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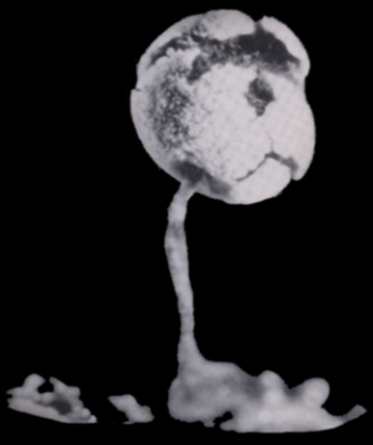

VOLUME I

Organisms, Nucleus, and Cell Cycle

Edited by

HENRY C. ALDRICH
JOHN W. DANIEL

Cell Biology of *Physarum* and *Didymium*

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Organisms, Nucleus, and Cell Cycle

This is a volume in
CELL BIOLOGY
A series of monographs

Editors: D. E. Buetow, I. L. Cameron, G. M. Padilla, and A. M. Zimmerman

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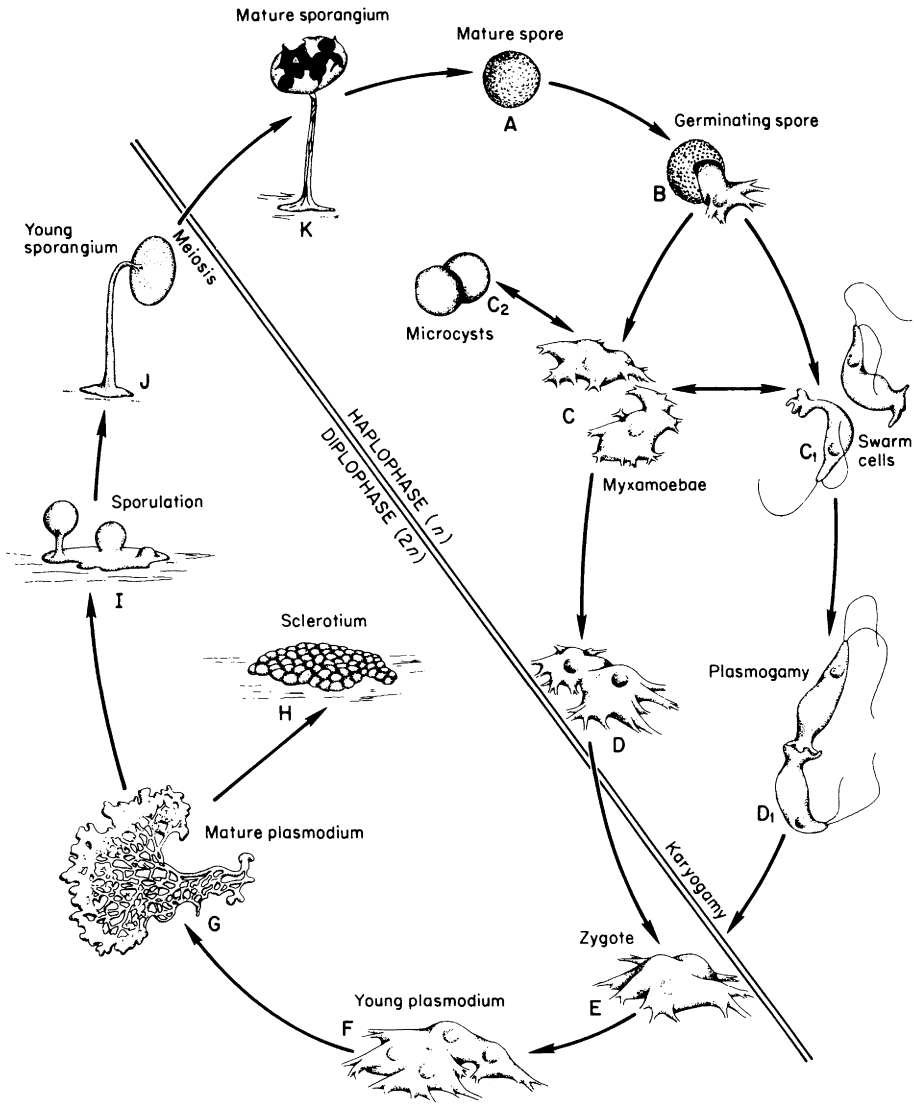

Life cycle of typical Myxomycete. From C. J. Alexopoulous and C. W. Mims (1979). "Introductory Mycology," 3rd ed., p. 69. Reprinted by permission of John Wiley & Sons, Inc.

Cell Biology of *Physarum* and *Didymium*

VOLUME I

Organisms, Nucleus, and Cell Cycle

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Preface

It is now more than twenty years since Dr. Daniel, working with Harold Rusch at the University of Wisconsin, developed the axenic culture methods and chemically defined media that enabled us to grow plasmodia of *Physarum polycephalum* in liquid shake culture. Since that time, the organism has become firmly established as an important experimental tool in cell biology. In the genetics area, a related organism, *Didymium iridis,* has also assumed importance because of its ease of manipulation in culture. This two-volume treatise summarizes important experimental research using these two organisms for developmental and cellular studies.

Because of the natural synchrony of the cell cycle events in the plasmodium, *P. polycephalum* has been used for dissecting the events of DNA, RNA, and protein synthesis. More recently, fundamental studies on the organization of chromatin have focused on the nucleus in the plasmodium. Studies on the spectacular shuttle streaming in the plasmodium have contributed greatly to our understanding of contractility and motility in nonmuscle systems. These examples suggest that these two species are experimental tools whose potential has just begun to be exploited.

This treatise was planned with several audiences in mind. It should serve as a frequent, single reference source to brief cell biologists on the primary research to date on *Physarum* and *Didymium*. To accomplish this aim, we have encouraged authors to organize their chapters as comprehensive reviews insofar as possible.

We frequently encounter cell biologists who are intrigued with the research possibilities of plasmodial slime molds but lack the familiarity with the basic biology of the organisms to handle them intelligently. To meet the needs of such scientists, we have included a general introductory chapter by the eminent taxonomist-morphologist C. J. Alexopoulos and a number of shorter chapters on experimental methods at the end of the second volume. The interest in these experimental methods chapters shown in our own laboratories indi-

cates that they will be of utility to researchers more familiar with the organisms as well.

The volumes will be a good source for graduate students in cell biology and perhaps may even be of use in other graduate courses. The contributors have not only reviewed work to date in their areas but have also pointed out areas and topics likely to be most fruitful for future research. This approach should prove stimulating to students searching for suitable dissertation and thesis topics.

We are great believers in plasmodial slime molds as research tools. Professors W. F. Dove and H. P. Rusch have recently published a volume on "Growth and Differentiation in *Physarum polycephalum*" in which they exhibit this same type of enthusiasm. Wider use of these organisms as research tools in cell biology will benefit us all.

We wish to acknowledge with gratitude the initial encouragement of Ivan Cameron, who urged us to organize this undertaking, and the aid of the staff of Academic Press in producing this work. We are also grateful for the cooperation and understanding of our families and laboratory associates during the time we have been occupied with the preparation of this book. All scientists active in the *Physarum* research group in the United States many of whom are chapter authors, have been generous with suggestions concerning the organization of this treatise. We thank them all!

Henry C. Aldrich
John W. Daniel

PART I

Introduction to the Organisms

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CHAPTER 1

Morphology, Taxonomy, and Phylogeny

CONSTANTINE J. ALEXOPOULOS*

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I. INTRODUCTION

The Myxomycetes are organisms with a unique life cycle. This consists of sporophores that bear the spores which, after meiosis takes place, give rise to haploid myxamoebae or anteriorly flagellate cells devoid of cell walls. Such cells behave as gametes, fusing in pairs to form zygotes. The zygote then grows into a free-living, multinucleate, diploid mass of protoplasm—the plasmodium—devoid of cell walls but generally enveloped by a slime sheath. The plasmodium feeds phagotrophically and eventually produces sporophores characteristic of the species. Many variations to this life cycle are known. Some strains, for example, are apogamic, completing the entire life cycle in a haploid condition; of the sexual strains, some are homothallic and some heterothallic, their gametes consisting of two mating types. For a comprehensive summary of the biology of Myxomycetes, see Alexopoulos (1966, 1973), Gray and Alexopoulos (1968), and Collins (1979).

Myxomycetes have been traditionally classified on the basis of their

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sporophore characteristics alone, but in recent years plasmodial characters and type of sporophore development have become important considerations in delimiting subclasses.

II. THE ORDER PHYSARALES

The genera *Physarum* and *Didymium* both belong to the order Physarales of the subclass Myxogastromycetidae of the class Myxomycetes. The order Physarales was established in 1922 by Thomas Macbride in the second edition of his monograph "The North American Slime Moulds." It corresponds to Lister's (1925) suborder Calcarineae, which is equivalent to Rostafinski's (1875–1876) subcohort Calcarineae.

The order, as now defined, contains myxomycete species with various types of sporophores, which are always of subhypothallic development (Alexopoulos, 1969, 1973), each covered by a typically calcareous peridium, except in *Protophysarum,* and with lime also often present in the hypothallus, stalk, columella, pseudocolumella, and capillitium when these structures are present. The spores, in mass, are dark purple-brown to black; by transmitted light, they appear purple-brown, brown, or violaceous, rarely pallid. The trophic stage is a phaneroplasmodium (Alexopoulos, 1960, 1969) of various colors, which is generally extensive but which may remain minute until it sporulates. The distinguishing feature of the order, along with the purple-brown spores, is the almost universal presence of lime in the peridium. This characteristic, however, is influenced by the environment, so that sporophores that are typically calcareous may sometimes be devoid of lime when they develop under certain conditions (Gray, 1961). The order consists of two families: the Physaraceae, of which *Physarum* is the largest genus, and the Didymiaceae, in which *Didymium* predominates.

A. Family Physaraceae

In the family Physaraceae, described by Rostafinski (1873), to which *Physarum* belongs, the peridial lime is granular except in *Protophysarum,* which is totally devoid of lime but which is placed here because of its subhypothallic development, its phaneroplasmodium, and its capillitial network, albeit devoid of lime nodes. The distinguishing feature of the family, which separates it from the Didymiaceae, is the presence of lime in the capillitium. This will be discussed in greater detail later.

Sporophores of the Physaraceae vary from minute, sessile spheres, through plasmodiocarps pendent on slender stalks, to stipitate simple or multilobed

sporangia. Such sporangia may be densely massed, forming pseudoaethalia. In the genus *Fuligo,* the sporangia are aggregated into an aethalium covered by a relatively thick calcareous cortex below a common peridium.

The peridium of the Physaraceae is typically sprinkled or covered with granular lime but varies from genus to genus and indeed even within genera in its makeup from a nearly limeless, thin, iridescent membrane; to a two-layered covering, the inner layer membranous, the outer calcareous; to a three-layered structure, as in *Leocarpus,* with a calcareous middle layer sandwiched between a cartilaginous outer and a membranous inner layer. In *Cienkowskia,* the membranous or cartilaginous peridium is densely covered with lime. In *Physarella* the peridium bears spinelike, calcareous trabeculae pointing inward.

Calcareous, rarely limeless, the capillitium in most genera of the Physaraceae (*Erionema, Fuligo, Physarella, Craterium,* and *Physarum*) typically consists of a network of limeless slender tubules connecting generally numerous, but sometimes few, limy nodes. In *Badhamia,* the capillitium is made up of more or less uniform calcareous tubules. In *Leocarpus* and *Cienkowskia,* it is of a duplex nature with limy, platelike partitions present in the plasmodiocarps of the latter genus. The aforementioned calcareous, spinelike tarbeculae extending inward from the peridium of *Physarella* have also been interpreted by some (Lister, 1925; Martin and Alexopoulos, 1969; Farr, 1976) as constituting one portion of a duplex capillitium, but it is probably better not to regard them as such. Capillitial lime nodes are often aggregated near the center of the sporophore, forming a globose or rod-shaped, calcareous pseudocolumella that, however, may be missing even in species of which it is considered to be characteristic.

There is nothing to distinguish the spores of the two families. In both they are typically globose, purplish-brown, violet or pallid, with variously ornamented walls. In the Physaraceae they vary in diameter from as small as 5 μm in *Physarum penetrale* Rex to as large as 22 μm in *Fuligo megaspora* Sturgis.

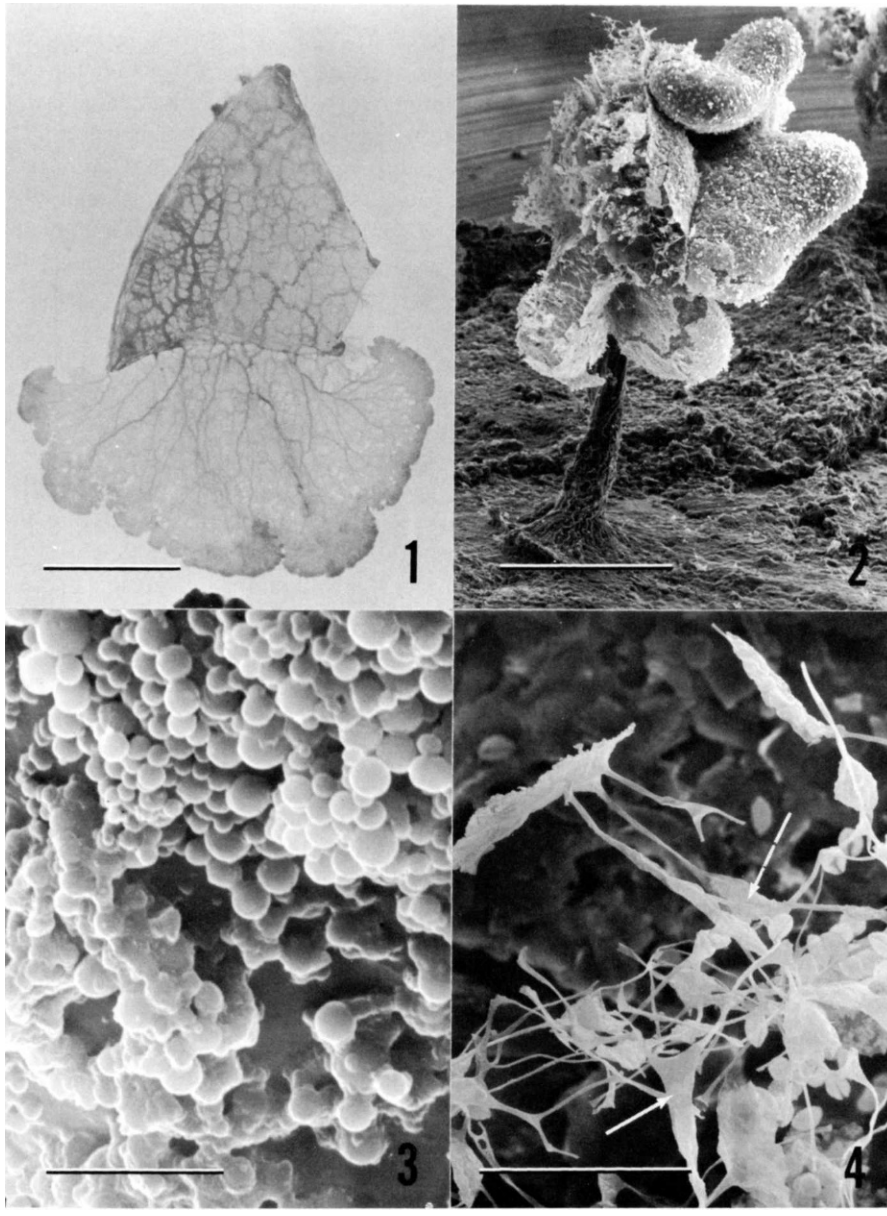
GENUS *PHYSARUM*

a. General Characteristics. The genus *Physarum* was described by Persoon (1794). The chief characteristic of the genus as now delimited is the capillitium, which consists of lime granules of different types connected in a network by fine, tubular filaments. Another characteristic of the genus is the absence of pseudocapillitium from the sporophores of any species. The lime deposits on and in the sporophores are granular or amorphous. "The infrequent occurrence of subcrystalline and partly crystalline lime is believed to be due to alternate wetting and drying," according to Farr (1976, p. 112). The sporophores may be sporangiate, plasmodiocarpous or, rarely, pseudoaethalioid. No species with truly aethalial sporophores is included in the genus.

b. The Plasmodium. Up to 1960, plasmodia of all Myxomycetes were generally regarded as essentially similar in form, and that of the much studied *Physarum polycephalum* was, in the minds of biologists in general, the prototype of all myxomycete plasmodia. To be sure, de Bary (1887) had observed that the plasmodia of *Stemonitis,* of the Trichiaceae, and of *Lycogala* were much more finely granular than those of the Physaraceae; Zukal (1893) had described the species "exceptional among Mycetozoa" (Lister, 1925, p. 185), plasmodium of *Licea (Hymenobolina) parasitica* (Zukal) Martin, and Celakowski (1892), Miller (1898), and Thom and Raper (1930 had pointed out how the growing plasmodium of *Stemonitis* differs from the "typical," i.e., physaraceous plasmodium, but it was not until 1960, when Alexopoulos described three general types of plasmodia—phaneroplasmodium, aphanoplasmodium, and protoplasmodium—and signaled the existence of a fourth type—that of the Trichiales, which he did not name—that the widespread occurrence of several major plasmodial types was emphasized and later generally accepted.

The plasmodium of *Physarum* is a phaneroplasmodium (Fig. 1), as, indeed, it is in all Physarales examined up to now. It consists of a usually robust, fairly extensive network of thick viens, each separate from the others or embedded in a continuous sheet of protoplasm that terminates in one or more fleshy fans. Each vein consists of an outer tubular gel layer (ectoplasm) in which no streaming can be observed, enclosing a core of endoplasm that exhibits more or less rhythmic, reversible streaming. The protoplasm of a phaneroplasmodium is very granular, and the ectoplasm and endoplasm are very distinct. The advancing fleshy fans are very conspicuous and have definite margins. The phaneroplasmodium is enveloped in a gelatinous sheath, or "envelope," as de Bary (1887) called it, containing microfibrils, that is shed as the plasmodium creeps over the substratum, leaving traces behind that are easily visible in nature on dead leaves or in the laboratory on the agar surface.

Physarum plasmodia may or may not be pigmented. In nature, the most conspicuous plasmodia are bright yellow to orange or white. Indeed, of the 84 species of *Physarum* listed in Martin and Alexopoulos (1969), 24 are described as having yellow plasmodia and 18 white, with those of most of the remaining species described as ochraceous, greenish, olive-green, orange, red, scarlet, maroon, violet, purple, blue, gray, and black or combinations of these colors such as orange-red, yellowish-green, and grayish-black. The plasmodia of 24 *Physarum* species are listed as unknown. Within a certain range, plasmodium color is more or less stable, but sometimes it varies. The plasmodium of *P. roseum* Berk. & Br., for example, is described as maroon or bright red, but in some cultures it is pink. Also, plasmodial color sometimes changes from colorless or white to pigmented (usually yellow) on exposure to light, as in *P. gyrosum* Rost. (Fergus and Schine, 1963; Koevenig, 1964). The nature of plas-



Figs. 1–4. *Physarum polycephalum*. Fig. 1. Phaneroplasmodium. Bar = 1 cm. Fig. 2. Sporangium with many heads on a single stalk. Bar = 500 μm. Fig. 3. Peridium covered with lime granules. Bar = 5 μm. Fig. 4. Capillitium showing fusoid, elongated lime nodes (arrows). Bar = 50 μm.

modial pigments remains unknown, but it appears that at least some pigments are confined in pigment granules (McManus, 1965).

In some species of *Physarum,* the plasmodium is capable of reaching a great size and may cover areas a square meter or more in extent on a well-watered lawn (*P. cinereum* Schum.) or a considerable portion of a large, decaying log [*P. polycephalum* Schw., *P. viride* (Bull.) Pers]. In other species, however, the plasmodium never attains a large size in nature, sporulating while still small (*P. roseum*). Whether the size a plasmodium is capable of reaching is genetically or environmentally controlled has not been determined, partly because only a small percentage of species have been cultured in the laboratory from spore to spore.

c. The Sporophore. *Physarum* sporophores vary from sessile to stipitate sporangia, which in *P. polycephalum* bear many heads (Fig. 2); to sessile plasmodiocarps to those with a weak, stalklike extension of the hypothallus; to pseudoaethalia consisting of crowded sporangia, as in some collections of *P. gyrosum* and *P. polycephalum*. In size, sporophores vary from the very small, sessile sporangia of *P. lateritium* (Berk. & Rav.) Morgan, 0.3–0.7 mm in diameter, to stipitate sporangia of *P. tenerum* Rex, which reach a height of 3 mm, and the pseudoaethalia of *P. gyrosum,* which attain a size greater than 3 mm across.

d. The Peridium. The peridium of *Physarum* is typically membranous but is sometimes cartilaginous (*P. nasuense* Emoto) or rugose [*P. melleum* (Berk. & Br.) Massee]. It is often double, consisting of a delicate inner wall covered by a thickly calcareous outer wall (*P. bivalve* Pers.), or sometimes triple, as in *P. bogoriense* Racib., with the two outer layers calcareous and closely attached. Typically the peridium bears lime granules (Fig. 3) and may be completely encrusted with granular lime. The amount of lime, however, varies even in different sporophores from the same collection and is probably influenced by the microenvironment at the time of sporulation. Species whose peridia are typically limy may be devoid of lime in humid atmospheres (Gray, 1961). The chemical composition of the membranous or cartilaginous walls is not known. The calcareous covering is lime, with some pigments incorporated in many species. *Physarum* sporophores "display spectacular array of colors and shades," as Collins (1979) puts it for Myxomycetes in general. Many paintings and color photographs of such sporophores have been published. Among the best known are the paintings in Crowder (1926), Lister (1911, 1925), Hattori (1935, 1964), Martin and Alexopoulos (1969), and the most recent and spectacular ones in Emoto's (1977) beautiful book. Color photographs have been published by Alexopoulos (1973). Sporophore pigments appear to be incorporated in the lime in some unknown way. The nature of the pigments is not known in any species. Of interest in this connection is Henney's (1968) study of *P. globuliferum* (Bull.)

Pers., the sporangia of which have been described as "white" (Lister, 1925, p. 27) or "white, pale ochraceous or pinkish" (Martin and Alexopoulos, 1969, p. 303). Henney showed that the blue sporangia of the so-called *P. bilgramii* Hagelst., formed from a blue plasmodium, are nothing more than a color variation of *P. globuliferum*.

The type of peridial dehiscence varies within the genus. Although irregular dehiscence, as in *P. polycephalum* (Fig. 2), is probably the rule, some species dehisce in a characteristic manner, which aids in identification. Thus, in the plasmodiocarpous *P. bivalve,* "dehiscence is by a more or less regular, formed longitudinal fissure" (Martin and Alexopoulos, 1969, p. 288); in *P. bogoriense,* the sides of the peridium characteristically dehisce in a stellate fashion into triangular reflexed lobes, making the identity of the plasmodiocarps almost unmistakable, even with the use of a hand lens in the field. Again, in the common *P. nutans,* Pers. dehiscence is typically petaloid or annulate.

e. The Stalk. The stalk, when present, is filled with debris from the substratum which it accumulates during its subhypothallic development. Its surface may be smooth or ridged. It may be plain, frosted with lime, or entirely calcareous. In many species the stalk extends into the sporangial sac as a generally short, conical columella. The latter, however, may be long and cylindric and may reach the apex of the sporangium, as in *P. crateriforme* Petch.

f. The Capillitium. Although the capillitium in *Physarum* varies in detail from species to species, it is built on the same pattern of a usually large number of calcareous nodes interconnected by a delicate tubular network (Fig. 4). Variations that distinguish the species mainly concern the relative abundance of capillitium in a sporophore; size, shape, and color of lime nodes; arrangement of capillitial tubules; origin of the capillitial network; and elasticity. The general structure of the capillitium may be detected under high magnification with a good dissecting microscope in a mature, dry sporangium still attached to the substratum. On removal of the peridium, the lime knots, usually abundant, are immediately evident as white, yellow, orange, red, or blue conspicuous nodes. The capillitium may appear as a dense network that completely fills the sporangium; or, in a few species, it may consist of sparingly branched threads; or, as in *P. rigidum,* it consists almost entirely of slender, rodlike tubules enclosing lime granules. In some species, notably *P. gyrosum,* the capillitium is elastic and expands instantly when the peridium is removed.

The variation in size, shape, color, and abundance of lime nodes is important in distinguishing among species. Lime nodes may be globose or flat, expanded and irregular in outline, or they may be elongated with several extensions, or fusoid or rodlike; they are usually concolorous with the lime granules on the peridium, but they may vary. In some species, many calcareous nodes are

massed in the center of the sporangial sac, forming a spherical or rod-shaped pseudocolumella.

g. The Spores. The spores of *Physarum* do not differ in the main from those of other members of the order Physarales. They are almost always globose, purplish-brown, violet-gray, or rarely pallid. They average 8–10 μm but may be as small as 5 μm in *P. penetrale* Rex or as large as 15 μm in *P. albescens* Ellis. The spore walls are seldom smooth, as in *P. laevisporum* Agnih. More often they are spinulose, as in *P. polycephalum* (Fig. 5) or verrucose. In a few species, such as *P. dictyosporum* Martin, they are conspicuously reticulate (Fig. 6). In *P. echinosporum* A. Lister, the spines, often united into prominent ridges, are very conspicuous.

h. Species of Physarum. Martin and Alexopoulos (1969) recognized 84 species of *Physarum*. Since then, at least 10 additional species have been described. With interest in myxomycete floristics revived throughout the world, many more will undoubtedly be described in the near future as the African continent, the tropics in general, the deserts, the far north and far south regions, and the alpine habitats are more thoroughly explored. The moist chamber method now being widely used is revealing many previously unknown species of Myxomycetes but, so far, relatively few undescribed species of *Physarum*.

i. Supposed Relationships. In the absence of fossil evidence or comparative chemical data, we must at present use morphological resemblances alone as indicating relationships. On that basis, *Physarum* is most closely related to *Badhamia* and *Craterium*. With the former, it is linked by the badhamioid capillitium of some species now placed in *Physarum* and the physaroid capillitium of some species until recently included in *Badhamia*, such as *P. decipiens* Curt. (Farr, 1961). With *Craterium*, *Physarum* is linked by intermediate forms which have a persistent, cuplike base characteristic of *Craterium*. Relationships could also be claimed between *Physarum* and *Physarella*, the latter often producing physaroid plasmodiocarps instead of the characteristically introverted, thimble- or bell-shaped sporangia. In 1954, Locquin suggested that *Physarum* be merged with *Fuligo* (Martin and Alexopoulos, 1969, p. 275), but this has not been accepted by myxomycete taxonomists. As for possible relationships among species of *Physarum*, one might consult Lister (1925) and Hagelstein (1944), who grouped species which resemble one another in certain characteristics. More recent monographs (Martin and Alexopoulos, 1969; Farr, 1976) list species alphabetically and do not attempt to indicate relationships. Farr's synoptic keys, however, are useful in grouping species that resemble one another. And, indeed, some recognized species merge with one another by intermediate forms, but until experimental evidence is obtained showing that such "species" interbreed, there