination: The myxamoebae of most species of Dictyostelium and Polysphondylium emerge by splitting the spore case longitudinally near one end (Raper 1935, 1941; Raper and Quinlan 1957; Bonner 1959) but, in the case of D. polycephalum Raper, the protoplasm swells and the spore wall is bulged out and bursts, or is completely dissolved in a median area (Raper 1956b). In the case of Acytostelium leptosomum Raper, the spore content swells and dissolves a spore in the spore wall (Raper and Quinlan 1957). Acrasis rosea L. S. Olive and Stoianovitch (1960) also emerges by a pore dissolved in the spore wall.

Protostelium mycophaga L. S. Olive and Stoianovitch (1960) sometimes produces spores which revert to the amoeboid state without leaving a trace of a spore wall or a thin-walled spore case is sometimes left behind. Olive (1902) states that Guttulinopsis also leaves no trace of a spore wall. He applied the term pseudospore to this type of spore.

Vegetative phase: The myxamoebae of species of Dictyostelium, Acytostelium, and Polysphondylium normally produce pointed or filose pseudopodia (Olive 1902; Raper 1935; Raper and Quinlan 1957). Lobose pseudopodia are produced by myxamoebae of species of Guttulina, Protostelium and Acrasis (Olive 1902; Olive and Stoianovitch 1960; Olive 1962). Although normally myxamoebae are colorless, the myxamoebae of G. rosea Cienkowsky, and A. rosea van Tieghem have a reddish color (Olive 1902; Olive and Stoianovitch 1960).

While bacteria are plentiful, the myxamoebae feed by invagination similar to the protozoa (Kudo 1946), and simple division occurs rapidly during this vegetative phase. Aggregation may be initiated by one or more of the following ways: 1) depletion of the food supply, 2) increase in incubation temperature, 3) decrease in relative humidity, and 4) increase in light (Raper 1939, 1940, 1956a; Bonner 1950; Bonner and Shaw 1957). Raper (1939) showed that aggregation began only after growth and cell division ceased and that the aggregation could be stopped or reversed by the addition of food. Sussman (1956a) further showed that when continued growth was prevented, even when newly germinated spores were used, aggregation commenced.

Aggregation. This phase forms a typical radial pattern as the myxamoebae move to a center of aggregation. In studies dealing with D. discoideum Raper, it has been found that the number of aggregative centers formed depended upon two things; the population density of the culture and the

total number of cells (Sussman and Noel 1952). Each aggregation required an initiator or I-cell and a proportional number of responder or R-cells. It was further noted that the ratio of I-cells to R-cells is 1:2200 for Dictyostelium discoideum, and 1:300 for D. purpureum Olive (Sussman and Noel 1952; Sussman 1952). The I-cell apparently becomes located at a center of aggregation for some time before aggregation begins. As mentioned later, Wilson (1953) believes that aggregations are initiated by zygote myxamoebae. It would be assumed that these would correspond to the I-cells described by Sussman.

A chemotactic stimulus was suspected by E. W. Olive (1902) to induce aggregation, but the existence of this substance was not proved until Bonner (1947) used an under-water technique in which myxamoebae placed downstream from an aggregative center moved toward the center in the direction of higher concentrations of the chemotactic stimulus. Bonner gave the substance the generic name "acrasin." This substance will be discussed in more detail in another section.

Since the publication of Olive's monograph (1902) it has been believed that the Acrasiales do not have a sexual stage in their life history. Olive stated that there was no mitotic division after the vegetative phase. In 1952 Bonner and Frascella using myxamoebae of D. discoideum found mitotic divisions in the aggregative phase. They also found that the haploid number of chromosomes in the myxamoebae of D. discoideum was four, three of which had double arms. However, Bonner (1959) later stated that he had observed seven chromosomes on several occasions. There are a number of sharp disagreements between the studies of Bonner and Frascella (1952) and those of Wilson (1952).

In Wilson's accounts (1952, 1953) of the sexuality of the Acrasieae, he described meiotic divisions in the aggregative phase and mitotic divisions just prior to spore formation, and counted seven chromosomes in the myxamoebae of Dictyostelium discoideum. He believes that zygotes initially form centers of aggregation and as each additional zygote is formed it migrates toward this center, forming the aggregation. Again, one would assume that these were the same as the I-cells described by Sussman and Noel (1952) and Sussman (1952). In 1957, Wilson and Ross published further data on sexuality. In this paper, five isolates of D. discoideum, two isolates of Polysphondylium violaceum Brefeld, and one isolate of P. pallidum Olive were used.

Migration: Several species of Acrasiales have migrating pseudoplasmodia. Two species, D. discoideum and D. polycephalum have stalkless migrating pseudoplasmodia. That of D. discoideum is slug-shaped while in D. polycephalum it is long and thin. These move about the substratum for a period of time, depositing a slime trail before culminating. Other species have migrating pseudoplasmodia which deposit a stalk as they move about. Eventually the anterior end of the pseudoplasmodium elevates and culmination is terminated. This type of culmination is found in D. mucoroides Brefeld, D. purpureum Olive, P. violaceum and P. pallidum. In D. minutum Raper, D. lacteum van Tieghem and Acytostelium leptosomum, culmination occurs with a migrating pseudoplasmodium.

Culmination: In this phase, sorocarps are formed from the pseudoplasmodia. The types of sorocarps differ considerably in their morphology among the various genera. Olive (1902) describes Guttulina as forming a stalk and sorus of similar cells. The mature fructifications of D. discoideum produce a sorocarp with a dilated base, a stalk, and a terminal sorus. Fructifications

of Dictyostelium minutum and D. lacteum are small (1 mm. or less) and gregarious so that they may be clumped together. D. polycephalum produces a coremiform fructification, and when D. mucoroides and D. purpureum are mature they produce terminal sori on long stalks. The only difference between these latter two species is the purple color of the sorus of D. purpureum. Acytostelium leptosomum produces a sorus on an acellular stalk, Protostelium spp. produce a single spore on an acellular stalk, and Acrasis rosea produces spores in a chain on a uniserate stalk of single cells.

Physiology

Germination seems to require some mineral nutrients. E. W. Olive (1902) found that better germination resulted when spores were placed in a drop of nutrient solution rather than in tap water. Russell and Bonner (1960) state that the percent germination of spores on non-nutrient agar is significantly lower than on nutrient agar.

Temperature for the best growth of most species of the cellular slime molds ranges from 20-25°C. (Raper 1940, 1956; Raper and Quinlan 1957). An increase in temperature will induce sporulation, though a sustained high temperature will have no effect on sporulation (Bonner and Shaw 1957). Relative humidity has little effect on the growing myxamoebae but a decrease in humidity will induce culmination in drier areas of plates. If humidity is maintained at a high level, prolonged migration occurs in various species of Acrasiales (Bonner and Shaw 1957), and D. polycephalum will fruit only in a humidity of from 30-40% (Whittingham and Raper 1957).

Harper (1932) studied the light requirements of D. mucoroides and Polysphondylium violaceum and found that light is not necessary to produce mature

sorocarps and that the sorocarps that were produced in the dark were larger than those grown in the light. However, more sorocarps were produced in lighted cultures. When light from one side was introduced, the sorocarps of Dictyostelium mucoroides inclined toward the light. Dictyostelium discoideum has been found to be positively phototrophic and the pseudoplasmodia will migrate toward a light source (Raper 1935). Bonner et al. (1950) found that the pseudoplasmodia of D. discoideum were positively thermotrophic in addition to being positively phototrophic.

The cellular slime molds will produce mature fructifications on a substrate with a hydrogen-ion concentration of from 4.5 - 8.0 (Raper 1939, 1951) but the best results occur in a pH range of from 5.5 - 6.5. L. S. Olive and Stoianovitch (1960) found that Acrasis rosea will grow best at a pH of 6.5, and D. polycephalum (Whittingham and Raper 1957) will produce best results at a pH of 6.2.

It has been suspected for some time that a chemotactic substance is produced by the cellular slime molds that initiates the aggregative phase of the life history. In 1947, Bonner obtained positive proof that such a substance was produced and gave it the generic name "acrasin." In 1953, Shaffer succeeded in isolating acrasin in in vitro cultures but found it to be very unstable at room temperature. When he passed the substance through cellophane, he found that it remained stable at room temperature for long periods of time. Shaffer then suspected an extracellular enzyme of inactivating the acrasin.

In 1956, Sussman et al. succeeded in extracting acrasin from cultures by using cold dilute HCl at pH 3.5. Sussman also suspected an extracellular enzyme, so selected a pH that would be below the activity range of the enzyme.

He then concentrated the preparation that resulted from the HCl treatment by vacuum distillation at 50°C. and passed the fluid through a cellulose powder column. When the resulting fluid was neutralized it remained stable.

When the fluid was dried and chromatograms were run, it was found that acrasin was formed of two fractions "A" and "B". When diluted with water and tested separately and in combination, the greatest biological activity resulted at a ratio of 2 parts "A" to 1 part "B"; In 1956, Shaffer succeeded in precipitating out the extracellular enzyme by using ammonium sulfate. The unidentified enzyme was found to inactivate the "B" fraction of acrasin, converting this fraction into a substance identical with fraction "A". Cultures of Dictyostelium discoideum were used in these studies since it produces acrasin in the migratory stage (Bonner 1949).

Phylogeny

Three of the four classes of the Myxomycophyta are well known. Two of these classes, the Myxomycetae and the Plasmodiophorae, have flagellate stages in their life history and because of this there is agreement among most workers (Bessey 1950, Alexopoulos 1962) that these classes have arisen from the Mastigophora. Kudo (1946) considers these protozoan ancestors to have been members of the Phytomonadina, a sub-class of the Mastigophora. He further believes that this sub-class gave rise to the Sarcodina. The third class of the Myxomycophyta, the Acrasieae does not have a flagellate stage in the life history and for this reason Bonner (1959) believes that this class has evolved from free-living soil amoebae. The fourth and least known class of the Myxomycophyta, the Labyrinthulae is believed by Smith (1955) to have originated in the Chrysophyceae. He even rejects Labyrinthulae from the Myxomycophyta

because the class shows such close similarities to the golden alga Chlorarachnion. Bonner (1959) is in agreement with Smith on the probable evolution of the Labyrinthulae but considers the class a member of the phylum Myxomycophyta, not, in spite of its differences, but because it is so different it fits in well with the heterogeneity of the other three classes. Two of these classes, the Myxomycetae and the Acrasieae will be considered here. The class Myxomycetae is characterized as having biflagellate zoospores in one stage of the life cycle, an amoeboid vegetative form, the formation of a true plasmodium, the formation of a fructification that has a true peridium around the spore mass, and in some a capillitium in the sorus to help in the liberation of spores.

The class Acrasieae does not have a flagellated stage but has a vegetative phase in which the myxamoebae feed in the same manner as the protozoan amoebae. The food particles are collected in food vacuoles and digested, much as in the Sarcodina (Kudo 1946). The Acrasieae have an aggregative phase during which a pseudoplasmodium is formed. This phase differs from the comparable stage in the myxomycetes in that the amoebae retain their individuality, and never fuse to form a coenocytic, multinucleate plasmodium.

Culmination in the class Acrasieae differs from that in the class Myxomycetae in many respects. The Acrasieae form mature sorocarps by producing stalks of a cellulose-like substance in which the cells are incorporated to give a cellular effect. The spore mass is held together by the production of a mucus-like substance and a true peridium with capillitium is not formed. At the present time, the order Acrasiales is composed of four families; Protosteliaceae, Guttulinaceae, Dictyosteliaceae, and Acytosteliaceae. Among

present day workers there is some disagreement on whether or not the order has arisen from one main line of development or two lines from a common ancestor (Olive 1960).

Raper (1960) and L.S. Olive (1960) agree that the most primitive family in the Acrasiales is the Protosteliaceae. This family is characterized as having a fructification consisting of a single spore at the tip of an acellular stalk. The family Acytosteliaceae is considered by Bonner (1959) to be less advanced than the Dictyosteliaceae. He believes that evolution within the class Acrasieae has been from the simple to the complex and he considers the fructification of Acytostelium with its acellular stalk and spherical spores to be more primitive than the Dictyostelia with their cellular stalks and capsule-shaped spores. Raper (1960) disagrees with Bonner's statements about the Acytosteliaceae and regards Acytostelium as more advanced than the Dictyostelia because in Acytostelium there is no loss of cells in the production of the stalk. Bonner (1959) thinks that the Guttulinaceae are degenerate forms of the Dictyostelia and should not be considered valid genera. Raper (1960) disagrees and believes that the members of the Guttulinaceae are valid genera although more primitive than the Dictyostelia. The Guttulinaceae have fructifications with little or no differentiation between the stalk and spore cells.

Bonner (1959) and Raper (1960) agree that the Dictyosteliaceae are highly advanced in the order Acrasiales. This family has all the characteristics associated with the class Acrasieae, namely, 1) aggregations forming radiate pseudoplasmodia, 2) highly differentiated stalk and spore cells, and 3) spores contained within a terminal sorus. Dictyostelium discoideum is thought by Bonner (1959) and Raper (1960) to be more highly evolved than D. mucoroides and D. purpureum because of the formation of the basal disk and the migrating

stage of the stalkless pseudoplasmodium. Dictyostelium polycephalum and the Polysphondylia are believed to be more complex species than D. discoideum because of the formation of branched fructifications. The genus Coenonia which is included in the Dictyosteliaceae has not been re-isolated since the publication of the original description. Although little is known about this genus, if it could be re-isolated, it would undoubtedly be the most complex member of the class Acrasieae because it is described as having a stalk with a hold-fast attachment at the base and a serrate-edged cupule at the tip in which the sorus rests (Raper 1960).

METHODS AND MATERIALS

During the spring and summer of 1961, 126 soil samples from 39 counties in Kansas were collected from wooded, cultivated, and uncultivated prairie areas from a depth of 0 to 3 inches. Samples of approximately 100 grams were placed in pint plastic bags and a 3 x 5 inch data card containing information as to location, date of collection, ecological habitat and the collector, was enclosed in each bag with the sample. These samples were stored in a refrigerator at 4°C. until isolations could be made from them.

Isolations were made on an agar using a hay infusion diluted to $\frac{1}{4}$ its original concentration as the base of the medium. It was prepared by using 35 grams of Bermuda grass clippings in 1000 ml. of distilled water which was autoclaved for $\frac{1}{2}$ hour at 15 lbs. pressure. This infusion was then filtered and reconstituted one liter. The $\frac{1}{4}$ hay infusion agar was then made by using 250 ml. of the infusion, 750 ml. of distilled water, 15 grams of Difco Bacto-Agar and 2 grams of $K_2 HPO_4$.

For isolations the medium was poured into sterile Petri plates and allowed to solidify. Approximately 10 grams of each soil sample was ground in a

sterile mortar with 10 grams of sterile distilled water. When a heavy suspension resulted, the liquid was cross-streaked in the form of an X onto two ¼ hay infusion agar plates. Directions for formulating this agar are given on the next page. These plates were marked with the sample number, and the data from each card were entered in a record book. The plates were placed in a plastic moist chamber and allowed to incubate at room temperature for five days

After five days, each plate was examined for mature fructifications of cellular slime molds and if any were located, spores from the sorus were transferred to a ¼ hay infusion agar plate that had previously been streaked with a suspension of Escherichia coli (Migula) Castellani et Chambers. If no fructifications were noted the plate was placed back in the moist chamber and again examined two days later. A final examination of all plates was made two weeks after initial inoculation and a transfer of each type of fructification was made to an E. coli streaked ¼ strength hay agar plate.

After a three-day period of growth on the E. coli streaked plates, tentative identification and transfers to stock tubes were made of each isolate. Cultures were maintained in screw-cap tubes containing approximately 10 cc. of ¼ strength hay agar slanted and streaked with E. coli.

A study was made of the comparative growth of ten isolates including seven species of Dictyosteliaceae using ¼ hay infusion agar (¼ HA), hay peptone agar (¼ HPA), dextrose peptone agar (DPA), galactose peptone agar (GPA), and ¼ carrot agar (¼ CA). The hay agar was prepared as outlined above. The hay peptone agar was prepared as the ¼ hay infusion agar with the addition of 5 grams of peptone. Dextrose peptone agar was prepared by using 5 grams dextrose, 5 grams peptone, 15 grams agar, and 2 grams K_2HPO_4 as a buffer per liter of distilled H_2O. Galactose peptone agar was prepared as the dextrose peptone

agar except that 5 grams of galactose were substituted for the dextrose. One-fourth carrot agar was prepared by autoclaving 300 grams of carrots in 1 liter of distilled H_2O for $\frac{1}{2}$ hour, filtering the liquid and "reconstituting" it to 1 liter. In preparing the $\frac{1}{4}$ carrot agar, 250 ml. of carrot infusion was used with 750 ml. distilled H_2O, 15 grams agar, and 2 grams K_2HPO_4 were a buffer. Two plates of each of these media were cross-streaked with Escherichia coli and inoculated with ten isolates of seven species of Acrasieae. After incubating at room temperature for five days, the results were recorded and are here presented in Table 2.

Growth studies were made of several of the Dictyosteliaceae that were isolated. Maturation cells were constructed from glass slides and sealed together with Canada balsam to form a chamber 12 x 22 x 2 mm., and 22 x 22 x 25 mm. These chambers were sealed onto 2" x 3" glass slides using Canada balsam; a small square of moist filter paper was placed on one vertical side, a small square of agar containing a pseudoplasm of a known species of cellular slime mold was placed on a piece of cork which was sealed on the opposite vertical side and a No. 1 22 x 22 mm. coverslip was sealed to the top of the chamber with silicone grease. The slide on which the chamber was mounted was tipped 90° and held vertically in a square, grooved, coplin jar. A plastic slide box was also used. When the slide was tipped vertically, the side of the chamber on which the square of agar was placed became the bottom of the chamber. In this way the fructifications would mature in a position so that they could be photographed. When photographs were to be taken, the slide was placed flat on the stage of the dissecting scope. When this was done, the agar square was then on the side of the chamber with the fructification in a horizontal position so that it could be photographed. A growth chamber regulated

at 30°C. and 40% relative humidity was used also for one of the species. This was used to incubate plates containing Dictyostelium polycephalum.

One of the microscopes used in examination of the Petri plates was an AO Spencer binocular dissecting 'scope equipped with a fluorescent illuminator mounted on the objective. Side illumination was provided by using a B & L spot light illuminator on an AO Spencer spot light illuminator, one placed on either side of the 'scope. The 'scope was also equipped with a Graphflex stereo camera which was used for taking all of the time interval growth pictures.

An AO Spencer Microstar Series binocular microscope equipped with a 35 mm. Kodak Pony IV camera attachment, and an AO Spencer Phasestar Series binocular phase microscope equipped with an ortho-illuminator and Kodak Pony IV attachment were used in taking photographs of the fructifications and of the spores and myxamoebae.

Slide mounts were made using lacto-phenoe with cotton blue and ringed with Zut to make a semi-permanent mount. Such slides of all species have been deposited in the K. S. U. herbarium.

RESULTS

Of the 126 soil samples used in this study, 75 samples contained at least one species of cellular slime mold. Table 1 is a list of the samples, the soil type and the identified species of Acrasieae that were isolated. Approximately half of the soil samples were collected in timbered areas; the rest were collected from cultivated and uncultivated prairie soils. Figure 1 shows the distribution of these samples. Twenty-nine of 39 counties were found to have slime molds and the majority of these counties are located in the northeastern quarter of the state (Fig. 1).

Leavenworth county (Table 1, Fig. 1) contributed 8 samples that contained seven of the nine species of Acrasieae that were isolated in this study. This was no doubt due to the type of forest covering in the area. Other counties which contributed soils that contained several species of cellular slime molds were Douglas county, from which six species were identified, and Allen and Saline counties from which five species were cultured.

The following four species were common to each of these counties: Dictyostelium mucoroides, D. purpureum, Polysphondylium pallidum, and P. violaceum. These species were also the most frequent isolates in the majority of the other counties. The remaining five species that were isolated less frequently were: D. polycephalum, D. minutum, D. discoideum, D. lacteum and D. brevicaule.

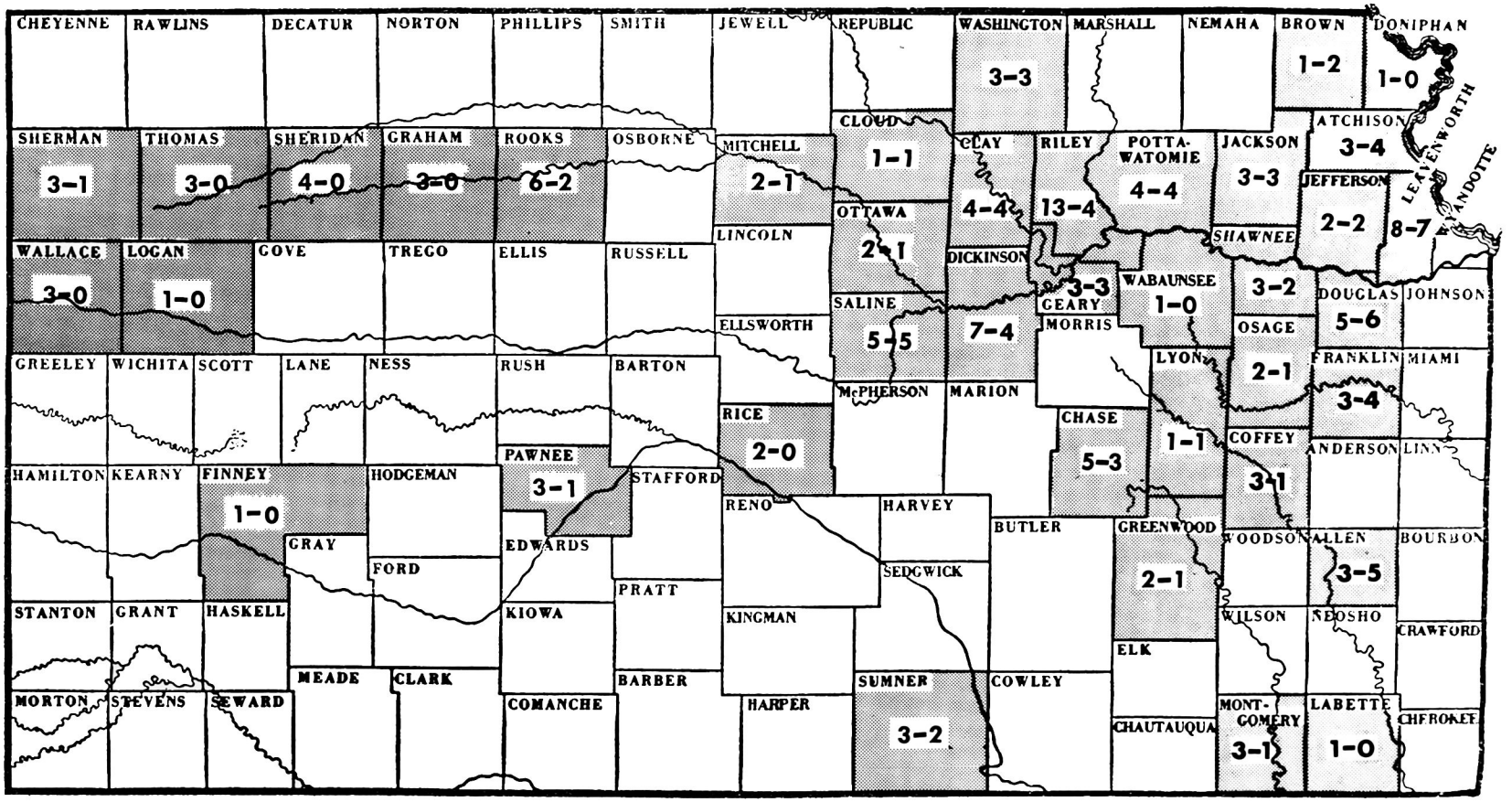
Dictyostelium mucoroides was first isolated in 1869 from dung by Brefeld. In 1902, Olive reported isolations of this species from various kinds of dung as well as from paper, decaying fleshy fungi, and other sources. Since 1902, D. mucoroides has been found to occur quite commonly in forest, cultivated and uncultivated soils by a number of workers such as Raper and Thom (1932), Singh (1947), Raper (1951), Bonner (1959), and L. S. Olive (1960). In Kansas, it was isolated mainly from timber topsoils although a few isolations were made from cultivated and uncultivated soils as well (Table 1, Fig. 1). This species is certainly one of the most common members of the Acrasieae. It was isolated from 56 of 126 soil samples which were collected in 27 counties (Table 1, Fig. 1).

The majority of the Kansas isolates represented a morphologic strain which produces numerous sorogens from the pseudoplasmodium and these mature into fructifications which have irregularly branched, yellowish stalks and milk-white sori. Raper (1951) also has isolated this strain quite frequently.

EXPLANATION OF PLATE I

Fig. 1. Distribution of soil samples used in the study of Acrasieae in Kansas. In the shaded counties, the first number represents the number of samples collected from that county while the second number following the hyphen represents the total number of species identified from that county.

PLATE I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SHERMAN
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THOMAS
3-0
SHERIDAN
4-0
GRAHAM
3-0
ROOKS
6-2
OSBORNE
MITCHELL
2-1
CLOUD
1-1
CLAY
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RILEY
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POTTA-
WATOMIE
4-4
JACKSON
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ATCHISON
3-4
JEFFERSON
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LEAVENWORTH
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WYANDOTTE
WALLACE
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LOGAN
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GOVE
TREGO
ELLIS
RUSSELL
LINCOLN
OTTAWA
2-1
DICKINSON
3-3
SHAWNEE
WABAUNSEE
1-0
3-2
DOUGLAS
5-6
JOHNSON
SALINE
5-5
7-4
GEARY
MORRIS
OSAGE
2-1
ELLSWORTH
GREELEY
WICHITA
SCOTT
LANE
NESS
RUSH
BARTON
McPHERSON
MARION
LYON
1-1
FRANKLIN
3-4
MIAMI
RICE
2-0
CHASE
5-3
COFFEY
3-1
ANDERSON
LINN
HAMILTON
KEARNY
FINNEY
1-0
HODGEMAN
PAWNEE
3-1
STAFFORD
RENO
HARVEY
BUTLER
GREENWOOD
2-1
WOODSON
ALLEN
3-5
BOURBON
GRAY
EDWARDS
FORD
SEDGWICK
PRATT
WILSON
NEOSHO
CRAWFORD
STANTON
GRANT
HASKELL
KIOWA
KINGMAN
ELK
MEADE
CLARK
BARBER
SUMNER
COWLEY
MONT-
GOMERY
3-1
LABETTE
1-0
CHEROKEE
MORTON
STEVENS
SEWARD
COMANCHE
HARPER
3-2
CHAUTAUQUA

Fig. 1

Table 1. Location of Soil Samples and the Isolated Species

Sample No.	County	Topsoil Type	Species Isolated
1	Riley	Timber humus	
2	Riley	Timber humus	P. pallidum
3	Riley	Timber humus	P. violaceum, D. mucoroides
4	Riley	Timber humus	
5	Riley	Timber humus	
6	Riley	Timber humus	D. mucoroides
7	Riley	Timber litter	
8	Riley	Timber humus	P. pallidum, D. mucoroides
9	Rice	Timber	
10	Rice	Uncultivated	
11	Saline	Timber humus	P. violaceum, D. brevicaule
12	Dickinson	Uncultivated	
13	Dickinson	Timber	
14	Chase	Timber	
15	Chase	Timber humus	D. purpureum, D. mucoroides
16	Sherman	Cultivated	
17	Sheridan	Uncultivated	
18	Mitchell	Uncultivated	
19	Rooks	Uncultivated	P. pallidum, D. mucoroides
20	Wallace	Uncultivated	
21	Allen	Uncultivated	
22	Thomas	Uncultivated	
23	Wallace	Cultivated	
24	Pawnee	Cultivated	
25	Jackson	Timber	
26	Jackson	Timber	P. pallidum, D. mucoroides
27	Chase	Uncultivated	
28	Shawnee	Timber	P. pallidum, D. mucoroides
29	Rooks	Uncultivated	P. pallidum
30	Graham	Timber clay	
31	Rooks[1]		P. pallidum
32	Leavenworth	Timber silt	P. violaceum, D. mucoroides, D. purpureum
33	Shawnee	Uncultivated	
34	Thomas	Uncultivated	
35	Graham	Uncultivated	
36	Pawnee	Uncultivated	D. mucoroides
37	Pawnee	Uncultivated	
38	Shawnee	Uncultivated	
39	Chase	Timber	P. pallidum, D. mucoroides, D. purpureum
40	Chase	Timber	D. purpureum
41	Allen		P. pallidum, D. mucoroides, D. purpureum
42	Jackson	Timber	P. violaceum
43	Wallace	Uncultivated	
44	Rooks		
45	Logan	Uncultivated	
46	Sheridan	Uncultivated	
47	Mitchell	Cultivated tops	D. mucoroides

Table 1. (cont.)

Sample No.	County	Topsoil Type	Species Isolated
48	Clay	Timber humus	P. pallidum, P. violaceum, D. mucoroides
49	Allen		P. pallidum, P. violaceum, D. polycephalum
50	Leavenworth	Timber humus	P. pallidum, P. violaceum, D. mucoroides, D. polycephalum
51	Dickinson	Timber	P. violaceum
52	Pottawatomie	Timber humus	P. pallidum
53	Ottawa	Timber	D. mucoroides
54	Sherman	Uncultivated	D. mucoroides
55	Riley	Timber sandy	P. violaceum, D. mucoroides, D. purpureum
56	Atchison	Timber humus	P. pallidum, P. violaceum, D. mucoroides
57	Pottawatomie	Timber humus	P. pallidum, D. mucoroides
58	Jefferson	Timber	P. pallidum, D. mucoroides
59	Leavenworth	Timber	P. violaceum, D. mucoroides
60	Clay	Timber humus	P. pallidum, D. mucoroides, D. polycephalum
61	Cloud	Uncultivated	D. mucoroides
62	Clay	Timber sandy	
63	Saline	Timber	D. mucoroides
64	Atchison	Timber	D. mucoroides, D. purpureum
65	Leavenworth	Timber	P. pallidum, P. violaceum, D. mucoroides
66	Leavenworth	Timber	P. pallidum, D. mucoroides
67	Clay	Timber	D. mucoroides
68	Ottawa	Timber	D. mucoroides
69	Leavenworth	Timber	P. pallidum
70	Riley	Timber sandy	P. violaceum, D. mucoroides
71	Dickinson	Timber silt	D. mucoroides, D. polycephalum
72	Sumner	Uncultivated	D. purpureum
73	Rooks	Uncultivated	
74	Graham	Cultivated	
75	Sheridan	Uncultivated	
76	Saline	Timber	P. pallidum, P. violaceum, D. purpureum, D. mucoroides
77	Sumner	Uncultivated	
78	Sumner	Timber clay	D. mucoroides
79	Thomas	Cultivated	
80	Finney	Uncultivated	
81	Labette		
82	Sheridan	Uncultivated	
83	Rooks	Uncultivated	
84	Douglas		P. pallidum
85	Coffey	Uncultivated	
86	Washington	Timber	P. pallidum
87	Douglas	Timber	P. violaceum, D. mucoroides, D. purpureum, D. discoideum

Table 1 (concl.)

Sample No.	County	Topsoil Type	Species Isolated
88	Franklin	Timber	P. pallidum, D. mucoroides
89	Pottawatomie		P. pallidum, D. minutum
90	Washington	Uncultivated	D. mucoroides
91	Douglas		
92	Coffey	Timber	D. mucoroides
93	Montgomery	Uncultivated	
94	Sherman	Uncultivated	
95	Lyon	Timber	D. mucoroides
96	Saline	Uncultivated	
97	Osage	Timber	D. mucoroides
98	Wabaunsee	Timber	
99	Leavenworth	Timber	P. pallidum, P. violaceum, D. mucoroides, D. minutum
100	Dickinson		P. pallidum, P. violaceum, D. mucoroides
101	Leavenworth	Timber	P. pallidum, D. mucoroides, D. lacteum
102	Montgomery	Uncultivated	
103	Greenwood		D. polycephalum
104	Douglas		
105	Dickinson	Uncultivated	
106	Atchison	Timber	P. pallidum, P. violaceum, D. mucoroides
107	Riley	Timber sandy	D. mucoroides
108	Pottawatomie		P. violaceum, D. mucoroides, D. purpureum, D. minutum
109	Coffey	Uncultivated	
110	Riley	Timber sandy	D. mucoroides
111	Geary	Silty clay	P. pallidum, D. purpureum, D. mucoroides
112	Greenwood		
113	Osage	Timber humus	D. mucoroides
114	Dickinson	Uncultivated	P. pallidum, D. mucoroides, D. polycephalum
115	Montgomery	Timber	P. violaceum
116	Brown	Timber	P. pallidum, D. mucoroides
117	Saline	Uncultivated	D. mucoroides
118	Riley	Timber sandy	D. mucoroides
119	Geary	Silt	P. pallidum, D. mucoroides
120	Geary	Uncultivated	
121	Jefferson	Timber	P. pallidum, D. mucoroides
122	Doniphan	Timber	
123	Douglas	Timber clay	P. pallidum, D. mucoroides, D. minutum
124	Washington	Timber	P. violaceum
125	Franklin	Timber clay	P. pallidum, P. violaceum, D. mucoroides
126	Franklin	Timber clay	D. purpureum

[1]Data on soil type missing

The morphologic strain which typifies the description of the species as presented in Olive's monograph (1902) was isolated relatively infrequently. This strain forms only one sorogen from the pseudoplasmodium and this stalked sorogen migrates along the substrate for a period of time before maturing into a fructification with a long stalk 1.0 cm. or more in length bearing a terminal sorus of a white or yellowish color. Other phases which seem to be intermediate between these forms have small white sorocarps with one or two branches. A strain of Dictyostelium mucoroides which produces numerous sorogens was used to study the development of the sorocarp of this species (Figs. 35-39).

Dictyostelium mucoroides was very easily isolated and cultured. It grew readily on $\frac{1}{4}$ hay infusion agar and Escherichia coli and repeated transfers did not alter the appearance of the fructifications. Also it is not difficult to maintain stock cultures in tubes for long periods of time. Raper (1951) has found that tubes of this species remained viable for a year when stored in a refrigerator at 3°-6°C.

An isolate of Dictyostelium believed to be D. brevicaule was obtained from a Saline county, Kansas, timber soil sample. This culture is believed to represent one of the four isolations of this species since the time of its original description by Olive in 1901 who isolated it from dung.

The Kansas isolate of D. brevicaule resembles a small sorocarp of D. mucoroides. When it is grown on fresh $\frac{1}{4}$ hay infusion agar using a 24-hour inoculum of E. coli as a food source, a single sorogen is produced from the pseudoplasmodium and this matures into a fructification 1.0-1.5 mm. in height with a fairly large white sorus 200-300u in diameter. However, when culture conditions are not ideal, i. e., when older media or an older bacterial

inoculum is used, abnormal appearing sorocarps are produced. These sorocarps have short, thick stalks and the entire fructification has a yellowish color and does not grow beyond 750μ in height. Often, 2 or 3 stalks can be seen on such a sorocarp. This phenomenon has been partially explained by the use of time-interval photography to show that if 2 or 3 sorogens mature close together the sori coalesce as the fructification is completed (Figs. 45-53). This did not occur when other species were observed under similar conditions. The importance of this phenomenon is not yet understood.

The second most frequently isolated species from Kansas soil was Polysphondylium pallidum Olive which was first isolated from dung in 1901 (Olive 1901, 1902). This species also has been found quite commonly in forest and cultivated soils (Raper 1951). It was isolated from 36 soil samples from 18 Kansas counties. Isolations were obtained mainly from timber topsoils and soils from uncultivated areas (Table 1, Fig. 1). Little variation has been noted between the isolates of this species. One to several sorogens may be produced from each pseudoplasmodium and these migrate along the substrate for a period of time before culminating to form regular sorocarps which measure 5.0 mm. to 10.0 mm. in height with a number of whorls of branches at intervals along the main axis. The sori of this species are white and average 70-125μ in diameter. Differences between isolates occur only in the number of whorls on a fructification or in the number of branches per whorl. Olive (1901, 1902) described another white spored species in 1901, P. album which formed larger fructifications with sori 100-200μ in diameter. This species is now considered doubtful by Raper (1951) and Bonner (1959). Because of the variations that occur in cultures of P. pallidum these authors consider P. album to be simply a larger strain of P. pallidum. One isolate of P. pallidum from Kansas soil

appeared to develop larger fructifications but this isolate would have to be studied rather extensively before any conclusions could be drawn. No difficulties were encountered in maintaining stock cultures of Polysphondylium pallidum in Escherichia coli streaked hay infusion agar tubes. Raper (1951) has found that stock cultures of this species remained viable for one year when stored at 3°- 6°C., but did not grow after 2 years storage at this temperature.

Brefeld first isolated P. violaceum in 1884 from dung. Olive (1902) also isolated this species from dung, but since that time P. violaceum has been found to occur quite commonly in cultivated and forest soils (Harper 1929, Raper and Thom 1932, Raper 1951, Bonner 1959). In this study all of the 22 isolates were obtained from timber soils collected in 12 counties (Table 1, Fig. 1) and usually occurred in association with one or more other Acrasieae.

Several variations were noted in the morphologic strains of P. violaceum that were isolated. Some strains have pseudoplasmodia that form stalked sorogens which migrate along the substrate for long periods of time. In this case only one or two incompletely formed whorls of branches are produced on the developing sorogen before the mature fructification is completed. Other strains form sorogens which produce a number of whorls of branches along the main axis before the sorocarp is completed (Figs. 93, 98-100). Most of the strains of P. violaceum are longer and less perfectly formed than those of P. pallidum. The stalk of P. violaceum is usually about 1.0 cm. or more in length with terminal sori of a pale violet color. This species is the only colored member of the genus Polysphondylium.

After repeated transfers, strains of this species form atypical fructifications. The stalked sorogens migrate along the substrate for extended periods of time and the stalk formation is often interrupted leaving gaps in

the stalk. Some sorocarps have sori which do not produce the typical violet color while others may not mature.

Dictyostelium purpureum Olive was isolated from dung in 1901 (Olive 1901, 1902). Olive isolated it numerous times from dung cultures but since that time this species has been found quite commonly in forest soils by Raper (1951) and Bonner (1959). The 13 isolates of this species were all obtained from timber soil samples collected in 11 Kansas counties (Table 1, Fig. 1). In 3 of the 13 samples, this species was the only cellular slime mold isolated. Little variation could be observed between these isolates.

The stalked sorogen of this species migrates along the substratum for a period of time before culminating into a sorocarp 1.0 cm. or more in height. The stalk is simple with a purple sorus at its apex. Frequently, however, fructifications are produced which form the sorocarps directly from the pseudoplasmodium without a migratory phase. Figures 83-88 show 4 such sorocarps.

The 13 isolates of D. purpureum retain their characteristics over repeated transfers. Raper (1951) has found that cultures of this species stored at 3°- 6°C. remain viable for periods up to 2 years.

Raper described D. polycephalum in 1956 from several isolates obtained from forest soils. In this study it was isolated from 6 samples of timber soils collected in 5 Kansas counties (Table 1, Fig. 1). These strains did not produce mature sorocarps in the soil-streaked plates until 10-14 days incubation. Dictyostelium polycephalum grew poorly on Escherichia coli streaked hay infusion agar plates and produced only typical stalkless pseudoplasmodia. Three different methods of inducing culmination were then tried so that these cultures could be maintained and studied. Galactose peptone agar (GPA) plates were used to produce an enriched substrate

and filter paper was taped inside the top valve of each plate to lower the relative humidity by absorbing the excess moisture, a requirement noted by Whittingham and Raper (1957). However, this method did not work well and very few mature sorocarps were produced.

A second attempt at inducing culmination of the pseudoplasmodia consisted of using three-membered cultures containing Aerobacter aerogenes (Kruse) Beijerinck and Dematium nigrum in addition to the cellular slime mold. However, again large numbers of pseudoplasmodia were formed but few mature sorocarps were produced.

A third method was tried using unglazed pottery lids in place of the regular glass Petri tops and using GPA and Aerobacter aerogenes as a food source. These plates were placed in a growth chamber which was regulated at 30°C. and 40% humidity. Good results were obtained and a number of mature sorocarps were produced.

Since the growth chamber was needed for other work, the isolates were maintained in a 30°C. incubator without a regulated relative humidity. As a result all of the 6 original isolations were eventually lost. However, one of these was re-isolated from the original soil sample and has been maintained in three-membered culture at 30°C. by making frequent transfers. This last method works sufficiently well so that a few fructifications are produced. Raper (1956) also had trouble in obtaining mature sorocarps of the species for this reason.

Whittingham and Raper (1957) studied the environmental factors affecting this species and found that optimum growth resulted at 30°C. and 40% humidity. The first and second methods of maintaining the Kansas isolates were tried using room temperature of 22-25°C. The first method did not work because the

filter paper did not absorb enough moisture to lower the relative humidity any great amount. The second method which was tried did not work although the relative humidity was lowered sufficiently. Apparently, the lower incubation temperature prohibited culmination of the pseudoplasmodia.

After the stalkless pseudoplasmodium migrates for a period of time, it rounds up and forms one to several sorogens. The sorogens mature (Figs. 8-15) forming a coremiform fructification 325-350u in height with a number of milk-white sori. In some respects the culmination of Dictyostelium brevicaule (Figs. 45-53) is similar to that of D. polycephalum. In the case of D. brevicaule, however, the various sorogens fuse during culmination to form only one sorus.

Raper first isolated D. minutum in 1941 from forest soil. Since then it has been isolated numerous times (Raper 1951). In this study it has been isolated 4 times in timber soils collected in three Kansas counties (Table 1, Fig. 1).

There is little variation among these strains although one isolate "D-123" produces microcysts in culture. Sorogens are produced directly from the pseudoplasmodium and these may develop singly or in clusters to form small fructifications 750u or less in height with spherical milk-white sori (Figs. 73-77). No difficulties were encountered in maintaining these cultures and they retain their culture characteristics after repeated transfers.

van Tieghem first described D. lacteum in 1880 from a culture isolated from decaying mushrooms (van Tieghem 1880; Olive 1902). This species was known only from the original isolate until Raper in 1951 obtained it several times from forest soil. In this study, D. lacteum was isolated only once from a Leavenworth county Kansas soil sample. This species produces a number of

sorogens from the pseudoplasmodium and these develop into fructifications 1.0 mm. or more in height with terminal milk-white sori (Figs. 59-68). Although both Dictyostelium minutum and D. lacteum produce sorocarps with stalks composed of a single row of cells, the sorocarps of D. lacteum are usually higher and more flexuous and tend to collapse and form a mat on the substratum, (Fig. 1) whereas those of D. minutum are usually fairly rigid with the sorus held erect at the tip of the stalk (Figs. 73-77).

Another difference between the two species is the shape of the spore that is produced. The spores of D. minutum are elliptical or capsule-shaped and measure 2.8 x 5.6 - 7.0u (Fig. 69) whereas those of D. lacteum are spherical and average 2.8 - 4.2u in diameter (Fig. 55). Raper (1941, 1951) has published the only observations on the development of these two species.

Dictyostelium discoideum was described by Raper in 1935. This species was isolated from a deciduous forest soil sample and was collected only twice prior to 1951 (Raper 1951). In this study it was isolated only once, this being from a Douglas county Kansas timber soil sample (Table 1, Fig. 1). This strain differs little from Raper's (1935) description although the Kansas strain seems to produce somewhat larger pseudoplasmodia when compared with a culture of Raper's isolate.

The stalkless pseudoplasmodium migrates for a period of time before rounding up. It then forms only one sorogen and this develops a simple unbranched stalk with an expanded base which bears a white to pale lemon colored sorus at the tip (Figs. 21-29). The mature fructification is usually 2.0 - 2.5 mm. high and the elliptical spores measure 2.8 x 8.4u. This species grows well on Escherichia coli streaked plates and Raper (1951) has found that cultures of this species remain viable for periods of 2 years or more.

In order to settle on a medium suitable for maintaining and studying these isolates, ten strains representing seven species of cellular slime molds were used in making a morphologic study of their development on various media. Two plates each of the following media were used in the study: one-quarter hay agar (¼HA), galactose peptone agar (GPA), dextrose peptone agar (DPA), ¼ hay peptone agar (¼HPA) and ¼ carrot agar (¼CA). The results are given in Table 2.

Table 2. Growth Study of Seven Species of Acrasieae on Various Media.

Species	Media				
	¼ HPA	GPA	DPA	¼ HPA	¼ CA
D. discoideum	+++	+++	+++	+++	++++
D. purpureum	+++	+++	+++	+++	++++
D. minutum (3 isolates)	+++	+	++++	++++	++++
D. mucoroides (2 isolates)	+++	+++	+++	++++	++++
P. pallidum	+++	++	++	++++	++++
P. violaceum	+++	++	++	++++	++++
D. brevicaule	+++	++	++	++++	++++

[2]+Poor growth, only a few abnormal sorocarps formed; ++light growth, small number of normal appearing sorocarps; +++good growth, large number of normal appearing sorocarps; ++++heavy growth, numerous, normal appearing sorocarps covering plate.

From this study, it was determined that ¼ hay agar gave the best results for a comparative study of the various isolates. On this agar, the bacterial population (Escherichia coli) was kept relatively low, and normal-appearing colonies of the cellular slime mold were usually abundant. Although ¼ carrot agar produced the most luxuriant growth, sufficient nutrients were available so that the bacteria grew well and fungi contaminated and overgrew the cellular slime molds. The other media, GPA, DPA, and ¼ HPA, all gave variable results and were not considered.

A study of the life history of each of the nine species was made using Petri plates containing ¼ hay agar and E. coli as a food source. After each

plate was inoculated with slime mold spores taken from a sorus, the plates were incubated at room temperature (22-25°C.) until aggregations were observed, usually after 24 hours. When this stage was noted, periodic examinations of the plates were made until mature sorocarps were observed in the culture. Semi-permanent slide mounts were made of many of the stages of each organism and photomicrographs were taken of numerous stages. Time-interval photographs were also taken of the culmination of each of the nine species as they developed in situ on small agar blocks placed in the maturation cell constructed as described above. A block of agar containing a pseudoplasmodium was mounted on a piece of cork which was attached to the side of the chamber and these pseudo-plasmodia were examined and photographed at hourly intervals until culmination resulted in a mature sorocarp.

The life history of these organisms is very short. The elapsed time between spore germination and the formation of a mature sorocarp is a matter of 24 - 48 hours except for species such as Dictyostelium discoideum and D. polycephalum which mature more slowly. There does not appear to be any need for a rest period or period of dormancy for the spores. Russell and Bonner (1960) have found no differences in the viability of spores 2 - 15 days in age and those recently formed. These workers, using nutrient and non-nutrient media, have found that germination begins $1\frac{1}{2}$ hours after inoculation and that nearly 100% germination occurs after 4 hours on nutrient media although germination on non-nutrient media is much lower. They also have found that the density of the spores affects percentage germination.

Myxamoebae of most species of Dictyostelium and Polysphondylium emerge by splitting the elliptical spore case longitudinally along one end (Raper 1935, 1941; Bonner 1959). The spore contents of D. polycephalum swell when water

enters and the spore wall bursts in a median area (Raper 1956). Germination in round spored species such as Acytostelium leptosomum Raper occurs when a pore is dissolved in the spore wall and the protoplasm emerges (Raper and Quinlan 1957).

Eight of the nine species of Dictyosteliaceae isolated in Kansas soils have elliptical spores (Figs. 2, 16, 30, 40, 69, 78, 89, 101). The ninth, Dictyostelium lacteum (Fig. 54) has spherical spores like those of A. leptosomum (Raper and Quinlan 1957). After germination of the spores of the Dictyosteliaceae, the myxamoebae move along the substrate by means of filose pseudopodia (Figs. 3, 17, 31, 41, 56, 70, 79, 90, 102). They feed on bacteria by a process of invagination similar to that of the Sarcodina (Kudo 1946). This vegetative phase usually lasts 24 - 36 hours before aggregation begins. Aggregation may be induced by several factors (Raper 1939, 1940, 1956a; Bonner 1950; Bonner and Shaw 1957). At least two of these, 1) decrease in relative humidity, and 2) depletion of food, were active in inducing aggregation in the Kansas isolates.

A typical radiate pseudoplasmodium is formed in seven of the nine Kansas species (Figs. 4, 18, 32, 42, 80, 91, 103). The other two species, D. minutum and D. lacteum usually form small round clumps from which the culminating sorocarps arise, although pseudoplasmodia with radiating arms have been observed in a few cases in cultures of these 2 species (Figs. 57, 71). Raper (1951) reported a radiate pseudoplasmodium for both D. minutum and D. lacteum but he used very thin bacterial streaks in his work and the myxamoebae had a low population density. Under these conditions if radiate pseudoplasmodia were formed, they could be easily observed.

Six of the nine Kansas species have definite migratory phases. Two of these species Dictyostelium discoideum and D. polycephalum form stalkless pseudoplasmodia which move about for a period of time before culmination. The pseudoplasmodium of D. polycephalum is long and thin with a very thin slime sheath (Fig. 6). As a result, it cannot be manipulated as well as the shorter, thicker, slug-shaped pseudoplasmodium of D. discoideum with its thicker slime sheath (Fig. 19). Another difference between the two species is that D. discoideum usually produces only one pseudoplasmodium from an aggregation (Fig. 18) whereas the aggregation of D. polycephalum produces a number of pseudoplasmodia (Figs. 4, 5).

Following the migration phase the pseudoplasmodium of D. discoideum orients itself in an upright position and produces only one sorogen which develops into a single sorocarp (Figs. 21-29), whereas the pseudoplasmodium of D. polycephalum rounds up and divides into a number of sorogens which form a coremiform fructification with several sori (Figs. 8-15).

The four other species with migratory phases are D. mucoroides, D. purpureum, Polysphondylium pallidum and P. violaceum. These species have stalked pseudoplasmodia which move along the substrate before culmination (Figs. 33, 80, 91, 103). These migrating pseudoplasmodia are formed when a central papilla arises from the aggregation and forms a slug-shaped pseudoplasmodium within which the myxamoebae orient themselves, secrete a stalk sheath and begin producing the stalk. As the stalk is produced the pseudoplasmodium moves forward along the substratum. These four species will also produce sorocarps directly from the pseudoplasmodium without a migratory phase. The pseudoplasmodia of four such organisms can be seen in Plates VI, XIV, XVI, and XVIII.

The migrating pseudoplasmodia of these four species will usually produce stalks 5.0 mm. - 10.0 mm. in length before culminating in simple, irregularly branched or branched (whorled) fructifications with terminal sori.

The three species of Dictyostelium which did not form migrating pseudoplasmodia in the Kansas isolates were D. brevicaule, D. minutum and D. lacteum. Dictyostelium brevicaule will form on occasion, a short-stalked pseudoplasmodium that will move along the substrate a short distance before culmination (Fig. 43), but this does not compare with the migratory phase of the other species.

The formation of the fruiting structure or sorocarp is referred to as the culmination phase of the life history. Fructifications vary from the small, single or gregarious sorocarps of D. minutum and D. lacteum to the complex coremiform fructifications of D. polycephalum and the multibranched sorocarps of the Polysphondylia. The small fructifications of D. minutum and D. lacteum have stalks composed of a single row of superposed or uniserate stalk cells at the tip of which is held the mass of spores in a mucus-like substance. The larger fructifications of D. mucoroides, and D. purpureum have a stalk which is 3 to several cells thick at the base but which tapers to a row of uniserate cells near the apex before forming the terminal sorus.

Dictyostelium discoideum has a fructification that forms a basal disc from which the stalk gradually tapers to a row of single cells before forming the sorus at the tip. Dictyostelium brevicaule forms a uniformly thick stalk several cells in diameter at the apex of which the sorus is held. Abnormal appearing fructifications of this species will sometimes have 2 or 3 stalks with only one sorus. This is partially explained by Figures 45 - 53 which show 3 sorogens fusing to form a single sorus. Dictyostelium polycephalum forms a coremiform fructification which is produced when a number of sorogens

mature close to one another and secrete a slime sheath around the stalks (Figs. 8-15). The stalks of each of the sorocarps in the fructification is 2 or 3 cells thick at the base and after diverging from the coremium, forms columns of uniserate cells at the apices of which the sori are borne. The Polysphondylia form branched fructifications (Figs. 93, 100, 105, 112). The main axis of the sorocarp is 2 - 3 cells thick at the base and tapers to a single row of cells at the tip of which the primary sorus is borne. At intervals along the main axis, however, whorls of branches are produced, and each of these consists of 1 or 2 cells at the point of attachment and then tapers to a uniserate sorus of cells at the tips of which the secondary sori are borne.

KEY TO THE ACRASIEAE

Nine species of cellular slime molds were identified by the use of various sources of information since a recent monograph does not exist. From these various sources, a key was constructed based on morphologic characteristics. Revisions were made when indicated from studies made of the Kansas isolates. Only members of the Dictyosteliaceae were isolated from Kansas soils but all of the valid species of Acrasieae were included in the key. Thirty-one species of cellular slime molds have been named, but at present only 27 are considered valid by most workers (Raper 1951, 1960; Bonner 1959; L. S. Olive 1960).

A description of the nine species obtained from Kansas soils based on these isolates is provided in the section of the key pertaining to species identification.

CLASS ACRASIEAE

Saprophytic, usually soil or coprophilous organisms having two definite phases: the vegetative phase of myxamoebae which move with the aid of

pseudopodia and multiply by simple cell division; and the fruiting phase in which the myxamoebae typically aggregate and form pseudoplasmodia which may or may not migrate before culminating in sessile or stalked sorocarps. The myxamoebae remain distinct and do not fuse during this stage. The spores or pseudospores are usually surrounded by a mucus-like substance on the sorocarp and are not contained within a peridium.

ORDER ACRASIALES

Only one order with the characteristics of the class.

Key to the Families of the Order Acrasiales

1. Myxamoebae with lobose pseudopodia, or myxamoebae without pseudopodia. . . . . Guttulinaceae
1. Myxamoebae with filose pseudopodia. 2
    2. Fructification a single encysted individual on a short acellular stalk . . . . . Protosteliaceae
    2. Fructification composed of more than one individual. 3
3. Stalk acellular, sorus containing a number of spores. . . . . Acytosteliaceae
3. Stalk of vacuolated, parenchyma-like cells. . . . . .Dictyosteliaceae

Family Guttulinaceae

Myxamoebae with rounded or lobose pseudopodia, or limax-shaped myxamoebae. Sori are irregular in shape or spherical and may be sessile or stalked; consisting of spores with a definite cell wall, or pseudospores which are encysted individuals without spore-walls (Olive 1902).

Key to the Genera of the Guttulinaceae

1. Myxamoebae limax-shaped, without pseudopodia. . . . . Guttulina
1. Myxamoebae with lobose pseudopodia. 2
   2. Sori sessile or stalked, consisting of pseudospores. . . . . Guttulinopsis
   2. Sori consisting of a chain of true spores on a uniserate stalk . . . . . Acrasis

Guttulina Cienkowsky

Trans. bot. sec. 4th meeting of Russ. nat. at Kazan, 1873.

TYPE SPECIES: Guttulina rosea Cienkowsky

Myxamoebae normally limax-shaped without pseudopodia. Sori irregular or spherical, sessile or stalked; composed of spores with a definite spore wall. The stalk cells are somewhat differentiated (Olive 1902).

Key to Species

1. Sorocarps white or yellowish. 2
1. Sorocarps of a definite color 3
   2. Sorus pure white, sessile; spores oval, colorless . . . . G. sessilis
   2. Sorus yellowish white, sessile or short stalked. . . . . G. protea
3. Sorus rose-colored, short-stalked . . . . . .G. rosea
3. Sorus golden yellow, short-stalked. . . . . .G. aurea

A fifth species, G. nivea, has been proposed by Raper (1960) but as yet sufficient information about it is lacking to include it in the key.

Guttulinopsis Olive

Proc. Amer. acad. arts and sci. 37: 335. 1901.

TYPE SPECIES: Guttulinopsis vulgaris Olive

Myxamoebae with lobose pseudopodia. Sori sessile or stalked: composed of pseudospores which lack definite cell walls. The individual simply encysts and upon becoming active the cyst wall is absorbed by the individual. All species of this genus are of a yellowish-white color (Olive 1902).

Key to Species

1. Sori stalked or sessile; sorocarps 55 - 150u tall, sori 150 - 400u in diameter. . . . . .G. vulgaris
1. Sori stalked 2
   2. Stalk approximately 800u long, some sorocarps as high as 3.0 mm. - 12.0 cm., sori 250u in diameter. . . . G. stipitata
   2. Stalk approximately 170 - 250u long, sori 100 - 400u in diameter. Stalk cells surrounded by mucus and slightly differentiated from those of the sorus . . . . . G. clavata

Acrasis van Tieghem

Bull. de la soc. bot. de France. 27: 317. 1880.

TYPE SPECIES: Acrasis granulata van Tieghem

Myxamoebae with lobose pseudopodia. Pseudoplasmodia not migrating. Sorocarps ranging from a single uniserate stalk to many branching chains; spores spherical, smooth or slightly roughened (Olive 1902; Olive and Stoianovitch 1960).

Key to Species

1. Sorocarps of a deep violet color. . . . . .A. granulata
1. Sorocarps pink. . . . . .A. rosea

Family Protosteliaceae

A sorocarp is formed when a myxamoeba secretes a non-cellular stalk and then encysts at the tip to become a spore. Spores develop singly although in luxuriant cultures several spores may coalesce and form heads of few to many spores with as many stalks below. One genus represents the family (Olive 1962).

Protostelium Olive and Stoianovitch

Bull. Torr. Bot. Club. 87: 1-20. 1960.

TYPE SPECIES: Protostelium mycophaga Olive and Stoianovitch

Myxamoebae with filose pseudopodia. Pseudoplasmodia lacking. Spores typically born singly at the tip of filiform, non-cellular stalks, occasionally several to a single stalk formed as described above (Olive 1960).

Key to Species

1. Spores bright orange. 2
1. Spores colorless. 3
   2. Spores typically globose 4.1 - 11.6u. . . P. mycophaga var. mycophaga
   2. Spores typically globose, 10.5 - 18u. . . . . P. mycophaga var. major
3. Spores spherical or nearly so 10 - 16u diameter . . . . . P. fimicola
3. Spores elongate, peanut-shaped, 8.8 - 9.3 x 20 - 46u. . . .P. arachispora

Family Acytosteliaceae

Myxamoebae with filose pseudopodia. Pseudoplasmodia radiate, developing into sorocarps composed of acellular stalks bearing masses of spores as terminal sori. There is only one genus in this family.

Acytostelium Raper

Mycologia 48: 179. 1956. Jour. gen. microbiol. 18: 16-32. 1957.

TYPE SPECIES: Acytostelium leptosomum Raper

Myxamoebae with filose pseudopodia producing radiate pseudoplasmodia. Sorocarps gregarious, unbranched, 750 - 1500u in height. Stalks acellular. Sori globose. Spores spherical. Mature fructifications are milk-white in color. Monotypic.

Family Dictyosteliaceae

Myxamoebae possess slender, filose pseudopodia. Pseudoplasmodia present; some forms with migrating, stalked, or stalkless pseudoplasmodia. Stalk is composed of highly differentiated, parenchyma-like cells with cellulose walls. The sori consist of spherical masses of spores; spores spherical or elliptical. Three genera are represented in the family.

Key to the Genera of the Dictyosteliaceae

1. Stalk expanded into a cupule at the tip in which the sorus rests. . . . . Coenonia
1. Stalk not expanded into a cupule at the tip. 2

2. Stalk simple or occasionally irregularly branched, luxuriant fructifications, frequently gregarious. . . . Dictyostelium

2. Stalks branching regularly in whorls along the main axis, only occasionally simple . . . . . Polysphondylium

Coenonia van Tieghem

Bull. de la soc. bot. de France. 31: 303-306. 1884.

TYPE SPECIES: Coenonia denticulata van Tieghem

Fructification simple or occasionally divided into three equidistant branches, consisting of a sorus borne in a cupule at the tip of the stalk. Stalk consists of cells in which the peripheral edges are papillate, it is attached to the substrate by a hold-fast, and the tip is expanded into a cupule with a serrated edge. Sorus is globose, yellowish. Spores are yellowish-white (Olive 1902; Raper 1960). Monotypic.

Dictyostelium Brefeld

Abd. d. Senck. nat. ges., 7: 85-107. 1869.

TYPE SPECIES: Dictyostelium mucoroides Brefeld

Fructifications are simple or gregarious. Sorocarps range in size from 300u to over 1 cm. in height. The sori are stalked. The stalk is usually simple or may occasionally branch irregularly, or a coremiform fructification may be formed. Sori are globose, consisting of spherical or elliptical spores.

Key to Species

1. Sorocarps typically forming a coremiform fructification. . . . . D. polycephalum

1. Sorocarps not typically forming a coremiform fructification. 2

2. Stalk arising from a basal disk . . . . . D. discoideum

2. Stalk not arising from a basal disk 3

3. Sorocarps white or colorless. 4

3. Sorocarps colored 8

4. Stalk long and flexible 1 cm. or more in height (Stalk over 5 cm. long, D. giganteum) . . . . . D. mucoroides

4. Stalk not long, less than 1 cm. in height 5

5. Stalk short, less than 5 mm. high 6

5. Stalk 6 - 10 mm. in height. 8

6. Sorocarp relatively large; sorophore rigid, from 1 - 3 mm. . . . . D. brevicaule

6. Sorocarps quite small, (1 mm. or less) may or may not be clustered. 7

7. Spores spherical 2.8 - 4.2u in diameter . . . . . .D. lacteum

7. Spores elliptical 2.8 x 5.6 - 7.0u. . . . . .D. minutum

8. Sorocarps light to golden yellow. . . . . D. aureum

8. Sorocarp of some other color. 9

9. Sorocarp bright rose. . . . . D. roseum

9. Sorocarp purple or almost black . . . . . .D. purpureum

1. Dictyostelium polycephalum Raper. Mycologia. 48: 169-205. 1956. Jour. gen. microbiol. 14: 716-732. 1956.

Spore germination is rapid and large numbers of myxamoebae can be observed in a culture within 24 hours. These myxamoebae are colorless and measure 8.5 - 15.4μ and have filose pseudopodia (Fig. 3). Aggregations are typically radiate and begin 36 - 48 hours after inoculation (Fig. 4). Long, thin stalkless, migrating pseudoplasmodia are formed from these aggregations and are present for periods of time up to two weeks (Figs. 5-6) before culmination is initiated. Culmination begins by the rounding up of the pseudoplasmodium (Fig. 8). Within hours, lobes begin to appear in the pseudoplasmodium (Fig. 9) and these slowly deepen and elongate (Figs. 10-11). The stalks of these lobes or sorogens are usually enclosed within a common slime sheath so that the sorogens are held together as culmination progresses (Figs. 12-13) forming a coremium. Often these coremia are composed of numerous sorocarps although the usual number is three or four (Fig. 14). Mature sorocarps may be simple but are typically coremiform (Figs. 7, 15) measuring 300 - 350μ in height. Sori are white or colorless and measure 40 - 80u in diameter. The spores of this species are colorless and elliptical and measure 2.8 x 5.6μ (Fig. 2).

2. Dictyostelium discoideum Raper. Jour. agr. res. 50: 135-147. 1935.

Spore germination is rapid and large numbers of myxamoebae can be observed in a culture within 24 hours of inoculation. Figure 17 shows the colorless myxamoebae which measure 5.6 - 11.2μ and have filose pseudopodia. Aggregations are formed within 36 - 48 hrs. and have atypical radiate pattern (Fig. 18). The pseudoplasmodia are stalkless, slug-shaped and migrate for a period of time before culmination (Fig. 19). Figures 21-29 show the various stages of

EXPLANATION OF PLATE II

Figs. 2-7. Stages in the life history of Dictyostelium polycephalum Raper.

Fig. 2. Spores x 1387.

Fig. 3. Myxamoebae x 1387.

Fig. 4. Aggregation x 29.

Fig. 5. Pseudoplasmodium formation x 38.

Fig. 6. Migrating pseudoplasmodium x 38.

Fig. 7. Mature fructification x 143.

PLATE II

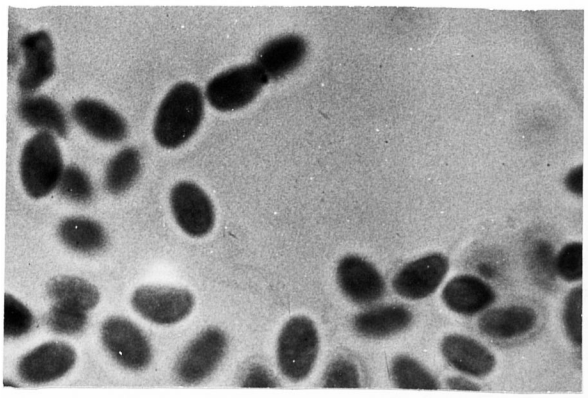


Fig. 2

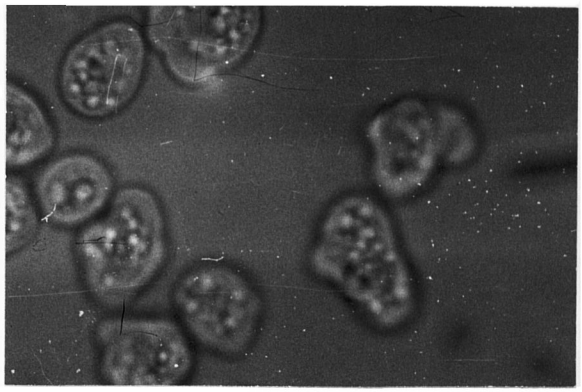


Fig. 3

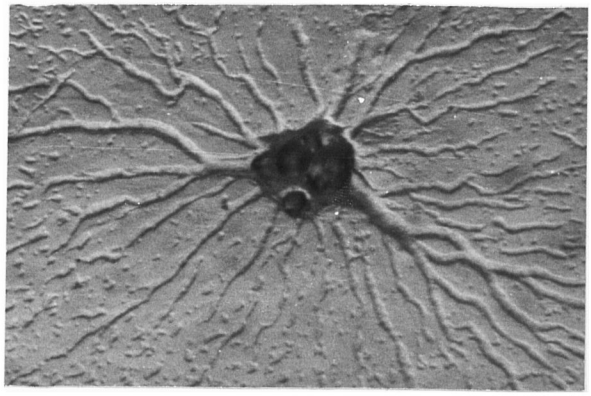


Fig. 4

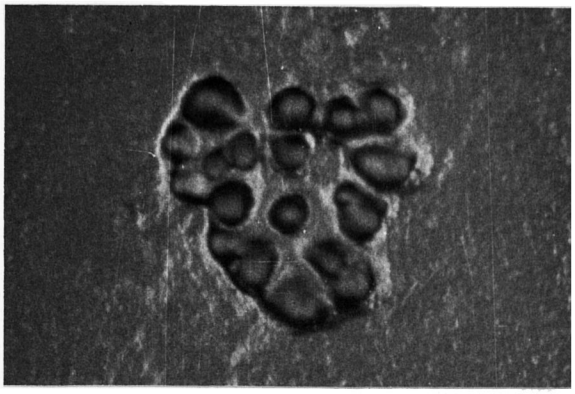


Fig. 5

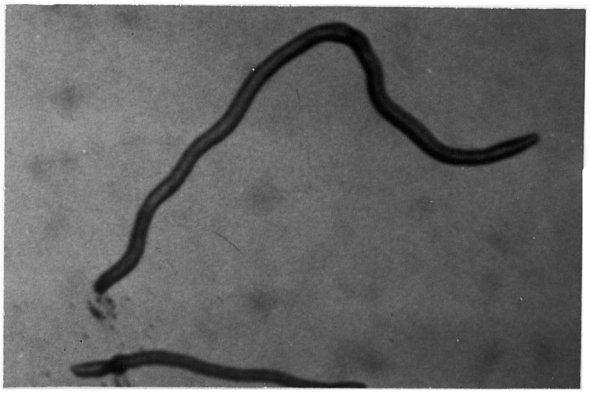


Fig. 6

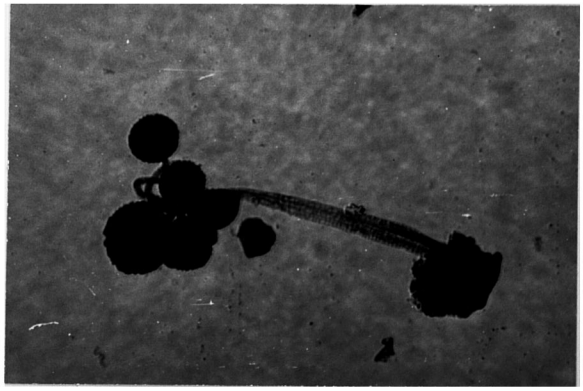


Fig. 7

EXPLANATION OF PLATE III

Figs. 8-15. Sorocarp formation in Dictyostelium polycephalum Raper photographed at hourly intervals except where indicated. Magnification x 72.

Fig. 8. Sorogen formation from pseudoplasmodium.

Fig. 9. Sorogen elongating to form coremiform stalk.

Fig. 10. Later stage in stalk elongation.

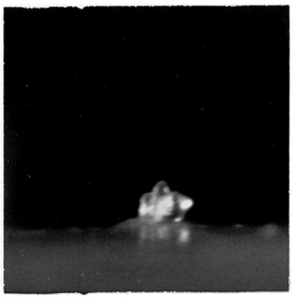
Fig. 11. Coremiform stalk formation nearly complete.

Figs. 12, 13. Sorogens separating above stalk sheath.

Fig. 14. Another sorogen elongating above sheath.

Fig. 15. Mature sorocarp of a different fructification.

PLATE III



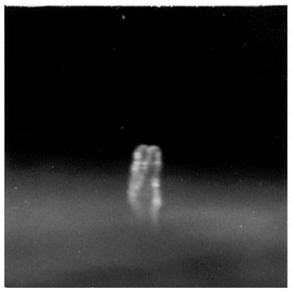


Fig. 9

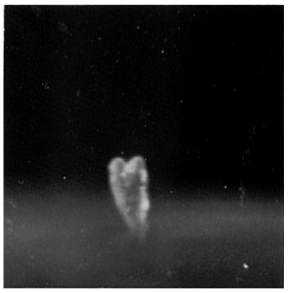


Fig. 10

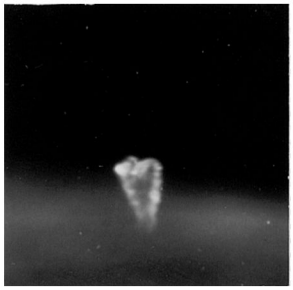


Fig. 11

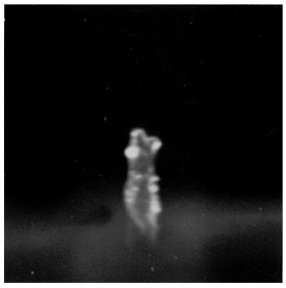


Fig. 12

Fig. 13

Fig. 14

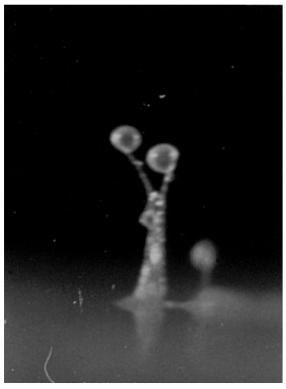


Fig. 15

EXPLANATION OF PLATE IV

Figs. 16-20. Stages in the life history of Dictyostelium discoideum Raper.

Fig. 16. Spores x 1387.

Fig. 17. Myxamoebae x 1387.

Fig. 18. Aggregation x 29.

Fig. 19. Migrating pseudoplasmodium x 29.

Fig. 20. Mature sorocarp x 143.

PLATE IV

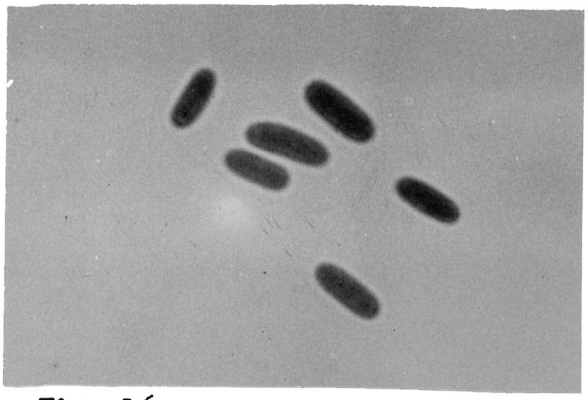


Fig. 16

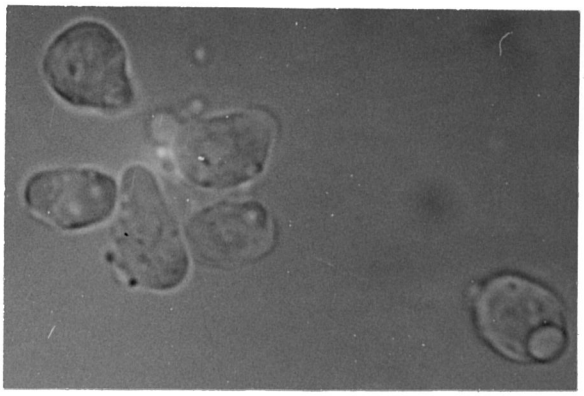


Fig. 17

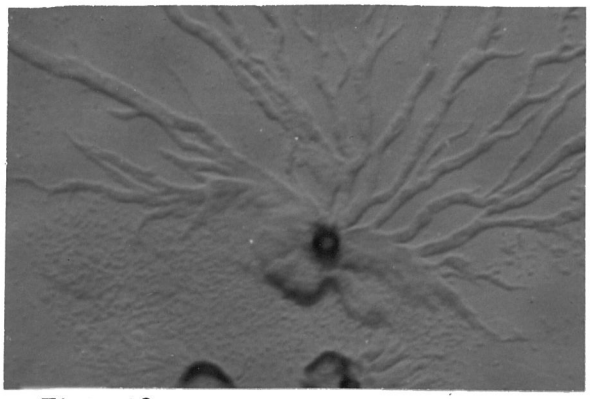


Fig. 18

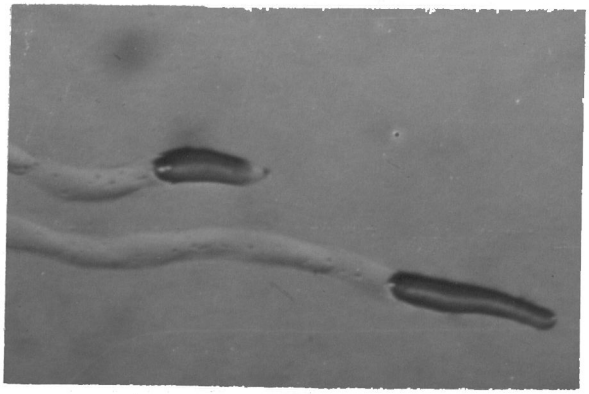


Fig. 19

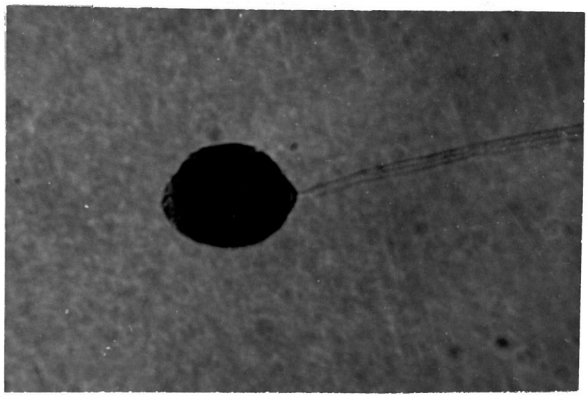


Fig. 20

EXPLANATION OF PLATE V

Figs. 21-29. Sorocarp formation in Dictyostelium discoideum Raper photographed at hourly intervals except where indicated. Magnification x 29.

Fig. 21. Migrating pseudoplasmodium.

Fig. 22. Tip of pseudoplasmodium elevating.

Fig. 23. Sorogen formed.

Fig. 24. Sorogen elevating as stalk is formed.

Fig. 25. Sorogen elevated to show basal disk and beginning of stalk formation.

Figs. 26-27. Stalk elongating.

Fig. 28. Sorocarp nearly complete, tip of stalk still visable.

Fig. 29. Mature sorocarp 18 hours after first photograph was taken.

PLATE V

Fig. 21

Fig. 22

Fig. 23

Fig. 24

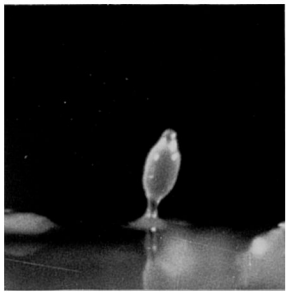


Fig. 25

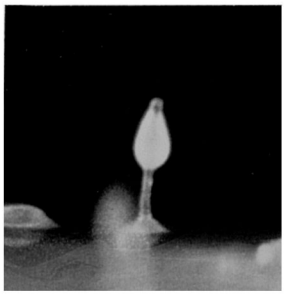


Fig. 26

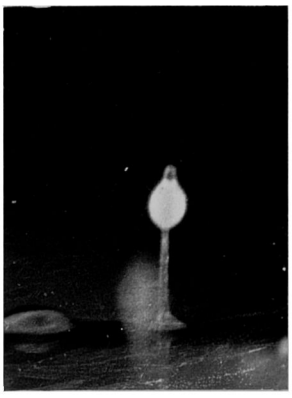


Fig. 27

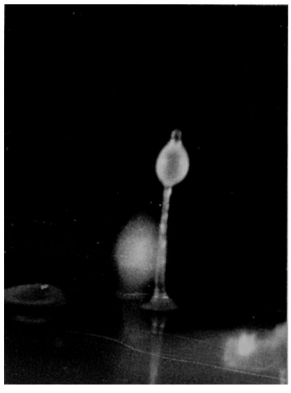


Fig. 28

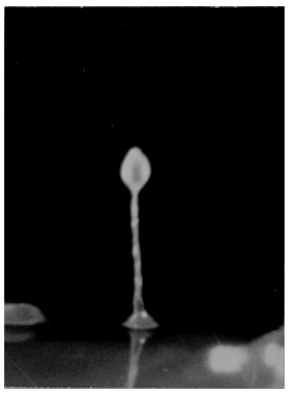


Fig. 29

culmination in this species. The pseudoplasmodium ceases migration, (Fig. 21), and the tip elevates (Fig. 22) and the pseudoplasmodium rounds up (Fig. 23). As the stalk is formed, it is pushed down through the pseudoplasmodium and eventually the sorogen starts to rise (Figs. 24-25). As culmination progresses, the stalk elongates and the mass of cells at the tip (Figs. 26-27) form cell walls and eventually develop into the sorus (Figs. 28-29). The sorocarp is simple and arises from an expanded base (Fig. 29). A fructification usually measures up to 2.4 mm. in height although this series of pictures shows a smaller fructification. Sori are greyish white to pale lemon color and measure 200u in diameter (Fig. 20). The spores are colorless or cream colored in mass and are elliptical and measure 2.8 x 8.4u (Fig. 16).

3. Dictyostelium mucoroides Brefeld. Abd. d. Senck. nat. ges., 7: 85-107. 1869.

Spore germination is rapid and myxamoebae can be observed in the culture within 24 hours. These myxamoebae are colorless, measure 8.4 - 9.9u and have filose pseudopodia (Fig. 31). Aggregations are formed in 24 - 36 hrs. and form atypical radiate pattern (Fig. 32). The stalked pseudoplasmodium (Fig. 33) migrates along the substratum for a period of time before culmination begins. Some strains of this species lack a long migrating period and culminate early into simple or irregularly branched fructifications. The culmination of such a strain is shown in Figures 35-39. Culmination begins by the breaking up of the pseudoplasmodium into a number of lobes or sorogens (Fig. 35). An hour later the stalks have elongated and culmination is proceeding (Fig. 36). Figures 37-38 show the culmination of this species one and two hours after the previous pictures. Figure 39 was photographed approximately 20 hours after culmination began and shows the mature fructification. Mature sorocarps

EXPLANATION OF PLATE VI

Figs. 30-34. Stages in the life history of Dictyostelium mucoroides Brefeld.

Fig. 30. Spores x 1387.

Fig. 31. Myxamoebae and spores x 1387.

Fig. 32. Aggregation x 29.

Fig. 33. Migrating pseudoplasmodium x 29.

Fig. 34. Mature sorocarp x 143.

PLATE VI

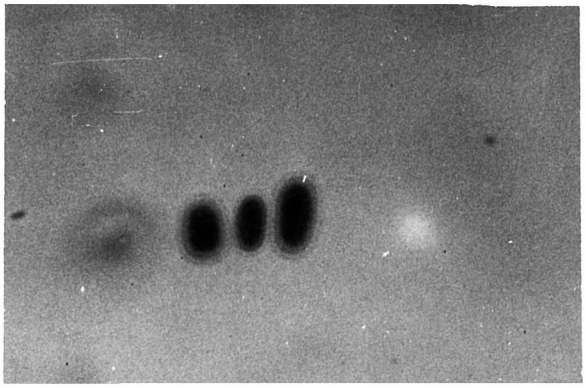


Fig. 30

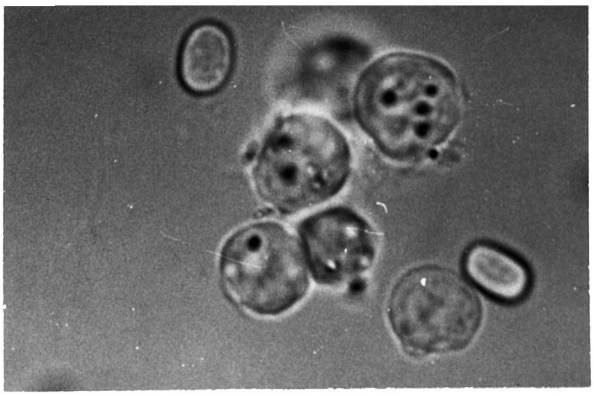


Fig. 31

Fig. 32

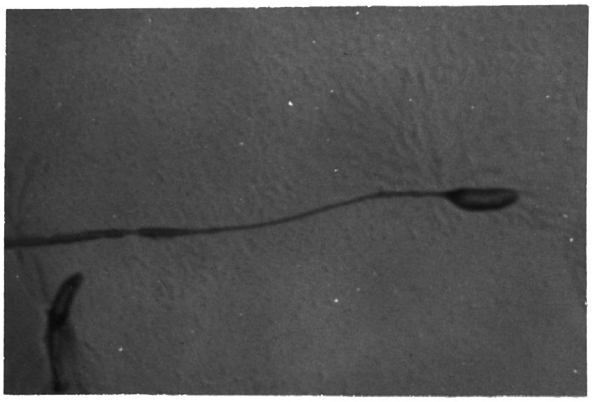


Fig. 33

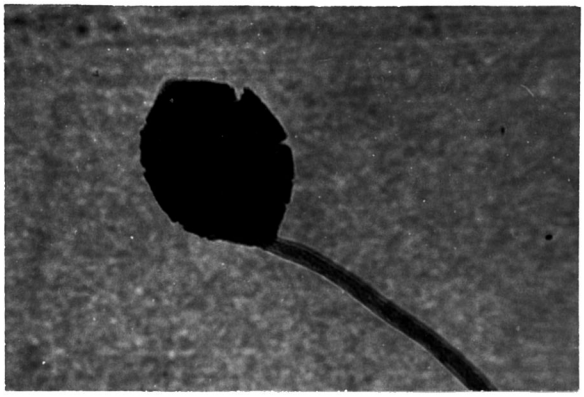


Fig. 34

EXPLANATION OF PLATE VII

Figs. 35-39. Sorocarp formation in Dictyostelium mucoroides Brefeld photographed at hourly intervals except where indicated. Magnification x 38.

Fig. 35. Sorogen formed from pseudoplasmodium.

Fig. 36. Sorogens elongating to form stalks.

Fig. 37. Development of sorogens two hours after previous photograph.

Fig. 38. Sorocarps nearly mature.

Fig. 39. Mature sorocarp 20 hours after the first photograph was taken.

PLATE VII

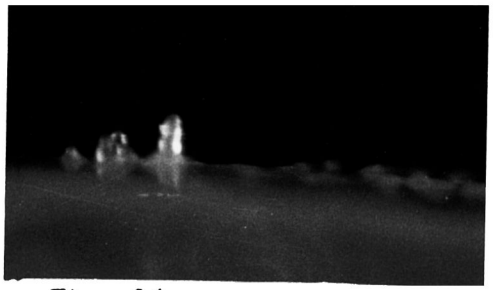


Fig. 35

Fig. 36

Fig. 37

Fig. 38

Fig. 39

may be simple or irregularly branched and measure 8 mm. - 1.5 cm. in height. These sorocarps are white or yellowish when old. Sori measure 200 - 300u in diameter (Fig. 34). Spores are colorless, elliptical and measure 2.8 x 5.6 - 7.0μ (Fig. 30).

4. Dictyostelium brevicaule. Olive. Proc. Amer. acad. arts and sci. 37: 340. 1901.

This isolate designated "D-11" may be a part of the D. mucoroides complex proposed by various authors, but it is believed to approximate Olive's concept of D. brevicaule. Spore germination is rapid and myxamoebae are observed in the culture within 24 hours. These myxamoebae are colorless, measure 9.9 - 13.2μ and have filose pseudopodia (Fig. 41). Aggregations form within 24 - 36 hours and have a typical radiate pattern (Fig. 42). The pseudoplasmodia (Fig. 43) culminate directly into mature sorocarps. Figures 45 - 53 show the culmination of this species. In this case three sorogens were formed from the pseudoplasmodium and these culminated close to one another (Figs. 45-48) so that eventually the sori coalesced and formed one sorus (Figs. 49-53). The mature sorocarps measure 500u - 1.5 mm. and the yellowish sori measure 200 - 300μ in diameter (Figs. 44, 53). The spores are colorless, elliptical and measure 2.8 x 7.0u (Fig. 40).

5. Dictyostelium lacteum van Tieghem. Bull. de la soc. bot. de France. 27: 317. 1880.

Spore germination is moderate although some myxamoebae can be seen in the culture within 24 hours. Myxamoebae are colorless, measure 8.4 - 10.0μ and have filose pseudopodia (Fig. 55). Aggregations form within 36-48 hours. The

EXPLANATION OF PLATE VIII

Figs. 40-44. Stages in the life history of Dictyostelium brevicaule Olive.

Fig. 40. Spores x 1387.

Fig. 41. Myxamoebae x 1387.

Fig. 42. Aggregation x 29.

Fig. 43. Pseudoplasmodium x 29.

Fig. 44. Mature sorocarp x 143.

PLATE VIII

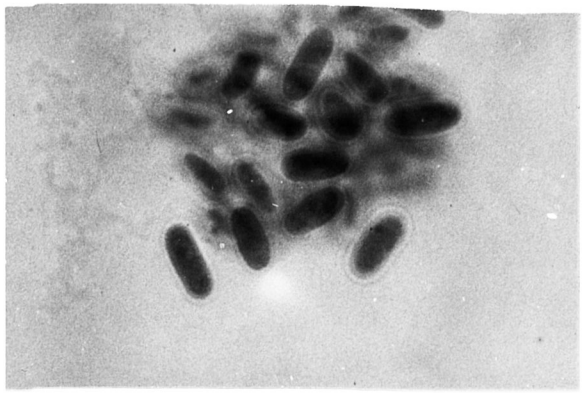


Fig. 40

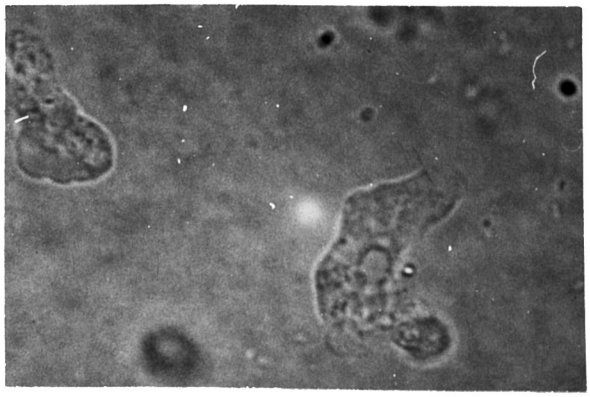


Fig. 41

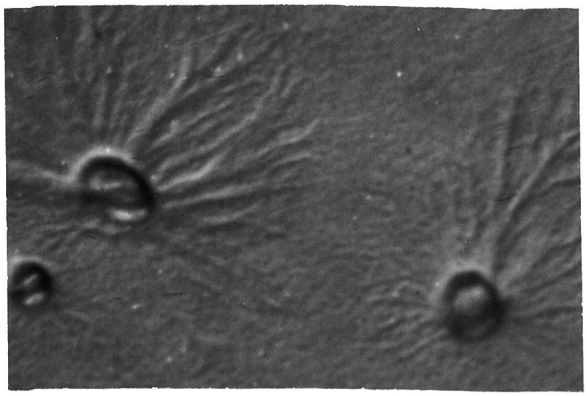


Fig. 42

Fig. 43

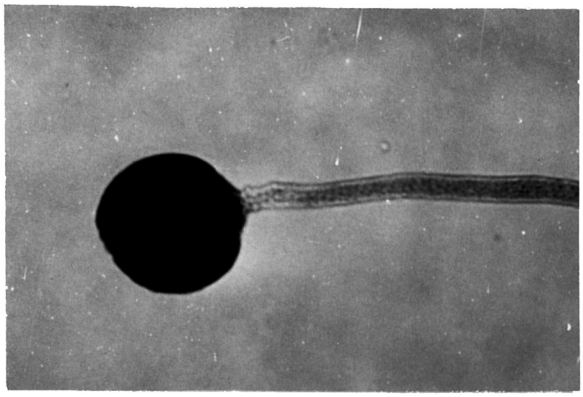


Fig. 44

EXPLANATION OF PLATE IX

Figs. 45-53. Stages in sorocarp formation of Dictyostelium brevicaule Olive photographed at hourly intervals except where indicated. Magnification x 29.

Fig. 45. Sorogen formed from the pseudoplasmodium.

Fig. 46. Three sorogens formed from the pseudoplasmodium.

Figs. 47-48. Sorogens elevating as stalk is formed.

Figs. 49-52. Sorogens beginning to fuse.

Fig. 53. Fusion of sorogens complete, sorocarp nearly mature.

Fig. 54. View of a mature sorocarp of another fructification.

PLATE IX

Fig. 45

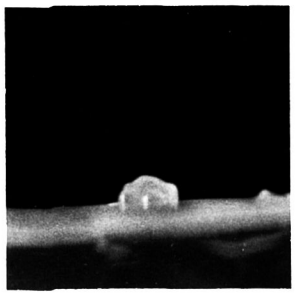


Fig. 46

Fig. 47

Fig. 48

Fig. 49

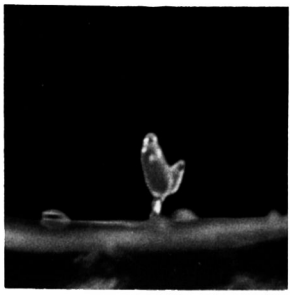


Fig. 50

Fig. 51

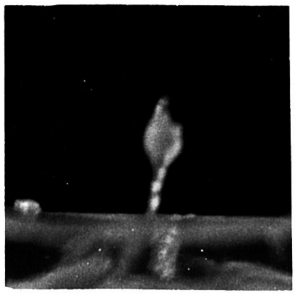


Fig. 52

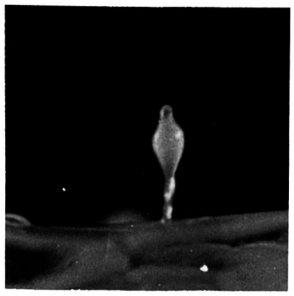


Fig. 53

Fig. 54

EXPLANATION OF PLATE X

Figs. 55-68. Stages in the life history of *Dictyostelium lacteum* van Tieghem.

Fig. 55. Spores x 1387.

Fig. 56. Myxamoebae x 1387.

Fig. 57. Aggregation x 38.

Fig. 58. Mature sorocarp x 143.

PLATE X

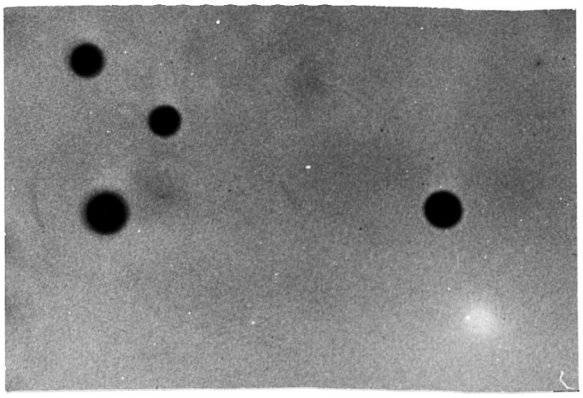


Fig. 55

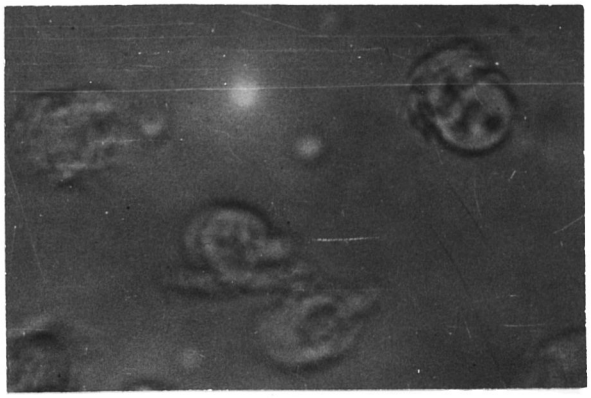


Fig. 56

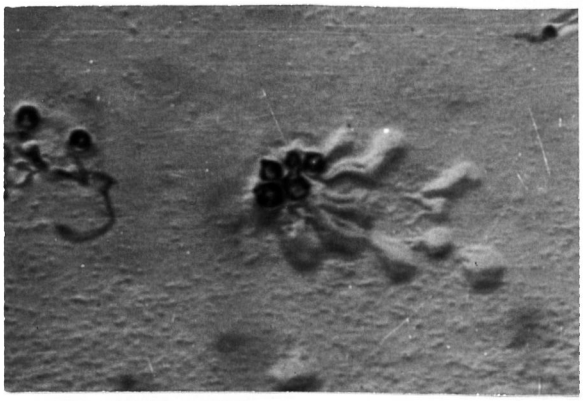


Fig. 57

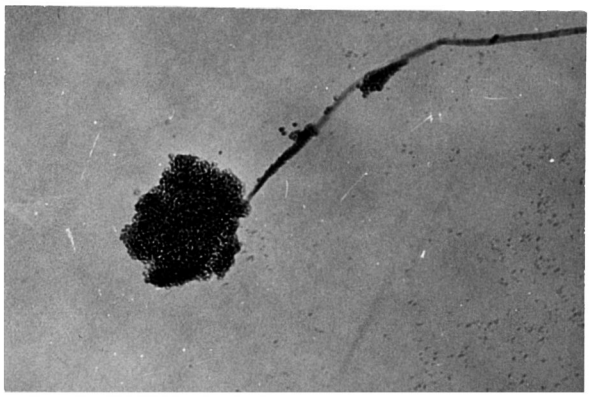


Fig. 58

EXPLANATION OF PLATE XI

Figs. 59-58. Stages in the sorocarp formation of Dictyostelium lacteum van Tieghem photographed at hourly intervals except where indicated. Magnification x 38.

Figs. 59-61. Sorogens formed from pseudoplasmodium.

Figs. 62-66. Sorogens forming a number of stalks which are closely oppressed.

Fig. 67. Sorocarp nearly mature.

Fig. 68. Mature sorocarps photographed 23 hours after initial picture was taken. The sorocarps have collapsed and form a mat close to the surface of the substratum.

PLATE XI

Fig. 59

Fig. 60

Fig. 61

Fig. 62

Fig. 63

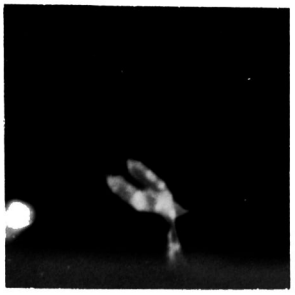


Fig. 64

Fig. 65

Fig. 66

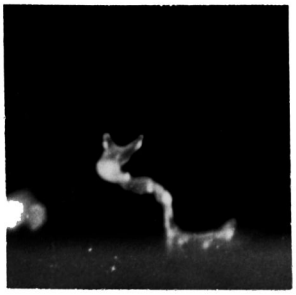


Fig. 67

Fig. 68

pseudoplasmodia may form in a radiate pattern (Fig. 56) but tend to form a central clump from which culmination begins directly. These clumps break up into numerous papillae or short projections (Fig. 58) which slowly elongate (Figs. 59-66), and eventually form sori on uniserate stalks (Fig. 67). These sorocarps do not remain erect but fall over and form a mat on the substrate. Sorocarps are clustered or sometimes single and measure less than 1 mm. in height. The sorus is milk white and measures 80 - 120u in diameter (Fig. 57). The spores are spherical and measure 2.8 - 4.2u in diameter (Fig. 54).

6. Dictyostelium minutum Raper. Mycologia. 33: 633-649. 1941.

Spore germination is rapid and myxamoebae are observed in the culture within 12 - 24 hours. These myxamoebae are colorless, measure 9.9 - 13.2u and have filose pseudopodia (Fig. 69). Aggregations form a pseudoplasmodium in a clump with no radiate arms (Fig. 70). The pseudoplasmodia break up into papillae or small sorogens which culminate directly into mature sorocarps. Figures 72, 73 show the pseudoplasmodium breaking up into a number of sorogens. These sorogens slowly elevate (Figs. 74-75) and eventually mature sorocarps are formed (Fig. 76). Sorocarps are clustered, or sometimes single and measure 250 - 750u in height. Sori are colorless to milk white and measure 80u in diameter (Fig. 71). Spores are colorless, elliptical and measure 2.8 x 5.6-7.0u (Fig. 68).

7. Dictyostelium purpureum Olive. Proc. Amer. acad. arts and sci., 37: 340. 1901.

Spore germination is rapid and myxamoebae can be seen in the culture within 12 - 24 hours. These myxamoebae are colorless, measure 5.6 - 7.0u and

EXPLANATION OF PLATE XII

Figs. 69-72. Stages in the life history of *Dictyostelium minutum* Raper.

Fig. 69. Spores x 1387.

Fig. 70. Myxamoebae x 1387.

Fig. 71. Aggregation x 38.

Fig. 72. Mature sorocarp x 143.

PLATE XII

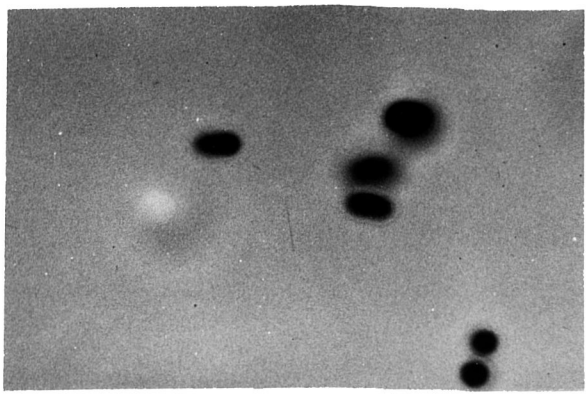


Fig. 69

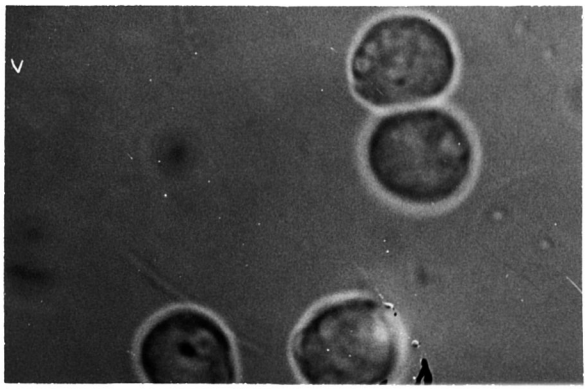


Fig. 70

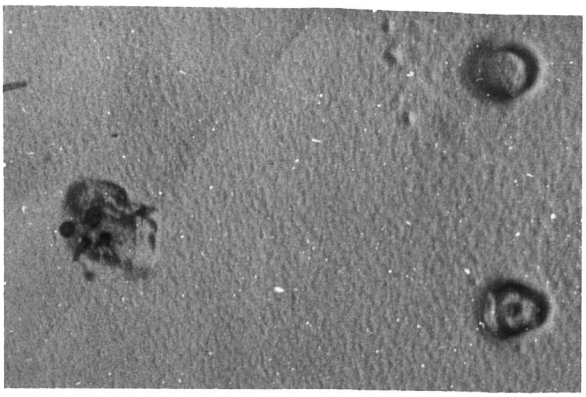


Fig. 71

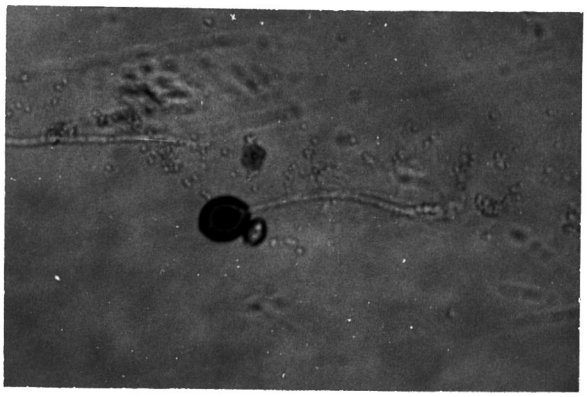


Fig. 72

EXPLANATION OF PLATE XIII

Figs. 73-77. Stages in the sorocarp formation of Dictyostelium minutum Raper photographs at hourly intervals except where indicated. Magnification x 38.

Fig. 73. Sorogen formation from pseudoplasmodium.

Fig. 74. Sorogens breaking up to form a number of papillae.

Fig. 75. Sorogens rising on stalks.

Fig. 76. Photograph of sorogen taken 2 hours after previous picture.

Fig. 77. Mature sorocarp of another fructification.

PLATE XIII

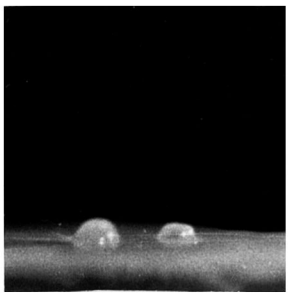


Fig. 73

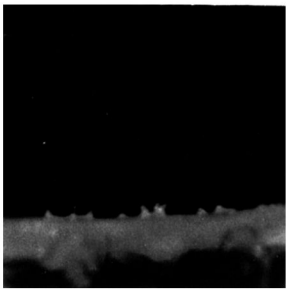


Fig. 74

Fig. 75

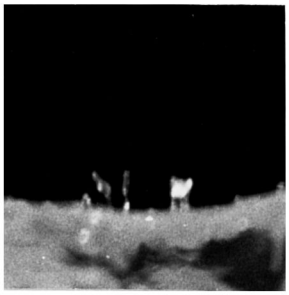


Fig. 76

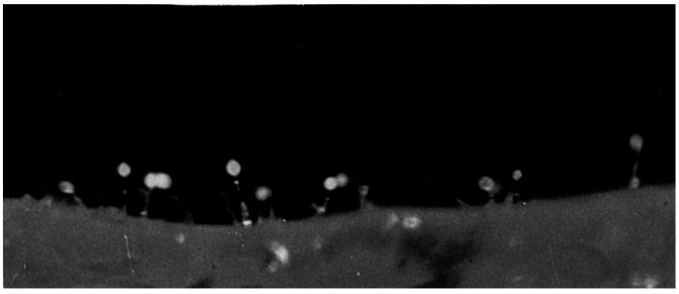


Fig. 77

EXPLANATION OF PLATE XIV

Figs. 78-82. Stages in the life history of Dictyostelium purpureum Olive.

Fig. 78. Spores x 1387.

Fig. 79. Myxamoebae x 1387.

Fig. 80. Aggregation x 29.

Fig. 81. Migrating pseudoplasmodium x 29.

Fig. 82. Mature sorocarp x 143.

PLATE XIV

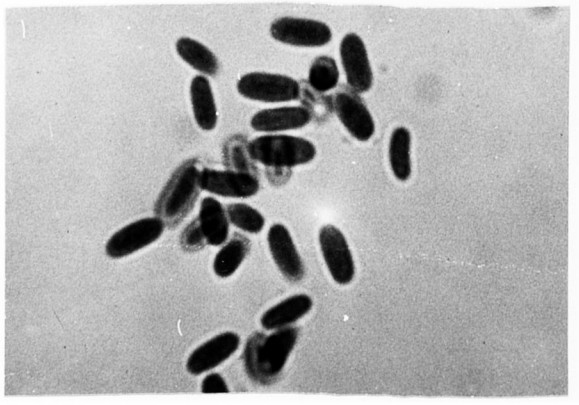


Fig. 78

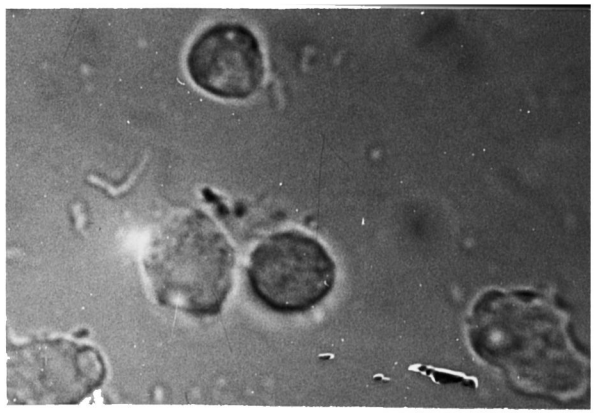


Fig. 79

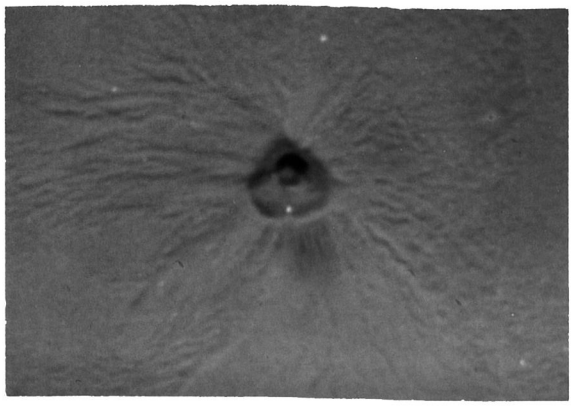


Fig. 80

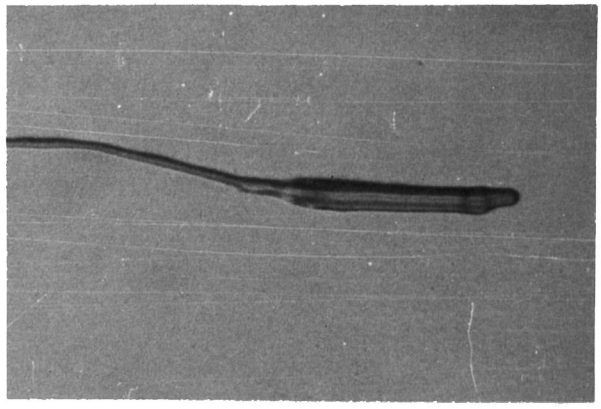


Fig. 81

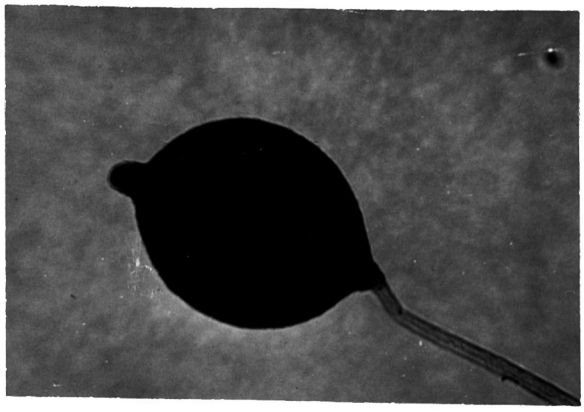


Fig. 82

EXPLANATION OF PLATE XV

Figs. 83-88. Stages in the sorocarp formation of Dictyostelium purpureum Olive photographed at hourly intervals except where indicated. Magnification x 38.

Fig. 83. Sorogens formed from the pseudoplasmodia.

Figs. 84-87. Sorogens elongating on stalks.

Fig. 88. Mature sorocarps photographed 24 hours after first picture was taken.

PLATE XV

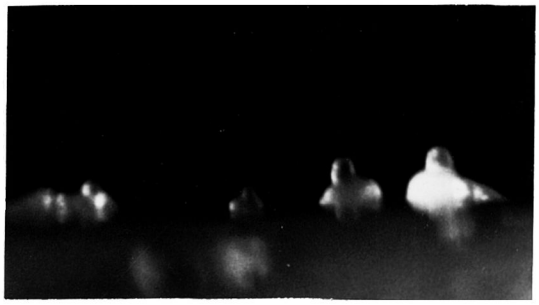


Fig. 83

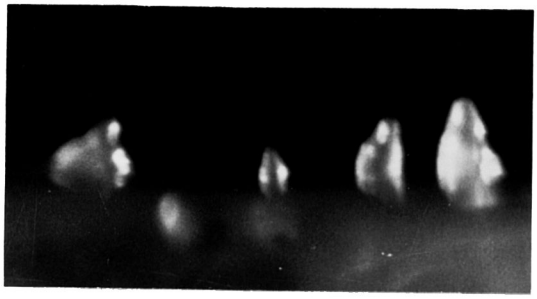


Fig. 84

Fig. 85

Fig. 86

Fig. 87

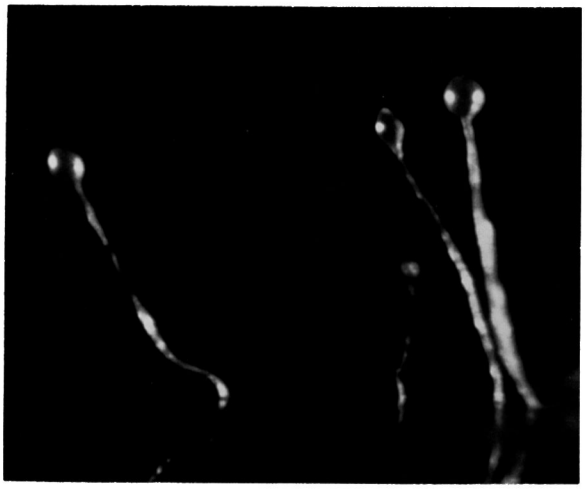


Fig. 88

have filose pseudopodia (Fig. 78). Aggregations are formed within 24 hours and form a typical radiate pattern (Fig. 79). The stalked pseudoplasmodium moves along the substrate for a period of time before culmination (Fig. 80) or may culminate immediately after pseudoplasmodium formation. Figures 82-87 are a series of time interval photographs showing culmination in this species. Figures 82-83 show the pseudoplasmodium beginning culmination. One hour later the sorogens have elevated slightly (Fig. 84). Figures 85-86 were photographed one hour apart and show the stalked sorogens. Figure 87 shows the mature sorocarp approximately 20 hours after the first picture was taken. These fructifications are not as large as usually occurs in this species but this may be due to conditions that they were subjected to during photography. Sorocarps are usually large, measuring 8 mm. to 1.5 cm. in height. Sori are purple or nearly black and measure 200 - 250μ in diameter (Fig. 81). Spores are purple in mass, elliptical, and measure 2.8 x 4.9 - 5.6μ (Fig. 77).

Polysphondylium Brefeld

Schimmelpilze. 6: 1-34. 1884.

TYPE SPECIES: Polysphondylium violaceum Brefeld.

Mature fructifications measuring 5.0 mm. to 10.0 mm. in height. The sori are spherical and are borne terminally on primary and secondary stalks, the latter branching in whorls at intervals along the main axis; fructifications occasionally simple as in Dictyostelium. (Olive 1902).

Key to Species

1. Sorocarps purple or violet. . . . . .P. violaceum

1. Sorocarps white (P. album). . . . . P. pallidum

1. Polysphondylium violaceum Brefeld. Schimmelpilze. 6: 3 - 34. 1884.

Spore germination is fairly rapid and myxamoebae can be seen in the culture within 24 hours. These myxamoebae are colorless, measure 9.8 - 21.0u and have filose pseudopodia (Fig. 89). Aggregations form within 24 - 36 hours and have a typical radiate pattern (Fig. 90). The stalked pseudoplasmodium moves along the substrate for a period of time before culmination (Fig. 91). Figures 93-99 illustrate the various steps in the culmination of this species. The pseudoplasmodium elevates on a stalk (Fig. 93) and eventually a few clumps of myxamoebae are left at intervals along the stalk as culmination progresses (Figs. 94-96). These clumps gradually form whorls of branches (Fig. 97) along the main axis and a terminal sorus is formed (Figs. 98-99). The fructifications of this species are less perfectly formed than P. pallidum and few whorls of branches may be formed. Mature sorocarps measure 1.0 cm. in height with terminal sori of a purple or violet color. The terminal sorus measures 100u, secondary sori measure 60u in diameter (Fig. 92). Spores are violet in mass, elliptical and measure 2.8 x 5.6u (Fig. 88).

2. Polysphondylium pallidum Olive. Proc. Amer. acad. arts and sci. 37: 342. 1901.

Spores of this species germinate quite readily and myxamoebae can be observed in a culture within 12 hours after inoculation. Myxamoebae are colorless, measure 8.4 - 14.0u and have filose pseudopodia (Fig. 101).

EXPLANATION OF PLATE XVI

Figs. 89-93. Stages in the life history of Polysphondylium violaceum Brefeld.

Fig. 89. Spores x 1387.

Fig. 90. Myxamoebae x 1387.

Fig. 91. Aggregation x 29.

Fig. 92. Migrating pseudoplasmodium x 29.

Fig. 93. Mature sorocarp x 143.

PLATE XVI

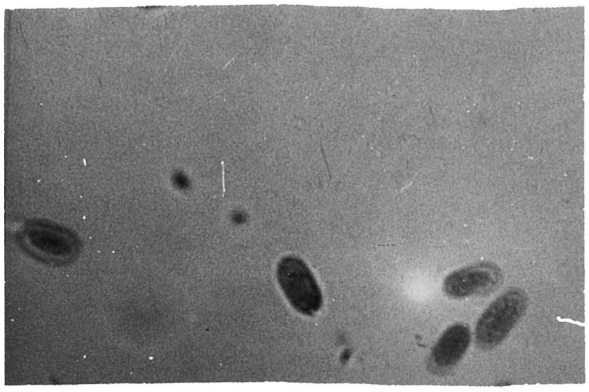


Fig. 89

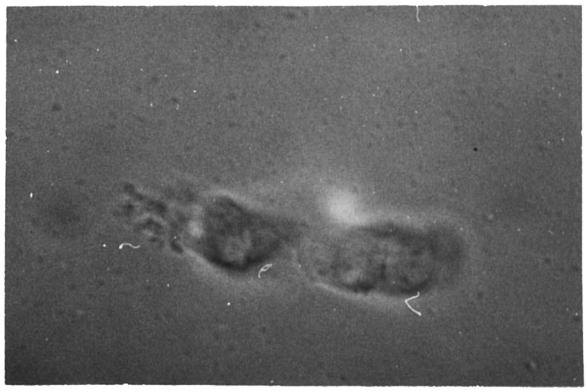


Fig. 90

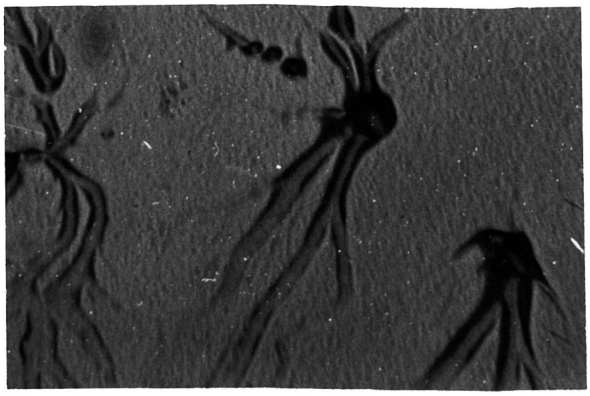


Fig. 91

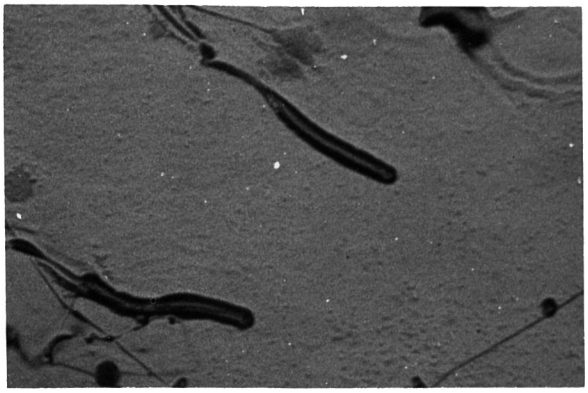


Fig. 92

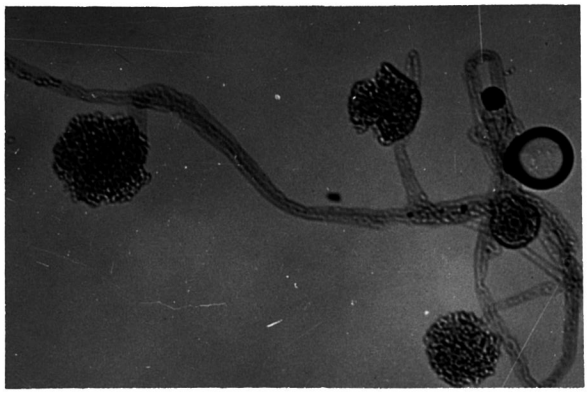


Fig. 93

EXPLANATION OF PLATE XVII

Figs. 94-100. Stages in the sorocarp formation of Polysphondylium violaceum Brefeld photographed at hourly intervals except where indicated. Magnification x 29.

Figs. 94-95. Sorogen elongating on stalk.

Figs. 96-98. Clumps of Myxamoebae left behind on stalk. These will form the whorls of branches.

Fig. 99. Mature sorocarp.

Fig. 100. Mature sorocarp of another fructification showing whorled branches.

PLATE XVII

Fig. 94

Fig. 95

Fig. 96

Fig. 97

Fig. 98

Fig. 99

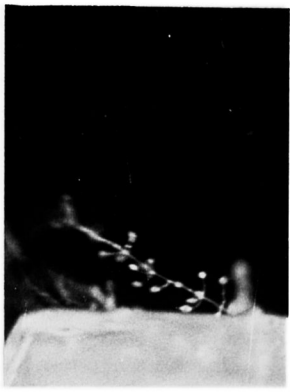


Fig. 100

EXPLANATION OF PLATE XVIII

Figs. 101-105. Stages in the life history of Polysphondylium pallidum Olive.

Fig. 101. Spores x 1387.

Fig. 102. Myxamoebae x 1387.

Fig. 103. Aggregation x 29.

Fig. 104. Migrating pseudoplasmodium x 29.

Fig. 105. Mature sorocarp x 143.

PLATE XVIII

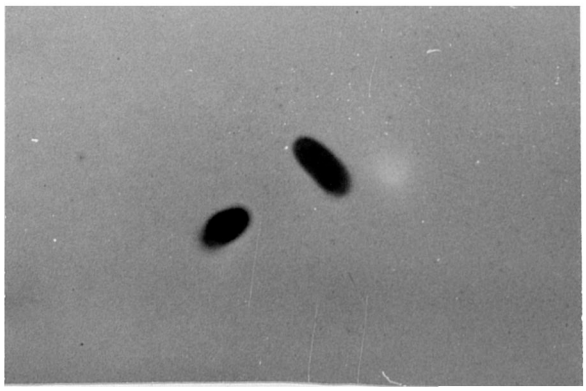


Fig. 101

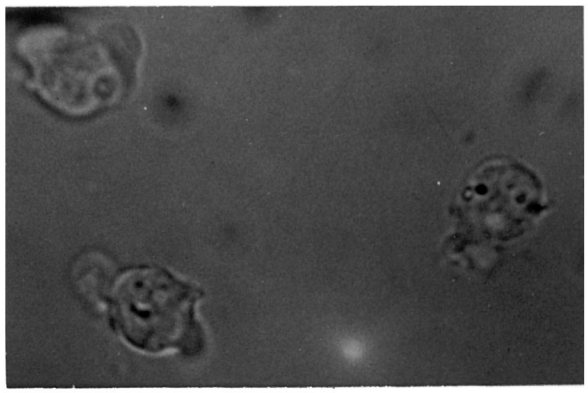


Fig. 102

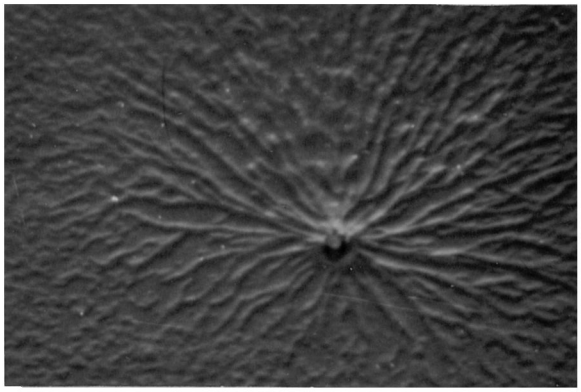


Fig. 103

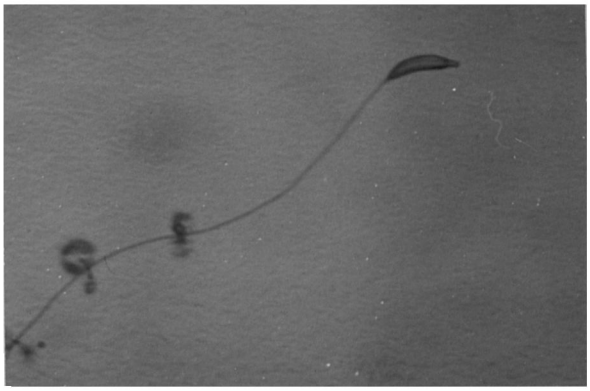


Fig. 104

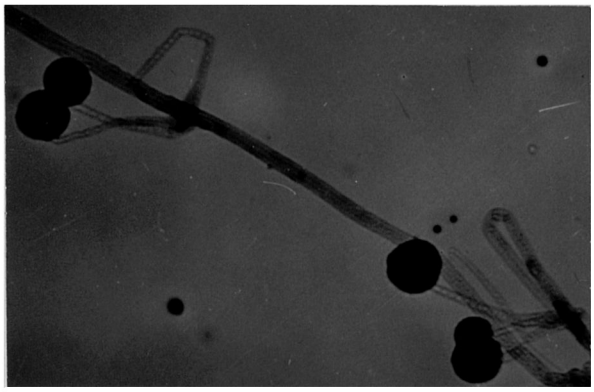


Fig. 105

EXPLANATION OF PLATE XIX

Figs. 106-112. Stages in the sorocarp formation of Polysphondylium pallidum Olive photographed at hourly intervals except where indicated. Magnification x 29.

Fig. 106. Sorogens forming from the pseudoplasmodium.

Figs. 107-110. Sorogens forming stalks. Clumps of myxamoebae which have been left behind on the main axis, forming whorls of branches.

Fig. 111. Mature sorocarps with a few branches.

Fig. 112. Mature sorocarp of another fructification with a number of branches.

PLATE XIX

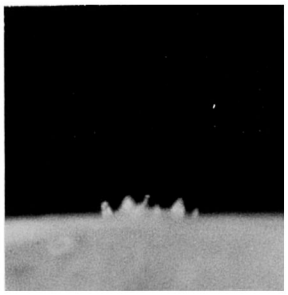


Fig. 106

Fig. 107

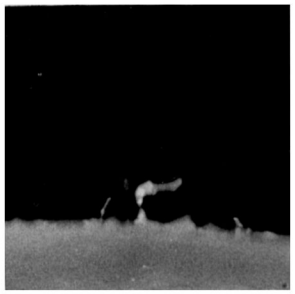


Fig. 108

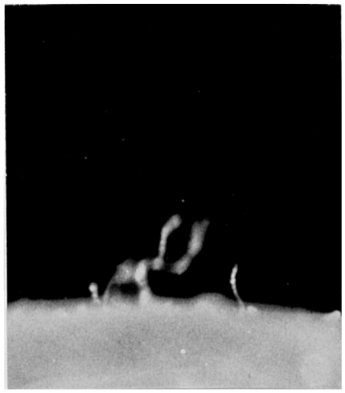


Fig. 109

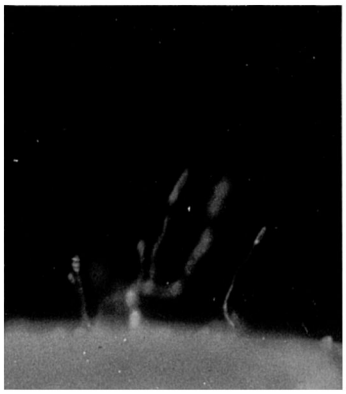


Fig. 110

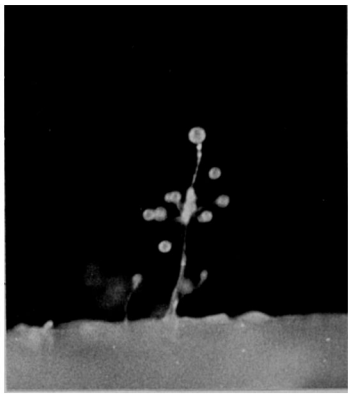


Fig. 111

Fig. 112

Aggregations are formed within 24 hours and form a typically radiate pattern (Fig. 102). Culmination begins when the tip of the pseudoplasmodium elevates, or, a number of sorogens are formed from the pseudoplasmodium each of which form mature sorocarps (Figs. 105-111). As culmination proceeds, clumps of myxamoebae are left behind and are spaced along the stalk at intervals (Figs. 105-109). These clumps mature and form whorls of branches along the main axis (Figs. 110-111). Sorocarps are white in color and measure 5.0 mm. in height. Sori are colorless to white, the terminal sorus measures 150u, the secondary sori are 70u in diameter (Fig. 104). The spores are colorless in mass, elliptical and measure 2.8 x 5.6u (Fig. 100).

DISCUSSION

The isolation of cellular slime molds from soil is a relatively simple process. Numerous media have been used as a substrate (Raper 1951) and many kinds of bacteria have been used as a food source (Raper 1937; Raper and Smith 1939). However, $\frac{1}{4}$ hay infusion agar with Escherichia coli has been found to be a very satisfactory, simple and convenient method.

Prior to 1902 the cellular slime molds were thought to be coprophilous organisms, as collections were first made from dung and decaying organic matter (Olive 1902). Harper (1929) was one of the first workers to isolate a member of the Acrasieae from soil. He cultured Polysphondylium violaceum on dung from cultivated soil collected in a New York City park. The present study and those of Raper and Thom (1932) and Raper (1941, 1951, 1956a) indicate that these organisms are very abundant in soils, particularly forest soils and soils from cultivated and uncultivated areas in many parts of the world.

This study and those of Raper (1951) and Bonner (1959) indicate that there are four species of cellular slime molds which are most commonly isolated from forest and cultivated soils. These are Dictyostelium mucoroides, D. purpureum, Polysphondylium violaceum and P. pallidum. The largest numbers of these species were isolated from forest soils from the northeastern quarter of the state, although they were isolated regularly from cultivated and uncultivated soils as well. Even dry topsoil samples from western Kansas counties yielded some isolates of these species.

Three species, D. polycephalum, D. minutum and D. lacteum which are reported to be isolated frequently from forest soils by Raper (1951, 1956a) and Bonner (1959) were isolated only a few times from Kansas forest soils. Raper isolated these species from deciduous forests of the eastern United States. The higher rainfall and deeper forests of the east may influence the frequency in the occurrence of these species.

The single isolation of D. discoideum from Kansas is apparently only the third time it has been observed since the original description in 1935 (Raper 1935, 1951).

An isolate of a Dictyostelium which gave some difficulty in identification was sent to Dr. K. B. Raper with the opinion that it might represent D. brevicaule. In personal communication, Raper stated that it was his belief that the Kansas isolate approximated E. W. Olive's (1901) concept of this species. Raper (1951) reportedly isolated several strains of Dictyostelia which might possibly be D. brevicaule. As far as is known, the Kansas isolate and those by Raper are the only collections that have been considered to represent the species since Olive's publication of the description in 1901.

It is considered on the basis of this study that cellular slime molds are common soil inhabitants in Kansas and can be easily isolated from soils of most any type obtained throughout the state. However, they are found more abundantly in the soils of eastern Kansas containing higher amounts of decaying organic materials.

SUMMARY AND CONCLUSIONS

In the spring and summer of 1961, 126 soil samples were collected in 39 counties in Kansas and these were used to isolate cellular slime molds. The isolations were made by streaking a suspension of a portion of each sample onto two $\frac{1}{4}$ hay infusion agar plates. After incubation the plates were examined and transfers of the isolated cellular slime molds were made onto Escherichia coli streaked $\frac{1}{4}$ hay infusion agar plates. In making a comparison of five different media, it was found that $\frac{1}{4}$ hay infusion agar was the most suitable medium for isolating and comparing all isolates of cellular slime molds.

Various stages of the life history of each species of Acrasieae were studied by making semi-permanent slide mounts. The culmination process of the species was studied by constructing maturation cells or chambers of glass slides. A block of agar containing a pseudoplasmodium of each species to be studied was mounted on the side of a chamber, the top of the chamber was covered, and the sorogen was allowed to mature in situ. Photographs of the culmination process were taken at hourly intervals to show the various stages of development.

For identification of the isolates, a key was constructed based on descriptions taken from the literature. Revisions were made where necessary according to information learned in the study of the Kansas isolates. In the course of this study, nine species of cellular slime molds were isolated from

soil samples obtained throughout the state. The most common member of Acrasieae in Kansas soils is undoubtedly Dictyostelium mucoroides Brefeld. This has been found to be true of other areas by several authors such as Raper and Thom (1932) and Raper (1951). In this study D. mucoroides was isolated 56 times from soil collected in 27 counties in Kansas. They were mainly from forest soils of eastern Kansas but isolates were also made from prairie soils of western Kansas.

Three other species which were found to be quite common in Kansas soils are Polysphondylium pallidum Olive which was isolated 36 times from soil collected in 18 counties in Kansas, P. violaceum Brefeld found in 22 samples from 12 counties and D. purpureum Olive which was obtained from 13 samples collected in 11 Kansas counties. These species also, have been most frequently isolated from forest soils of eastern Kansas. However, several isolates of each have been obtained from soils from cultivated and uncultivated prairie areas.

A species of Dictyostelium which forms a long, thin stalkless pseudoplasmodium, D. polycephalum, was isolated 6 times from forest soils collected in 5 Kansas counties. Raper (1956) has found this species to occur quite commonly in forest soils. In this study, difficulties were encountered in maintaining cultures as mature fructifications were formed slowly or infrequently.

The species D. discoideum which was first isolated and described by Raper in 1935, has been reported only once since then, this again being Raper in 1951. Both of these were from deciduous forest soils of eastern United States. The present report is apparently only the third time that this species has been isolated, this also being from a forest soil sample of eastern Kansas.

A species of cellular slime mold that has been reported numerous times from forest soils is D. minutum which was first isolated and described in 1941

(Raper 1941, 1951). This species was isolated 4 times from forest soils collected in 3 Kansas counties.

One of the species which was isolated only once in a Kansas soil sample was Dictyostelium lacteum van Tieghem. This species was isolated and described in 1880 but was known only from this description until 1951 when Raper reported isolating it several times from forest soil (Raper 1951). This Kansas isolate was obtained from a forest soil sample which was collected in Douglas county.

A species approximating D. brevicaule Olive was isolated from a Saline county Kansas forest soil sample. This species was first isolated and described in 1901 (Olive 1901, 1902). Raper (1951) reportedly isolated several strains of a Dictyostelium which he believes approached Olive's concept of this species and he (Raper, personal communication) believes the Kansas isolate also approximates the description of this species.

From this study it was learned that the first phase of the life history of each of these species is similar and differences are not apparent until pseudoplasmodium formation. Four of the isolated species formed stalked migrating pseudoplasmodia, two species formed stalkless migrating pseudoplasmodia and two species culminated directly without forming an intermediate migrating phase. In addition, a ninth species approximating D. brevicaule was isolated that has a short migrating phase before culmination.

Mature fructifications vary from the small sorocarps of D. minutum and D. lacteum to the coremiform fructification of D. polycephalum and the multibranched sorocarps of the Polysphondylia.

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I would also like to thank Dr. Lloyd C. Hulbert who kindly collected numerous soil samples for this study. Other soil samples were provided by Dr. S. M. Pady, Mr. C. L. King and numerous county agents.

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Dr. Kenneth B. Raper of the Departments of Bacteriology and Botany, University of Wisconsin provided cultures of several isolates of Acrasieae and confirmed the identification of one of the Kansas isolates. And Dr. Lindsay S. Olive, Department of Botany, Columbia University sent cultures of two of his new species.

I acknowledge the Department of Botany and Plant Pathology, Kansas State University for providing the facilities for this study.

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STUDIES IN THE ACRASIEAE FROM KANSAS SOILS

by

BONNIE JEANNE WILEY

B. S., Western Illinois University, 1959

AN ABSTRACT OF A THESIS

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requirements for the degree

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Manhattan, Kansas

1963

The class Acrasieae, which is often referred to as the cellular slime molds, is a group of organisms commonly found in soils. The members of this group have two phases to their life history: a vegetative phase which is myxamoeboid and feeds on soil bacteria and decaying organic material; and a fructifying stage in which the sorocarp containing the spores is formed. The sorocarp is composed of a cellular stalk which may be branched or unbranched, with a terminal sorus composed of a mass of spores held together by a mucus-like substance. Cellular slime molds have been isolated from a variety of substrates but occur commonly in forest soil.

The purpose of this study was to isolate and identify species of Acrasieae which occur commonly in forest soil, and to study portions of the life history of each of these. In addition, the morphology of these organisms was studied, especially the development of those which have not been studied in detail by earlier workers.

In the spring and summer of 1961, 126 soil samples were collected in 39 counties in Kansas and these were used to isolate cellular slime molds. By grinding a portion of each sample with an approximately equal amount of distilled water, a soil suspension resulted which was streaked onto $\frac{1}{4}$ hay infusion agar plates. The plates were incubated at room temperature for one week and any observed cellular slime molds were transferred to fresh $\frac{1}{4}$ hay infusion agar plates streaked with Escherichia coli.

Ten morphologic strains of seven species of Acrasieae were used in studying growth on five different media. One-quarter hay infusion agar gave the results best suited for making comparative studies of the various isolates.

Different stages in the life history of each species were studied by making semi-permanent slide mounts. The culmination process of each species was also studied by constructing maturation cells or chambers of glass slides. Blocks of agar containing pseudoplasmodia were placed in these chambers and allowed to mature in situ. Photographs of the culmination process were taken at hourly intervals to illustrate the various stages of development.

In order to identify the isolates obtained, a key was constructed based on descriptions taken from the literature. Revisions were made where necessary according to information learned in the study of the Kansas isolates.

In the course of this study, nine species of cellular slime molds were isolated from soil samples obtained throughout the state. The most commonly isolated species was Dictyostelium mucoroides Brefeld which was obtained 56 times from samples collected in 27 counties. It was isolated mainly from forest soils of eastern Kansas but isolates also were made from prairie soils of western Kansas.

Three other species which were found to be quite common in Kansas soils are Polysphondylium pallidum Olive which was isolated 36 times from soils collected in 18 Kansas counties, P. violaceum Brefeld found in 22 samples from 12 counties, and D. purpureum Olive which was obtained from 13 samples which were collected in 11 Kansas counties. These species also have been most frequently isolated from forest soils of eastern Kansas. However, several isolates of each have been obtained in soils from cultivated and uncultivated prairie areas.

Two species which were isolated less frequently were Dictyostelium polycephalum Raper which was isolated 6 times from soils obtained from 5 Kansas counties and D. minutum Raper which was isolated from 4 samples collected in 3 Kansas counties. These isolates were made from forest soils of eastern Kansas.

Three species were identified which were represented by single isolations. Dictyostelium discoideum Raper was obtained from a Douglas county forest soil sample. Dictyostelium lacteum van Tieghem was isolated from a Leavenworth county forest soil sample and a species which is considered to be D. brevicaule Olive was obtained from a Saline county forest soil sample.

Mature fructifications vary from the small sorocarps of D. minutum and D. lacteum to the coremiform fructification of D. polycephalum and the multibranched sorocarps of the Polysphondylia.

It is concluded on the basis of this study that cellular slime molds are common soil inhabitants in Kansas and can be easily isolated from soils of almost any type obtained throughout the state. However, they are found more abundantly in the soils of eastern Kansas which contain higher amounts of decaying organic material.