RESEARCHES ON FUNGI

VOLUME VI

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THE BIOLOGY AND TAXONOMY OF PILOBOLUS,
THE PRODUCTION AND LIBERATION OF SPORES
IN THE DISCOMYCETES, AND PSEUDORHIZAE
AND GEMMIFERS AS ORGANS OF CERTAIN
HYMENOMYCETES

BY

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WITH TWO HUNDRED AND THIRTY-ONE FIGURES IN THE TEXT

PREFACE

THE object of this volume is to make contributions to our knowledge of certain Phycomycetes, Ascomycetes, and Basidiomycetes.

Part I is devoted to a study of Pilobolus. The ocellus function of the subsporangial swelling has been treated of in detail. A new species of Pilobolus has been described, and a final Chapter on the Systematics of the Pilobolidae has been contributed by my friend, Mr. W. B. Grove.

Part II is concerned with the production and liberation of spores in the Discomycetes. The phenomenon of puffing has been discussed; and attention has been called to the fact that the asci of many Discomycetes are heliotropic, so that light is of great importance in directing the ascus guns toward the mouths of cup-shaped fruit-bodies and toward the openings of the hymenial depressions in Morchellae. Finally, a simple method for making audible the puffing of Discomycetes has been described.

The first two Chapters of Part III treat of the pseudorhizae of *Collybia radicata*, *C. fusipes*, *Coprinus macrorhizus*, and other Hymenomycetes, while the final Chapter treats of *Omphalia flavida* as a luminous and gemmiferous Coffee leaf-spot fungus. The gemmae of this fungus are unique, for they appear to have been derived from pilei which have become sterile and detachable. With a view to enabling the reader to compare the gemmifers of *O. flavida* with those of *Sclerotium coffeicola* (another Coffee leaf-spot fungus), a brief account of *S. coffeicola* based in the main on Stahel's investigations has been appended.

This volume contains two hundred and thirty-one illustrations

in the text, including one hundred and thirty-four drawings and ninety-seven photographs. Forty of the drawings have been borrowed from other authors. The other drawings were executed by my own hand or in conjunction with Miss Ruth Macrae. Of the ninety-seven photographs fifty-six were made under my direction, ten have been borrowed from other authors, and the rest were kindly contributed by friends and correspondents : one each by S. F. Ashby. Jessie S. Bayliss Elliott, W. S. Odell, and H. H. Thornbury, two by F. Dickson and W. R. Fisher, two by Somerville Hastings, three each by B. O. Dodge, G. L. Fawcett, and W. H. Long, five by A. E. Peck, and nine by the Photographic Division of the Geological Survey of Canada. The source of each borrowed illustration is acknowledged in the text.

For the session 1933–1934 I was granted leave of absence from the University of Manitoba, and this period has been spent at the Herbarium of the Royal Botanic Gardens, Kew, in completing for the press the MS. and illustrations of Volumes V and VI of these *Researches*. For the facilities placed at my disposal for carrying out this work I here desire to express to Sir Arthur Hill, Director of Kew Gardens, and Mr. A. D. Cotton, Keeper of the Herbarium, my hearty appreciation.

My best thanks are due once more to the Canadian National Research Council for grants in aid of the work. These grants have enabled me to employ in succession two research assistants, Miss Ruth Macrae, M.Sc. (McGill), and Miss Eleanor S. Dowding, Ph.D. (Manitoba). The investigations on the gemmifers of *Omphalia flavida* and on the heliotropism of the asci of *Ascobolus magnificus* were carried out in conjunction with Miss Macrae, and certain investigations upon the life-history and structure of Pilobolus in conjunction with Dr. Dowding. I here desire to express my indebtedness to these two ladies for their valuable services. Once again Mr. W. B. Grove, M.A., has been kind enough to give me the benefit of his assistance in reading the proofs.

A. H. REGINALD BULLER.

KEW, *August* 19, 1934.

TABLE OF CONTENTS

CHAPTER III

PILOBOLUS UMBONATUS, A NEW SPECIES, WITH REMARKS ON THE PILOBOLIDAE

CHAPTER IV

A SYSTEMATIC ACCOUNT AND ARRANGEMENT OF THE PILOBOLIDAE

Contributed by W. B. Grove

PART II

THE PRODUCTION AND LIBERATION OF SPORES IN THE DISCOMYCETES

CHAPTER I

THE PHENOMENON OF PUFFING IN SARCOSCYPHA PROTRACTA AND OTHER DISCOMYCETES

PART I

THE BIOLOGY AND TAXONOMY OF PILOBOLUS

RESEARCHES ON FUNGI

CHAPTER I

INTRODUCTORY AND HISTORICAL

Pilobolus and its Subsporangial Swelling—The Discovery, Structure, Taxonomy, and Life-history of Pilobolus—Sex in the Pilobolidae—The Cytology of Pilobolus—Crystalloids—The Orange-red Pigment—Parasites of Pilobolus—The Excretion of Drops by the Sporangiophore and its Cause—Premature Discharge of the Sporangia—Influence of External Conditions on the Breadth of the Subsporangial Swelling—The Ballistics of the Projectile—Pilobolus in its Relations with Light—The Effect of Light on Fruit-body Development—The Heliotropic Response of the Fruit-body to Light of Various Colours—Allen and Jolivette's Investigations—The Subsporangial Swelling as an Ocellus—The Solution of the Problem of the Non-resultant Heliotropic Reaction of Pilobolus to Two Beams of Light—The Discharge of the Sporangium.

Pilobolus and its Subsporangial Swelling.—The genus Pilobolus belongs to the Phycomycetes, is a close relative of Mucor, and includes about sixteen species. Most of these species come up along with those of Mucor and other moulds upon the solid excrement of herbivorous animals, such as the horse and the cow, while one species occurs upon river mud. The general appearance of a Pilobolus, when growing on horse dung, is shown in Figs. 1, 5 (pp. 2, 8), and 13 (p. 37).

Pilobolus differs from Mucor in that, as a preliminary to the dissemination of the spores, the sporangium is shot away from the sporangiophore, whereas in Mucor this does not take place. The sporangiophore of Mucor is an organ which serves merely to raise the sporangium above the substratum, and thus to put it in a favourable position for the passive dispersion of the spores by insects and other agencies. On the other hand, the sporangiophore

of Pilobolus not only raises the sporangium above the substratum but is also adapted for shooting the sporangium away to a considerable distance. With this difference of function is associated a very marked difference in the structure of the two sporangiophores, that of Pilobolus being morphologically much more highly specialised than that of Mucor.

Pilobolus, owing to its very common occurrence, the beauty of its form, its unique subsporangial swelling, its very pronounced heliotropism, the beads of water with which its sporangiophore is usually ornamented, and the great distance to which its sporangium is discharged, has attracted the attention of mycologists for more than two centuries, and to-day it is described in many text-books of Botany. As a contribution to our knowledge of a fungus which is of such perennial interest, there

Fig. 1.—*Pilobolus longipes*. A group of sporangiophores which came up spontaneously on horse dung in the laboratory at Winnipeg. They were all directed toward the source of brightest daylight. Natural size.

will be presented : in this Chapter a historical review of the literature on Pilobolus ; in Chapter II an account of the author's researches on Pilobolus, of which a brief report [1] was made in 1921 ; in Chapter III a description of a new species, *Pilobolus umbonatus* ; and in Chapter IV a taxonomic description of all the known species of Pilobolus, drawn up by Mr. W. B. Grove. In Chapter II, from the point of view of the organism as a whole, it will be shown : (1) that the subsporangial swelling, in addition to acting as part of a squirting apparatus, has the function

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[1] A. H. R. Buller, " Upon the Ocellus Function of the Subsporangial Swelling of Pilobolus," *Trans. Brit. Myc. Soc.*, Vol. VII, 1921, pp. 61–64.

of an optical sense-organ or simple eye (ocellus) ; (2) that this simple eye, owing to the manner in which it responds to helio-tropic stimuli, causes the Pilobolus gun to be so laid that the sporangium with its load of spores is shot away as far as possible into an open space ; (3) that this mode of discharge causes the sporangium to lodge on herbage where it may be swallowed with the herbage by some grazing herbivorous animal ; and (4) that the sporangium fastens itself to a grass-stem or grass-leaf, etc., in such a way that it cannot be washed away from its place of attachment even during a heavy shower of rain.

The Discovery, Structure, Taxonomy, and Life-history of Pilobolus.—The earliest record of Pilobolus appears to have been made in 1688 by John Ray [1] in his *Historia Plantarum* from a description sent to him from Virginia by John Banister. The fungus was illustrated (Fig. 97, p. 192) by Plukenet [2] in 1691, was " observed on horse dung about London " by Petiver [3] in 1696, and was observed again by Henry Baker [4] in 1744 on mud brought from the Thames.[5]

In 1778, and more fully in 1782, Otto F. Müller,[6] a Danish zoologist who studied the lower animals, described a Pilobolus as a new kind of zoophyte. He regarded the glistening subsporangial swelling as a crystalline body, and a little worm which, doubtless, was crawling over its outside he thought was inside, swimming

[1] John Ray, *Historia Plantarum*, Vol. II, 1688, p. 1928.

[2] L. Plukenet, *Almagestum botanicum*, London, 1696, p. 164 ; also *Phytographia*, London, 1691, Plate CXVI, Fig. 7. There can be but little doubt that this Figure is a reproduction of Banister's original drawing made in Virginia.

[3] In John Ray's *Synopsis methodica stirpium britannicarum*, London, ed. II, 1696, p. 322.

[4] Henry Baker, *Natural History of the Polype Insect*, 1744, Chap. XI, Plate XXII, Figs. 9, 10.

[5] Coemans, in his *Monographie du genre Pilobolus* (p. 1), remarks that Baker's description and illustrations leave no doubt that his Pilobolus was *P. oedipus*, this conclusion being strengthened by the fact that the fungus was found on mud, a substratum upon which *P. oedipus* has often been observed. Nising (Coemans' *Monographie*, p. 60) observed it on mud of the river Oder, and I have observed it on mud obtained from the Red River at Winnipeg.

[6] Otto F. Müller, " Von der Entdeckung eines neuen Geschlechts von Thier-pflanze," *Berlinische Sammlungen zur Beförderung der Arzneiwissenschaft*, Stück I, 1778, pp. 41–52 ; also " Von einem Kristallschwämmchen," *Kleine Schriften a.d. Naturhistorie*, herausg. v. J. A. E. Goeze, Bd. I, 1782, pp. 122–132.

about in any direction it pleased " as if in a tiny ocean " (*cf.* Fig. 2). Hence Müller described his Pilobolus as a plant which enclosed a living worm in a crystalline body and he, therefore, regarded the fungus as a marvellous organism combining characteristics of all the three realms of nature—animal, vegetable, and mineral. Müller's conception of Pilobolus, which is reminiscent of the fabulous monsters of antiquity, received widespread attention ; but it was shown by later workers to have been based on erroneous observation. Tode, Persoon, Currey, and Coemans all found the worms on the outside of the sporangiophore and never inside as Müller and his followers Durieu de Maisonneuve and Léveillé had supposed.[1]

Curiously enough, Müller's error about the position of the
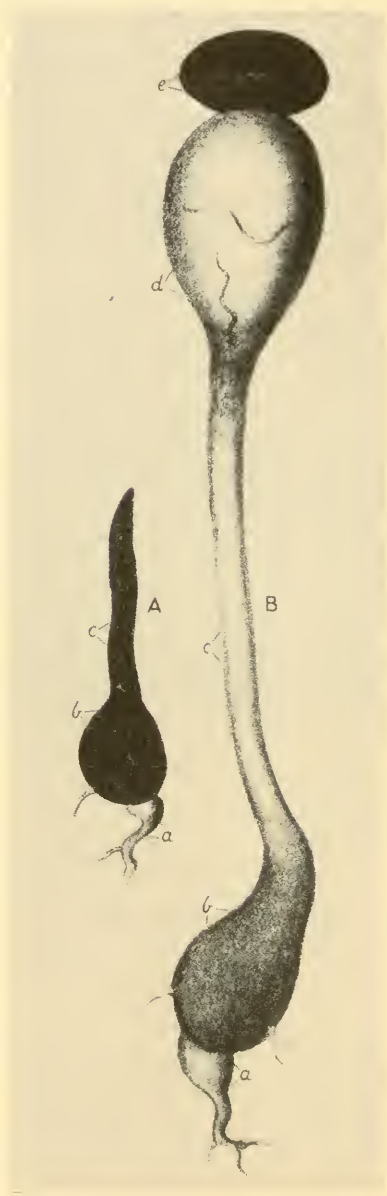
Fig. 2.—*Pilobolus Kleinii.* A, a rudimentary sporangiophore : *a*, the mycelium ; *b*, the basal reservoir ; *c*, the stipe elongating apically ; the whole filled with dense orange-red protoplasm. B, a mature sporangiophore : *a*, the mycelium ; *b*, the basal reservoir ; *c*, the stipe now fully elongated ; *d*, the subsporangial swelling, erroneously supposed to contain two worms swimming in its vacuole ; *e*, the sporangium about to be discharged. Drawn by É. Boudier ; photographically copied from Plate 582 of his *Icones Mycologicae* ; letters added by the author. Magnification, 25.

[1] For the literature on this controversy *vide* Coemans, *Monographie,* pp. 48–49.

worm has been repeated in recent years by Boudier [1] who, in one of the Plates of his splendid *Icones Mycologicae* (1905–1910), illustrates *Pilobolus Kleinii* in colours and shows us two worms in the middle of a subsporangial swelling enlarged twenty-five times (Fig. 2). The worms look as though they might have been on the exterior of the under side of the swelling, but that Boudier intended us to think that they were inside the great vacuole is shown by his description of the drawing : " Autre adulte, on remarque dans la vésicule supérieure 2 anguillules qui s'y sont introduites." *A priori* it seems most unlikely that two worms should succeed in penetrating into a sporangiophore without injuring or killing it or leaving any trace of their mode of entrance : and, in view of the critical observations of Tode, Persoon, Currey, and Coemans, already cited, it must be concluded that Boudier, like Müller one hundred and thirty years earlier, was the victim of an optical illusion.

When a fruit-body of a Pilobolus has been removed from its substratum and has been placed horizontally on a glass slide, any worm which may be in the film of moisture where the subsporangial swelling is in contact with the slide is magnified by the swelling which acts as a lens. It may well be that this optical effect originally suggested the idea that a wriggling worm, which in reality is on the exterior of the swelling, seems to be moving about inside.

Müller's worm was probably a larval Roundworm (one of the Nematoda) and, if it was, it may have belonged to a species of the genus Strongylus. There are several species of Strongylus, *e.g.* *S. vulgaris*, which are parasites of horses. They deposit their eggs in great numbers in the caecum and colon of the alimentary canal of the horses concerned, and the eggs pass to the exterior in the faeces. After a mass of dung has been dropped upon the ground, the eggs hatch within twenty-four hours and, after seven days, the larvae (Fig. 3), which have fed on the faecal matter, begin to swarm on to grass blades, etc., where they pass through a few moults and are in a favourable position to be swallowed by grazing horses. Within these animals they grow to a length of two inches or more and become sexually mature. In unsterilised horse-dung cultures, such as are

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[1] É. Boudier, *Icones Mycologicae ou Iconographie des Champignons de France principalement Discomycètes*, Paris, 1905–1910, Pl. 582, c.

so often made in mycological laboratories, it is during the swarming time that the larvae of Strongylus species find their way on to Pilobolus fruit-bodies, up bits of straw, the tips of which they often cover, and up the sides of the dish upon which, when very numerous, they may arrange themselves so as to form a curious retiform pattern (Fig. 4). Persoon, Tode, Grove, the writer, and many others have seen one or more of the little worms wriggling about in drops or films of moisture on the exterior of a subsporangial swelling ;

Fig. 3.—Strongylus larvae (Nematode worms) which swarmed up the side of a glass culture-dish from horse dung, about seven days after the dung (fresh) was placed in the dish (cf. Fig. 4). They were separated from one another by immersing them in water on a slide, where they wriggled violently. With the addition of iodine, they came to rest, straightened, died, and took on a yellow colour, whereupon they were photographed. Magnification, 51.

but, hitherto, mycologists may not have realised that the worms are but larvae and that, when climbing on to a Pilobolus or up the side of a dish, they are only following a swarming instinct which, when it leads them under natural conditions to ascend grass-blades, gives them a chance to be swallowed by a horse and thus to enter an animal in which they may continue their development.[1] When a Pilobolus fruit-body explodes, any Strongylus larvae which may be present on the sporangiophore must be thrown down violently on to the surface of the dung, while, if a larva should happen to be on the sporangium, it will be carried away with the projectile. O. F.

[1] For the facts concerning the life-history of the Roundworms of the horse, I am indebted to Dr. G. Hadwen of the Ontario National Research Foundation.

Müller, according to Coemans,[1] depicts along with a discharged sporangium a worm. This may have been carried with the projectile during its flight through the air.

To test the supposition that Nematode worms may travel through the air upon a discharged sporangium, I placed a sheet of

Fig. 4.—Strongylus larvae (Nematode worms) swarming up the side of a glass culture-dish from horse dung, seven days after the dung (fresh) was placed in the dish. Fruit-bodies of *Pilobolus longipes* appeared on the dung a few days before and, to the left, some of their black discharged sporangia can be faintly seen sticking to the surface of the glass. Some of the larvae swarmed up the sporangiophores, and those which reached the sporangia may have been shot away with the sporangia when these were discharged. At Winnipeg. Natural size.

clean dry glass in front of a large number of fruit-bodies of *Pilobolus longipes* that had appeared on horse dung infected with Nematodes which had begun to swarm. Soon fifty or more sporangia struck and stuck to the glass plate. On examining the discharged sporangia I found that worms were associated with two of them. Under the free turned-out edge of one of these sporangia two worms were partly hidden. The other sporangium was in contact with one end

[1] E. Coemans, *Monographie*, p. 49.

of a worm which was freely exposed in the liquid drop that had accompanied the sporangium during its flight. Thus conclusive evidence was obtained that Nematode larval worms may occasionally be shot through the air along with a Pilobolus projectile.

In 1772 Scopoli [1] recognised the true affinities of Pilobolus, as is indicated by the fact that he gave the name *Mucor obliquus* to the species which he studied. The first good description
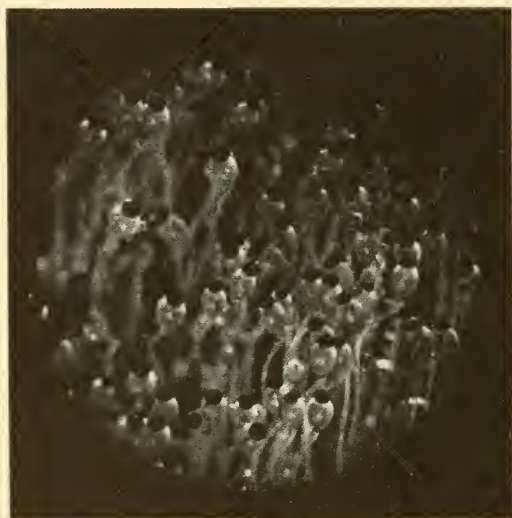
Fig. 5.—*Pilobolus* (*Kleinii ?*). A photomicrograph of a group of fruit-bodies growing on dung in the laboratory, showing black sporangia, subsporangial swellings, stipes, and drops of mucilaginous fluid excreted on the swellings and stipes. Photographed by B. O. Dodge. Magnification, about 3·5.

of the genus was given in 1784 by Tode,[2] who named one of its species *Pilobolus crystallinus*—a name which is still retained. Among the later observers who contributed most to our knowledge of the species of Pilobolus are Persoon,[3] Montagne,[4] Cohn,[5]

[1] J. A. Scopoli, *Flora Carniolica*, Vienna, ed. II, 1772, Vol. II, p. 494.

[2] H. J. Tode, " Beschreibung des Hutwerfers (Pilobolus)," *Schrift. der Naturf. Berlin. Gesell.*, Bd. V, 1784, p. 46, Plate I.

[3] C. H. Persoon, *Synopsis methodica fungorum*, 1801, Part I, pp. 117–118.

[4] J. F. C. Montagne, " Note sur le genre Pilobolus et description d'une espèce nouvelle," *Mém. de la Soc. Linn. de Lyon*, 1836, 7 pp., 1 Plate. The species described was *P. oedipus*.

[5] Ferdinand Cohn, " Die Entwicklungsgeschichte des Pilobolus crystallinus," *Nova Acta Acad. Caes. Leop.*, Bd. XXIII, 1851, pp. 495–534, Taf. LI and LII.

Coemans,[1] Klein,[2] van Tieghem,[3] Bainier,[4] Grove,[5] Palla,[6] and Morini.[7]

In 1875 van Tieghem [8] founded the genus Pilaira on two mould-species which have simple cylindrical non-exploding sporangiophores, like those of Mucor, and dehiscent adhesive sporangia, like those of Pilobolus. The genus Pilaira, as is evident, serves to connect Pilobolus with Mucor.[9]

In 1884 appeared Grove's *Monograph of the Pilobolidae*,[10] which contained a history of the family, a full discussion of the synonymy involved, and a key to the ten species then known. The species were arranged as follows :

Order, **MUCORINI**, de Bary.

Family, PILOBOLIDAE, van Tieghem.

Genus I—PILOBOLUS, Tode.

1. Pilobolus oedipus, *Montagne.*

2. ,, exiguus, *Bainier.*

[1] E. Coemans, " Monographie du genre Pilobolus Tode, spécialement étudié au point de vue anatomique et physiologique." *Mém. cour. et des Sav. étrang. Acad. roy. de Belgique*, T. XXX, 1861, 68 pp., 3 tab.

[2] J. Klein, " Zur Kenntnis des Pilobolus," *Pringsh. Jahrb. f. wiss. Bot.*, Bd. VIII, 1872, pp. 305–381, Taf. XXIII–XXX.

[3] P. van Tieghem : (1) " Sur la structure et le mode de déhiscence du sporange des Pilobolées et sur deux espèces nouvelles de Pilobolus," *Bull. Soc. Bot. France*, T. XXII, 1875, p. 274 ; (2) " Nouvelles recherches sur les Mucorinées," *Ann. Sci. Nat.*, 6 sér, T. I., 1875, pp. 41–61 ; (3) " Observations au sujet d'un nouveau travail de M. Brefeld sur les Mucorinées et en particulier sur les Pilobolus," *Bull. Soc. Bot. France*, T. XXIII, 1876, p. 35 ; (4) " Troisième mémoire sur les Mucorinées," *Ann. Sci. Nat.*, 6 sér., T. IV, 1876, pp. 335–349.

[4] G. Bainier, *Étude sur les Mucorinées*, Paris, 1882, 126 pp., 11 tab.

[5] W. B. Grove, " Monograph of the Pilobolidae," *The Midland Naturalist*, Birmingham, England, 1884. Reprint, pp. 1–39, Pl. IV and VI.

[6] E. Palla, " Zur Kenntniss der Pilobolus-Arten," *Oesterr. bot. Zeitschr.*, Bd. L, 1900, pp. 349–370, 397–401, tab. col. Under the title " Contribution à la connaissance des espèces du genre Pilobolus " Palla's paper is reviewed and translated by R. Ferry in *Rev. Mycol.*, T. XXVI, 1904, pp. 19–33, tab.

[7] F. Morini, " Materiali per una monografia delle Pilobolee," *Mem. della R. Accad. delle Sci. dell'Istituto di Bologna*, ser. VI, T. III, 1906 ; T. VI, 1909.

[8] P. van Tieghem, *loc. cit.*, pp. 51–61.

[9] The name *Pilobolus* is derived from πῖλος, a hat, and βάλλω, I throw ; while *Pilaira* is derived from πῖλος, and αἴρω, I raise.

[10] W. B. Grove, *loc. cit.* The arrangement of the species of Pilobolus in Saccardo is based on Grove's Monograph.

3. Pilobolus crystallinus, *van Tieghem*.
4.   ,,   Kleinii, *van Tieghem*.
5.   ,,   longipes, *van Tieghem*.
6.   ,,   roridus, *Persoon*.
7.   ,,   nanus, *van Tieghem*.

Genus II—Pilaira, van Tieghem.

1. Pilaira Cesatii, *van Tieghem*.[1]
2.   ,,   nigrescens, *van Tieghem*.
3.   ,,   dimidiata, *Grove*.

Cohn[2] (1851) traced the life-history of *Pilobolus oedipus* (his *P. crystallinus*). He observed the germination of the spores, the branched unicellular mycelium, the basal swelling cut off from the rest of the mycelium by a wall, stages in the development of the stipe, sporangium, and subsporangial swelling, the formation of the columella, the ripening of the spores, and the discharge of the sporangia. He estimated the number of spores in a sporangium at 15,000–30,000.

Further observations on the life-history of *Pilobolus oedipus* and *P. crystallinus* were made by Coemans[3] (1861). He observed that some of the sporangia were shot to a height of 105 cm. (= 3 feet 5·3 inches),[4] and he was the first to perceive that the violent discharge of the sporangia and their adhesion to grass by means of their gelatinous base provide for the swallowing of the spores by herbivorous animals and lead to the germination of the spores in dung-plats. He says: "But do not believe that the discharged globule is left to chance, its fall is calculated and everything is provided for: nature has endowed it with an adhesive wall which permits of its attaching itself to anything on which it alights. As Pilobolus in the open grows in meadows or in the midst of herbage frequented by herbivora, its sporangia naturally attach themselves to the surrounding grass; if there comes along a cow or any other

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[1] According to the modern rules of nomenclature, this species is now known as *Pilaira anomala* (Cesati) Schröter.

[2] F. Cohn, *loc. cit.*   [3] E. Coemans, *loc. cit.*

[4] *Ibid.*, p. 39.

herbivorous animal which eats the herbage and sporangia together, the reproduction of the fungus is assured. The sporangia open in the stomach, the spores are mixed with the food, the heat of the animal favours their germination, and the spores, on passing out with the residue of digestion, thus find themselves germinating in a substratum indispensable for their development. Throughout the summer of 1860, I thus saw two cows, which I had at my disposal, quite unknown to themselves sow and propagate *Pilobolus crystallinus* and spread it in all the meadows into which I had them driven ; it is beautiful to see here the two organic realms acting together and assisting one another to assure the reproduction and conservation of a delicate little fungus." [1]

Both Cohn and Coemans, as indeed all later workers, were impressed with the periodic development of Pilobolus, and with the fact that each fruit-body grows to maturity and discharges its sporangium within twenty-four hours. Grove (1884) [2] remarks with truth that " Pilobolus is the Ephemeron of plant life," and he adds, *à propos* of the diurnal succession of crops of fruit-bodies : " While contemplating the saucer in which I grew my specimens, after listening to the mimic bombardment which raged so furiously an hour before, standing as it were on the field of battle with nothing but dead and dying soldiers stretched around me, I have felt as Wellington might have felt after Waterloo, but with a consolation denied to that gallant hero. I knew that even then around my feet another army was growing up among the mangled remains without any help from me, and would be ready the next day with full equipment to march with me to victory again."

Brefeld,[3] in 1881, as a result of investigations made upon pure cultures, described and illustrated the life-history of a Pilaira—then known as *Pilobolus anomalus* Ces. but subsequently as *Pilaira Cesatii* van T.—and of four species of Pilobolus. As Grove [4] has pointed out, none of these four species was correctly named ; Brefeld's *P. crystallinus* is *P. Kleinii* van T. ; his *P. oedipus* is

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[1] E. Coemans, *loc. cit.*, p. 53.   [2] W. B. Grove, *loc. cit.*, p. 18.
[3] O. Brefeld, *Untersuchungen über Schimmelpilze*, Heft IV, Leipzig, 1881, pp. 60–80, Taf. III and IV.
[4] W. B. Grove, *loc. cit.*, p. 30.

P. Kleinii forma *sphaerospora* Grove ; his *P. microsporus* is *P. crystallinus* Tode ; and his *P. roridus* is *P. longipes* van T.

Sex in the Pilobolidae.—We still know comparatively little about sex in the Pilobolidae. Van Tieghem [1] (1875) found the zygospores of *Pilaira anomala* (Cesati) Schröter in two of his cell-cultures, in one of which three spores had been sown and in the other five, while Brefeld [2] (1881) found about fifty zygospores of the same fungus on
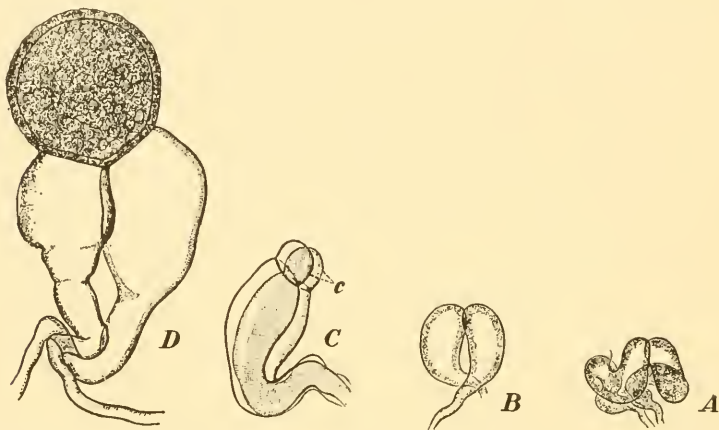
Fig. 6.—*Pilobolus crystallinus.* Stages in the formation of a zygospore. A, two aerial zygophores from two different hyphae have approached one another and have come into contact. B, the zygophores are increasing in size and have become progametes. C, the progametes have each become divided by a septum into a terminal gamete *c* and a proximal suspensor. D, the gametes have fused and have become converted into a large, thick-walled, oil-bearing zygospore to which the enlarged suspensors are still attached. Drawn by W. Zopf. From his *Die Pilze* (1890, p. 84). Magnification : A, B, and C, 160 ; D, 165.

horse dung. Zopf [3] (1888) observed the formation of the zygospores of *Pilobolus crystallinus* (Fig. 6) and (1892) of *P. Kleinii* in cultures in which the Piloboli were attacked by parasites [4] and suggested that their formation was due to the action of the parasites ; but this

[1] P. van Tieghem, " Nouvelles recherches sur les Mucorinées," *Ann. Sci. Nat.*, 6 sér., T. I, 1875, pp. 57–58.

[2] O. Brefeld, *loc. cit.*, p. 65.

[3] W. Zopf, " Zur Kennt. d. Infectionskrankh. nied. Thiere u. Pflanzen," *Nova Acta Acad. Leop.*, Bd. LII, Nr. 7, 1888, pp. 352–358, Taf. VI, Figs. 8–17 ; *Beiträge z. Morph. u. Physiol. niederer Organismen*, Heft II, 1892, pp. 5–10, Taf. I, Figs. 4–7.

[4] *P. crystallinus* and *P. Kleinii* were both attacked by *Pleotrachelus fulgens*, and the former species also by an undetermined species of *Syncephalis*.

view, according to Blakeslee,[1] cannot be accepted, because Thaxter subsequently found the zygospores of *P. Kleinii* on sheep dung but without the parasites. Morini [2] (1906) observed zygospores in a species which he has named *P. Borzianus*. He found them a little below the surface in cultures several months old. From Blakeslee's discussion [3] (1904) of the conditions under which zygospores have been observed in *Pilaira anomala*, *P. crystallinus*, and *P. Kleinii*, it is clear that at that time we did not know whether these species are homothallic or heterothallic and that exact experiment alone could throw light on this problem. The thick-walled tuberculate " spores durables " which van Tieghem [4] described as developing terminally on short curved stalks from the mycelium of *Pilobolus nanus* (Fig. 106, G, p. 212), are regarded by Fischer [5] as azygospores.

Recently (1931), Krafczyk [6] has solved the problem of the conditions required for the formation of zygospores in *Pilobolus crystallinus*. He isolated single sporangia from goat dung and made pure cultures from each one on a decoction of hay and dung solidified with agar.[7] Then he paired mycelia obtained from different sporangia, two by two, and soon found a pair which yielded zygospores along the line where they met. Then, using the $(+)$ and $(-)$ strains as testers, he was able to determine the sexual nature of a large number of other mycelia derived from sporangia collected in various localities. Thus he has shown conclusively that *P. crystallinus* is heterothallic.[8]

[1] A. F. Blakeslee, " Sexual Reproduction in the Mucorineae," *Proc. Amer. Acad. Arts and Sci.*, Vol. XL, 1904, p. 238.

[2] F. Morini, " Materiali per una monografia delle Pilobolee," *Memorie della R. Accad. delle Sci. dell'Ist. di Bologna*, Ser. VI, T. III, 1906, pp. 120–123.

[3] A. F. Blakeslee, *loc. cit.*, pp. 238–239.

[4] P. van Tieghem, " Troisième mémoire sur les Mucorinées," *Ann. Sci. Nat.*, 6 sér., T. IV, 1876, pp. 312–398, Pl. X–XIII.

[5] A. Fischer, in Rabenhorst's *Kryptogamen Flora von Deutschland*, Bd. I, Abt. IV, p. 268.

[6] H. Krafczyk, " Die Zygosporenbildung bei Pilobolus cristallinus," *Ber. d. D. bot. Gesell.*, Bd. XLIX, 1931, pp. 141–146, Figs. 1 and 2.

[7] This medium was suggested by the work of E. Bersa (" Kultur und Ernährungsphysiologie der Gattung Pilobolus," *Sitzungsber. Akad. Wissensch. in Wien*, math.-nat. Klasse, Bd. CXXXIX, 1930, pp. 355–371) who cultivated *Pilobolus Kleinii* and *P. sphaerosporus* on various media and found that they would grow very easily on horse-dung agar.

[8] Krafczyk holds that, judging by his illustrations, Zopf did not find zygospores in *Pilobolus crystallinus*, but in *P. Kleinii*. *Loc. cit.*, p. 141.

The Cytology of Pilobolus.—Harper [1] in 1899, as the result of an investigation made with modern technical methods, gave an account of the cytological changes which take place during the formation of the sporangiophore and sporangium of *Pilobolus crystallinus*, *P. oedipus*, and *P. microsporus*. His observations were as follows.

In seven to eight days after the spores have been sown on sterilised horse dung, the sporangiophores begin to appear. The yellow bulb-like swelling of the mycelium, from which a sporangiophore arises, appears in the afternoon and at that time the vegetative nuclei within it divide rapidly. After these divisions have been completed, the sporangiophore grows out from the swelling and most of the cytoplasm flows upwards into the sporangiophore carrying nuclei with it. The end of the sporangiophore swells up to form the sporangium, and cytoplasm and nuclei pass upwards into it (Fig. 7, A–C). The protoplasm in the sporangium at first forms a spongy framework in whose meshes is a considerable amount of cell-sap ; but, later, it becomes truly vacuolated in that it comes to contain rounded cavities whose outlines are determined by surface tension. A layer of larger flattened vacuoles comes to lie in the curved surface which marks the outline of the future columella (Fig. 7, D, *v*). Then protoplasmic cleavage starts from the edge of the sporangiophore and pushes upwards (Fig. 7, D, *c c*) ; and this cleavage, aided by the fusion of certain of the flattened vacuoles, results in the formation of a dome-shaped furrow (Fig. 7, E, *c*) which separates the sporangium from the sporangiophore. The cell-wall is then deposited in the cleft between the two membranes.

A jelly-like substance is excreted by the spore-plasma and is deposited as a layer on the inside of the lower part of the wall of the sporangium, and there is an extension of it up the wall of the columella (Fig. 7, E, *b*, and H, *a*, and Fig. 8, D, *d*). The subsporangial swelling begins to be formed beneath the sporangium late in the afternoon. When full-grown its wall is lined by a thin layer of protoplasm containing many nuclei.

Cleavage of the spore-plasma begins shortly after the columella is complete (Fig. 7, E). The protoplasm becomes somewhat vacuolar and the nuclei are rather evenly distributed through its mass. Cleavage

[1] R. A. Harper, " Cell-Division in Sporangia and Asci," *Annals of Botany*, Vol. XIII, 1899, pp. 490–503, Plates XXIV–XXVI.
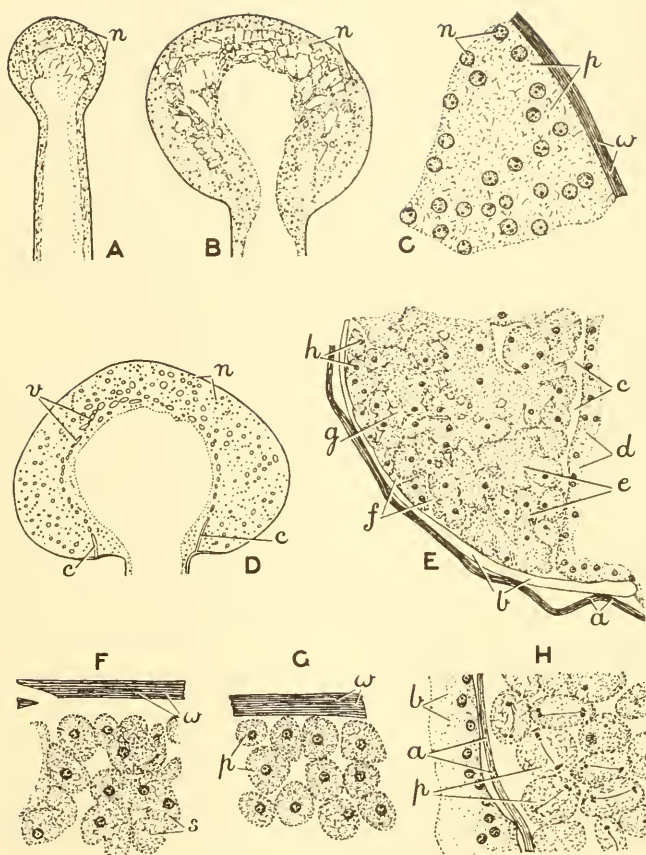
FIG. 7.—*Pilobolus crystallinus.* Cytology of the sporangium. A, median longi-
tudinal section of a sporangiophore bearing a young sporangium ; the cyto-
plasm is vacuolated and contains numerous nuclei, *n*, shown as dots. B, median
section of an older sporangium showing the course of protoplasmic flow from
the mouth of the sporangiophore ; protoplasm highly vacuolated and containing
many nuclei, *n*. C, section of sporangial wall *w* and spore-plasma *p* just before
cleavage begins ; *n*, the nuclei. D, median section of a sporangium at a stage
when the columella is forming, showing cleavage furrows *c c* at the base and
flattened vacuoles *v* above ; *n*, the nuclei ; small rounded vacuoles throughout
the cytoplasm. E, section of spore-plasma *e* from base of sporangium during
cleavage, showing : *a*, the sporangial wall ; *b*, the gelatinous collar ; *c*, the
columella-cleft between the columella and the spore-plasma ; *d*, the protoplasmic
lining of the columella ; *e*, the spore-plasma undergoing cleavage to form pro-
tospores ; *f*, superficial cleavage furrows ; *g*, an angular vacuole of the spore-
plasma ; *h*, nuclei. F, section through upper part of a sporangium showing
sporangial wall *w* and irregular sausage-shaped bodies *s* formed by cleavage of
the spore-plasma, a little older than E. G, similar to F but older, showing
uninucleate protospores *p* formed by cleavage of the spore-plasma ; *w*, spo-
rangial wall. H, protospores *p* with dividing nuclei, young daughter nuclei
connected by remains of spindle fibrils ; *a*, gelatinous collar ; *b*, protoplasm of
columella. Magnification : A, B, D, not stated ; C, 824 ; E–H, 500. Copied
by the author from R. Harper's *Cell-Division in Sporangia and Asci* (*Annals of
Botany*, Vol. XIII, 1899, Plates XXIV and XXV).
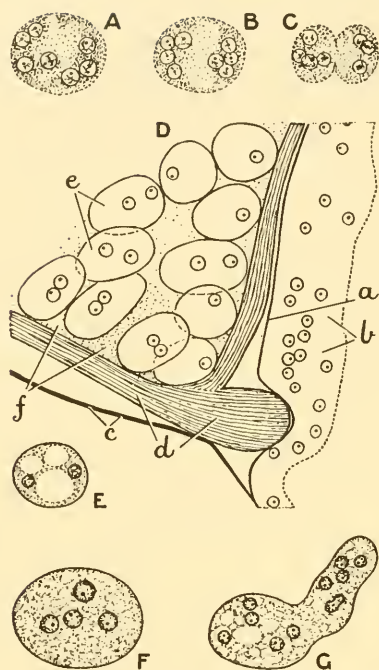
Fig. 8.—*Pilobolus crystallinus*. Cytology of
the sporangium (continued) and of ger-
minating spores. A, a multinuclear
cell (embryonic cell) developed from a
protospore, about to undergo constric-
tion ; nuclei arranging themselves in
two groups and a central zone be-
coming hyaline. B, a similar cell,
slightly older. C, a still older cell
undergoing constriction into two
halves. D, outline drawing of part of a
median section through a sporangium
with binucleated spores (formed by
growth and constriction of proto-
spores) : *a*, wall of columella ; *b*, proto-
plasm of columella containing many
nuclei ; *c*, sporangial wall ; *d*, gela-
tinous collar with extension upwards
on the columella ; *e*, binucleated spor-
angiospores; and *f*, intersporal homo-
geneous slime. E, living spore about
to germinate in a nutrient medium.
F, spore swollen and germinating in
a nutrient medium. G, germinated
spore with its germ-tube, with
numerous nuclei. Magnification :
A–C, E–G, 824; D, not stated. Copied
by the author from R. Harper's *Cell-
Division in Sporangia and Asci*
(*Annals of Botany*, Vol. XIII, 1899,
Plate XXV).

furrows then appear around
the base of the sporangium (*f*)
cutting the surface of the
protoplasm into irregular poly-
gonal areas. The rounded
vacuoles in the interior of the
protoplasm become angular (*g*),
and their edges cut through the
protoplasm to meet similar
cleavage furrows from adjacent
vacuoles. The surface furrows
grow deeper and meet and be-
come continuous with the edges
of the vacuoles. The spore-
plasma thus becomes roughly
marked into blocks of irregular
size containing a variable num-
ber of nuclei. Further furrowing
cuts these first-formed blocks
into oblong rounded sausage-
shaped masses generally con-
taining two to four nuclei in a row
(Fig. 7, F). These oblong bodies
are now divided transversely to
form rounded or spherical masses
each with one or a few nuclei
(Fig. 7, G). The *protospores* so
formed now begin to grow,
and their nuclei divide rapidly
(Fig. 7, H) so that the masses
once more become multinucle-
ated (Fig. 8, A). Then each cell
divides by constriction, the nuclei
being separated into two groups
in the halves so formed (Fig. 8,
A–C). The nuclei may then
divide further, their division
being followed by further cell-

divisions but, finally, nuclear division ceases. Cell-division, however, continues until the masses are cut up into regular oblong binucleate cells (Fig. 8, D). The primary cleavage resulting in protospores is complete about 4 A.M., and the period of embryonic growth and division lasts until about 7 A.M. The binucleated spores (e), which at first are naked masses of protoplasm, soon become covered by a wall, and drops of oil appear in their interior. The ripe spores lie embedded in a shining mass of intersporal substance (f) which can be stained readily with gentian violet and which appears to be nothing more than an excretion of the protoplasm made during the ripening of the spores.

The spores (Fig. 8, E), when sown, swell tremendously (Fig. 8, F) before pushing out a germ-tube, and the two nuclei soon divide to form eight or more which may be seen in the sporeling when the germ-tube is still very short (Fig. 8, G). The mycelium is unicellular and multinucleate. When a sporangiophore is to be formed, the protoplasm collects at a point in the mycelium and there forms a barrel-shaped swelling. This swollen portion is cut off from the rest of the mycelium (in the species studied) by both peripheral and proximal walls. The division of the protoplasm is accomplished by simple constriction furrows ; and in the bulb so formed, as already mentioned, a rapid multiplication of nuclei takes place.

FIG. 9.—*Pilobolus roridus.* Diagrammatic representation of octohedral crystalloids in the stipe of a fruit-body : *a*, the cell-wall ; *b*, the parietal protoplasm ; *c c*, the cell-sap of the great vacuole ; *d*, a crystalloid in the cytoplasm ; *e e*, crystalloids in the cell-sap ; *f*, a crystalloid which is hollow in the centre. Some of the crystalloids have concave faces. Magnification not stated. Copied by the author from P. van Tieghem's *Nouvelles recherches sur les Mucorinées* (*Ann. Sci. Nat.*, T. I, 1875, Plate 1) and reproduced on a larger scale.

Crystalloids.—In 1872, Klein [1] discovered crystalloids in the sporangiophore of Pilobolus ; and, in 1875, van Tieghem [2] found that these bodies are present not only in Piloboli (Fig. 9) but in a large number of other Phycomycetes, *e.g. Phycomycetes nitens*,
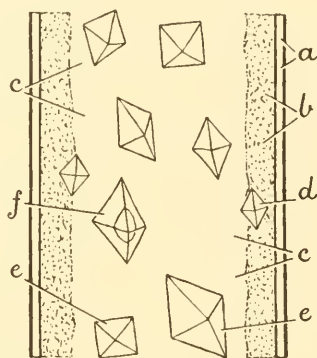
[1] J. Klein, *loc. cit.*, p. 337, Taf. XXIV, Fig. 23.
[2] P. van Tieghem, " Nouvelles recherches sur les Mucorinées," *Ann. Sci. Nat.*, 6 sér., T. I, 1875, pp. 24–32.

Spinellus fusiger, *Mucor plasmaticus*, and *Sporodinia grandis*.
There is no evidence that van Tieghem used a *camera lucida* in
making his drawings and, doubtless, he represented the crystalloids
shown in his diagrammatic illustration reproduced in Fig. 9 as being
larger, relatively to the diameter of the stipe, than they are in nature.

The Orange-red Pigment.—In 1892 Zopf [1] investigated the
orange-red pigment which colours the protoplasm of Pilobolus and
found that it consists of carotin which is held within minute oil-
drops. Zopf expressed the opinion that the carotin is merely a
reserve food-substance ; but, as we shall see later, in the mature
sporangiophore carotin is especially concentrated in the mass of
protoplasm which forms a perforated septum at the base of the sub-
sporangial swelling and which is intensely illuminated when the
sporangiophore is in heliotropic equilibrium. It is therefore possible
that the carotin plays some part in the response of the sporangiophore
to heliotropic stimuli.

Parasites of Pilobolus.—The Piloboli, when growing wild on
horse dung, etc., are not infrequently attacked by parasitic fungi
which may attach themselves to or enter the young sporangiophores,
stop their growth, and entirely prevent the production of sporangia
and spores. Among these parasites, as shown in the accompanying
Table, are species of Pleotrachelus, Piptocephalis, Syncephalis, Mortie-
rella, and Dimargaris.

Parasites of Pilobolus

Group	Parasite	Host Pilobolus	Authority
Chytridiales.	Pleotrachelus fulgens	P. Kleinii .	Zopf.
Mucoraceae	Piptocephalis microcephala .	P. roridus	van Tieghem
	Piptocephalis arrhiza .	P. oedipus .	van Tieghem
	Syncephalis reflexa	P. roridus / P. crystallinus	van Tieghem
	Syncephalis nodosa	P. roridus / P. longipes / P. Kleinii .	van Tieghem / Buller
	Syncephalis sp. .	P. crystallinus	Zopf
	Mortierella polycephala	P. roridus / P. crystallinus	van Tieghem
	Dimargaris crystalligena	P. sp. .	van Tieghem

[1] W. Zopf, " Zur Kenntniss der Färbungsursachen niederer Organismen, No. III.
Phycomyceten-Färbungen," *Beiträge zur Physiologie und Morphologie niederer
Organismen*, Leipzig, Heft II, 1892, pp. 3–12.

The mode of attack of an unnamed Syncephalis on *Pilobolus crystallinus* (Fig. 10) was described by Zopf.[1] The same author[2] discovered *Pleotrachelus fulgens*, a chytridiaceous species, in *P. Kleinii* and showed that, in its turn, the Pleotrachelus is attacked by a minute parasite which he called *Endobiella destruens*.

The parasitism of *Piptocephalis microcephala*,[3] *P. arrhiza*,[4] *Syncephalis reflexa*,[5] *S. nodosa*,[6] *Mortierella polycephala*,[7] and *Dimargaris crystalligena*[8] on Pilobolus species was observed by van Tieghem in the course of his researches on the Mucoraceae. In treating of the genus Mortierella, he states that the species grow very vigorously as saprophytes on sterilised dung, but that when
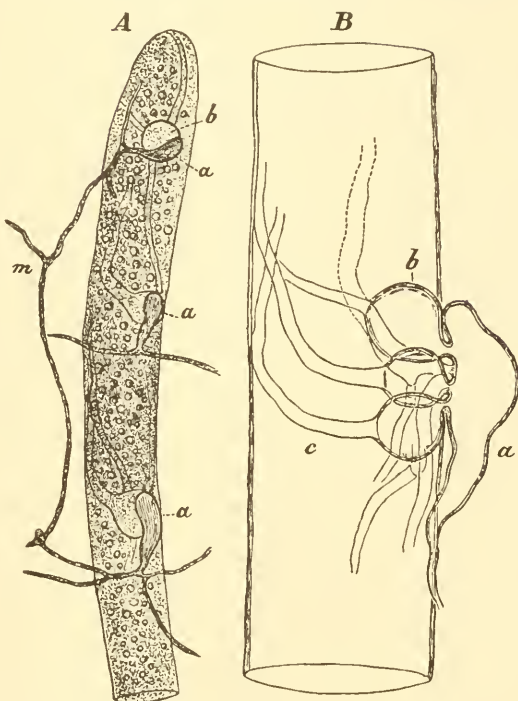
Fig. 10.—The sporangiophore of a fruit-body of *Pilobolus crystallinus* parasitised by the mycelium of a Syncephalis. A, the mycelium of the parasite *m* has grown over the sporangiophore and has produced club-shaped appressoria *a a a* at its surface. Beneath each appressorium inside the sporangiophore is a haustorial bladder *b* from which haustorial hyphae have been produced. B, a piece of a large sporangiophore : *a*, an appressorium of the Syncephalis; *b*, haustorial bladders; *c*, haustorial hyphae. Drawn by W. Zopf. From his *Die Pilze* (1890, p. 15). Magnification : A, 250 ; B, 900.

[1] W. Zopf, "Zur Kenntniss der Infectionskrankheiten niederer Thiere und Pflanzen. No. IV. Einfluss von Parasitismus auf Zygosporenbildung bei *Pilobolus crystallinus*," *Nova Acta Acad. Caes. L.-C. Nat. Cur.*, Bd. LII, 1888, pp. 352–358.

[2] W. Zopf, "Zur Kenntniss der Färbungsursachen niederer Organismen," 1892, *loc. cit.*, pp. 7–8.

[3] P. van Tieghem, *loc. cit.*, p. 148.   [4] *Ibid.*, p. 138.   [5] *Ibid.*, p. 136.

[6] *Ibid.*, p. 118.   [7] *Ibid.*, p. 97.   [8] *Ibid.*, p. 157.

they meet with a Pilobolus or a Mucor they at once parasitise it. Thus the parasitism of Mortierella, so far as Pilobolus and Mucor are concerned, is purely facultative.[1] Syncephalis [2] is also a facultative parasite, while the parasitism of Piptocephalis [3] and Dimargaris [4] is obligatory.

At Winnipeg, cultures of *Pilobolus longipes* and *P. Kleinii* in some years are much parasitised by *Syncephalis nodosa*. This species, originally described by van Tieghem [5] in 1875 and recorded by Seymour [6] as a parasite on undetermined Mucorineae in North America, is characterised by having two or three nodular thickenings on the mature and collapsed sporangiophore.

My own observations on *Syncephalis nodosa* as a parasite of Pilobolus may be here briefly recorded. The spores of *S. nodosa* germinate readily in hanging drops of dung-agar. The mycelium, as found on young Pilobolus sporangiophores, consists of very fine hyphae which by means of hyphal fusions form a reticulum with enlargements where two or more hyphae are joined together (Fig. 11, C–E).[7] It creeps over the surface of a Pilobolus stipe and there forms irregularly swollen appressoria (*cf.* Fig. 10, *a*). From an appressorium one or sometimes two hyphae are sent into the interior of the stipe. These hyphae, which may have haustorial swellings just inside the wall of the stipe, form a mycelium which grows within the interior of the stipe, soon invades the subsporangial swelling if this has been formed and, finally, stops the growth of the host-plant (Fig. 11, A and B). The interior mycelium at various points then makes its way out of the stipe. On the surface of the stipe, here and there, a hypha swells up, branches and rebranches, and forms what we may call a *mat of basal hyphae* (D and E). From these thickened hyphae cylindrical sporangiophores grow outwards into the air. When the sporangiophores have attained a height of about 0·2 mm., each of them becomes clavately swollen at its apex and then sends out terminally three or four processes which become

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[1] P. van Tieghem, *loc. cit.*, p. 97.   [2] *Ibid.*, p. 116.
[3] *Ibid.*, p. 138.   [4] *Ibid.*, p. 157.   [5] *Ibid.*, pp. 131–133.
[6] A. B. Seymour, *Host Index of the Fungi of North America*, Cambridge, Mass., U.S.A., 1929, p. 5.
[7] Cotton-blue dissolved in lactic acid was used as a stain to show up the Syncephalis mycelium against the stipe of the Pilobolus.

branched (E and F). Each final branch develops into a cylindrical
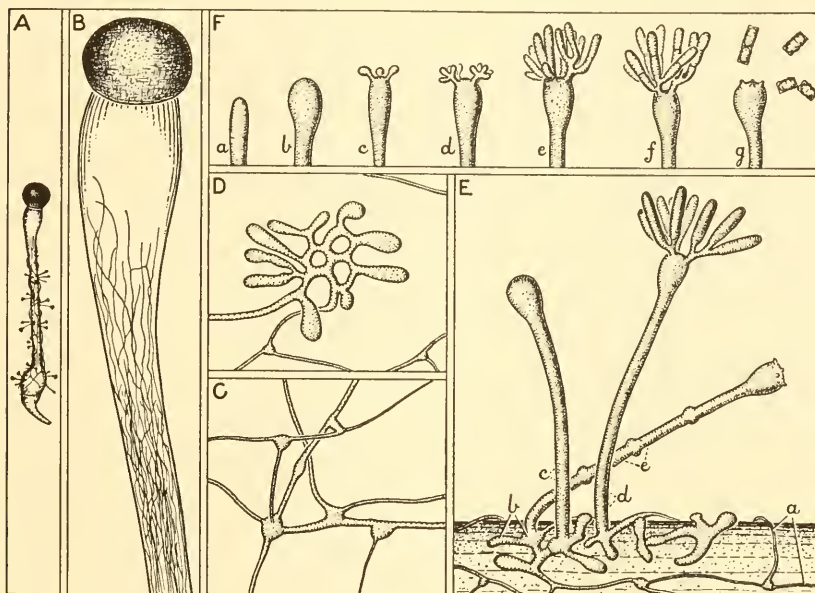sporangium, and each sporangium forms within itself a chain of

FIG. 11.—*Syncephalis nodosa* as a parasite on *Pilobolus Kleinii* and *P. longipes.*
A, an immature fruit-body of *P. Kleinii* attacked by *S. nodosa*; the subsporan-
gial swelling has ceased to develop, and the fruit-body is dying or already dead;
the parasite has produced sporangiophores on the basal swelling and stipe.
B, another young fruit-body of which the subsporangial swelling ceased to de-
velop owing to the invasion of the fruit-body by *S. nodosa*; hyphae of the para-
site are growing upwards inside the stipe and subsporangial swelling. C,
mycelium of *S. nodosa*, which was observed growing about a Pilobolus fruit-
body, showing characteristic swellings and hyphal anastomoses. D, a mat of
basal hyphae, which was observed on the surface of a Pilobolus stipe. E, part
of a stipe of *P. longipes* with *S. nodosa* growing upon it; *a*, ordinary hyphae of
S. nodosa; *b*, a basal mat of *S. nodosa* hyphae from which have sprung three
sporangiophores; *c*, a young sporangiophore with its swollen head; *d*, a spo-
rangiophore bearing nine cylindrical sporangia; *e*, an old sporangiophore,
from which the sporangia have fallen away, with a shaft showing three nodular
thickenings. F, a series of stages in the development of sporangia and spores of
S. nodosa. The end of the sporangiophore: is at first cylindrical, as at *a*; then
becomes swollen, as at *b*; and then sends out three or four processes, as at
c and *d* respectively. The processes soon branch, as at *d*. These branches
become cylindrical sporangia, as at *e*. Two or three spores develop in each
sporangium, as at *f*. Finally, the cylindrical sporangia break away from the
sporangiophore and break up into segments, so that each segment contains
one spore, as at *g*. Magnification: A, 8; B, 40; and C–F, 350.

two or three spores (F, *f*). At maturity the sporangia, of which
there are often nine on each sporangiophore, separate from the
sporangiophore and from one another, and then each sporangium

breaks across transversely into two or three pieces. Each piece encloses a single spore and appears as a short-cylindrical, wrinkled, amber-coloured structure (F, *g*). As a sporangiophore collapses, certain portions of it do not decrease appreciably in diameter and these become the nodular thickenings so characteristic of the species (E, *e*).

The Excretion of Drops by the Sporangiophore and its Cause.— Everyone who has studied Pilobolus has had occasion to notice the numerous drops of clear liquid which are excreted from the sporangiophore (Fig. 12).

Wilson,[1] in 1881, working in Pfeffer's laboratory, observed : (1) that, if sporangiophores of *Pilobolus crystallinus* are allowed to stand for a short time in a dry atmosphere, the drops vanish and in the place of each there can be seen a group of radiating crystals,[2] visible to the naked eye ; (2) that, if the same plants are then placed in a moist atmosphere, many of the drops reappear and precisely in those places where the crystals were ; (3) that, if the sporangiophores are very carefully washed with the most delicate brush and distilled water and are then placed in moist air, the drops, as a rule, do not quickly reapppear and often do not reappear at all; and (4) that, if the sporangiophore is washed and afterwards minute particles of sugar are placed upon its wall, large drops of water quickly form about the sugar particles. Wilson came to the conclusion that the formation of the drops is due, not to the internal pressure of the cell-sap, but, as in nectaries, to the osmotic action of substances which are present on the surface of the cell-wall.

In 1897 Pfeffer,[3] in a discussion of the excretion of water from uninjured plants, remarked : "Bearing in mind all these possibilities it is impossible to say whether the drops which appear upon

[1] W. P. Wilson, "The Cause of the Excretion of Water on the Surface of Nectaries," *Unters. a. d. bot. Inst. zu Tübingen*, 1881, p. 15.

[2] Each drop contains a colloidal gelatinous substance (*vide infra*, Figs. 42 and 43, pp. 86 and 87) and, when the drop dries up, this substance contracts so as to form a tiny, often angular, lump of solid matter. I have never seen the "radiating crystals" of which Wilson speaks nor, apparently, did he realise that a drop contains colloidal matter. It seems probable that he mistook angular lumps for groups of crystals.

[3] W. Pfeffer, *Pflanzenphysiologie*, 1897, English edition, Oxford, Vol. I, 1900, p. 275.

the sporangiophore of Pilobolus, when it is kept in a saturated atmosphere, are formed by the active or the plasmolytic excretion of water. As a matter of fact a drop of the fluid leaves a crystalline

FIG. 12.—*Pilobolus Kleinii.* Sporangiophores in various stages of development represented semi-diagrammati-cally in one drawing : *h,* the substratum of horse dung ; in *a* the stipe is elongating ; in *b* the sporangium is de-veloping terminally ; in *c* each sporangiophore has developed a large subsporangial swelling beneath the sporangium, and the sporangium is about to be dis-charged : in *d* the sporangium has been discharged and only the collapsed stipe and subsporangial swelling remain behind. The sporangiophores are bedecked with numer-ous watery drops which glisten in the light like dew-drops. Drawn by É. Boudier ; photographically copied from Plate 582 of his *Icones Mycologicae* ; letters added by the author. Magnification, 16.

deposit when allowed to dry upon a cover-slip. It is therefore possible that the excretion of water is plasmolytic in this case, for any osmotic substance must tend to withdraw water from a fully turgid cell with which it is in contact."

Lepeschkin,[1] in endeavouring to elucidate the mechanism of active water-excretion in plants, used Pilobolus as his chief material for experimentation. Among his numerous observations on water excretion by Pilobolus and the factors which influence it were the following.

The young naked stipe, that grows upwards from the basal swelling, usually excretes at its apex a large transparent drop. If this drop is removed with a capillary pipette, it is replaced by a new drop in the next 30–50 minutes. With further growth of the stipe, smaller drops appear at intervals down the whole length of the stipe. After separation of the sporangium by a wall, excretion of water from the sporangium proceeds very slowly or ceases. Three to five hours before the sporangium is shot away, the excretion of water from the sporangiophore diminishes gradually and then ceases.[2] (*Cf.* Fig. 42, C, p. 86.)

The excretion of water from the sporangiophore was studied under the microscope. The fruit-bodies were enclosed in a damp-chamber (with the coverglass smeared with glycerine). The drops were measured with an ocular-micrometer and were drawn off with a capillary pipette already introduced into the chamber. It was found that water excretion always occurs at the same places on the surface of the sporangiophore and quite regularly and continuously. The drops became about 0·2 mm. in diameter and were drawn off five to ten times in succession. The time taken for the renewal of a drop at a particular place on the sporangiophore was very constant and varied with the place from seven to twelve minutes. At different places on the sporangiophore the amount of water excreted is very unequal. The most energetic excretion of water takes place directly under and above the orange-yellow zone which lies at the top of the stipe and just beneath the subsporangial swelling.[3]

The chemical composition of the cell-sap in the great vacuole of the sporangiophore of *Pilobolus longipes* and that of the excreted drops were determined and compared. The turbid liquid expressed from a number of sporangiophores was dried at 60° C. and the

[1] W. W. Lepeschkin, " Zur Kenntnis des Mechanismus der aktiven Wasseraus-scheidung der Pflanzen," *Beihefte z. Bot. Centralb.*, Bd. XIX, 1906, pp. 409–452.

[2] *Ibid.*, pp. 412, 418–419. [3] *Ibid.*, pp. 412–413.

deposit was treated with cold water.   Only two-thirds of the deposit
was dissolved.   The percentage composition of the soluble portion
of the deposit was found to be : $38 \cdot 8$ organic substances (no carbo-
hydrates) ; $20 \cdot 5$ K_2O and Na_2O (chiefly K_2O) ; $19 \cdot 3$ Al_2O_2 and
Fe_2O_2 ; $1 \cdot 5$ SO_3 ; $14 \cdot 5$ P_2O_5 ; $4 \cdot 2$ Cl ; $1 \cdot 4$ CO_2 and, finally,
traces of SiO_4.   Altogether, the sap contained $1 \cdot 2$ per cent. of
soluble and $2 \cdot 9$ per cent. of insoluble substances.   It was found
that the excreted drops contain $0 \cdot 5$ per cent. of mineral salts of the
same nature as those contained in the cell-sap, but no organic sub-
stances.   The drops have an alkaline reaction, as may be determined
with litmus paper, and this reaction is due to K_2CO_3.[1]

Lepeschkin's statement that the excreted drops are completely
devoid of organic substances cannot be accepted ; for, as Knoll [2]
subsequently discovered, the drops contain a colloid in consequence
of which, as they dry up, their surface assumes an irregularly
wrinkled appearance (Fig. 42, C, p. 86).   However, it may well
be that this colloidal substance is not excreted along with mineral
salts from the vacuole of the sporangiophore but, as Knoll believes,
is formed by local mucilaginisation of the cell-wall.[3]   (Cf. Fig. 43,
p. 87.)

If the sporangiophores are transferred to dry laboratory air,
even although the substratum contains plenty of water, they at
once begin to dry, soon become flaccid, and eventually bend and fall.
With the diminution of cell-turgor, the energy of excretion becomes
smaller and smaller and soon the excretion of drops ceases com-
pletely.   To revive a flaccid sporangiophore, it is necessary to place
it in air which has at least 90 per cent. moisture in it.[4]

Tufts of Pilobolus (*Pilzrasen*) were set on salt solutions which
diminish the cell-turgor.   In spite of very great air-moisture, a 1 per

[1] W. W. Lepeschkin, " Zur Kenntnis des Mechanismus der aktiven Wasseraus-
scheidung der Pflanzen," *Beihefte z. Bot. Centralb.*, Bd. XIX, 1906, p. 421.

[2] F. Knoll, " Untersuchungen über den Bau und die Funktion der Cystiden und
verwandter Organe," *Jahrb. f. wiss. Bot.*, Bd. L, 1912, pp. 488–489.

[3] Knoll (*loc. cit.*, pp. 453–500) found that the cystidia of a number of Hymeno-
mycetes excrete potassium oxalate which crystallises out in the slime formed from
the cell-wall.   Whether or not the drops excreted by Pilobolus contain traces of
organic substances other than colloidal slime, which Lepeschkin failed to detect,
remains to be determined by further analyses.

[4] W. W. Lepeschkin, *loc. cit.*, p. 413.

cent. solution of sodium chloride caused water excretion to cease, and even a 0·5 per cent. solution brought about a very much diminished excretion.[1]

The fruit-body primordia (the red bulbils, *cf.* Fig. 2, A, p. 4), if cut away from the rest of the mycelium in the evening and supplied with water, develop into normal fruit-bodies during the night, and the next morning it is found that water excretion from the sporangiophores has taken place normally. This shows that water absorption and water excretion take place in Pilobolus in one and the same cell.[2]

The water-excretion energy (the volume of the fluid excreted from ten sporangiophores during one hour in divisions of the collecting pipette [3]) increases almost proportionally to the temperature. It was found to be : at 0° C., 0·0 ; at 3°–4°, 0·2 ; at 18°, 1·9 ; at 25°, 2·8 ; at 30°, 3·6 ; and at 35°, 4·5. At 0° C. the stipes do not swell at their apex and there is no excretion. At 35° C. the excretion is so great that it is not covered by the absorption of water by the basal swelling. An exposure to 35° C. for a few hours results in so great a loss of turgor that the sporangiophores bend and fall.[4]

In the evening a tuft of Piloboli was set in a glass tube and the air in the tube was replaced with pure nitrogen. The sporangiophores continued their development and next morning the excretion of drops was found to have taken place quite normally. Oxygen therefore appears to have little or no effect on the development of a fruit-body primordium into a mature fruit-body or on the energy of water excretion.[5]

[1] W. W. Lepeschkin, *loc. cit.*, p. 413.   [2] *Ibid.*, p. 414.

[3] The volume of each division of the graduated pipette was 0·03 cubic mm.

[4] W. W. Lepeschkin, *loc. cit.*, pp. 414–416.

[5] *Ibid.*, p. 416. Verification of Lepeschkin's statement that fruit-bodies of Pilobolus can develop from primordia to maturity without any oxygen supply seems desirable. There is the possibility that a very small amount of oxygen diffused out of the substratum on which the fruit-bodies grew after his experimental tube was closed. One evening I covered some primordia of *Pilobolus longipes* fruit-bodies and a small mass of horse dung on which they were growing with *liquidum paraffinum* (depth upwards of one inch). Next morning I found that a number of the primordia had developed during the night into normal mature fruit-bodies. The presence of the *liquidum paraffinum* doubtless much reduced the access of oxygen to the fruit-bodies, but whether or not it cut it off completely is uncertain.

When chloroform or ether vapour is introduced very slowly into the air surrounding the sporangiophores and the first amounts are small enough, water excretion ceases, but with larger doses water excretion is increased. Sufficient vapour of alcohol, hydrochloric acid, ammonia, or sulphuretted hydrogen also increases the energy of excretion. An increased excretion can be obtained if one sets the fungus tuft on a solution of any of these substances or caffeine. When some of the sporangiophores of *Pilobolus longipes* were exposed to the vapour of alcohol for five minutes, the energy of water excretion, which during the night had been $4 \cdot 1$, was raised to 250 ; and when a tuft of sporangiophores of *P. Kleinii* was set on a $0 \cdot 5$ per cent. solution of caffeine for two minutes, the energy of excretion, which during the night had been 2, was raised to 42. The increase of water excretion caused by anaesthetics and other poisonous substances is accompanied by a decrease in the cell-turgor. The drops excreted under the action of alcohol vapour contained $1 \cdot 9$ per cent. of dissolved substances instead of the usual $0 \cdot 6$ per cent.[1]

Diffused light has no influence on the excretion of water, but direct sunlight causes its cessation.[2]

On the basis of his experimental results Lepeschkin discussed various theories devised to explain how it is that a cell, such as the sporangiophore of Pilobolus, is able to absorb water in one part and excrete water in another part. Since the excretion of water in the form of a drop at one spot in the sporangiophore is continuous and steady, he rejected the hypothesis of Godlewski which postulates alternating periods of greater and less osmotic pressure in the cell and corresponding periods of non-excretion and excretion due to the expansion and contraction of the protoplasm.[3] Having observed that a sporangiophore which has been washed by dipping in water and has been again brought into moist air continues to excrete water in the form of drops,[4] he also rejected the theory of Wilson

[1] W. W. Lepeschkin, *loc. cit.*, pp. 416–418, 434.
[2] *Ibid.*, p. 418.   [3] *Ibid.*, p. 419.
[4] Wilson (*loc. cit.*) washed sporangiophores in water with a brush, then set them in moist air, and thereafter observed that, often, the sporangiophores did not excrete any more drops. Hence he concluded, according to Lepeschkin erroneously, that washed sporangiophores do not excrete water. Lepeschkin states that washing

that the formation of drops on the sporangiophore, like the forma-
tion of drops on the epidermis of nectaries, is due to a substance
which issues from the cell-walls and by osmosis attracts water to
itself.[1]   Having observed that there is a most plentiful discharge
of water from the sporangiophore and that the excretion of water
from the sporangium at the time of ripening of the spores is small
or has entirely ceased, Lepeschkin refused to accept the view of
Brefeld according to which the excretion of water is due to the
protoplasm becoming denser during the formation of the spores.[2]
Of the two hypotheses of Pfeffer, (1) that the excretion of water in
a cell is due to the unequal distribution of osmotic substances in the
vacuole, water being absorbed where osmotic substances are in
greatest concentration and excreted where the osmotic substances
are in least concentration, and (2) that the excretion of water is due
to the unequal permeability of the protoplasmic membrane for
water and dissolved salts, water being absorbed where the membrane
is less permeable and best able to withstand osmotic pressure and
excreted where the membrane is more permeable and least able to
withstand osmotic pressure, Lepeschkin accepted the latter.[3]   He
concluded that the facts elucidated by his investigations justify the
conclusion that water excretion in Pilobolus is due to the difference
in permeability of the plasma-membrane for dissolved substances
in its upper and lower parts, and that all the phenomena caused by
the action of anaesthetics, poisonous substances, temperature, and
strong light are due to the great changeableness of the plasma-
membrane in respect to permeability.[4]

That differential permeability may lead to excretion of drops
seems to be proved by some experiments of Weis [5] who lowered the
permeability of one part of a cell by means of alcohol.   Weis sub-
jected one half of a long (30–50 mm.) intact internodal cell of
Nitella to alcohol vapour or bathed it in a 10 per cent. alcohol

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with a brush removes a coat of fat from the exterior of the sporangiophore, that such
a washed sporangiophore excretes water, and that the excreted water instead of
forming drops runs at once over the surface of the cell-wall.

[1] W. W. Lepeschkin, *loc. cit.*, pp. 419–421.   [2] *Ibid.*, p. 421.

[3] *Ibid.*, pp. 421–425.   [4] *Ibid.*, pp. 434–435.

[5] A. Weis, " Zur Mechanik der Wasserausscheidung aus lebenden Pflanzenzellen,"
Planta, Bd. II, 1926, pp. 241–248.

solution. The treated half of the cell was allowed to project into the air while the untreated half was immersed in water. Under these conditions the treated half excreted drops. These drops were of so large a volume (62 per cent. of the cell volume when alcohol vapour was employed) that it could be concluded with certainty that, while the treated half of the cell had been excreting water, the untreated half had been absorbing water. In this experiment, however, while the absorbed water was doubtless pure, the excreted water may well have been nothing more than escaped cell-sap becoming more and more diluted.

The excretion of water from the sporangiophore of Pilobolus is, as we have seen, not general over the whole surface but at certain definite places on the wall. These places are very small in area and may be over one hundred in number. They become covered by as many drops, each of which seems to issue at a point. The drops do not run down over the surface of the sporangiophore because the wall is covered with a thin layer of fat.[1] According to Lepeschkin's theory of excretion : (1) the plasma-membrane just beneath each point of the cell-wall where a drop exudes must be regarded as exceptionally permeable for water and dissolved substances ; and (2) the driving force causing exudation is the osmotic pressure of the cell-sap.

As Lepeschkin's analyses indicate, the drops excreted by Pilobolus are more watery than the cell-sap. In 1921, V. H. Blackman [2] showed that " the claim of Lepeschkin that the osmotic pressure of the stronger cell-contents is responsible for the exudation from the cell of a weaker solution cannot be substantiated " and pointed out that Pfeffer himself [3] in 1890 had withdrawn this hypothesis as physically unsound. Blackman suggested other

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[1] No proof has been offered that the sporangiophore is covered with a layer of fat or wax. There is the possibility that the drops do not run down the sporangiophore because the surface of the latter bears numerous fine crystals of calcium oxalate.

[2] V. H. Blackman, " Osmotic Pressure, Root Pressure, and Exudation," *The New Phytologist*, Vol. XX, 1921, pp. 106–115, with three diagrams.

[3] W. Pfeffer proposed this hypothesis (along with others) in 1877 in his *Osmotische Untersuchungen*, Leipzig, pp. 224–225, and repudiated it first in 1890 in his " Zur Kenntnis der Plasmahaut und Vacuolen," *Abh. K. Ges. d. Wiss.*, Leipzig, p. 302, and again, in 1897, in his *Pflanzenphysiologie* (English edition, Oxford, 1900, pp. 271–272).

mechanisms for excretion but felt that without more knowledge of cell-dynamics it was impossible to treat the problem of exudation satisfactorily.

Weis [1] set a Pilobolus fruit-body on a slide, placed a glass thread and some vaseline across the sporangiophore just under the sub-sporangial swelling, pressed the glass thread down with a cover-glass, and thus compressed the sporangiophore into two parts. This enabled him to apply plasmolytic solutions to the subsporangial swelling by itself and to the stipe by itself. He found that, with a 0·4–0·5 gram-molecule cane-sugar solution, plasmolysis took place in the swelling slowly or not at all and in the stipe considerably faster. From this he concluded that the osmotic value of the cell-sap (or the resistance to filtration of the wall as a whole) must be greater in the swelling than in the stipe, but added that his conclusion still leaves us free to regard the tiny places in the wall where drops are excreted as more permeable than the rest of the wall.[2]

Ursprung and Blum [3] submerged a Pilobolus fruit-body in paraffin oil (*liquidum paraffinum*) and with a compression apparatus applied pressure to the liquid. As the pressure was slowly increased, drops came out of the wall of the subsporangial swelling and the adjacent part of the stipe at about the same time. If the screw of the pressure apparatus was not further tightened, the sporangiophore sucked in the drops again. With renewed increase of the pressure, the drops appeared again, but none were observed on the basal part of the stipe. From this experiment the authors concluded that, if the turgor pressure is the same throughout the whole of the sporangiophore, the suction pressure of the contents in the upper part is smaller than in the lower part, and they add that this difference may be due to differences in the osmotic values of the

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[1] A. Weis, "Zur Mechanik der Wasserausscheidung aus lebenden Pflanzenzellen," *Planta*, Bd. II, 1926, p. 247.

[2] Weis, unfortunately, does not state whether or not the compression of the sporangiophore into two parts injures the cell in any way nor whether or not his sporangiophores had ceased to excrete water before they were used for his experiment.

[3] A. Ursprung and G. Blum, " Eine Methode zur Messung Polarer Saugkraft-differenzen," *Jahrb. f. wiss. Bot.*, Bd. XLV, 1925, p. 11.

contents in different parts of the cell or to the plasma-membrane differing in permeability in different places.

In 1919, Stern [1] suggested that bleeding from wounds in flowering plants is due to electro-osmosis ; and, in 1921, Blackman [2] discussed electro-osmosis as a possible cause of the excretion of drops in Pilobolus. Hitherto, however, the electrical phenomena which accompany the excretion of drops in Pilobolus have not been investigated.

From the review of the literature given in the foregoing pages it appears that we are still far from understanding the mechanism by means of which Pilobolus excretes its drops. It is possible that further light might be thrown on the problem : (1) by measuring, with the help of Barger's or Ursprung and Blum's capillary-tube method,[3] the osmotic pressure of a series of drops exuded from one and the same point on the sporangiophore ; (2) by comparing the osmotic pressure of the drops and of the cell-sap ; (3) by obtaining more accurate chemical analyses of the drops and cell-sap than hitherto has been attempted ; and (4) by investigating the suction force of the basal swelling and of the subsporangial swelling by the method used by Ursprung and Blum [4] in their measurements of the difference in the suction force exerted through opposite walls of endodermis and other cells.

[1] K. Stern, " Über elektroosmotische Erscheinungen und ihre Bedeutung für pflanzenphysiologische Fragen," *Zeit. f. Botanik*, Bd. XI, 1919, p. 600.

[2] V. H. Blackman, *loc. cit.*, p. 114. He cites the work of Bartell and Madison who found that with various solutions the normal osmotic tendency of gold-beaters' skin membranes might be increased, decreased, or reversed. " The results can be explained by the electrical relations of the membrane ; a difference of potential between the faces of the two membranes is developed if electrolytes are used and this electro-endosmosis may aid or retard the normal process of osmosis ; we thus have an additional force superimposed on the ordinary osmotic relations." Among the most recent papers treating of electro-osmosis through organic membranes with numerous citations of the relevant literature is that of L. Bruner, "Untersuchungen über die Elektrolyt-Permeabilität und Quellung einer leblosen natürlichen Membrane," *Jahrb. f. wiss. Bot.*, Bd. LXXIII, 1930, pp. 513–632. His experiments were made with the testa of the Horse Chestnut.

[3] For Barger's method *vide infra*, pp. 141–142. For the method of Ursprung and Blum, *vide* their " Zwei neue Saugkraft-Messmethoden," *Jahrb. f. wiss. Bot.*, Bd. LXXII, 1930, pp. 257–306.

[4] A. Ursprung and G. Blum, " Eine Methode zur Messung Polarer Saugkraft-differenzen," *loc. cit.*

Premature Discharge of the Sporangia.—In the evening Lepeschkin [1] placed a tuft of fruit-body primordia on a 2 per cent. solution of sodium chloride. Next morning it was found that some of the fruit-bodies had not developed sporangia and that others had developed to maturity in the usual way. Plasmolytic experiments showed that, whereas in the previous evening plasmolysis of the fruit-bodies began with a 1·4 per cent. sodium-chloride solution, in the morning it began with a 3·5 per cent. solution. Thus the osmotic pressure had increased two and one-half times during the night. Lepeschkin, in the morning, removed the fruit-bodies from contact with the 2 per cent. salt solution and set them on water. Two phenomena were then noticed : (1) the imperfectly developed fruit-bodies greatly increased their excretion of water ; and (2) the perfectly developed fruit-bodies, which had ceased or were ceasing to excrete water, did not increase their excretion of water but, instead, within 1–5 minutes exploded and shot away their sporangia with a bang.[2] These phenomena were doubtless due to the increase of the turgor pressure in the sporangiophore brought about by the transference of the fruit-bodies from the 2 per cent. salt solution to water.

Lepeschkin also observed that, when mature fruit-bodies of Pilobolus are subjected to chloroform or ether vapour by the gradual method beginning with very small amounts, the discharge of the sporangia is prematurely hastened. Thus by the gradual method he subjected 70 fruit-bodies to chloroform or ether and in other vessels kept 95 fruit-bodies in ordinary moist air. The experiment began at 9 A.M. Of the 70 fruit-bodies treated with narcotics, 20 had shot away their sporangia by 9.30 A.M., 65 by 10 A.M., and all by 11.30 A.M., whereas of the 95 untreated sporangia not one had shot away its sporangium by 11.30 A.M. Lepeschkin explained the earliness of discharge of the sporangia under the influence of chloroform and ether by supposing that these narcotics diminish the permeability of the plasma-membrane of the sporangiophore and so increase the turgor of the sporangiophore to the degree required for the discharge of the sporangium before the usual time.

There are two forces to be taken into account in the discharge

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[1] W. W. Lepeschkin, *loc. cit.*, p. 429.   [2] *Ibid.*, pp. 432–433.

of a sporangium of Pilobolus or of the eight spores from the ascus of a Peziza : (1) the turgor pressure of the cell-sap, and (2) the resistance of the wall to breakage. Both in Pilobolus and in an ascus the wall is so constructed that it breaks along a particular line of ultimate weakness. It is possible that shortly before discharge the wall, along its break-line, is gradually weakened and that the turgor pressure of the cell-sap is incapable of causing discharge of the sporangium or spores until the weakening has proceeded far enough. Under natural conditions, as Lepeschkin [1] found, the concentration of the cell-sap of the sporangiophore of Pilobolus steadily diminishes as development proceeds ; but whether or not this concentration, and with it the turgor pressure, is markedly increased just before the discharge of the sporangium remains to be determined by experiment.

I have observed that the application of poisonous substances, such as iodine, mercuric chloride, silver nitrate, copper sulphate, sulphuric acid, acetic acid, and alcohol, to the hymenium of *Peziza vesiculosa* (my *P. repanda*) lying in water at once causes the asci to explode and discharge their spores.[2] It seems unlikely that the discharge of the asci, which takes place within a few seconds of the application of the reagents, can be due to increase of cell-turgor. It was found that asci which had contracted after treatment with a neutral salt may be caused to explode when brought into contact with iodine.[3] It is possible that the early explosion of the sporangiophores of Pilobolus in Lepeschkin's experiments with chloroform and ether was in part due to the action of the anaesthetics on the protoplasm immediately beneath that part of the wall which eventually ruptures. It may be that this protoplasm is stimulated by anaesthetics to weaken the wall through enzyme action prematurely.[4]

Influence of External Conditions on the Breadth of the Subsporangial Swelling.—Lepeschkin [5] at 9 P.M. placed tufts of young fruit-bodies on water and on 4·5, 9, and 18 per cent. sugar solutions.

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[1] W. W. Lepeschkin, *loc. cit.*, p. 422. Lepeschkin observed that a young sporangiophore (no sporangial swelling) and a ripe sporangiophore of *Pilobolus Kleinii* plasmolyse with a 3·7 and a 2·3 per cent. solution of potassium nitrate respectively.

[2] These *Researches*, Vol. I, 1909, p. 238.

[3] *Ibid.*, p. 239.   [4] *Cf. ibid.*, pp. 239–240.

[5] W. W. Lepeschkin, *loc. cit.*, p. 423.

These liquids were contained in vessels filled with glass beads. Next morning it was found that the fruit-bodies had developed normal sporangia and stipes of normal length and that the average width of the subsporangial swelling in ocular-micrometer divisions for the fruit-bodies grown on water and on the 4·5, 9, and 18 per cent. sugar solutions was 49, 38, 29, and 14 respectively. It thus appears that the width of the subsporangial swelling is determined in part by the osmotic value of the substances dissolved in the water permeating the substratum.

Lepeschkin [1] washed off the " fat layer " from some young fruit-bodies which had not yet developed subsporangial swellings, isolated the fruit-bodies from the mycelium, and then set half of the fruit-bodies with only the basal swelling in water (normal apical end in the air) and the other half of the fruit-bodies with only the apical end in water (basal swelling in the air). Next day he found that the fruit-bodies which had had the basal swelling in water had subsporangial swellings of normal diameter (46 micrometer divisions), whilst the fruit-bodies which had had their apical ends in water had subsporangial swellings of much less than normal diameter (9–15 micrometer divisions).[1] These results suggest that immersion in water prevents the subsporangial swelling from developing to its normal size.

The Ballistics of the Projectile.—Link,[2] in 1809, was the first to attribute the projection of the sporangium to its true cause, the tension in the swelling below the sporangium ; and this explanation was endorsed by de Bary [3] in 1866. The osmotic pressure in the cell-sap of the sporangiophore just before the discharge of the sporangium is effected has been measured by the writer (*vide infra*) and has been found equal to a pressure of several atmospheres.

Allen and Jolivette,[4] in the course of their studies of the helio-

[1] W. W. Lepeschkin, *loc. cit*, p. 424.

[2] H. F. Link, " Observationes in Ordines plantarum naturales," *Magaz. d. Ges. naturf. Freunde*, Berlin, Bd. Ill, 1809, p. 32. He says : " Explosio fieri mihi videtur, dum suprema pars stipitis bullata, sporangium inferne ambiens, contrahitur."

[3] A. de Bary, *Morphologie und Physiologie der Pilze, Flechten, und Myxomyceten*, Leipzig, 1866, p. 146.

[4] Ruth F. Allen and Hally D. M. Jolivette, " A Study of the Light Reactions of Pilobolus," *Trans. Wisconsin Acad.*, Vol. XVII, 1914, pp. 533–598.

tropic reaction of Pilobolus to white light and light of different colours (*vide infra*), grew sporangiophores in the dark and observed the accuracy with which the projectiles struck a small circular window or two such windows. An account of their work was published in 1914.

In 1921, I[1] pointed out that, when discharge of the sporangium takes place, the neck of the subsporangial swelling just beneath the sporangium is ruptured transversely, the wall of the swelling and stipe contracts elastically, and the cell-sap is squirted out of the top of the swelling, so that the sap carries the sporangium with it through the air. I also stated that *Pilobolus Kleinii* and *P. longipes* can both shoot their largest sporangia upwards to a maximum height just exceeding six feet and to a maximum horizontal distance just exceeding eight feet. The experimental evidence upon which this statement rests will be given in the next chapter.

In 1927, Pringsheim and Czurda,[2] in recording their investigations on the ballistics of Pilobolus, again called attention to the fact that a drop of sap expelled from the subsporangial swelling travels with the sporangium on its journey through the air, but they were unable to suggest the true form of the projectile during its flight. A solution of this problem will be found in the next chapter.

The other results of Pringsheim and Czurda's ballistic investigations were summarised by those authors[3] as follows. The horizontal range of Pilobolus is greatest when the sporangiophores are inclined obliquely upwards, and the extreme range observed was about 2 metres. The accuracy of the shooting depends on the brightness of the source of light and especially on the range : the greater the distance of the target aimed at, the more pronounced the scattering. With a not too distant disc of light the middle is hit more often than the edge. The speed of the projectile just after discharge, on the average, was found to be 14 metres per second. With the help of a ballistic oscillating pendulum, on the basis of the amount of

[1] A. H. R. Buller, " Upon the Ocellus Function of the Subsporangial Swelling of Pilobolus," *Trans. Brit. Myc. Soc.*, Vol. VII, 1921, p. 61.

[2] E. G. Pringsheim and V. Czurda, " Phototropische und ballistische Probleme bei Pilobolus," *Jahrb. f. wiss. Bot.*, Bd. LXVI, 1927, pp. 869–872.

[3] *Loc. cit.*, p. 900.

movement caused by the projectile, the mass of the projectile was found to be 0·011 mg. By weighing, the dry weight of the projectile was found to be 0·0057 mg. The force of the discharge was calculated to be 10·8 ergs or 26×10^{-8} calories.

Pilobolus in its Relations with Light.—The response of Pilobolus to light has been studied by Sorokin (1873), Fischer von Waldheim (1875), Brefeld (1881), Regel (1881), Allen and Jolivette (1914), Parr (1918), Buller (1921), Pringsheim and Czurda (1927), and Van der Wey (1929).

The Effect of Light on Fruit-body Development.—Brefeld,[1] for *Pilobolus crystallinus* (his *P. microsporus*), found : (1) that, in the absence of light, no sporangia are formed but the sporangiophores continue to grow for 10–14 days until they become 8–10 inches long, when they wither ; (2) that a two-hour exposure to light is sufficient to cause a rudiment of a fruit-body to complete its development in the dark ; and (3) that the fruit-bodies develop normally in blue light but cannot develop their sporangia in red-yellow light. There can be no doubt, therefore, that light stimulates *P. crystallinus* in a formative or morphogenic manner.

The Heliotropic Response of the Fruit-body to Light of Various Colours.—The sporangiophores of Pilobolus exhibit marked heliotropism (*cf.* Fig. 13). Sorokin,[2] Fischer,[3] and Brefeld studied the heliotropic effect of light passed through (1) a solution of potassium bichromate and (2) an ammoniacal solution of copper oxide. The results were divergent. Sorokin found that Pilobolus gives no heliotropic reaction in blue light and is negatively heliotropic in red-yellow light, while Fischer obtained a strong positive response in blue light but no response in red-yellow light. Subsequent work has shown that these conclusions were erroneous.

Brefeld[4] obtained a positive response in both blue and red-

[1] O. Brefeld, *Untersuchungen über Schimmelpilze*, Leipzig, Heft IV, 1881, pp. 76–78 ; Heft VIII, 1889, p. 276, Taf. XII, Figs. 1–6.

[2] N. Sorokin, " Ueber die Wirkung des Lichtes auf die Pilze," *Beiläge zu d. Protocollen der Sitz. d. Naturf.-Ges. an d. Univers. z. Kasan*, 1875 (in Russian, cited from Parr's paper).

[3] A. Fischer von Waldheim, " Ueber den Heliotropismus niederer Pilze," *Arb. d. bot. Lab. d. k. Univ. Warschau*, Warschau, 1875, Heft I (*vide* Just's *Bot. Jahresbericht*, Vol. III, 1875).

[4] O. Brefeld, *loc. cit.*, Heft IV, p. 77.

yellow light, with a better reaction in blue light. Gräntz [1] repeated Brefeld's experiments and confirmed them. According to Regel,[2] Pilobolus is positively heliotropic in white, blue, yellow, and red light at all the intensities and temperatures employed. The con-clusions of Brefeld, Gräntz, and Regel, as we shall see, have been substantially confirmed by the more recent work of Miss Parr.

The first attempt to express the photic sensibility of plants in quantitative terms was made by Wiesner [3] (1879) in his classic work on heliotropism. The next considerable advance in this direction was made by Blaauw [4] (1909), who for the first time employed modern physical methods. Blaauw investigated the heliotropic response of *Phycomyces nitens*.

Fig. 13.—*Pilobolus* (*Kleinii ?*). A photomicrograph of a group of fruit-bodies growing on dung in the laboratory showing long slender stipes bearing drops of mucilaginous fluid, beautifully symmetrical pear-shaped subsporangial swellings, and jet-black disc-shaped sporangia. The diameter of each subsporangial swelling considerably exceeds that of the sporangium which crowns it. Owing to the heliotropic curvature of the stipes, the sporangia all faced the source of strongest light. Photographed by B. O. Dodge. Magnification, about 3·5.

Miss Rosalie Parr [5] (1918), realising that the chief reason for the

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[1] F. Gräntz, *Ueber den Einfluss des Lichtes auf die Entwicklung einiger Pilze*, Inaug. Diss., Leipzig, 1898 (cited from Allen and Jolivette).

[2] K. Regel, " Ueber die Einwirkung des Lichtes auf Pilze," *Sitzungsber. d. Bot. Sect. d. St. Petersburger Naturf.-Ges.*, Jan., 1881 (in Russian) ; summary in *Bot. Zeit.*, 1882, p. 29 (cited from Allen and Jolivette).

[3] J. Wiesner, " Die heliotropischen Erscheinungen im Pflanzenreiche," *Denkschriften d. K.-k. Acad. Wien*, Vol. XXXIX, 1879.

[4] A. H. Blaauw, " Die Perception des Lichtes," *Rec. d. Trav. bot. néerl.*, Vol. V, 1909.

[5] Rosalie Parr, " The Response of Pilobolus to Light," *Annals of Botany*, Vol. XXXII, 1918, pp. 177–205.

divergent results which had been obtained by others in their studies of the heliotropic response in plants lay in the lack of accurate measurements of the quantity and quality of the light employed, undertook the task of attempting to correlate all previous results (1) by studying the heliotropic response of Pilobolus to carefully calibrated light of different wave-lengths and intensities, and (2) by a determination of the energy relation, if any, between this light and heliotropic response.

In carrying out her work, Miss Parr made use of very delicate physical instruments which could be calibrated in standard units. The light sources were two, a Nernst lamp of single glower type and a nitrogen-filled tungsten Mazda lamp. A beam of light from one of these lamps was broken up by means of a lens and prism. The relative energy values of the light in the different spectral regions employed expressed in ergs per sec. per square cm. were determined with the aid of a thermopile and galvanometer, the standard light energy used for comparison being a Hafner lamp kept at a distance of 2 metres from the thermocouple ($2 \cdot 075$ ergs). The spectral regions used in the experiments were tested with a spectrometer and the limits of the wave-lengths were thus obtained. Cultures of Pilobolus, grown in coal-gas-free air under known conditions of temperature, humidity, ventilation, etc., were kept in absolute darkness for three hours preceding the formation of the sporangiophores. The sporangiophores, whilst still pointed and showing no trace of the formation of sporangia at their ends, were then exposed to measured spectral regions for a definite period of time which varied from about 50 to 100 minutes (the presentation time). Each culture, after exposure, was again placed in the dark and kept there for an hour (the transmission time). Then with a reading glass the number of curved sporangiophores was recorded. The presentation time which was required, before a transmission time of an hour, to produce a curvature in approximately one-half of the cultures was taken as the standard for the reaction of Pilobolus to light. To obtain this presentation time with a wave-length of any one frequency it was necessary to make several experiments ; for, with too short an exposure, none of the sporangiophores showed any curvature while, with too long an exposure, all the sporangio-

phores showed curvatures. The mean wave-lengths to which the cultures were exposed were as follows : 708 and 667 (red) ; 631 and
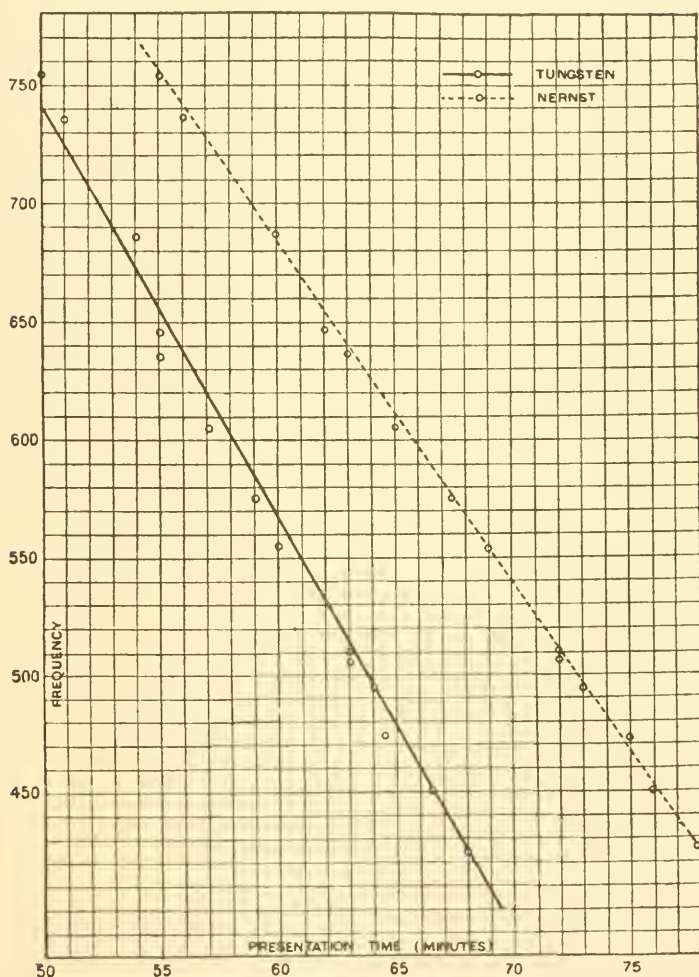
FIG. 14.—Graph showing presentation times in relation to *frequency* (not wave-length) of light waves in heliotropic experiments on Pilobolus. The time required to cause one-half of the spor- angiophores in a culture to bend toward the light is seen to decrease as the light employed changes from red (long waves with *low* fre- quency) through orange, yellow, green, blue, and indigo to violet (short waves with *high* frequency). The graph also shows that Pilobolus responds heliotropically to the light of all the regions of the visible spectrum. Reproduced from Rosalie Parr's *The Response of Pilobolus to Light* (*Annals of Botany*, Vol. XXXII, 1918).

612 (orange) ; 589 and 585 (yellow) ; 540 and 525 (green) ; 496 and 470 (blue) ; 465 and 438 (indigo) ; 414 and 398 (violet).

Miss Parr's investigation, carried out with so much care and precision, yielded a series of important results (Fig. 14), some of which may be summarised as follows. (1) Pilobolus responds heliotropically to the light of all the regions of the visible spectrum from red to violet. (2) The presentation time required to produce a heliotropic curvature decreases gradually from red to violet or, conversely, the heliotropic response of the fungus increases gradually from red to violet. The minimum response is in the red and the maximum in the blue. There are no intermediate maxima or minima such as others have supposed to exist. (3) The presentation time does not vary in direct ratio with the measured value of the energy of the light in the different regions of the spectrum. (4) The presentation time varies in inverse ratio to the square roots of the wave frequency. (5) While light energy is a factor in the relative time required for heliotropic excitation, yet the quality of the light, *i.e.* the frequency of the waves, is of more importance. (6) The divergent views held by previous investigators regarding the spectral region of maximum response may be explained on the basis of the energy value and frequency of the light employed.[1]

Allen and Jolivette's Investigations.—A series of interesting experiments upon the reactions of Pilobolus to light were described by Allen and Jolivette [2] in 1914. They studied the accuracy of aim of the fungus when influenced by (1) a single source of white light, (2) two sources of white light used simultaneously or successively, and (3) two sources of light differing in colour.

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[1] Miss Parr (*loc. cit.*, p. 203) summarised her results in seven paragraphs, the first four of which are substantially identical with (1) to (4) as given above. Her other paragraphs were as follows. (5) The product of the square root of the frequency times the presentation time decreases with the decrease in the energy value of the spectral regions, and is an approximate constant for a given light source. (6) The spectral energy in its relation to the presentation time may be expressed approximately in the Weber-Fechner formula, if the wave-frequencies be made a function of the constant. (7) The relation of the spectral energy to the presentation time may also be approximately expressed in the Tröndle formula, the wave-frequencies being a function of the constant.

[2] Ruth F. Allen and Hally D. M. Jolivette, " A Study of the Light Reactions of Pilobolus," *Trans. Wis. Acad.*, Vol. XVII, 1914, pp. 533–598.

Allen and Jolivette remark : " Whatever the mechanism in Pilobolus for the perception of light may be, it is certainly efficient. For example, in the white light 95 per cent. of the sporangia struck a four-centimeter (circular) opening (in the dark chamber) when the culture was twenty centimeters distant from the light ; and, with one or two exceptions, the remaining 5 per cent. struck within one or two centimeters of the opening. It is plain that the aiming has been done with remarkable precision."

Among the conclusions of Allen and Jolivette are the following. (1) Pilobolus aims point blank at a light and makes no allowance for the distance through which the sporangia must travel. (2) Pilobolus fires its sporangia very accurately toward white and blue light, much less accurately toward yellow light, and very inaccurately at red light.

Allen and Jolivette also made the following interesting, unexpected, and important discovery : when a culture is exposed to *two equal beams of white light* coming from two sufficiently different directions (angle between the two beams greater than about 10°), the sporangium of each Pilobolus fruit-body is aimed at one or the other source of light and *the aim is as accurate at the source of light chosen as if the other source did not exist (cf.* Fig. 15). The authors were at a loss to explain their discovery and remark " apparently there is nothing to prevent these simultaneous stimuli from acting together to produce a resultant reaction. But this does not occur. The visible reaction is to one and one only of the two possible sources of illumination." That a Pilobolus fruit-body aims at one or the other of two equal well-separated sources of light and not midway between them is due, as we shall see shortly, to the peculiar properties of the subsporangial swelling.

The Subsporangial Swelling as an Ocellus.—In 1921, I [1] published a short paper entitled " Upon the Ocellus Function of the Subsporangial Swelling of Pilobolus " in which it was pointed out that the subsporangial swelling acts not only as part of a squirting apparatus but as a lens and thereby plays an important part in heliotropic response. A model of a Pilobolus fruit body, which can

[1] A. H. R. Buller, " Upon the Ocellus Function of the Subsporangial Swelling of Pilobolus," *Trans. Brit. Myc. Soc.*, Vol. VII, 1921, pp. 61–64.

be used for demonstrating the way in which light is refracted through a subsporangial swelling, was also described, and it was announced that a fuller description of my observations, accompanied by illustrations, was in preparation for the press. The more elaborate
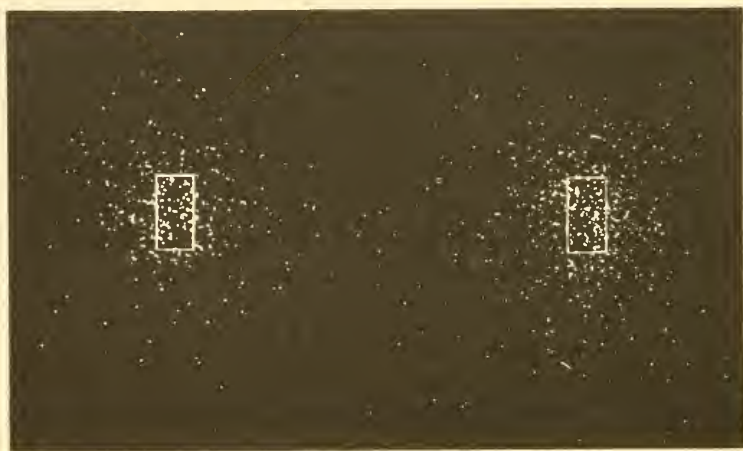
FIG. 15.—*Pilobolus Kleinii.* Result of a heliotropic experiment in which the sporangiophores were illuminated *with two equal beams of white light* which converged on each fruit-body at a relatively *wide angle* (about 25°). Natural-size reproduction of a photographic print made directly from a sheet of glass which covered two openings in the side of a dark chamber. The positions of the openings, each $1 \cdot 0 \times 0 \cdot 5$ cm., have been indicated by white lines. The discharged sporangia, which adhered to the sheet of glass and were black, appear as white dots. Direction of shooting was horizontal. Distance of the vertically placed surface of the culture medium from the glass plate was 12 cm. The distance between the two windows, measured from centre to centre, was $5 \cdot 5$ cm. The sporangiophores were continuously lighted during the whole course of their development. From the distribution of the discharged sporangia it is evident that the sporangiophores directed themselves *toward one or other source of light* and not in the resultant direction midway between them. Photograph by Van der Wey (*Proc. K. Akad. v. Wetensch. te Amsterdam,* Vol. XXXII, 1929, Plate I, Fig. 5).

treatment of the ocellus function of the subsporangial swelling promised thirteen years ago was completed at that time ; but, owing to my pre-occupation with other investigations, its publication has been delayed until now : it will be found in the next chapter of this book.

The Solution of the Problem of the Non-resultant Heliotropic Reaction of Pilobolus to Two Beams of Light.—In 1927, Pringsheim

and Czurda [1] repeated the experiments of Allen and Jolivette and confirmed their statement that, when the fruit-bodies of Pilobolus are exposed to two beams of light coming from sufficiently different directions, the sporangia are shot to one or other of the two sources of light and not in an intermediate direction. They also observed that, before the development of the sporangium and the sub-sporangial swelling, the young pointed stipe, when subjected to two beams of light coming from different directions, is affected by both beams and takes up a resultant phototropic position. In attempting to explain the non-resultant response of a mature fruit-body to two beams of light, Pringsheim and Czurda made two assumptions : (1) light here hinders growth, and (2) the refraction of the light that enters the subsporangial swelling at a small angle on one side is such that the wall on the same side at the base of the swelling is brightly lighted. As Van der Wey has rightly pointed out, the first of these assumptions is unjustifiable and the second is contrary to facts.

Van der Wey,[2] dissatisfied with the conclusions of Pringsheim and Czurda, re-investigated the response of mature fruit-bodies of *Pilobolus Kleinii* to two beams of light and, in 1929, in a paper containing numerous statistical observations, graphs, reproductions of photographs, and a construction diagram very similar to one of my own (*vide infra*, Fig. 59, p. 124), gave an explanation of the phenomenon which seems to be very satisfactory. He states that we know : (1) that the orientation of the ripe sporangiophores does not follow the resultant law (Fig. 15), and (2) that light falling at an acute angle on the subsporangial swelling has a greater heliotropic influence the smaller the angle ; and he assumes that the pigment zone at the base of the subsporangial swelling is the region of the protoplasm where perception of the light takes place. He then presents a picture of the course of the reaction of Pilobolus to two beams of light as follows.

" Let us suppose that a sporangiophore during its first

[1] E. G. Pringsheim and V. Czurda, " Phototropische und ballistische Probleme bei Pilobolus," *Jahrb. f. wiss. Bot.*, Bd. LXVI, 1927, pp. 863–901.

[2] H. G. Van der Wey, " Ueber die phototropische Reaktion von Pilobolus," *Proceedings, Koninklijke Akademie van Wetenschappen te Amsterdam*, Vol. XXXII, 1929, pp. 1–13.

development has grown in a resultant direction between two direc-
tions of light, A and B.   What must happen in the second period of
reaction ?   Let us assume that the subsporangial swelling has then
beome fully developed.   So long as the sporangiophore stands in the
resultant direction, the distribution of light is quite symmetrical ;
the light cannot therefore cause a
reaction. The sporangiophore is in
a condition of equilibrium which,
however, is unstable. For, as soon
as it inclines towards A, it receives
the light of A at a smaller angle
and that of B at a greater angle,
in consequence of which the in-
fluence of A becomes greater and
that of B smaller. As a result of
this the sporangiophore will bend
toward A until finally a position
of stable equilibrium has been
attained. This final position is
expressed in illustrations of the
results of shooting-experiments
(*Schuss-bilder*) and in Figs. 8
and 9 (graphs) can be read for
every angle."

Van der Wey has also shown :
(1) that, when the angle between
the two beams of light is small
(about $7°-10°$), the sporangiophores

Fig. 16.—*Pilobolus Kleinii.* Result of
another heliotropic experiment in
which the sporophores were illu-
minated with *two equal beams of
white light*, but here the beams
converged on each fruit-body at a
relatively *narrow angle* (about
$4·5°$). Conditions otherwise the
same as given in the description of
Fig. 15. The sporangia are most
densely disposed between the two
windows, from which it is evident
that the sporangia directed them-
selves not toward the one or the
other source of light but in
directions *between the two sources of
light*. Photograph by Van der Wey
(*loc. cit.*, Fig. 2).

take up a position which indicates that they are influenced strongly
by one beam of light and also to a slight degree by the other beam,
and (2) that, as the angle becomes very small ($2°-4°$), the effect of
the two beams becomes more and more nearly equalised.   Evidence
of the almost equal effect of the two beams converging on the fruit-
bodies at an angle of about $4·5°$ is shown in the photograph
reproduced in Fig. 16.

Already, in 1920, I had solved the problem of the non-resultant
reaction of Pilobolus to two beams of white light in essentially the

same manner as Van der Wey has done in his recent paper, and my solution was included in an address on the heliotropism of Pilobolus which I gave in Canada [1] and in the United States [2] in 1920 and in England [3] in 1921. The address was accompanied by lantern slides, among which were the construction diagrams shown in Figs. 46, 47, and 59 (pp. 91, 92, and 124). In my paper [4] on Pilobolus published in 1921 I treated of the heliotropic reaction of the sporangiophore to one beam of light only and, in the expectation of publishing a much fuller account of the fungus in the near future, did not even mention my solution of the two-beam problem. Since 1921 until now I have published nothing further on Pilobolus. Thus Van der Wey, through the publication of his excellent paper in 1929, has rightly obtained the credit for being the first to solve the two-beam problem.

My own solution of the two-beam problem was written several years before Van der Wey's paper came into my hands, and I have therefore included it without alteration in a section of the next chapter.

The Discharge of the Sporangium.—In 1932, Ingold,[5] in a brief paper on the sporangiophore of *Pilobolus Kleinii*, described the mode of discharge of the sporangium. He rightly affirmed that the columella is shot away with the sporangium and that a drop of cell-sap is attached to the gelatinous side of the sporangium as this travels through the air. However, he failed to account correctly for the fact that a sporangium becomes *attached by its gelatinous side to any object that it strikes.* He says : " The columella probably begins to tear away at a point on the circumference of the line of dehiscence, and the tear rapidly spreads. Through the aperture thus produced water exudes, forming a drop which, as it grows, increases the tear. This drop is moving with great velocity and

[1] At Guelph, at the second annual meeting of the Canadian Branch of the American Phytopathological Society, Dec. 10, 1920.

[2] At Chicago, before the Physiological Section of the Botanical Society of America, Dec. 28, 1920.

[3] At London, before the Linnean Society of London, June 16, 1921 (*vide Proceedings of the Linn. Soc.*, 1921, p. 63).

[4] *Loc. cit.*

[5] C. T. Ingold, " The Sporangiophore of Pilobolus," *The New Phytologist*, Vol. XXXI, 1932, pp. 58–63.

as it rounds off, in separating from the sporangiophore, tears away the sporangium completely, so that in the projectile the sporangium is at the bottom of the drop with the black cap undermost forming an unwetted base to the drop." Thus Ingold holds that, from the moment the sporangium is discharged, it trails behind the drop which carries it forward. It seems most unlikely that the projectile should rotate through 180° and no further. Rather we must suppose that the sporangium and the drop begin to rotate at the moment of discharge and continue to rotate as they progress through the air. To become stuck to an obstacle by its gelatinous side it is not necessary for the sporangium to strike the obstacle by that side. As will be explained more fully in the next Chapter, the sporangium is forced round into its final position—wettable gelatinous side toward the obstacle and unwettable black convex side away from the obstacle—by the drop at the moment the drop strikes the obstacle and flattens out upon it.

Ingold says that " the stalk and upper bulb of the sporangio-phore contain a clear liquid except where the stalk joins the bulb ; here a conspicuous zone of oil is invariably found. This oil appears to the naked eye as a minute orange spot at the base of the sub-sporangial swelling." These statements give the impression that the oil, like the cell-sap, is contained within the vacuole. As a matter of fact the oil is located in a biconcave mass of protoplasm (*vide infra*, Fig. 28, *g*), and it consists not of one large drop but of numerous very tiny drops embedded in the protoplasm.

Ingold also states that, when the sporangium is discharged, the lower part of the sporangium-wall " partially breaks down." My own observations do not support this view. Ingold failed to notice that, when the sporangium dehisces, its wall splits transversely into two pieces : (1) a lower narrow band which remains attached to the columella, and (2) a much larger convex cap (*vide infra*, Figs. 29 and 30). He correctly illustrated the lower band of sporan-gium-wall attached to a columella isolated from a discharged sporangium, but failed to recognise it as such.

CHAPTER II

Culture Methods—Germination of Spores, Growth of Mycelium, and Formation of
Primordia of Fruit-bodies—A Colourless Sporangial Wall as an Abnormality
in *Pilobolus longipes*—Species observed—General Description of the Pilobolus
Gun and its Projectile—The Discharge of the Projectile—Development of
the Pilobolus Gun and its Projectile—The Heliotropism of the Pilobolus Gun
demonstrated by a Simple Experiment—The Range of the Pilobolus Gun—
The Structure of the Sporangiophore and Sporangium illustrated by *Pilobolus
Kleinii* and *P. longipes*—The Two Functions of the Subsporangial Swelling—
The Heliotropism of the Sporangiophore with special reference to the Ocellus
Function of the Subsporangial Swelling—The Mechanism of Heliotropic
Response in Pilobolus and in the Leaves of certain Flowering Plants—The
Ocellus of Pilobolus and the Eye-spots of Volvox—The Ocellus of Pilobolus
and the Human Eye—A Heliotropic Experiment made on *Pilobolus longipes*—
A Solution of the Problem of the Reaction of the Sporangiophore of Pilobolus
to Two Equal Beams of White Light—A Model for illustrating the Pilobolus
Fruit-body in its Relations with Light—The Periodicity in the Development
of Pilobolus Fruit-bodies—The Subsporangial Swelling and the Discharge of
the Pilobolus Gun—The Osmotic Pressure of the Cell-sap of Pilobolus—Factors
in the Efficient Working of the Pilobolus Gun—An Analysis of the Cell-sap
of *Pilobolus longipes*—The Landing of the Pilobolus Projectile and the Attach-
ment of the Sporangium to Herbage—The Relations of Pilobolus with Flowering
Plants and with Herbivorous Animals.

Culture Methods. — At Winnipeg, during the winter months,
Pilobolus was usually obtained as follows. Horse-dung balls,
collected in the frozen condition from the streets or obtained fresh
from a stable, were placed unbroken in a compact layer on the floor
of a large culture chamber which was exposed to daylight on a table
in the laboratory. The chamber was 3 feet long, 1·5 feet wide, and
2 feet high, its four sides and its roof were made of glass, while its
floor was covered with a sheet of zinc having upturned edges. The
air of the chamber was kept moist by means of a standing beaker

of water and also by occasionally spraying the dung-balls or pouring water on the zinc floor.

After the dung had been in the chamber for a few days, usually but not always Piloboli began to appear upon it ; and often many hundreds or even thousands of fruiting structures came to maturity each day for several days in succession. The sporangiophores exhibited the usual positive heliotropism, in consequence of which

FIG. 17.—Sporangia of *Pilobolus longipes* which were shot on to a sheet of white paper set against the best-illuminated side of a large glass chamber in which numerous fruit-bodies had come up on dung introduced there a few days before. The large drop of cell-sap which accompanied each sporangium was absorbed by the paper, so that the sporangia have not run together in groups as they do on a glass surface but have remained where they landed. Natural size.

they shot off their sporangia toward the side of the chamber through which the strongest light entered. These projectiles adhered to the glass where they struck, and thus a very large number of them came to dot its surface (Fig. 17).

In other cultures horse-dung balls were spread over the floor of an almost cubical glass chamber (base 20×21 inches, height 24 inches). Here, as in the other large glass chamber, there was a considerable body of air above the culture medium, and thus aeration of the dung was provided for. The cultures in this chamber were very successful. As an example one may be described. On

February 13, 1929, fresh horse-dung balls taken from a stable were spread over about three square feet of the chamber floor. Four days later, upwards of twenty thousand fruit-bodies of *Pilobolus longipes* had appeared—every dung ball was covered with them. At 2 P.M. I put my ear to the glass wall of the chamber nearest to the source of daylight, and I could hear more than one hundred sporangia strike the glass per minute. This miniature bombardment had been in progress for some time and it persisted through the mid-day hours until every Pilobolus gun had exploded.

Still other cultures were made by placing dung-balls in large crystallising dishes (10 × 3 inches) each covered with a glass plate (*cf.* Fig. 26, A, c, p. 64). A slight space was provided between each dish and its plate, so as to permit deleterious gases emanating from the dung to escape from the chamber and fresh air to enter.

It was observed that dung-balls which produced Mucors freely usually produced but relatively few Piloboli, and *vice versa*, and also that promising-looking crops of Piloboli were sometimes utterly ruined by the attacks of parasitic moulds of which one species proved to be *Syncephalis nodosa*.[1]

The natural cultures of Pilobolus just described were so successful that they were used to provide the supply of normal mature fruit-bodies required for the study of (1) the structure, heliotropism, and explosion of the Pilobolus gun and (2) the structure and characteristics of the Pilobolus projectile.

A few pure cultures were also made. Sporangia of *Pilobolus longipes* were caught on sterilised slides or cover-glasses and then sown on sterilised horse dung in a large crystallising dish covered with a glass plate. The spores germinated, and, after a few days, a crop of fruit-bodies began to appear. Similar results were obtained when sporangia or isolated spores were sown on sterilised dung-agar[2] in Petri dishes. The pure cultures on horse dung were employed merely for the study of the production of abnormal fruit-bodies (*vide infra*), while the dung-agar cultures were used to permit of observations on the germination of the spores, the

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[1] *Cf.* p. 21.

[2] For the technique of making dung-agar *vide* Vol. IV, 1931, pp. 195–197.

development of the mycelium, and the early stages in the formation and growth of the fruit-body.

Germination of Spores, Growth of Mycelium, and Formation of Primordia of Fruit-bodies.—Sporangia of *Pilobolus longipes* (*cf.*

Fig. 18) were caught on a clean glass plate and then crushed in warm water, and some of the spores were then transferred with the help of a platinum loop to a drop of dung-agar hanging in a van-Tieghem cell. In other cultures .the powder from crushed sporangia was sown and, in still other cultures, sporangia were sown as wholes. In each culture, at room temperatures, a small percentage of the spores germinated.

On germinating, a spore swells up considerably and then puts out one or sometimes two germ-tubes (Figs. 19 and 20, A–G). The germ-tube then grows rapidly in length and branches monopodially. A well-grown mycelium has rather thick main hyphae from which come off lateral hyphae which are much thinner and which in their turn give rise to still thinner branches (Fig. 20, G). The main hyphae (Fig. 21, A) come to contain numerous red particles, doubtless oil-drops containing carotin, and it is these branches alone which are destined to form the primordia of fruit-bodies. Two days

FIG. 18.—*Pilobolus longipes.* A group of fruit-bodies in air, just before the discharge of the sporangia. In each fruit-body are shown the upper part of the slender stipe, the large subsporangial swelling filled with cell-sap, and the terminal black sporangium. The red protoplasm at the junction of the stipe and subsporangial swelling appears black and may be readily observed in the fruit-body on the right. Magnification, 20.

after the spores have been sown, here and there in a well-grown mycelium a thick main branch for some distance along its length begins to swell laterally ; and, in the course of a few hours, the swelling develops into a fruit-body primordium or *tuber* (Figs. 20, H, and 21, B–D). As the primordium develops, protoplasm flows into it from the apical and basal portions of the main hypha and from all the adjacent smaller lateral hyphae. As a result of this flow, the tuber
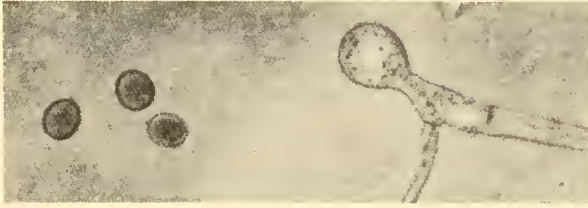
Fɪɢ. 19.—*Pilobolus longipes*. Photomicrograph of spores
sown in dung-agar. Left, three spores which have not
germinated. Right, a spore which swelled up and
germinated. There are red particles in it and in the
mycelium. Magnification, about 400.
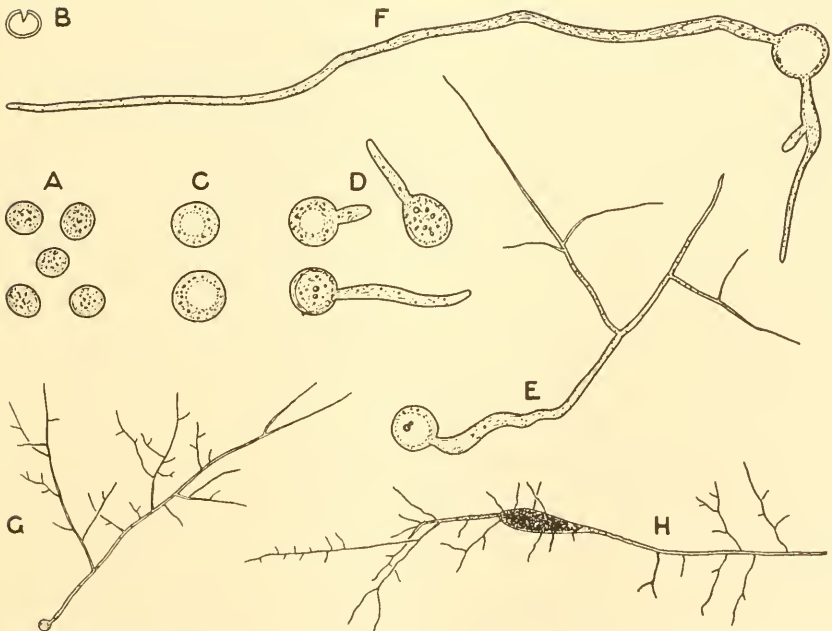
Fɪɢ. 20.—*Pilobolus longipes*. Germination of spores in cleared dung-agar and the
production of mycelia. A, spores, just placed in the culture medium ; they are
reddish-yellow, owing to the presence of carotin dissolved in oil-drops. B, one
of the spores which has been crushed, to show the thick cell-wall. C, 14 hours
after sowing, two spores which have swollen. D, 24 hours after sowing, three
spores which have emitted germ-tubes. E, 25 hours after sowing, a young
mycelium produced from a single germ-tube. F, 25 hours after sowing, a young
mycelium produced from two germ-tubes. G, 26 hours after sowing, a more
advanced mycelium developed from a single germ-tube. H, 63 hours after
sowing the spores, part of a mycelium showing a thick main hypha and slender
secondary hyphae. The main hypha has swollen locally and has formed a
fruit-body primordium (tuber or trophocyst) filled with dense red protoplasm.
Magnification : A–F, 293 ; G and H, 66.
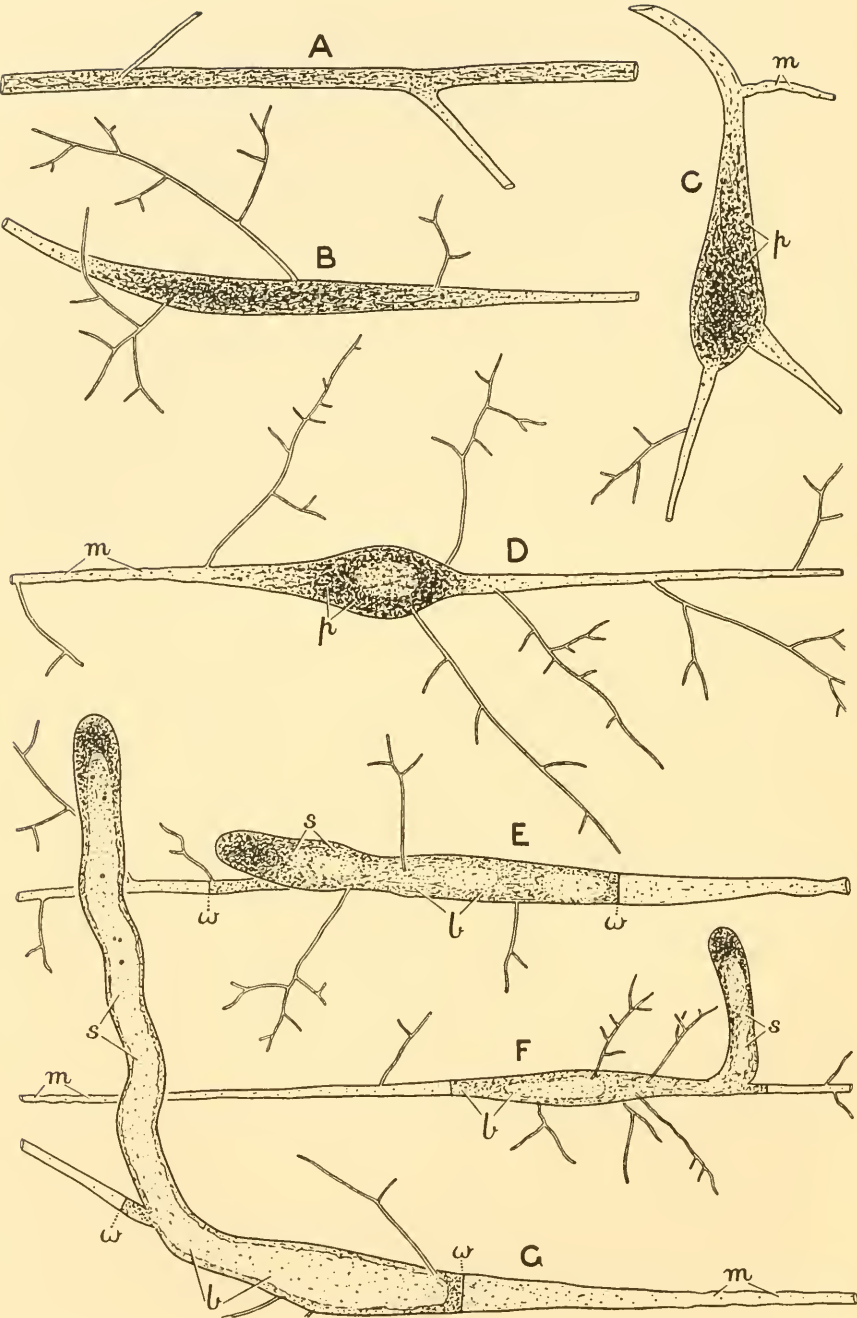
comes to contain a considerable mass of red protoplasm. This protoplasm contains vacuoles, and a streaming movement can be readily observed in it. As a tuber is forming, the main hypha adjacent to the tuber may become somewhat moniliform in outline (Fig. 21, C–G, *m*). On attaining maturity, each tuber becomes separated from the rest of the main hypha on which it is situated by two septa, one at each of its ends (Fig. 21, E–G). The lateral hyphae attached to a tuber were produced by the mycelium before the tuber came into existence and are therefore not comparable to rhizoids. Each of them eventually becomes separated from the tuber by a septum. As shown by Lepeschkin's experiments with isolated tubers, a mature tuber contains all the materials, except sufficient water, necessary for the formation of a fruit-body.[1] On this account Morini has called it a *trophocyst*.[2]

Soon after a tuber has been formed and cut off from the hypha with which it is connected, it begins to germinate : at one end it develops a coarse hypha which grows up into the air (Fig. 21, E–G) and eventually becomes differentiated into the stipe, the sub-sporangial swelling, and the sporangium. The tuber itself becomes

Fig. 21.—*Pilobolus longipes*. Origin and early development of the fruit-body primordium (tuber or trophocyst). Culture medium, cleared dung-agar. A, part of a thick main hypha of a well-developed mycelium ; it is reddened with carotin. B, another main hypha which is forming a fruit-body primordium by swelling laterally for some distance along its length ; red protoplasm is collecting in the swelling. C, a primordium, *p*, which has attained full size, it is filled with red protoplasm ; one of the main hyphae, *m*, which has passed its contents into the primordium, has become moniliform. D, a primordium which has developed to full size in the middle of a main hypha ; the main hypha has become moniliform at *m* ; the secondary branched hyphae have ceased to grow in length. E, a primordium, cut off from the rest of a main hypha by the cross-walls *w w* ; it has begun to form a fruit-body and is now differentiated into a basal swelling *b* and a young stipe *s*. F, similar to E, but more advanced ; at *m* the main hypha, which has now lost most of its contents, has become moniliform. G, similar to E and F, but more advanced : *w w*, two cross-walls which separated the fruit-body primordium from the rest of the main hypha ; *m*, a part of the main hypha which has become moniliform ; *b*, the basal swelling of the fruit-body ; *s*, the young stipe with red protoplasm densely aggregated at its apex which is still immersed in the culture medium ; the basal swelling and stipe contain a large central vacuole. The basal swelling of G is about 0·25 mm. long. Under natural conditions on horse dung, basal swellings often attain a length of 1–2 mm. Magnification, 240.

[1] *Cf. supra*, p. 26.

[2] F. Morini, " Ricerche sopra una nuova Pilobolea," *Mem. R. Accad. Sci. Ist. Bologna*, ser. 5, Vol. VIII, 1900, p. 86. *Vide* also his " Materiali per una monografia delle Pilobolee," *ibid.*, ser. 6, Vol. III, 1906, p. 118.

the *basal swelling* or *basal reservoir* of the fruit-body and, owing to its mode of origin, it is usually much elongated (under natural conditions on horse dung 1–2 mm. long). It is on account of the unusual length of the basal swelling that *P. longipes* has received its specific name. As the coarse hypha grows into the air, most of the protoplasmic contents of the basal swelling flow into it, and a thick mass of red protoplasm always accumulates at the growing point (Fig. 21, F, G). As soon as the hypha has attained a length of several millimetres (under natural conditions on horse dung often 2–3 cm.), it ceases to grow in length and develops a sporangium at its apex, and thereafter it develops a subsporangial swelling. Thus the order of formation of the four parts of the fruit-body is as follows : basal swelling, stipe, sporangium, and subsporangial swelling.

A Colourless Sporangial Wall as an Abnormality in Pilobolus longipes.—Some normal fruit-bodies of *Pilobolus longipes*, having the usual long cylindrical foot, rounded spores, and black sporangial wall, came up on horse dung spontaneously in the laboratory ; and some of the sporangia, after they had landed on a sterilised glass slide, were used to inoculate sterilised horse-dung balls contained in a large crystallising dish. The dung consisted of fresh balls obtained from a stable and it covered the base and filled up about half the space in the dish. The dish, after the dung had been inoculated. was closed by means of a well-fitting glass plate. About a week after inoculation, fruit-bodies of *P. longipes* began to appear on the dung. They had the characteristic cylindrical foot and rounded spores, but many of them, although by no means all, were otherwise abnormal. The development of the abnormal fruit-bodies took place so slowly that the sporangia were not ripe in the mornings and many of them were not discharged. The subsporangial swellings and sporangia were remarkably small—not much more and very often less than one-half the usual size ; and many of the sporangia never turned black and remained bright orange-yellow. The persistent orange colour of the sporangia was due to the fact that the *sporangial wall had failed to develop any black pigment*, so that it was colourless, thus permitting the orange-yellow spores to show their colour through it. Many of the orange-yellow sporangia were discharged, so that they struck and stuck to the side of the dish

nearest to the light. Some of these discharged sporangia were mounted in water on a slide, and then the individual spores could be seen through the colourless sporangial wall.

Suspecting that the abnormal development of the fruit-bodies was due to the fumes emitted by the sterilised dung-balls, which were unable to escape from the crystallising dish, I removed the covering plate and set the dish under a large bell-jar. As a result of the change in external conditions, the succeeding crops of fruit-bodies became quite normal in time of development, in the size of the subsporangial swellings and sporangia, and in the development of the intensely black pigment in the sporangial wall. Thus the supposition that the abnormal development of the fruit-bodies in the pure culture of *P. longipes* was due to gaseous emanations from the horse dung seems to have been well founded. As supporting this view it may be mentioned that fruit-bodies of *Coprinus curtus* became sterile when subjected to the fumes of fresh horse dung.[1]

In 1876, van Tieghem remarked that he had observed fruit-bodies of *Pilobolus oedipus* in which the sporangium-wall had failed to blacken, so that, just as in the abnormal *P. longipes* fruit-bodies described above, the orange-yellow spores showed through the uncoloured sporangium-wall and thus gave to the sporangium as a whole an orange-yellow appearance.[2]

Van Tieghem,[3] also in 1876, gave the name *Pilobolus nanus* to a minute species, not more than 1 mm. high, which he found on rat dung (Fig. 106, p. 212). This species, according to van Tieghem, differs from all other Piloboli in having a *yellow* (not black) sporangium-wall when the sporangium is projected. *P. nanus* has not been seen again since 1876. For the present, therefore, the possibility is not excluded that the yellowness of the sporangium-wall in this species may have been abnormal.

Species observed.—Among the species of Pilobolus which occur at Winnipeg the following have been identified : *P. longipes* (Figs. 18 and 24), *P. Kleinii* (Fig. 27), and *P. umbonatus* nov. sp. (Fig. 105,

[1] These *Researches*, Vol. IV, 1931, p. 9.
[2] P. van Tieghem, " Troisième Mémoire sur les Mucorinées," *Ann. Sci. Nat.*, 6 sér., T. IV, 1876, p. 342.
[3] *Ibid.*, pp. 340–342, Pl. X, Figs. 16–22.

p. 210), all of which came up in the horse-dung cultures just described, and *P. oedipus* which fruited freely on a cake of drying mud brought to the laboratory from the banks of the Red River. The species chiefly used for observation were *P. Kleinii* and *P. longipes. Pilobolus umbonatus* will be described in Chapter III. *Pilobolus crystallinus*, so far as I know, has never come up in any of my cultures.

General Description of the Pilobolus Gun and its Projectile.— As is well known, the sporangiophore of Pilobolus shoots away its sporangium to a distance of several feet and therefore acts as a gun. The gun, as shown in Fig. 2 (p. 4), consists of three parts : (1) a *basal swelling* which, with the aid of mycelial hyphae, serves to fix the gun firmly to the substratum ; (2) a slender cylindrical *stipe*, several millimetres long, which by means of a heliotropic response serves to lay the gun in the direction in which it is to be discharged ; and (3) a large oval *subsporangial swelling* which acts as part of a squirting apparatus and also, as will be shown later, as an ocellus which perceives the direction of the incident light and thereby assists the stipe in its heliotropic movements. The projectile—the *sporangium*—is seated on the free end of the subsporangial swelling, is discoid, is covered with an intensely black membrane, and contains many thousands of spores. Photographs of some Pilobolus guns which are about to discharge their projectiles are shown in Figs. 5, 13, and 18 (pp. 8, 37, and 50).

In every species of Pilobolus, the diameter of the subsporangial swelling much exceeds that of the sporangium and still more that of the stipe. This may be realised by reference to Fig. 22 which shows a plan of cross-sections of these parts for typical large fruit-bodies of *Pilobolus longipes* and *P. Kleinii.*

The Discharge of the Projectile.—*Pilobolus Kleinii* and *P. longipes* can both shoot their largest sporangia vertically upwards to a maximum height just exceeding six feet and to a maximum horizontal distance just exceeding eight feet. It is therefore evident that the Pilobolus gun gives its projectile a high initial velocity.

When a Pilobolus gun is fully developed and ready to discharge its projectile (Fig. 18, p. 50), the wall of the sporangiophore, *i.e.* of the basal reservoir, stipe, and subsporangial swelling, is greatly

distended by the osmotic (turgor) pressure exerted by the cell-sap
in the large central vacuole. This pressure, as will be shown
subsequently, is equal to that of about $5 \cdot 5$ atmospheres.   Since
the inward pressure of the cell-wall must be equal to the outward
pressure of the cell-sap, we must suppose that, when a Pilobolus
gun is about to be discharged, the distended cell-wall of the
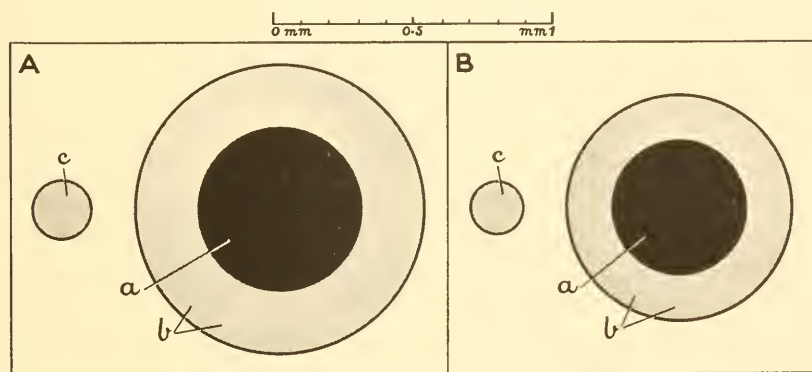
Fig. 22.—To show the relative diameters of the black sporangium a, the subspor-
angial swelling b, and the stipe c in two large wild fruit-bodies of two species
of Pilobolus. A, *Pilobolus longipes* (same fruit-body as that in Fig. 57).   B,
P. Kleinii.   In each drawing is represented an apical view of a fruit-body
facing the strongest rays of light and a cross-section of the stipe.   In A the
radius of the sporangium is 56 per cent. of the radius of the subsporangial
swelling and in B 64 per cent.   The light rays which strike the surface b are
refracted into the subsporangial swelling and form a spot of light which, when
the fruit-body is in heliotropic equilibrium, rests symmetrically upon the ring
of red protoplasm at the top of the stipe (*cf.* Fig. 50, D).   As may be seen by
reference to the mm. scale, the diameters of the sporangium, subsporangial
swelling, and stipe in A are $0 \cdot 58$, $1 \cdot 03$, and $0 \cdot 21$ mm. respectively.

sporangiophore is compressing the cell-sap with a pressure equal
to that of about $5 \cdot 5$ atmospheres.

When discharge of a sporangium takes place, the neck of the
subsporangial swelling just beneath the sporangium is ruptured
transversely (*vide* the dotted line a in Fig. 28, p. 70), the wall of
the swelling and the stipe contracts elastically, and the cell-sap
is squirted out of the top of the swelling, with the result that a large
drop of sap carries the sporangium with it through the air.   As
the projectile begins to describe its trajectory, the collapsing gun
(since action and reaction are equal and opposite) flies backwards
and immediately strikes the dung-ball to which it is attached.

However rapidly the projectile may be travelling, on striking a blade of grass or any other obstacle, it always sticks where it strikes. Under natural conditions, Pilobolus guns are usually situated on the dung of herbivorous animals in pastures, etc., in consequence of which the projectiles usually land on, and adhere to, the surrounding herbage.

Development of the Pilobolus Gun and its Projectile.—In a good natural culture of Pilobolus a crop of Pilobolus guns and projectiles is produced daily for several days in succession. Each crop takes about 24 hours for its development which culminates in the discharge of the sporangia between 9 A.M. and early afternoon. In the late afternoon a new crop can be seen beginning to develop.

The successive development of the stipe, the sporangium, and the subsporangial swelling of *Pilobolus Kleinii* is shown on a larger scale in Fig. 23, while a series of successive stages in the development of a diurnal crop of fruit-bodies of *P. longipes*, as affected by external conditions, particularly light and darkness, is represented diagrammatically on a smaller scale in Fig. 24.

The primordia of a new crop of Pilobolus guns and projectiles consist of much swollen cells which are filled with dense orange-red protoplasm and look like little tubers. These primordia, the *trophocysts* of Morini,[1] are formed in the mycelium at the surface of the substratum by mid-day (Fig. 24, A). During the afternoon, each primordium puts out a coarse cylindrical hypha which grows away from the substratum into the air (Fig. 23, A). The red protoplasm of the primordium flows up into the hypha which thereby becomes reddened in its turn, particularly at its free end just below the apical growing-point (Fig. 12, *a*, p. 23). The primordium becomes the basal swelling of the new sporangiophore and the coarse hypha the stipe. From the first the young sporangiophore is positively heliotropic, and from early afternoon to dark, as it grows in length, it keeps its long axis parallel to the strongest incident rays of light (Fig. 24, B and C). If, as happens in the depth of winter, darkness supervenes before the sporangium begins to develop, the sporangiophore continues to grow in length orthotropically (Fig. 24, C and D). Toward evening, the end of

[1] *Vide supra*, p. 53.

the sporangiophore begins to develop into a sporangium (Figs. 12,
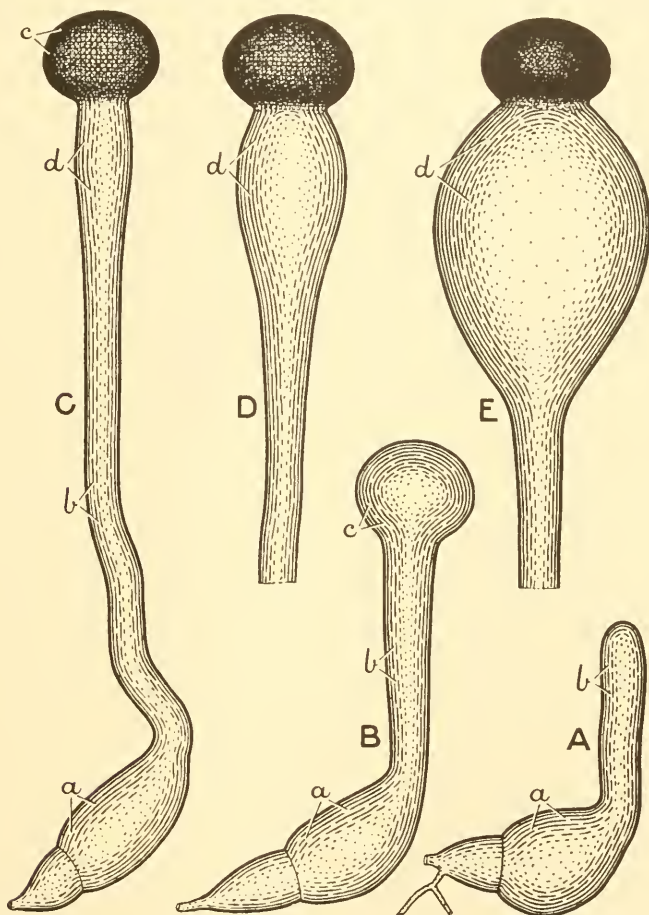
FIG. 23.—*Pilobolus Kleinii*, wild, on unsterilised horse dung in a culture chamber. Stages in the development of a fruit-body and, in particular, of the subsporangial swelling: *a*, basal swelling; *b*, stipe; *c*, sporangium; *d*, subsporangial swelling. A, in the afternoon: a tuber (trophocyst) has begun to develop into a fruit-body: the tuber *a* is now to be regarded as a basal swelling and its apical outgrowth *b* as a stipe. B, at 6 P.M., a very short fruit-body: a sporangium *c* has developed at the end of the stipe; as yet there is no trace of a subsporangial swelling. C and D at 12.20 A.M. and E at 9 A.M., showing successive stages in the development of the subsporangial swelling. Magnification, 27.

b, 23, B, and 24, D). The sporangium attains its full size before midnight. At first, it is orange owing to its content of orange-

coloured protoplasm ; but, subsequently, as its wall matures and becomes pigmented, it turns black (Figs. 12, *c*, and 23, C–E).   The conical columella, which separates the sporangium from the stipe, is developed early in the evening ; but the spores are not formed until after midnight.   Shortly after midnight, the top of the stipe immediately under the sporangium begins to become transformed into a subsporangial swelling.   The development of the swelling and the ripening of the spores take place simultaneously between 12 midnight and 6 A.M., *i.e.* while in winter it is still dark (Figs. 23, C–E, and 24, D–F).   In the morning, after daylight has appeared, the top of the stipe just beneath the subsporangial swelling bends heliotropically, so that the sporangium comes to face the strongest incident rays of light with great precision (*cf.* Figs. 13, p. 37, and
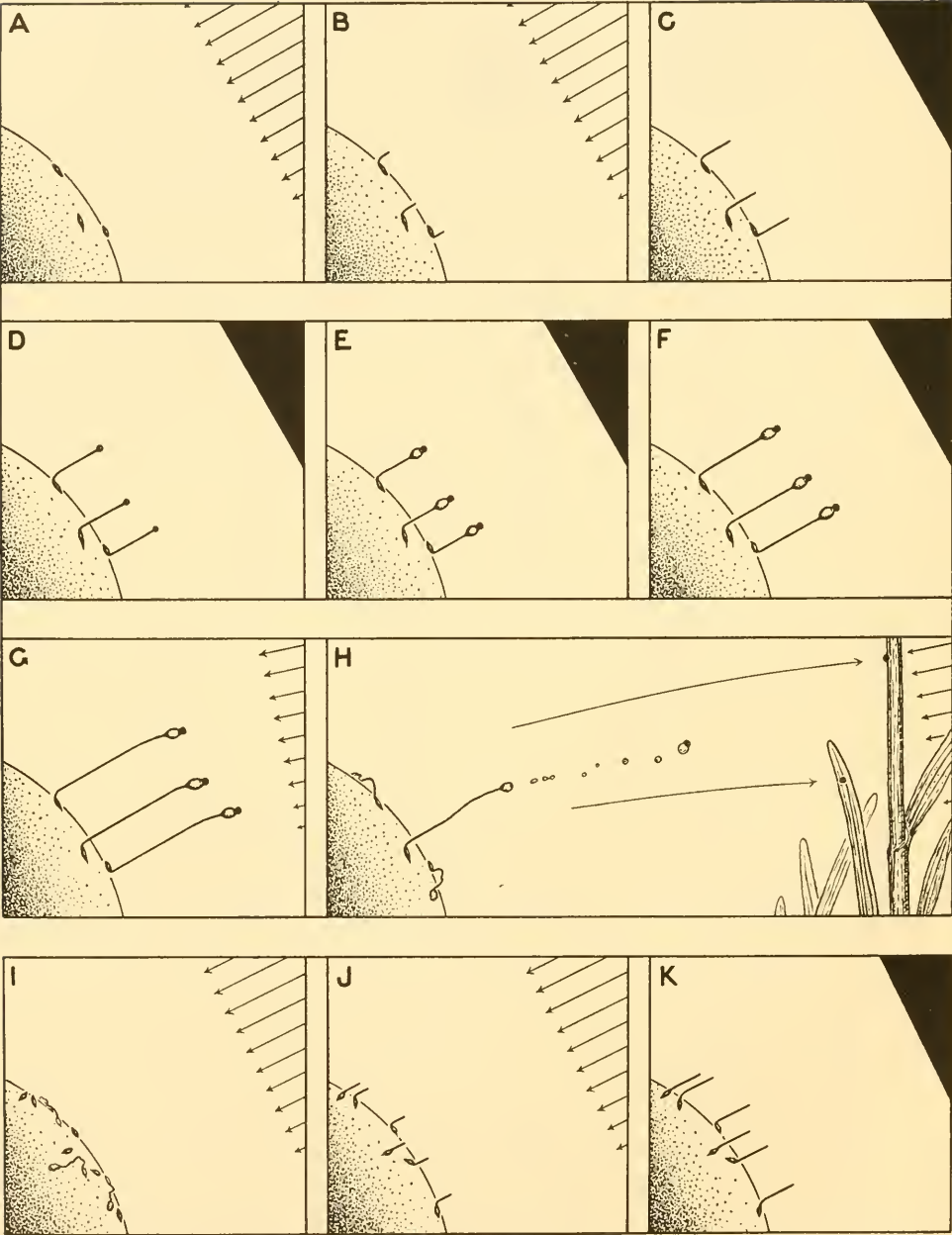
FIG. 24.—*Pilobolus longipes.*   Rhythmic development and heliotropism of the fruit-bodies, represented diagrammatically.   In the right-top corner of a drawing, arrows indicate daylight and the direction of the strongest incident rays, and black triangles indicate night and the absence of effective heliotropic radiation. A–H, successive stages in the development of three fruit-bodies of the first diurnal generation ; I–K, successive stages in the early development of six fruit-bodies of the second diurnal generation.   The fruit-bodies are supposed to be developing on a horse-dung ball in a field.   The times given are those which were observed in a laboratory culture (fresh unsterilised horse dung) late in November.   A, at 3 P.M. ; three red tubers (trophocysts) have appeared on the surface of the dung-ball.   B, at 4 P.M. ; each tuber, now to be regarded as a basal swelling of a fruit-body, has given rise to a stipe which is elongating apically and is positively heliotropic.   C, at 6 P.M. ; darkness has set in ; the stipes are still elongating ; in the absence of light they are growing straight forward in the direction they took up in the afternoon.   D, at 10 P.M. ; the stipes temporarily have ceased to elongate ; each of them has given rise to a terminal sporangium.   E, at 12.30 A.M. (midnight) ; each fruit-body has now developed a subsporangial swelling ; the stipes are about to resume their growth in length.   F, at 4 A.M. (still dark) ; the stipes have elongated considerably.   G, at 12 A.M. (noon) ; the direction of the strongest incident rays of light, as indicated by the arrows, has changed since the previous afternoon ; as daylight dawned, the fruit-bodies, with the help of their subsporangial swellings, readjusted themselves heliotropically and so their stipes are now curved.   H, at 1 P.M. ; the climax of development has arrived ; two of the fruit-bodies have discharged their sporangia in the directions indicated by the long curved arrows ; the discharged sporangia have struck and stuck to near-by grass ; the third fruit-body has just exploded ; the jet of sap squirted out from the mouth of the subsporangial swelling has broken up into a series of drops of which the largest is attached to the wettable gelatinous under side of the sporangium ; the projectile (sporangium and sap-drop) may be rotating in its flight ; the two sporangiophores which have discharged their projectiles are lying on the dung-ball ; owing to action and reaction being equal and opposite, they were of necessity forced backwards as the projectiles were forced forwards. I, at 3 P.M. ; the three discharged fruit-bodies of the first diurnal generation can be seen lying on the surface of the dung-ball where they are rapidly disintegrating ; six new tubers (trophocysts) of the second diurnal generation have now appeared (*cf.* A).   J, at 4 P.M., and K, at 6 P.M., show stages in the development of the second diurnal generation of fruit-bodies resembling stages B and C for the first diurnal generation.   Magnification, 1·5.

24, G). The Pilobolus gun continues to mature (Fig. 12, c), until at last it explodes (Figs. 12, d, and 24, H). When this happens, the gun dies instantly and is thrown backwards on to the substratum of dung, while the projectile, with its freight of living spores, travels forward, describes a parabolic trajectory, and lands safely on some object which may be several feet away from the gun which has discharged it (Fig. 24, H). As soon as one crop of fruit-bodies becomes exhausted, another begins its development (Fig. 24, I–K).
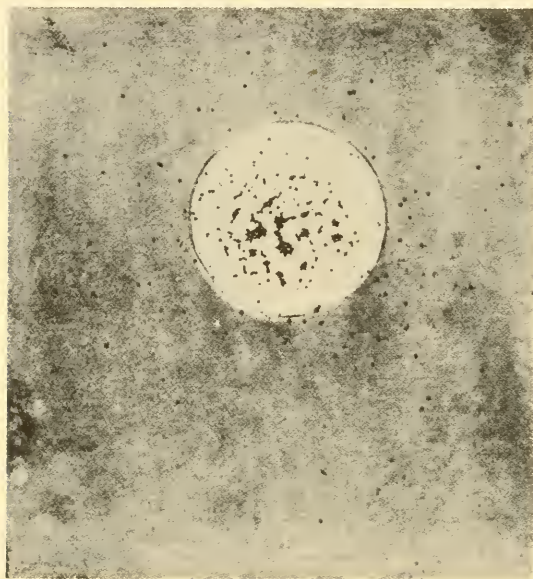
FIG. 25.—Result of a simple heliotropic experiment with *Pilobolus Kleinii*. The fungus came up on dung balls spread on the floor of a large dark chamber which was illuminated with daylight solely by a circular glass wall-window one inch in diameter. The photograph here reproduced shows the window and part of the surrounding interior wall, after the end of the experiment. Most of the projectiles hit the window as if it were a target. The local aggregations of the sporangia in the centre of the window were caused by sporangia running together in the drops of cell-sap which were shot on to the glass with the sporangia. Natural size.

The Heliotropism of the Pilobolus Gun demonstrated by a Simple Experiment.— Some fresh dung balls were spread over the surface of a large cubical culture chamber which was illuminated with daylight solely by means of a circular glass wall-window one inch in diameter.

After a few days, a number of fruit-bodies of *Pilobolus Kleinii* appeared on the dung about a foot from the window. The sporangiophores bent heliotropically toward the source of light and discharged their sporangia at the window. The photograph reproduced in Fig. 25 shows the window and part of the surrounding interior wall of the chamber at the end of the experiment, after the chamber wall had been illuminated to enable the photograph

to be made.   An inspection of the photograph shows that most of the projectiles hit the window as if it had been a target.   The local aggregations of sporangia in the central part of the window were caused by sporangia running together in the drops of cell-sap which were shot on to the glass with the sporangia.

The Range of the Pilobolus Gun.—Experiments on the range of the Pilobolus gun have been recorded by Coemans [1] in 1861, by Grove [2] in 1884, by myself [3] in 1909, and by Pringsheim and Czurda [4] in 1927.   Coemans observed a maximum vertical range for *P. oedipus* of just over one metre (107 cm.) ;   Grove observed a maximum horizontal range for *P. Kleinii* of 4 feet 2 inches ; I observed a maximum horizontal range for *P. longipes* of 6 feet 2 inches, whilst Pringsheim and Czurda observed a maximum horizontal range for *P.* sp. (? *P. crystallinus*) of 2 metres (6 feet, 7 inches).   All these records have been broken in a series of experiments which I have made since 1909 and which will now be described.

The vertical range of *Pilobolus longipes* and *P. Kleinii* was determined with the help of the apparatus shown in Fig. 26.   The chamber A, made of beaver-board attached to a wooden frame and painted black within, was so constructed that its upper part *a*, which had a large round hole *h* in its roof, surrounded and enclosed the upper half of the lower part *b* above which it could be raised to various heights by inserting within it at its corners sticks of various lengths such as those shown at I.   To change a set of four shorter sticks for four longer ones, the panel *d* was first removed, the part *a* was then raised to the desired height, then the four shorter sticks which had stood vertically between the corner frame-posts of *b* and the roof of *a* were removed and the four longer sticks were inserted in their place.   To permit of a culture dish containing dung balls bearing Piloboli (A, *c*) being put into or taken out of

[1] E. Coemans, " Monographie du genre Pilobolus, spécialement étudié au point de vue anatomique et physiologique," *Mém cour. et des Sav. étrang. Acad. Roy. de Belgique*, T. XXX, 1861, p. 39.

[2] W. B. Grove, " Monograph of the Pilobolidae," *The Midland Naturalist*, Birmingham, England, 1884, p. 16.

[3] A. H. R. Buller, these *Researches*, Vol. I, 1909, p. 259.

[4] E. G. Pringsheim and V. Czurda, " Phototropische und ballistische Probleme bei Pilobolus," *Jahrb. f. wiss. Bot.*, Bd. LXVI, 1927, p. 873.
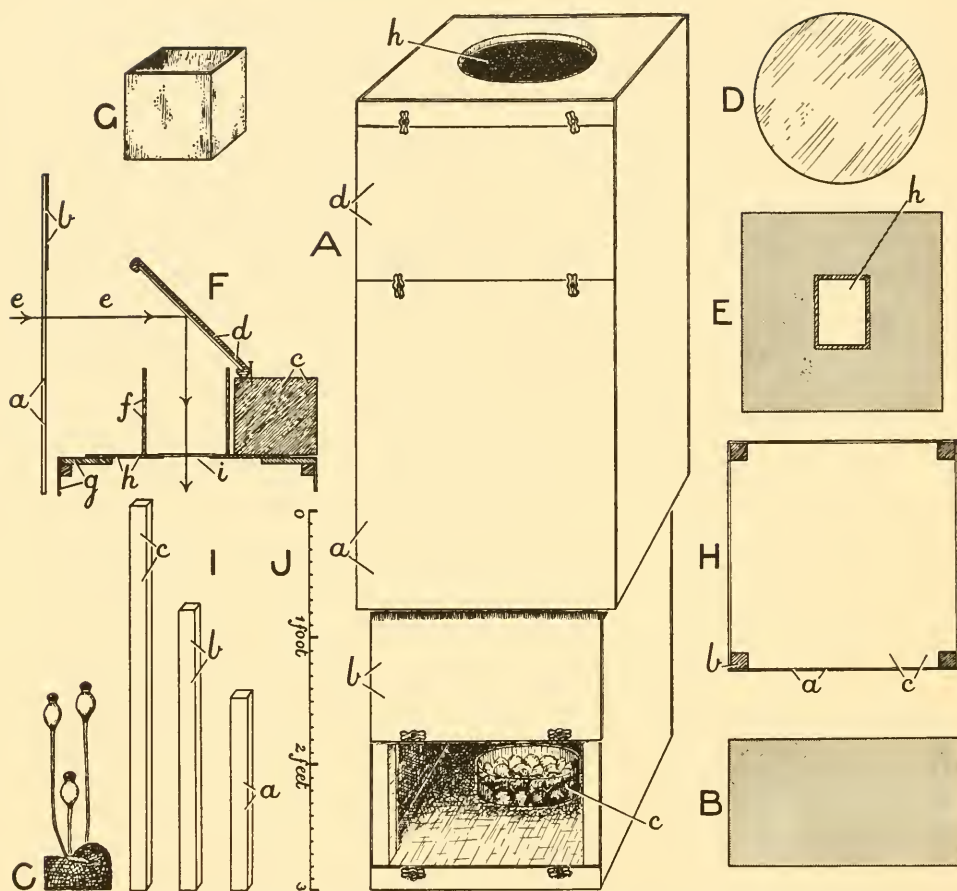
Fig. 26.—Experimental chamber used for finding the greatest height of projection of the sporangia of *Pilobolus longipes* and of *P. Kleinii.* A, a case constructed of beaver-board and wood, the upper part of which, *a*, can be moved up or down about the lower part, *b*, which it partly encloses and conceals. B, a panel which has been removed from the base of A, so as to show the culture-dish containing horse-dung balls beset with Pilobolus fruit-bodies. C, three *Pilobolus longipes* fruit-bodies which were looking upwards on the dung-balls in the dish at the base of A : in one fruit-body the long basal swelling at the base of the stipe can be seen. In the roof of the case A is a large circular aperture, *h*. D, a circular sheet of glass which can be set over the top of A so as to cover the aperture and catch any projectiles which may strike it from below. E, a piece of beaver-board with a central rectangular aperture *h* over which can be set a rectangular sheet of glass. E can be used instead of D to cover the aperture in the roof of A and catch projectiles. When E has been set on the top of A, light is reflected vertically downwards through its sheet of glass by means of a mirror. The vertical section F shows in detail how the light is reflected : *a*, a window facing a clear sky ; *b*, black paper ; *c*, a block of wood to which is fixed (by lateral attachment not here shown) the mirror *d* which reflects light, as shown by the arrows *e e*, through the sheet of glass, *i*, at the top of the case vertically downwards to the Pilobolus fruit-bodies on the dung in the dish ; *f*, a cardboard box open above and below (shown separately

the chamber, the lower part of the chamber was provided with a removable panel shown at B.

To catch any Pilobolus projectiles that might be shot vertically upwards, the hole h in the top of the chamber was covered either with a circular sheet of glass (like that shown at D) or with a sheet of beaver-board (like that shown at E) having a slotted rectangular aperture in its centre into which could be inserted a sheet of glass of the same size as a photographic half-plate. In the critical experiments the cover E was always used in preference to D.

To ensure that bright light rays should pass vertically downwards through the sheet of glass on the top of the chamber, a four-sided box G without top or bottom was set over the sheet of glass as shown at f in F, and then light coming from the sky through a window a was reflected by means of a mirror d vertically downwards through the sheet of glass as indicated by the arrow e.

About 10 A.M. on the day on which an experiment was to be made, the air of the experimental chamber was first moistened by spraying it with water and by enclosing in the chamber dishes of water and wet sheets of blotting paper. As soon as the air of the chamber had become sufficiently laden with water-vapour, the panel B was removed, a culture dish (A, c) was set in the middle of the floor of the chamber, and then the panel B was put back in its place. The Pilobolus guns growing on the dung-balls in the culture dish were then illuminated solely by rays of light coming vertically downwards to them through the sheet of glass at the top of the chamber. Under these conditions the guns adjusted themselves heliotropically and soon came to look vertically upwards in the manner shown at C. Later in the morning the guns discharged their sporangia vertically upwards toward the glass window at the top of the chamber. Since every sporangium sticks to glass where it comes into contact with it, it was always possible to tell whether

FIG. 26—*cont.*

at G); g, the top of the case A; h, the beaver-board cover containing in its slot a sheet of glass i. H, a cross-section of the lower part of the case A seen from above when the upper part a has been removed: a, beaver-board; b, wood; c, air. In A the roof of the upper part a rests on four sticks of equal length (*cf.* a in I) which in turn rest on the four wooden corners of the lower part b (*cf.* b in H). To enable one to change four sticks of the length a (in I) to four of the length b, and so raise the height of the case, one takes away the panel d in A.

or not any sporangia had been shot up as high as the sheet of glass or the beaver-board sheet holding the glass by simply examining the under surfaces of these structures and noting whether or not any sporangia had become attached there. As a rule, not more than one experiment was made on any one day.

In a series of experiments in which the top of the experimental chamber was raised by successive increments (usually of 6 inches each) from 3 feet to 6 feet 6 inches, it was found that the sporangia of *Pilobolus Kleinii* and of *P. longipes* were shot from the top of the dung-balls vertically upwards: in considerable numbers to a height of 4 feet; in smaller numbers to a height of 5 feet ; and in still smaller numbers to a maximum height of 6 feet 0·5 inch. We may conclude, therefore, that both *P. Kleinii* and *P. longipes* can shoot up their sporangia to a maximum height greater than the average height of a man.

In one experiment with *P. Kleinii*, the total number of sporangia which were shot upwards from the top of the dung-balls to a height of 5 feet 6·5 inches exceeded one hundred and twenty. Of these sporangia fifteen had struck and stuck to the plate of glass (Fig. 26, F, *i*) and over one hundred had struck and stuck to the sheet of beaver-board (*h*) which held the glass and covered much of the hole in the roof of the chamber.

The total number of *P. Kleinii* sporangia which were shot up to a height of 6 feet 0·5 inch in the course of three successive daily experiments was twenty. All of the twenty sporangia were found to be of the largest size [1] and, from the unusually large diameters of the haloes of precipitated matter by which they were surrounded, it was evident that each one of them had been carried from its sporangiophore to the roof of the experimental chamber by a very large drop of expelled cell-sap. That the largest Pilobolus projectiles should be shot to the greatest height was to be expected from dynamical considerations ; for, the initial velocities being equal, the larger the projectile, the greater is its momentum when shot away and the higher will it be carried upwards through the air.

From the equation :

$$v^2 = 2gs$$

where v = the initial velocity, g = the acceleration due to gravity,

[1] One of them had a diameter of 0·54 mm.

and s = the vertical height to which a projectile is discharged when shot vertically upwards, *neglecting the resistance offered by the air* it can be calculated that a Pilobolus projectile, if shot vertically upwards to a maximum height of 6 feet, has an initial velocity of 19·6 feet per second.

When the resistance offered by the air to the flight of a Pilobolus projectile is taken into account, it is clear that the projectiles of *P. Kleinii* and of *P. longipes* which were shot up to a height of 6 feet 0·5 inch must have had an initial velocity which exceeded 20 feet per second.

Employing the equation :

$$s = \tfrac{1}{2}gt^2$$

where s = the vertical distance of rise or fall, g = the acceleration due to gravity, and t = the time of rise or fall in seconds, *neglecting the resistance offered by the air* it can be calculated that, when a body is shot up from rest to a height of 6 feet so that it falls back again to where it started from, the total time of rise and fall = $2t = 2 \times 0·61 = 1·22$ seconds. Because of the resistance of the air, the projectiles of *P. Kleinii* and of *P. longipes* which rise to a height of 6 feet and then fall to earth must take upwards of 1·22 seconds to complete their movement.

The horizontal range of the guns of *P. Kleinii* and of *P. longipes* was investigated by putting the culture dish in a special experimental chamber 12 feet long and 3 feet wide built of beaver-board about a window in the laboratory. The culture dish was placed on the floor under the window and tilted to an angle of 45°–50° from the horizontal and light from an upper window-pane was reflected to it at an angle of about 40°–45° from the vertical by means of a vertical mirror hanging four feet above the floor. The chamber was painted black inside and its air was moistened by spraying it with water. The floor of the chamber was covered with sheets of white paper to receive any sporangia which might fall on it. The sporangiophores, in consequence of their heliotropism, became directed toward the light at an angle of 40°–45° with the horizontal before they began to discharge their sporangia.

In a series of experiments made in the chamber just described, the maximum horizontal distance to which any sporangium was

discharged was observed to be : for *P. Kleinii*, 8 feet 0·5 inch ; and for *P. longipes*, 8 feet 7·5 inches.

Theoretically, if the resistance due to the air be neglected, a gun can shoot a projectile twice as far horizontally as it can vertically. If, therefore, a Pilobolus gun could shoot a projectile in a vacuum to a height of 6 feet, it would be able to shoot it under the same conditions to a maximum horizontal distance of 12 feet.[1] In actuality, however, the air offers a considerable resistance to the flight of a projectile less than 1 mm. in diameter ; and it is on account of air-resistance that *P. Kleinii* and *P. longipes*, which shoot their sporangia to a maximum height of about 6 feet, shoot their sporangia to a maximum horizontal distance of only about 8 feet instead of 12 feet.[2]

Since the calculations just recorded were made, Pringsheim and Czurda,[3] from measurements made with the help of a pair of rotating slotted discs, have calculated that the velocity of the projectiles of the Pilobolus used in their experiments, just after discharge, is approximately 46 feet per second (14 metres per second). Using the equation given on page 66, it can be calculated that, if the air offered no resistance, the height to which a projectile having an initial velocity of 46 feet per second would rise, if shot vertically upwards, is 33 feet. Since my own observations show that the strongest Pilobolus guns shoot their projectiles vertically upwards to a height of only 6 feet, it is obvious that, assuming the correctness of Pringsheim and Czurda's observations and calculations, the air must offer a very considerable resistance to the flight of the Pilobolus projectile.

The Structure of the Sporangiophore and Sporangium illustrated by Pilobolus Kleinii and P. longipes.—The sporangiophore of *P. Kleinii*, like that of other Piloboli, consists of a *basal swelling* (which differs from that of *P. longipes* in being bulbous instead of much

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[1] *Cf.* these *Researches*, Vol. V, 1933, Fig. 163, p. 328.

[2] The smaller the projectile, the more nearly equal to the vertical range does the horizontal range of the gun become. It is for this reason that the vertical and horizontal ranges of a hymenomycetous basidium, which shoots away four tiny basidiospores, are approximately equal. *Cf.* these *Researches*, Vol. I, 1909, p. 186 and Fig. 65.

[3] E. G. Pringsheim and V. Czurda, *loc. cit.*, pp. 879–882.

elongated), a cylindrical *stipe*, a large pear-shaped *subsporangial swelling*, and a *columella* which is surrounded and hidden from external view by the sporangium (Fig. 27). The sporangium is a black discoid body filled with thousands of orange-yellow oval spores.

A fruit-body of *Pilobolus Kleinii*, which was of typical form, full-grown, and just about to shoot away its sporangium, was placed on a slide in water under a raised cover-glass, and its upper part was carefully drawn with the *camera lucida*. With the help of this drawing, a median vertical section of the upper part of a typical fruit-body was constructed semi-diagrammatically, and the drawing which resulted is reproduced in Fig. 28.

As shown in Fig. 27, the basal swelling, the stipe, the subsporangial swelling,

FIG. 27.—*Pilobolus Kleinii*. Diagram of an optical longitudinal section of a living fruit-body, together with part of the mycelium to which the fruit-body is attached in the substratum. The substratum (dung, dung-agar, etc.), *s*. The mycelium : *a–b*, a main hypha from which the fruit-body originated ; *c c*, thinner secondary hyphae, branches of the main hypha ; *d*, an apophysis (swelling on the main hypha), separated from the fruit-body by a septum. The fruit-body consists of a unicellular sporangiophore *e*, *f*, *g*, *h* and of a sporangium *m*, *n*, *o*. The sporangiophore : *e*, the basal swelling ; *f*, the cylindrical stipe : *g*, the pyriform subsporangial swelling; *h*, the columella ; *i*, the great vacuole of the sporangiophore filled with clear cell-sap ; *j*, drops of clear mucilaginous liquid excreted from the sporangiophore and sporangium ; *k*, a red, carotin-containing, biconcave, perforate, protoplasmic septum at the top of the stipe ; *l*, a shallow reddish ring of protoplasm at the top of the subsporangial swelling. The sporangium : *m*, the black sporangial wall ; *n*, colourless jelly situated in the lower part of the sporangium between the spore-mass and the wall of the sporangium and columella ; *o*, numerous oval orange-yellow spores. Magnification, about 23.
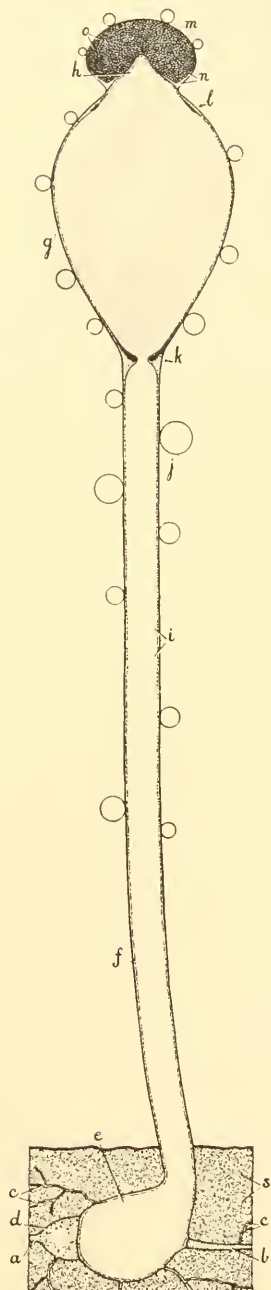
and the columella are four parts of a single cell.   Their wall is exceedingly thin, so thin indeed that, even in so large an illustration as Fig. 28, it has to be represented by a single thin black line.   The wall is lined by a layer of protoplasm which encloses a huge vacuole filled with perfectly clear cell-sap.

The protoplasm of the basal swelling, subsporangial swelling, and columella is in general colourless and disposed in a layer which is no thicker than, or scarcely thicker than, the cell-wall against which it presses ; but, around the top of the subsporangial swelling (Fig. 28, *f*) and around the top of the stipe where this passes into
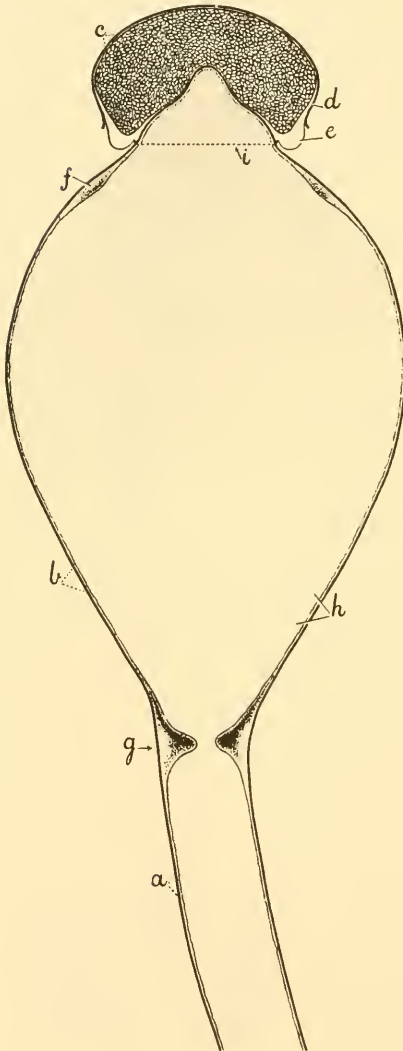
FIG. 28.—*Pilobolus Kleinii.*   A median-longitudinal section through the upper part of a fruit-body, just before the discharge of the projectile : *a*, the stipe ; *b*, the subsporangial swelling ; and *c*, the sporangium.   The sporangium is filled with spores and covered with an intensely black wall *d* which has split open circumscissilely, and has thus allowed a thick gelatinous inner ring *e*, present only around the base of the sporangium, to bulge outwards.   The subsporangial swelling, which is pyriform, has a thin elastic wall lined by a layer of protoplasm which is very thin except at *f* where it is slightly thickened and at *g* where it bulges inwards so as to form a large biconcave septum which is perforated in the centre.   The protoplasm at *f* is reddish and at *g*, as indicated by the shading, very red, especially on its upper side.   The subsporangial swelling is continued above into a conical columella and below into the cylindrical stipe.   The protoplasm of the stipe, swelling, and columella contains one large continuous vacuole *h* filled with clear cell-sap.   The broken line *i* passes through the plane of abscission and indicates where the Pilobolus projectile, consisting of the sporangium and columella, separates from its attachment when the Pilobolus gun is discharged.   Magnification, 69.

the subsporangial swelling (Fig. 28, *g*), it is coloured red and heaped up. The redness of these thicker masses of protoplasm, as Zopf [1] has pointed out, is due to their containing carotin held within minute oil-drops.

The upper heap of protoplasm (Fig. 28, *f*) is relatively shallow and pale red in colour. It is so situated that, when the Pilobolus gun faces the light, it receives the full force of the incident rays. It is probably photosensitive, and it is possible that its photochemical reactions may serve to bring about chemical changes which increase the osmotic pressure of the cell-sap or weaken the wall of the subsporangial swelling at the place (*Riss-stelle*) where it is to be ruptured transversely when the projectile is shot away. Since Coemans published his *Monographie* in 1861, it has been known that, when maturing Pilobolus fruit-bodies are placed in the dark, the discharge of their sporangia is delayed for several hours.[2] There must be some photochemical mechanism by which the light of the sun is employed in preparing the Pilobolus gun for discharge, and it is possible that in the working of this mechanism the upper heap of protoplasm plays an important part.

The lower heap of protoplasm, shortly before the discharge of the sporangium, usually has the form of a biconcave lens with a rounded vacuolar passage-way in its centre, as shown at *g* in Fig. 28. The passage-way varies in width but is never absent, so that the protoplasm at the top of the stipe never forms a complete septum and the vacuole of the subsporangial swelling is always continuous with that of the stipe.

The lower heap of protoplasm contains numerous oil-drops, with carotin dissolved in them, which, as indicated by shading at *g* in Fig. 28, are : (1) very densely packed in its upper surface layer which, in consequence, is coloured bright orange-red ; (2) are sparsely distributed in its lower surface layer which, in consequence, is coloured very pale orange-red ; and (3) are practically absent from its inner core which, in consequence, is colourless.

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[1] W. Zopf, " Zur Kenntniss der Färbungsursachen niederer Organismen, No. III, Phycomyceten-Färbungen," *Beiträge zur Physiologie und Morphologie niederer Organismen*, Leipzig, Heft II, 1892, pp. 3–12.

[2] E. Coemans, " Monographie du genre Pilobolus," 1861, *loc. cit.*, pp. 45–46.

The function of the lower heap of protoplasm will be discussed in the Section which treats of the heliotropism of the sporangiophore.

A sporangium which is still young and intact (Fig. 27) is covered externally on its upper and outer sides by a thin black convex cell-wall which is continuous with the cell-wall of the top of the sub-sporangial swelling, while below it is separated from the columella by the wall of the columella, which is a thin, almost colourless, convex septum. The wall of the sporangium is remarkable for three special qualities : (1) its toughness and persistence as compared with the sporangium-wall of Mucor, which is diffluent ; (2) its intense
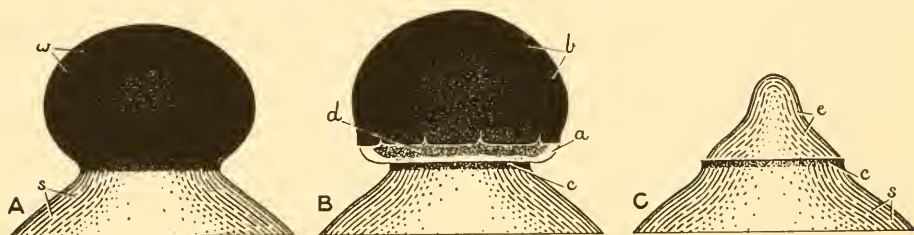
Fig. 29.—*Pilobolus Kleinii.* Dehiscence of the sporangium. A, a ripe sporangium shortly before dehiscence ; it crowns the top of a subsporangial swelling *s* and is covered by a black wall *w*. B, the sporangium just after dehiscence : a layer of jelly *a* lining the interior of the lower part and sides of the sporangial wall has swollen and thereby caused the sporangium-wall to break transversely into two parts, an upper cap-like part *b* and a basal band *c* ; *d*, spores which can be seen through the protruding jelly. C, what is left of B after the sporangium has been stroked away under water with a needle : *s*, the subsporangial swelling ; *e*, the columella ; and c, the band of sporangium-wall encircling the base of the columella. Magnification, 66.

blackness ; and (3) its complete resistance to being wetted by water. As we shall see later, all these qualities are biologically significant.

The contents of a sporangium which is still young and intact are two : (1) a mass of several thousand orange-yellow oval spores packed closely together and embedded in a matrix that swells up somewhat when brought into contact with water ; and (2) a solid transparent mass of jelly which is disposed in the form of a ring around the base of the columella between the sporangium-wall and the spores (Figs. 27 and 30, A).

The spores are *dichroic* : when mounted in water under a cover-glass and viewed with the low-power objective of a microscope, they are *orange-yellow* in *transmitted* light and *greenish* in *reflected* light.

Shortly before the sporangium is to be discharged, the gelatinous ring absorbs water, swells up considerably, presses strongly against the sporangium-wall, and thus causes the wall to split transversely into two very unequal parts : (1) a relatively small lower part in the form of a narrow band which remains attached to the columella and the subsporangial swelling (Figs. 29, B, *c*, and 30, B) ; and (2) a relatively large upper part in the form of a free convex cap.   The gelatinous ring pushes the sides of this cap outwards and somewhat
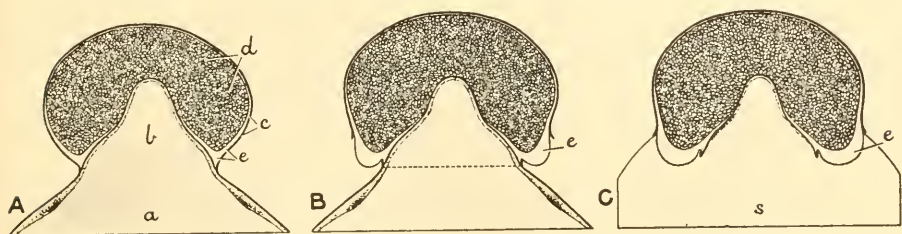
Fig. 30.—*Pilobolus Kleinii.*   Dehiscence and discharge of a sporangium, illustrated semi-diagrammatically with median vertical sections.   A, before dehiscence : *a*, the top of a subsporangial swelling and *b*, the columella, both lined with a thin layer of protoplasm and having the great central vacuole filled with cell-sap ; *c*, the black sporangial wall ; *d*, the spores ; and *e*, a layer of jelly between the spores and the wall of the sporangium and columella, now swelling and about to cause the dehiscence of the sporangium.   B, after dehiscence : the layer of jelly *e* has swollen to such an extent that it has caused the sporangium-wall to break transversely into two parts, an upper cap-like part and a basal band (*cf.* Fig. 29, B) ; the jelly now protrudes through the gap in the wall ; the broken transverse line at the top of the subsporangial swelling indicates where abscission will take place when the sporangium is discharged. C, the sporangium just after it has been shot into the air : *e*, the protruding jelly which will serve to attach the sporangium to some substratum ; *s*, part of a globule of cell-sap which has been shot out of the subsporangial swelling and is attached to the gelatinous, wettable, under side of the sporangium. Magnification, 66.

upwards and thus itself becomes exposed to view.   After the sporangium-wall has thus been pushed outwards and broken, the sporangium has the appearance shown in Figs. 28, 29, B, and 30, B.

The breaking of the sporangium-wall into two parts a short distance above its base and the exposure of the gelatinous ring has been referred to by van Tieghem and other systematists as a process of *dehiscence*.   Usually, when a sporangium dehisces, *e.g.* in Mucor, Saprolegnia, and Ascobolus, its spores are immediately liberated, but this is not so in Pilobolus ; for, when the sporangium-wall of Pilobolus breaks, the spores are prevented from escaping from the sporangium by the gelatinous ring which fills the gap between the

edge of the wall and the columella (Fig. 30, B and C). The real significance of dehiscence in Pilobolus lies in this : that, whereas it does not permit of the escape of the spores from the sporangium, it leads to the exposure of the gelatinous ring which, as we shall see later, has the function of attaching the sporangium with its enclosed spores to the herbage on which herbivorous animals feed. The escape of the spores from a Pilobolus sporangium does not take place when the sporangium dehisces but only when the sporangium, having been eaten with grass by horses or cows, etc., is moistened and compressed in an alimentary canal. It is of interest to note that the swelling up and exposure of the gelatinous ring on the outside of the sporangium is beautifully timed, for it takes place only a few minutes before the sporangium is discharged and the ring is to function in the service of spore-dispersion.

It was observed that, when a pane of glass is set just in front of a large number of fruit-bodies of *Pilobolus longipes*, so that the sporangia are shot against it, the force of the impact is so great that sometimes a sporangium is slightly shattered to the extent that (1) a few spores may be forced out of the sporangium owing to the displacement of the gelatinous ring and (2) fragments of the peripheral less darkly-coloured portion of the black sporangial wall may be broken away from the main mass of the wall. The escaped spores and the broken wall-fragments can then be seen lying in the drop of cell-sap which forms a halo around the sporangium (*cf.* Fig. 33). Under natural conditions in the open, the sporangia are shot not against substances as hard and inelastic as glass but on to herbage and, normally, after they have landed they contain their full complement of spores. There is every reason to suppose that, in pastures and woods, the partial rupture of a Pilobolus sporangium due to impact on landing occurs either not at all or only as a rare accident.

The number of spores contained in the sporangium of *Pilobolus oedipus* (Cohn's *P. crystallinus*) was estimated by Cohn [1] to be 15,000–30,000, and Coemans [2] remarked that this estimate did not

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[1] Ferdinand Cohn, " Die Entwicklungsgeschichte des Pilobolus crystallinus," *Nova Acta Acad. Caes. Leop.*, Bd. XXIII, 1851, p. 513.

[2] E. Coemans, " Monographie du genre Pilobolus," 1861, *loc. cit.*, p. 25.

appear to him to be too large. I have estimated the number of spores in a very large sporangium of *P. Kleinii*, which was 0·54 mm. in diameter and had been shot up to a height of 6 feet 0·5 inch, as follows.

The sporangium was mounted in water and the cover-glass was rubbed over it in such a way that the spores were arranged approximately in a layer two spores thick. Then the area occupied by this

Fig. 31.—*Pilobolus longipes*. Two fruit-bodies from which the sporangium has been pulled off under water, so as to expose the columella. The columella crowns the sub-sporangial swelling and has a central peak. One of the black sporangia is seen to the right below. Magnification, 51.

spore-layer was measured and the upper spores occupying one-four-hundredth of one square mm. were drawn and counted. With the help of these data it was estimated that the sporangium had contained approximately 90,000 spores. Smaller sporangia of *P. Kleinii* probably contain not more than half this number. In *P. Kleinii*, therefore, the number of spores contained within the sporangium varies with the size of the sporangium from about 30,000 to about 90,000.

A sporangium which has dehisced and has its gelatinous ring protruding around its base can be stroked away from the sporangio-

phore under water with the help of a needle without much difficulty. After such an operation has been effected, the columella, while still attached to the subsporangial swelling, becomes exposed to view as shown in Figs. 29, C, and 31. The narrow band of sporangium-wall, left attached to the columella and subsporangial swelling and surrounding the base of the columella when the sporangium dehisced, can then be clearly seen (Fig. 29, C, c). It has a dark appearance owing to its being covered externally with numerous minute
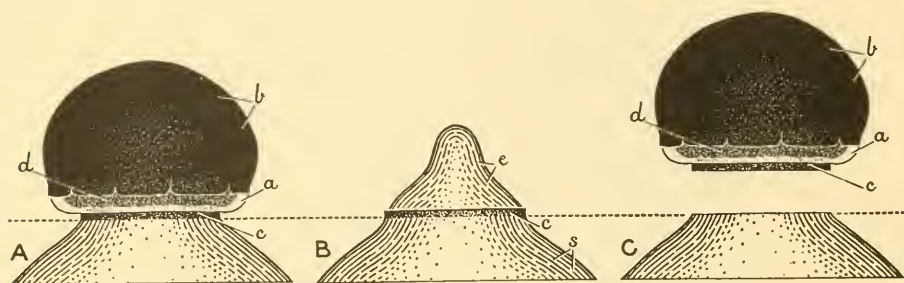
Fig. 32.—*Pilobolus Kleinii.* Level of abscission of the sporangium indicated by a broken line. A, the top of a subsporangial swelling crowned by a ripe sporangium which has dehisced; the sporangium-wall has split transversely into a convex upper portion b and a lower band c, and in the gap one can observe the jelly a and the spores d. B, like A, but the sporangium has been stroked off under water, leaving behind the columella e and the basal band of the sporangium-wall c ; s, the subsporangial swelling. C, like A, but with the sporangium shown separate from the subsporangial swelling ; the separation has been effected at the level of abscission ; the drawing serves to indicate that the basal band of the sporangium-wall remains attached to the sporangium as a whole when this is shot away. Magnification, 66.

particles which, as shown by microchemical reactions, are crystals of calcium oxalate. The particles extend over the surface of the subsporangial swelling below the band of the sporangium-wall, but are less closely packed and less conspicuous there.

At the moment when a sporangium is discharged from the sporangiophore, the wall of the subsporangial swelling just under the junction of the band of sporangium-wall and the columella suddenly splits transversely, so that the sporangium and the columella are shot away together. The level of abscission of the sporangium is indicated by a broken line in Figs. 28 (p. 70), 30, B (p. 73), and 32. A large drop of cell-sap remains attached to the gelatinous ring and the columella as the projectile travels through

the air (*vide infra*), and the projectile, after landing, has the appearance shown in Figs. 33–35.

An upper view of two projectiles which have settled on glass is
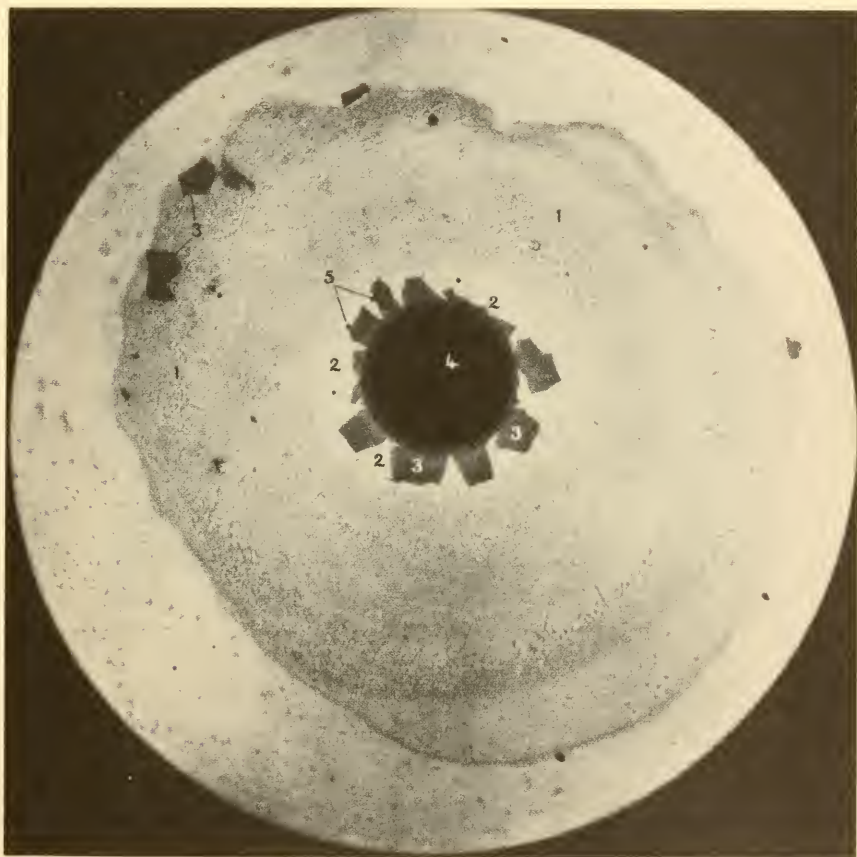
Fig. 33.—*Pilobolus longipes*. Photomicrograph of the upper side of a discharged sporangium and of the drop of cell-sap (now dried) which accompanied it. The projectile struck and stuck to a sheet of glass. No. 1, the precipitate of the cell-sap, in part crystalline ; No. 2, a broad ring-layer of jelly in contact with the glass ; No. 3, the peripheral part of the sporangium-wall which is now radially split and flattened out above the ring of jelly, isolated bits of it can be seen at the periphery of the drop to the left ; No. 4, the convex, very black, main portion of the sporangium-wall covering many thousands of spores ; No. 5, two of four spores which were forced out of the sporangium as this struck the glass. Magnification, 51.

shown in Figs. 33 and 34. Here one can distinguish : No. 1, a precipitate of amorphous granules and small crystals produced by

the drying of the cell-sap ; No. 2, the gelatinous ring which causes the sporangium to adhere tightly to its substratum ; No. 3, the paler peripheral part of the cap-like portion of the sporangium-wall, now flattened, radially split, and in part fragmented ; No. 4, the intensely black convex main part of the cap-like portion of the
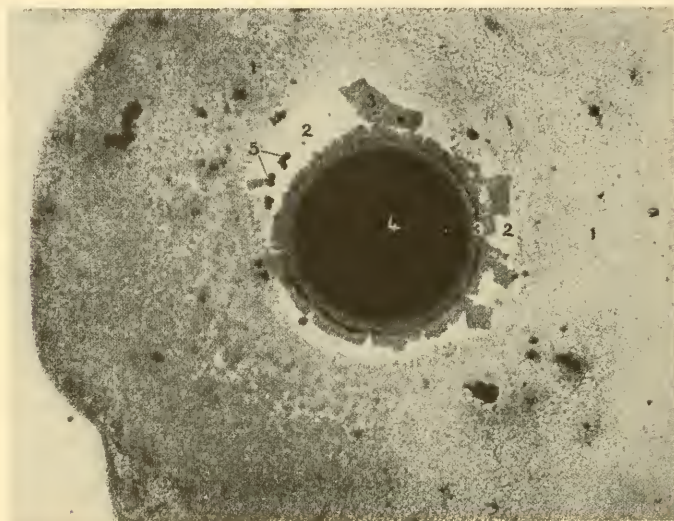
FIG. 34.—*Pilobolus longipes.* Photomicrograph of the upper side of another discharged sporangium and of the drop of cell-sap (now dried) which accompanied it. No. 1, the precipitate of the cell-sap ; No. 2, a broad clear ring-layer of jelly in contact with the glass ; No. 3, the peripheral part of the sporangium-wall, now flattened and broken and overlying the jelly ; No. 4, the convex, very black, main portion of the sporangium-wall covering many thousands of spores ; No. 5, a few isolated spores lying under the jelly ; they were forced out of the sporangium when this struck the glass. Magnification, 51.

sporangium-wall, which covers and hides many thousands of spores ; and No. 5, a few spores which, owing to the violence with which the projectile impinged on a rigid piece of glass, were pressed out of the sporangium between the wall and the gelatinous ring.

A lower view of a projectile obtained through a cover-glass to which the projectile is attached is shown in Fig. 35. Here one can distinguish : No. 1, a precipitate of amorphous granules and large branched crystals produced by the drying-up of the cell-sap ; No. 2, the gelatinous ring, the outer limit of which is more easily seen here

than in Figs. 33 and 34 ; No. 3, the peripheral paler part of the cap-
like portion of the sporangium-wall, now flattened, more or less
radially split and in part fragmented ; No. 4, the black tuck of the
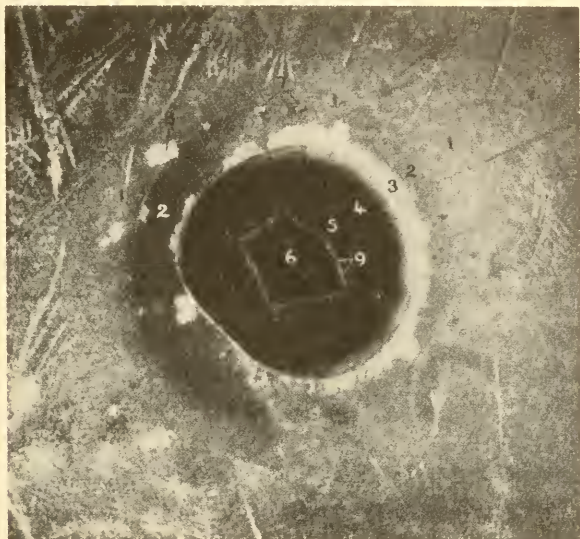
FIG. 35.—*Pilobolus longipes*.. Photomicrograph of the *under*
side of a discharged sporangium and of the drop of cell-
sap (now dried) which accompanied it, taken through
a cover-glass to which the sporangium was attached.
No. 1, dried cell-sap containing long branched crystals ;
No. 2, a broad ring-layer of jelly attached to the glass
and covering a few isolated spores, No. 7, which were
forced out of the sporangium when this struck the glass ;
No. 3, the flattened peripheral part of the sporangium-
wall, seen through the jelly, a piece of which, No. 8, broke
away as the sporangium hit the glass ; No. 4, the black
flattened tuck of the sporangium-wall obscuring spores
from view (*cf.* Fig. 41, *g*) ; No. 5, the main mass of the
spores, seen through the inner zone of the ring of jelly ;
No. 6, the columella flattened down on to the glass, its
peripheral band No. 9 (part of the sporangium-wall)
now forming a pentagon.   Magnification, 51.

sporangium-wall (*cf.* Fig. 41, *g*, p. 85), seen through the inner zone
of the gelatinous ring ; No. 5, thousands of spores massed together ;
No. 6, the columella pressed down on to the glass, with its periphery
now pentagonal in form ; No. 7, a few spores which, owing to the
violence with which the projectile impinged on the cover-glass,
were pressed out of the sporangium so that they lie between the

gelatinous ring and the surface of the cover-glass ; No. 8, a fragment of the peripheral paler part of the cap-like portion of the sporangium-wall (No. 3) ; and No. 9, a narrow band of sporangium-wall attached to the rim of the columella.

The somewhat brittle, paler, radiately split, outer portion of the sporangial wall of a discharged sporangium (No. 3 in Figs. 33, 34, and 35) may be conveniently referred to as the *fringe* of the sporangium. It is to be noted in a dried-up discharged sporangium that the pale fringe rests directly on the film of jelly and that the
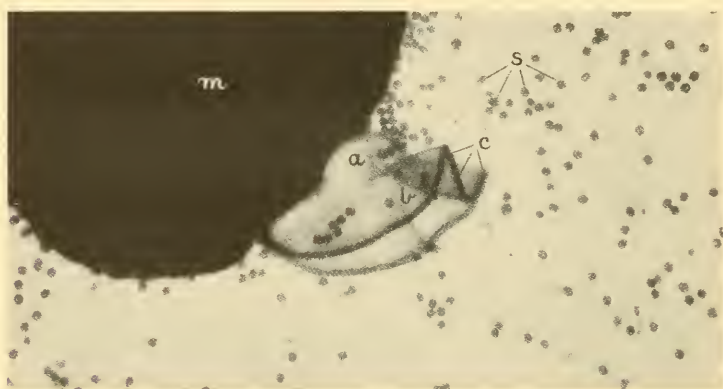
Fig. 36.—*Pilobolus longipes*. Photomicrograph of a discharged sporangium which has been rubbed laterally in water under a coverglass : *m*, a mass of orange-red spores pressed out from under the black sporangium-wall ; *s*, single spores ; in the centre the isolated cap-shaped columella consisting of a peak *a*, a brim *b*, and a marginal band (part of the sporangial wall) *c*. Magnification, 105.

covering of the spores and their protection from light-rays, etc., is restricted to that part of the sporangium-wall which is very tough and intensely black.

Cohn,[1] in 1851, thought that, when a Pilobolus gun is discharged, the wall of the columella does not travel with the sporangium but remains behind upon the top of the subsporangial swelling ; but this view was shown to be erroneous by Coemans[2] in 1861. That, normally, the columella remains attached to the sporangium when this is discharged can be readily proved by breaking up a dis-

[1] Ferdinand Cohn, " Die Entwicklungsgeschichte des Pilobolus crystallinus," *Nova Acta Caes. Leop.*, Bd. XXIII, 1851, pp. 516–517, Taf. LII, Figs. 12, 13.

[2] E. Coemans, *loc. cit.*, pp. 42–43, Plate II, Fig. 8.

charged sporangium and examining the fragments under the microscope. If one mounts a newly discharged moist sporangium in water on a slide and then presses on the cover-glass so as to give it a lateral movement, one can often separate the sporangium into its component parts, namely : (1) the orange-yellow spores; (2) the intensely black, free, convex, cap-like portion of the sporangium-wall ; (3) the annular mass of jelly ; and (4) the obtusely conical wall of the columella, to the rim of which is attached the narrow band of sporangium-wall (covered with fine particles) which separated from the cap-like portion of the sporangium-wall when the wall, owing to pressure from the annular mass of jelly, was split transversely into two parts. Photomicrographs of columellae isolated in the manner just described are shown in Figs. 36 and 37.

Fig. 37.—*Pilobolus longipes*. Photomicrograph of a columella and some isolated spores obtained by rubbing a discharged sporangium laterally in water under a cover-glass. The columella is cap-shaped : *a*, its central peak, here flattened irregularly ; *b*, its brim ; and *c*, its marginal band (part of the sporangial wall). Isolated spores, *d*. Magnification, 105.

An examination of isolated columellae in side view, like that shown in Fig. 36, revealed the fact that each columella is shaped like a cap and that, whereas the wall of the brim (*b*) is relatively thin, the wall of the peak (*a*) is thick and apparently gelatinously swollen. There can be but little doubt that, as a discharged projectile dries up and the columella comes to press against the substratum, the wall of the columella assists the annular mass of jelly in causing the sporangium to become adherent to the substratum.

That the mass of jelly produced within a sporangium is truly in the form of a hollow ring was established by dissection carried out as follows. A ripe fruit-body of *Pilobolus longipes* was submerged in a drop of water, and its sporangium was pulled away from the columella with the help of a needle. A cover-glass was then placed over

the sporangium and moved laterally so as to disperse the spores in the water. The cover-glass was now removed. Then pieces of the sporangial wall were lifted from the jelly and the jelly was moved about until it was free from spores. From three of the sporangia treated in this way as many complete rings of jelly were obtained (Fig. 38). It is clear that in a sporangium the jelly does not extend over the top of the columella but is annular in form.

At the moment a sporangium with an attached drop lands upon a grass-leaf or other object, its ring of jelly is fully distended, the cavity of the cone-shaped columella is filled with cell-sap, and the contents of the sporangium (spores and substance lying between them) are very watery. Immediately thereafter, the drop and the sporangium begin to lose water by evaporation and, in the course of a few minutes, they become air-dried. From the flattened drop there are deposited on the substratum amorphous particles and branched crystals, which form a halo around the sporangium (Figs. 33–35, pp. 77–79). As the sporangium dries, the gelatinous ring contracts to a very thin hard flat film (Fig. 35, No. 2, and Fig. 41, b) which sticks the sporangium tightly to the surface of the object on which it lies. As the gelatinous ring dries and flattens, the pale radiately-split fringe of the sporangial wall settles down on the top of the jelly and thus comes to lie parallel to the surface of the substratum (Figs. 39 and 41). The cell-sap in the cavity of the columella is bounded by the conical wall of the columella and the surface of the substratum, and it is free from air-bubbles As this cell-sap evaporates, the columella cavity (into which air cannot enter) necessarily becomes more and more contracted, with the result that the columella becomes compressed both laterally and from above downwards. The lateral compression results in the free edge of the

Fig. 38.—*Pilobolus longipes.* A gelatinous ring isolated from a sporangium that had been stroked away from the sporangiophore and columella under water. Magnification, 75.

columella becoming more or less drawn together or becoming polygonal (Fig. 35), while the compression from above downwards causes the more or less swollen conical wall of the columella to flatten and to come directly into contact with the surface of the substratum. The columella-wall, when dry, adheres to the substratum (Fig. 41, c), and thus to some extent assists the gelatinous ring in attaching the sporangium to a grass-leaf or other object.
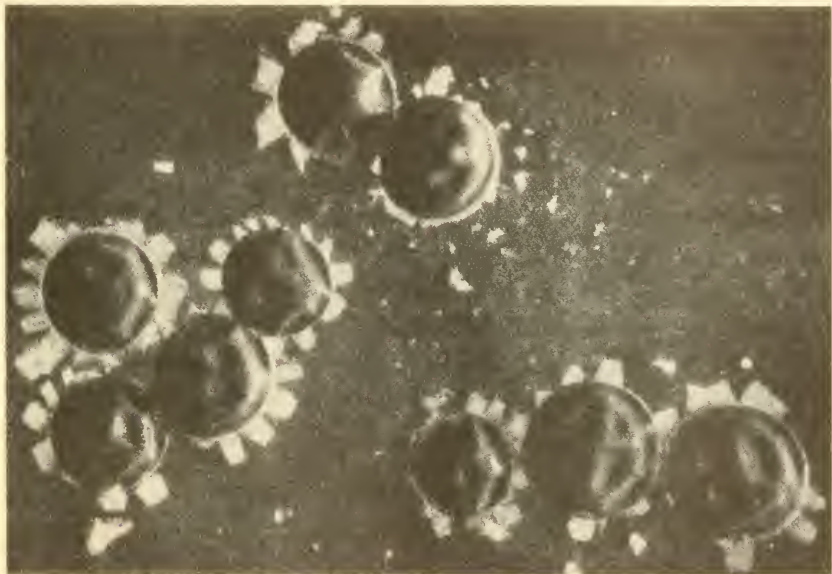
Fig. 39.—*Pilobolus Kleinii.* Discharged sporangia, from a pure culture, attached to a sheet of glass, photographed by reflected light. To show the characteristic rounded depressions (dimples) which were developed in the sporangium-wall as the sporangia dried up. Magnification, 60.

The sporangium as a whole, as it dries after discharge, contracts considerably, perhaps to one-quarter of its original volume. This contraction is due in part to the shrinkage and disappearance of the large central columella cavity, as already described, and in part to the loss of water from the spores and from the substance lying between them. Loss of water from the interior of the sporangium results in : (1) a flattening of a lower zone of the originally convex part of the sporangium-wall, so that this zone becomes added to the fringe (*vide* Figs. 33 and 34, between Nos. 3 and 4, and Fig. 41,

between f and g) ; (2) the formation of a tuck in the wall beneath the edge of the convex part of the sporangium ; (3) the formation of depressions or dimples in the upper part of the sporangium-wall (Figs. 39 and 40) ; and (4) the contraction of the spore-mass, the adherence of the spores to one another, and a change of the shape of each spore from rounded to polygonal (Fig. 41).

The existence of a tuck in the sporangium-wall of a dried sporangium (Fig. 41, g) has not been previously recorded by any other observer, and it was discovered only as a result of a detailed

FIG. 40.—*Pilobolus longipes.* Discharged sporangia, from a pure culture, attached to a sheet of glass, photographed by reflected light. To show the characteristic wrinkles developed in the sporangium-wall as the sporangia dried up. Magnification, 60.

investigation on dried and drying sporangia. If a sporangium which has dried on a glass surface is pried loose from its substratum, the fringe of the sporangial wall and the gelatinous ring are left behind on the glass, but the wall-tuck remains attached to the under side of the sporangium. The tuck extends centripetally to a distance of about one-fifth to one-quarter the radius of the sporangium as a whole. A sporangium was allowed to land on a cover-glass and dry there, and then the cover-glass, with the sporangium beneath, was set on a drop of sulphuric acid on a glass slide (*cf.* Fig. 35). With the microscope the action of the sulphuric acid could be followed. The acid dissolved away the sporangium-wall progressively from the free edge inwards, until the whole wall disappeared leaving the mass of spores behind. The fringe of the wall disappeared first, and soon,

as the acid made the remaining part of the wall more and more translucent, the tuck of the wall under the edge of the convex sporangium became clearly revealed.

The depressions or dimples formed in the upper black portion of the sporangial wall as the sporangium dries up vary in number with the size of the sporangium and in pattern with the species. As a rule, the larger the sporangium, the more numerous are the depressions. The difference between the pattern of the depressions on the sporangia of *P. Kleinii* and that on the sporangia of *P. longipes* can be readily realised by comparing Fig. 39 with Fig. 40 : the depressions of *P. Kleinii* are rounded, those of *P. longipes* irregular and somewhat gyrose.

As a fruit-body from which the sporangium has been stroked off (Fig. 31, p. 75) loses its turgidity, the wall of the subsporangial swelling just beneath the columella contracts and becomes very thick, whereas the wall of the columella
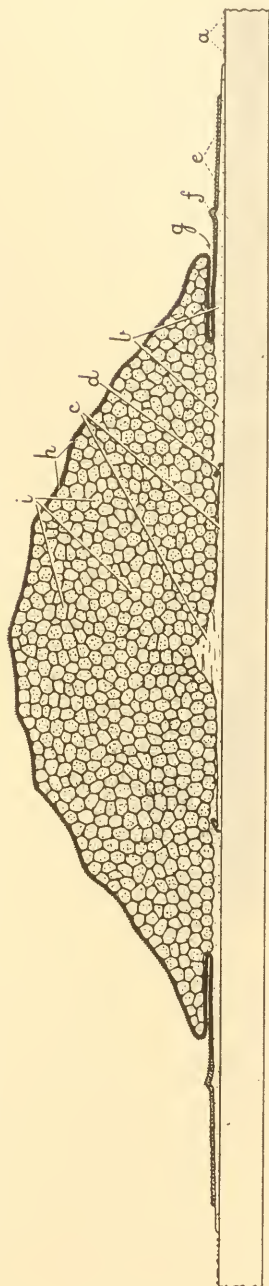
FIG. 41.—*Pilobolus longipes*. Diagrammatic representation of a median vertical section through a discharged dried-up sporangium attached to a thin sheet of glass : *a*, precipitate of amorphous particles and crystals derived from the drop that was discharged with the projectile ; *b*, the gelatinous ring, attaching the projectile to the substratum ; *c*, the columella, flattened out and adhering to the substratum ; *d*, a band of sporangium-wall attached to the rim of the columella ; *e*, the fringe of the sporangium-wall overlying part of the gelatinous ring ; *f*, a crinkle in the sporangium-wall and *g*, a tuck in the sporangium-wall, both formed as the sporangium dried up and settled down on its substratum ; *h*, the black sporangial wall, bearing numerous fine crystals of calcium oxalate, now wrinkled ; *i*, the spores which, owing to mutual pressure, have become polygonal in form. Drawn by A. H. R. Buller and Eleanor S. Dowding. Magnification, 194.
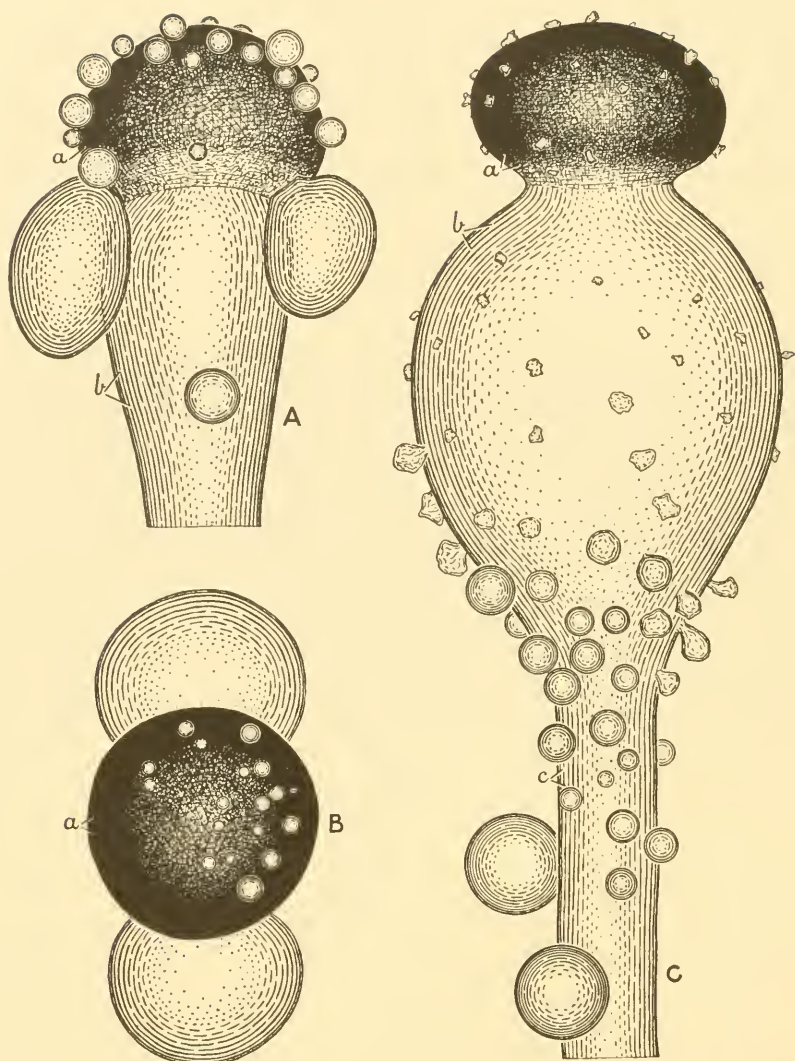
Fig. 42.—*Pilobolus Kleinii*. The excretion and drying of drops. A, the upper part of a young fruit-body : *a*, the sporangium ; *b*, the subsporangial swelling beginning to expand. Numerous small drops have been excreted by the sporangium and two very large drops have appeared at the junction of the sporangium and the subsporangial swelling. B, another young fruit-body, similar to A, but seen from above. The drops attached at the junction of the sporangium and the young subsporangial swelling are of the maximum size. C, a mature fruit-body shortly before the discharge of the sporangium. The drops on the sporangium *a* and the upper part of the subsporangial swelling *b* are drying up and in doing so are becoming irregular in form, thus revealing the fact that they contain a gelatinous substance. The excretion of drops has been especially active in the lower part of the subsporangial swelling, but the largest drops are on the stipe, *c*. Drawings made with the help of a *camera lucida*. Magnification, 90.

does not.   This indicates that the upper part of the wall of the subsporangial swelling, just under what is normally the line of rupture when the sporangium is shot away, is highly elastic whereas the wall of the columella is not.   It may be added that with iodine the thickened wall of the subsporangial swelling takes on a much deeper red colour than the relatively thin wall of the columella.

The globular watery drops which are formed in large numbers on the sporangium and sporangiophores are well shown in Boudier's drawing reproduced in Fig. 12 (p. 23), and they are also shown freshly excreted and uncontracted in the diagrammatic Fig. 27 (p. 69).   These drops, as Knoll [1] discovered, resemble those on the pileal hairs of *Coprinus ephemerus* and *Psathyrella disseminata*, etc., and on the gills of various Hymenomycetes, in that they contain a

Fig. 43.—*Pilobolus longipes*.   From left to right, diagrammatic representation of two drops excreted from a sporangium, and of successive stages in their drying up.   As the drops dry, they shrink in size, become irregular in form (owing to their gelatinous contents), and finally become as black as the sporangium-wall.   Magnification, 100.

pellucid colloidal substance or slime—readily soluble in water but insoluble in alcohol—which gives to the surface of the drops, as these dry up, an irregularly wrinkled appearance.[2]   With the help of the microscope I myself have often observed the wrinkling of the drops of Pilobolus as they lose water by evaporation (Figs. 42, C, and 43).

The drops on the sporangium and upper part of the subsporangial swelling usually dry up, leaving in their places tiny irregular gelatinous masses, before the sporangium is discharged (Fig. 42, C). Doubtless, whilst they exist as large watery spheres, they interfere to some extent with the course of the rays of light entering the subsporangial swelling.   Their early disappearance must therefore be of advantage in that it enables the sporangiophore to direct the

[1] F. Knoll, "Untersuchungen über den Bau und die Funktion der Cystiden und verwandter Organe," *Jahrb. f. wiss. Bot.*, Bd. L, 1912, pp. 453-501.

[2] For drops on the pileal hairs of *Coprinus curtus* and their mode of drying *vide* these *Researches*, Vol. IV, 1931, Fig. 12, p. 18.

sporangium with greater accuracy toward the source of brightest light.

The drops excreted by the sporangium differ from those excreted by the sporangiophore in that as they dry up they become brown and ultimately as black as the subjacent sporangial wall (Fig. 43).
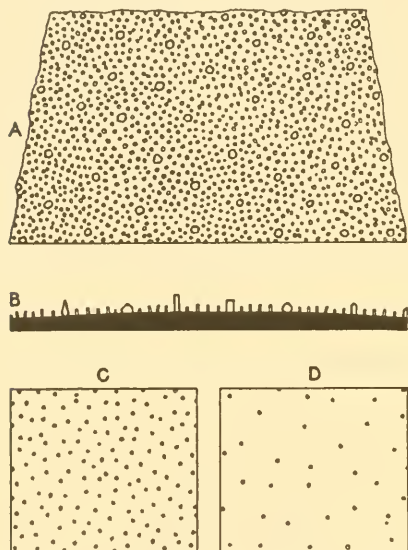
Fig. 44.—*Pilobolus longipes.* Crystals of calcium oxalate on : A, a piece of the fringe of the wall of a discharged sporangium, seen in face view ; B, the black part of the sporangium-wall, seen in tangential view ; C and D, the wall of the subsporangial swelling and of the stipe respectively, seen in face view. Magnification, 1130.

This fact, which does not seem to have been previously recorded, lends support to Knoll's view that the mucilage in the drops excreted by the pileal hairs and cystidia of Hymenomycetes, the fruit-bodies of Pilobolus, etc., is produced as a result of local mucilaginisation of the cell-wall.

Knoll [1] supposed that the drops are forced out of the sporangiophore of Pilobolus chiefly by turgor pressure, and he suggested that their excretion is a result of the operation of a mechanism of the nature of a valve which serves to prevent the continual rise of the turgor pressure and thereby to prevent a premature discharge of the sporangium. It may well be that the excretion of drops is concerned with the regulation of the water and salt content and the pressure equilibrium of the sporangiophore ; but that it is specially concerned with preventing the premature discharge of the sporangium seems very doubtful, for the drops begin to be excreted on the naked stipe of the very young fruit-body long before the sporangium or subsporangial swelling has been formed and some twenty hours before the sporangium is to be discharged (Fig. 12, *a* and *b*, p. 23). Moreover, similar drops are commonly excreted

[1] F. Knoll, *loc. cit.*, p. 489.

from the sporangiophores of other Mucorineae which do not discharge their sporangia, *e.g. Mucor Mucedo* [1] and *Sporodinia grandis*.

The wall of a Pilobolus fruit-body bears numerous very minute crystals of calcium oxalate. In *P. longipes* these crystals are arranged : most densely on the sporangium-wall, less densely on the wall of the subsporangial swelling, and far less densely on the wall of the stipe (Fig. 44, A, C, and D). They can be most readily

observed on the translucent fringe (*cf.* Fig. 33, No. 3, p. 77) of a discharged sporangium mounted in chloral hydrate. On the wall of a piece of fringe, in face view (Fig. 44, A), the crystals can be seen to be of two sizes : (1) less numerous larger crystals of various shapes, and (2) far more numerous smaller columnar crystals. Some of these crystals are shown in profile in Fig. 44, B. The larger crystals are 2–3 μ high and the smaller ones about 1 μ high. Van Tieghem, Brefeld, Zopf, and others, in their somewhat diagrammatic drawings of
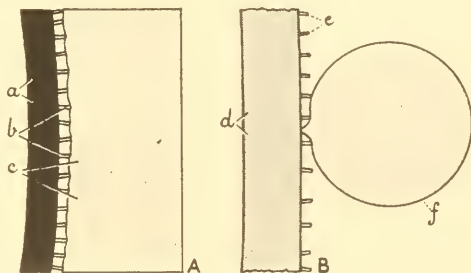
Fig. 45.—*Pilobolus longipes.* Water and the cell-wall. A, diagram showing : *a*, the black sporangial wall ; *b*, crystals of calcium oxalate protruding from the wall ; and *c*, water in which the sporangium has been immersed. Owing to the presence of the crystals and the surface tension of the water, it is supposed, as here represented, that a layer of air is held between the water and the cell-wall so that the latter cannot readily be wetted. B, diagram showing : *d*, the wall of a subsporangial swelling ; *e*, crystals protruding from the wall ; and *f*, a drop of water, still very small, which is being excreted. The drop has a very small base and it is supposed that it touches the crystals in the manner shown. Magnification, about 2250.

Pilobolus sporangia (Figs. 100, 101, 106, 107, and 110, pp. 203–219), have represented the crystals projecting from the sporangium-wall as being much longer than they actually are. It may well be that the unwettability of the sporangium-wall is due to the numerous, closely-set crystals imprisoning air and thus preventing water from coming into contact with the wall's surface (Fig. 45, A). It is also probable that the drops excreted from the sporangium,

[1] O. Brefeld, *Untersuchungen über Pilze*, Heft I, Leipzig, 1872, p. 12. Brefeld remarks that the numerous drops excreted from the surface of the young sporangiophore of *Mucor Mucedo* have a weak acid reaction.

subsporangial swelling, and stipe, as they grow larger, come to touch the crystals in the manner shown in Fig. 45, B.

The Two Functions of the Subsporangial Swelling.—The subsporangial swelling of the Pilobolus gun functions in two entirely different ways : (1) *as an ocellus* which receives the heliotropic stimulus which causes the stipe to direct the free end of the gun toward the source of the brightest light ; and (2) *as part of a squirting apparatus* which, by violently expelling cell-sap, shoots away the sporangium from the sporangiophore.

The squirting function of the subsporangial swelling was recognised by Link [1] as long ago as 1809, but the ocellus function remained unknown until I [2] called attention to it in a brief paper published in 1921. In what follows these functions will be treated of in detail.

The Heliotropism of the Sporangiophore with Special Reference to the Ocellus Function of the Subsporangial Swelling.—When a Pilobolus fruit-body is exposed to unilateral light, the upper part of its stipe just beneath the subsporangial swelling makes a positively heliotropic curvature, with the result that, within about an hour, the free end of the fruit-body, *i.e.* the subsporangial swelling and sporangium, comes to point in the direction of the brightest incident rays of light. The heliotropic reaction of the stipe, as we shall see, is controlled by the subsporangial swelling which, in its mode of refracting and collecting light, acts as a lens.

One morning in February, 1919, on looking at a large number of fruit-bodies of *Pilobolus longipes* which were growing on horse dung in a culture chamber, I observed that, although they all pointed toward a distant window so that their free ends were parallel to the incident rays of light (*cf.* Fig. 46), the top of each stipe glowed with a reddish light. It at once became obvious in respect to each fruit-body : (1) that, but for the existence of the subsporangial swelling, the top of the stipe would be in the shadow of the black sporangium directly in front of it ; and (2) that the glow must be

[1] H. F. Link, " Observationes in Ordines plantarum naturales," *Magaz. d. Ges. naturf. Freunde,* Berlin, Bd. III, 1809, p. 32.

[2] A. H. R. Buller, " Upon the Ocellus Function of the Subsporangial Swelling of Pilobolus," *Trans. Brit. Myc. Soc.,* Vol. VII, 1921, pp. 61–64, no illustrations.

Fig. 46.—A median longitudinal section through the upper part of a fruit-body of *Pilobolus Kleinii*, just before the discharge of the projectile. The gun is shown directed toward the source of the brightest light, in which position it is, heliotropically (or photochemically), in a condition of physiological equilibrium.

The sporangium is filled with spores and covered with an outer intensely black wall *o* which is now broken below, thus allowing a thick gelatinous inner ring, *g*, which is only present around the base of the sporangium, to bulge outwards. The subsporangial swelling, which is pear-shaped, has a thin elastic wall lined by a layer of protoplasm which is very thin everywhere except at *r*, where it is slightly thickened, and at *p*, where it bulges inwards so as to form a large biconcave septum which is perforated in the centre. The protoplasm at *r* is reddish and at *p*, as indicated by the shading, very red, especially on its upper side where it receives the light rays. The subsporangial swelling is continued above into a conical columella *c*, and below into the upper part of the cylindrical stipe or shaft *sh*. The protoplasm of the stipe, swelling, and columella contains one large continuous vacuole filled with clear cell-sap. The broken line *a* passes through the plane of abscission and indicates where the Pilobolus projectile, consisting of the sporangium and the columella, separates from its attachment when the Pilobolus gun is discharged.

Twenty-one parallel rays of light are shown diagrammatically, by means of arrows, striking the fruit-body head-on in a direction parallel to the longitudinal axis of the subsporangial swelling. Nine of these rays strike the black cell-wall of the sporangium, which they cannot penetrate. The other twelve rays strike the upper surface of the subsporangial swelling and are there refracted through the wall into the interior of the swelling and, as shown by the arrows, converge upon the red perforated protoplasmic septum. The septum, like the retina of an ocellus, comes to be lit up with a spot of light, in consequence of which it gives out a red glow which in living fruit-bodies can be seen with the naked eye. *s, s, s* are parts of the subsporangial swelling which are not pierced by any direct rays of light and are therefore in the shade. Some of the light of all of the rays Nos. 1–6 is reflected at the surface of the wall of the subsporangial swelling; and, in passing in succession from ray No. 1 to No. 6, more and more light is reflected and less and less refracted. The size of every part is shown by the scale. Magnification, 69.

due to light refracted through the subsporangial swelling and focussed on the mass of red protoplasm heaped up in the stipe just where the stipe joins the swelling (Fig. 46, *p*). The discovery that the subsporangial swelling acts as a lens and that, when the fruit-body is in heliotropic equilibrium, it focusses its light on the mass of red protoplasm at its base formed the basis of all my further studies on the heliotropism of Pilobolus.

The lens action of the subsporangial swelling was further investigated by means of construction diagrams which, as a result of precise mathematical calculations, show how the swelling refracts light (1) when its axis is parallel to the direction of the incident rays of

Fig. 47.—A median longitudinal section through the upper part of a fruit-body of *Pilobolus Kleinii*, just before the discharge of the projectile. The gun is shown directed not toward the source of light but at an angle of 19° thereto, in which position it is not, heliotropically (or photochemically), in a condition of physiological equilibrium.

The section is exactly the same as that shown in Fig. 46 and there fully described. Again we have : the sporangium filled with spores and covered by an outer black cell-wall *o*, now broken and allowing an inner basally-situated thick gelatinous ring *i* to bulge outwards ; the subsporangial swelling, capped by the columella *c* and passing below into the stipe, its thin cell-wall covered by a layer of protoplasm which is everywhere thin except at *r* where

light (Fig. 46) and (2) when its axis is inclined to the direction of the incident rays at an angle of 19° (Fig. 47).

In constructing Figs. 46 and 47, an outline of a typical fruit-body of *Pilobolus Kleinii* was first very carefully drawn with the *camera lucida*. Then the parallel lines Nos. 1, 2, 3, 4, etc., were added to represent the paths of a few rays of light directed toward the fruit-body. Each ray was now treated separately : its angle of incidence at the surface of the subsporangial swelling or stipe was measured with instruments ; its angle of refraction into the swelling or stipe was then calculated ; and, finally, the angle of refraction being known, a line representing the path of the ray through the interior of the subsporangial swelling or stipe was drawn. In calculating the paths of the refracted rays of light, the following refractive indices were used : air into cell-sap,[1] 1·34 ; air into cell-wall and protoplasm taken together,[2] 1·5 ; cell-wall and

Fig. 47—*cont.*

it is somewhat thickened and at *p* where it bulges inwards to form a large biconcave perforate septum containing red particles massed especially near its upper surface ; the stipe ; the large clear vacuole enclosed by the protoplasm of the stipe, subsporangial swelling, and columella ; and the abscission line *a*.

Twenty-three parallel rays of light are shown diagrammatically, by means of arrows, all inclined at an angle of 19° to the axis of the subsporangial swelling. Of these rays : eight strike the black cell-wall of the sporangium, which they cannot penetrate ; eight strike the right-hand wall of the subsporangial swelling and are there refracted through the wall into the interior of the swelling, so that, as shown by the arrows, they converge to form a spot of light on the left-hand wall at *st* ; and six strike the right-hand wall of the stipe and are there refracted as shown by the arrows. *s, s, s* are areas in the subsporangial swelling which are not pierced by any of the rays of light and which therefore are in the shade. The concentration of the rays of light at the spot *st* presumably gives a photochemical stimulus to the protoplasm, which stimulus is conducted down to *m*, the motor region of the stipe. The stipe grows faster on the side *m* than on the opposite side, with the result that the subsporangial swelling and the sporangium are turned about the top of the stipe through an angle of 19°, *i.e.* until their axis becomes parallel to the direction of the incident rays of light. As the subsporangial swelling and sporangium are turned, the spot of light *st* descends to the base of the swelling until, finally, it comes to rest symmetrically upon the red perforate protoplasmic septum, as shown in Fig. 46. Heliotropically (or photochemically) the position of stable physiological equilibrium has then been reached and no more turning takes place. The part of the stipe below the motor region *m*, at and below *u*, does not alter its position whilst the motor region is making its curvature. The scale serves to indicate the size of each part. Magnification, 69.

[1] The cell-sap was regarded as pure water. The refractive index from air to water is 1·34.

[2] The refractive index 1·5 here used is the same as that found by Senn for the refraction from air into the cell-wall of Vaucheria, etc. *Vide* G. Senn, *Die Gestalts- und Lageveränderung der Pflanzen-Chromatophoren*, Leipzig, 1908, pp. 363–366.

protoplasm taken together into cell-sap,[1] 0·893. Where the proto-
plasm formed a thin even layer on the cell-wall, the cell-wall and
protoplasm were treated as forming a very thin negligible plate,
and the rays were regarded as being refracted directly from the air
into the cell-sap (refractive index 1·34). On the other hand, where
the protoplasm was heaped up around the top of the subsporangial
swelling (rays 1, 2, and 3 in Figs. 46 and 47), the path of each ray
was calculated first from the air into the cell-wall and protoplasm
taken together (refractive index 1·5) and then from the cell-wall
and protoplasm taken together into the cell-sap (refractive index
0·893).

An experiment which proves that the refractive index from air
into the cell-sap of Pilobolus is, as assumed above, approximately
equal to the refractive index from air into water, namely, 1·34, was
made by comparing the *actual* width with the *calculated* width of
the central illuminated part of a median cross-section of a sub-
sporangial swelling when the swelling is placed with its axis hori-
zontal in air under the microscope, is illuminated from below with
a beam of parallel light rays, and is viewed from above. In Fig. 48,
the cross-section of the swelling is represented by a circle, and the
observer is supposed to be looking downwards on it in the direction
shown by the arrow o and to be observing the width of its illuminated
central part. When an *actual* beam of parallel light rays was
reflected upwards from the mirror, the illuminated central part of
the median cross-section of the swelling had a width of $k–l$ and the
lateral dark parts widths of $i–k$ and $l–j$. When a *theoretical* beam
of parallel light rays was represented as being refracted into the
interior of the swelling and when, in calculating the paths of the
rays through the cross-section, 1·34 was used as the refractive
index from the air into the cell-sap, the illuminated central part of

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[1] Knowing the refractive index from air into cell-wall and protoplasm and
from air into cell-sap, the refractive index from cell-wall and protoplasm into cell-
sap can be readily calculated from the equation :

$$a \times b \times c = 1$$

where a = the refractive index from air into cell-wall and protoplasm (1·5),
    b = the refractive index from cell-wall and protoplasm into cell-sap, and
    c = the refractive index from cell-sap into air (1/1·34).
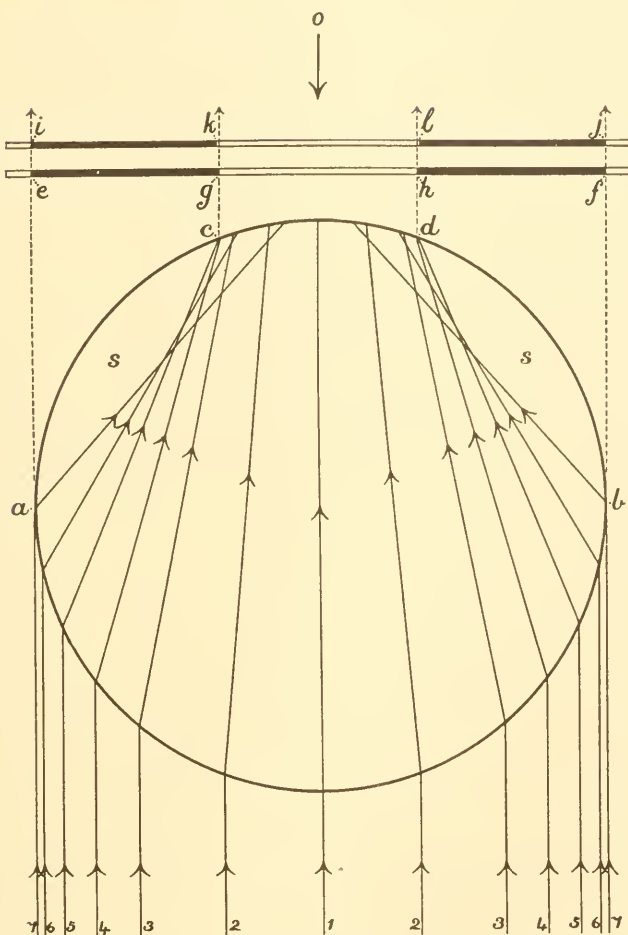
FIG. 48.—Diagram showing how a beam of parallel light-rays is refracted within the subsporangial swelling of *Pilobolus Kleinii*. The disc represents a much enlarged transverse section of a swelling which had its axis horizontally directed. The rays of light 1–7 are passing upwards from the plane mirror of a microscope. The rays on entering the swelling, assuming the refractive index from the air to the cell-sap is 1·34, must be refracted as shown, so that they all emerge between *c* and *d*, the parts of the swelling *s* and *s* receiving no light whatever. An observer looking down the microscope in the direction of the arrow *o* should, therefore, theoretically, perceive the upper wall of the swelling brightly lighted between *c* and *d* and black between *a* and *c* and between *b* and *d*. The lighted and unlighted portions of the wall are shown projected above, the dark regions being represented by *e–g* and *h–f* and the illuminated one by *g–h*. When actual observations were made with a beam of parallel light rays, it was found that the dark regions of the wall were as projected at *i–k* and *l–j* and the illuminated one as projected at *k–l*. The theoretical results shown in the line *e–f* agree very well with the practical results shown in the line *i–j*. This agreement justifies one of the assumptions underlying the construction of Figs. 46, 47, and 59, namely, that the refractive index for light in passing from air to cell-sap is approximately 1·34.

the median cross-section had a width g–h (corresponding to c–d in the constructional diagram below) and the lateral dark parts had widths of e–g (corresponding to a–c) and h–f (corresponding to d–b). It will be seen by inspection that the *actual* illuminated central part of the cross-section, namely, k-l, is approximately equal to the *calculated*, namely, g–h ; and this equality may be taken as justifying the assumption that the refractive index from air into cell-sap is approximately the same as that from air into water, namely, 1·34.

The subsporangial swelling has the optical properties of a biconvex lens. When sunlight strikes upon one side of it, the rays are refracted through it and converge so as to form a spot of light on the opposite side. The spot can be seen under the microscope in living fruit-bodies which are unilaterally illuminated and its mode of formation can readily be inferred from a study of the diagrams reproduced in Figs. 46, 47, and 48.

When the incident rays of light strike the sporangium and subsporangial swelling head on and are exactly parallel to the long axis of the swelling as shown in Fig. 46, the spot of light which is formed by the rays entering that part of the swelling which bulges out beyond and around the sporangium is symmetrically placed at the base of the swelling. Under these conditions the sporangiophore is in physiological equilibrium and no heliotropic reaction is possible. When, however, the incident rays of light strike a sporangium and subsporangial swelling obliquely, say at an angle of 19° as shown in Fig. 47, the spot of light is formed on one side of the wall of the swelling in a manner which is asymmetrical for the swelling as a whole. Under these conditions the sporangiophore is in physiological inequilibrium, in consequence of which it reacts heliotropically : the top of the stipe bends toward the source of the light and swings the subsporangial swelling and sporangium round until they face the light head on.

The heliotropic reaction just described may be explained as follows. The patch of protoplasm which is strongly illuminated by the spot of light (*st* in Fig. 47) undergoes a photochemical change, in consequence of which it sends out a stimulus which travels down the protoplasm lining the wall of the base of the subsporangial swelling to the protoplasm surrounding the top of the stipe. The

stimulus may be nothing more or less than a diffusible growth-promoting substance ; but, whatever it is, in response to it the top of the stipe (*m* in Fig. 47), which is the motor region of the sporangiophore, reacts by growing in length and by growing in length most rapidly on the side which is nearest to the spot of light, *i.e.* on the side which receives the strongest stimulus. As a result of this differential growth-reaction of the stipe, the sub-sporangial swelling is turned about its base through an angle, and the spot of light gradually passes downwards on the wall of the swelling until it comes to be symmetrically placed at the base of the swelling, as shown in Fig. 46. As soon as the spot of light reaches this symmetrical position, a physiological state of equilibrium becomes established in the sporangiophore and a further heliotropic reaction of the stipe is impossible. At the end of the turning movement the Pilobolus gun is directed toward the source of the brightest light.

In support of the explanation of the heliotropic reaction of the sporangiophore just given, which involves the passage of a stimulus from the rigid subsporangial swelling to the plastic motor region of the stipe, may be cited the fact (1) that, under conditions like those represented in Fig. 47, the motor region of the stipe, *m*, when the heliotropic reaction begins, is in a shadow, *s*, and receives no direct rays of light whatever, and (2) that the most strongly illuminated protoplasm is the patch shown at *st* which receives the light concentrated upon it by the body of the subsporangial swelling which acts as a lens.

We may now enquire to what degree, if any, the light falling on the stipe acts as a stimulus which assists in bringing about the heliotropic reaction of the stipe. It is true that in such a tilted fruit-body as that shown in Fig. 47 the rays of light falling on the stipe (rays 9–14) converge so as to form a bright band of light along the middle of the back of the stipe ; but, as shown in Fig. 47, the highest ray strikes the stipe at *u* some distance below the motor region *m* ; and, as may be inferred by comparing Fig. 47 with Fig. 46, as heliotropic bending takes place and becomes more and more complete, the subsporangial swelling shades the upper part of the stipe to a greater and greater degree from the direct rays of the sun,

so that during the last stages of the bending, in such a fruit-body as that shown in Fig. 46, the upper part of the stipe for some distance back from the motor region receives no direct rays of light whatever. It is further to be emphasised that, as the heliotropic reaction of the sporangiophore becomes more and more complete, whereas the spot of light in the subsporangial swelling approaches nearer and nearer to the motor region of the stipe until finally it comes to rest upon the annular heap of protoplasm just above it, the band of light in the stipe recedes more and more from the motor region.

The facts brought forward in the above discussion justify the conclusion that the heliotropic reaction of the sporangiophore of Pilobolus, at least in its final stages, is not due to the action of the light which falls on the motor region or any other part of the stipe but to the action of the light which falls on the subsporangial swelling and, in particular, to the action of the light which forms an asymmetrically situated light-spot on the protoplasm lining the cell-wall of the swelling.

In *Pilobolus Kleinii* the protoplasm in the lower part of the subsporangial swelling and at the top of the stipe, as shown in Figs. 46 and 47 by shading, is strongly pigmented with carotin ; and, as we have seen, at the top of the stipe just above the stipe's motor region, the protoplasm is usually heaped up so as to form a strongly biconcave, very red, centrally perforated septum. Direct observations with the microscope and a study of the course of the rays of light represented in Fig. 46 make it clear that the protoplasmic septum, owing to its peculiar shape and to its distance from the top of the subsporangial swelling, is admirably adapted for receiving the rays of light as they begin to diverge from one another after coming to a focus in the cell-sap when the sporangiophore is in a position of complete or almost complete physiological equilibrium. From the point of view of general structure and function, the layer of protoplasm lining the lower part of the subsporangial swelling and forming the incomplete septum at the top of the stipe—in its concave shape, its strong pigmentation, its position in respect to the subsporangial lens, and its mode of functioning by sending a stimulus to the motor region of the stipe—is comparable with the

retina, while the pear-shaped body of the subsporangial swelling is comparable with the *lens*, of the eye of certain Mollusca.   In this connexion one may compare Fig. 46 which shows a median vertical section through a subsporangial swelling and stipe of *Pilobolus Kleinii* with Fig. 49 which shows a median vertical section through the ocellus of a Snail, *Helix pomatia*.   Since the subsporangial swelling is so like the ocellus of a Mollusc and is used as an organ for detecting the direction of the incident rays of light, there seems no reason why we should not regard it as a very simple eye which functions like an ocellus.

The fact that the photochemically sensitive protoplasm in the basal part of the subsporangial swelling and at the top of the stipe is so rich in carotin and that this pigment is so densely aggregated in the upper surface layer of the perforate protoplasmic septum (Fig. 46, *p*) suggests that the carotin absorbs light and thereby plays an important part in the heliotropic reaction of the sporangiophore.   Zopf came to the conclusion that the carotin of Pilobolus is simply a reserve food material, but this explanation does not account for the aggregation of the carotin particles around the base of the subsporangial swelling and at the top of the stipe nor for the fact that the pigment

FIG. 49.—The eye of a snail, *Helix pomatia*, retracted : *ep*, epidermis ; *c*, cornea ; *l*, lens ; *r*, retina ; *op. n.*, optic nerve. For comparison with the subsporangial swelling of Pilobolus. From Vol. III of the *Cambridge   Natural History*. By courtesy of Macmillan and Co.

persists in this position until the Pilobolus gun is discharged. When discharge of the gun takes place in water on a slide under a cover-glass, the ring-like mass of orange-red protoplasm at the top of the stipe is often shot out through the mouth of the subsporangial swelling, and I have several times identified it as it lay some distance from the mouth of the sporangiophore not merely by its form but by its high content of carotin (Fig. 65, D–F, p. 137).

The diameters of the sporangium, the subsporangial swelling, and the motor region of the stipe below the swelling in a typical large fruit-body of *Pilobolus Kleinii* (the one shown in Fig. 46) were

observed to be 0·43 mm., 0·76 mm., and 0·16 mm. respectively. A simple calculation based on these data shows that the sporangium, when head on to a beam of parallel light rays, casts a shadow which has an area 7·2 times that of a cross-section of the stipe.   If there were no subsporangial swelling, the shadow of the sporangium would cut off the light from the top of the stipe before the ortho-heliotropic position had been completely attained.   This would prevent the gun from being accurately directed toward the source of the strongest light.   Evidently, the difficulty of supplying the stipe with the delicate heliotropic stimulus which it requires has been overcome in the course of evolution by the intercalation, between the sporangium and the stipe, of the large light-collecting subsporangial swelling.   That part of the swelling which bulges out laterally beyond the black sporangium receives the light and, by refraction, concentrates it upon the base of the swelling.   The asymmetrical position of the spot of light so produced provides the condition for the despatch of a stimulus from the swelling to the motor region of the stipe, and to this stimulus the motor region of the stipe can react with great precision, even when it lies in the shadow of the sporangium and subsporangial swelling and is receiving, as shown in Fig. 47, no direct rays of light whatever.

The spot of light which is formed within the subsporangial swelling by sunlight and is of so much physiological importance for the heliotropic reaction of the sporangiophore is not constant in size, shape, and brightness, but varies in all these qualities with the position in which it happens to be formed.   A study of this variability will now be attempted.

When the incident rays of light strike the subsporangial swelling transversely, *i.e.* perpendicularly to its long axis, the spot of light formed by the convergence of the rays on the back wall of the swelling is oval in outline, of maximum size, and of minimum brightness (Figs. 50, A, and 51, p. 105).   Its oval shape is due to the fact that the main mass of the swelling is oval like an egg, its relatively large size to the fact that the swelling is not nearly so wide as it is long, and its paleness to the fact that the rays of light fall on the back wall of the swelling at a considerable distance in front of their focal point.   As may be seen by reference to Figs. 48

(p. 95) and 51 (p. 105), the width of the oval spot of light is about equal to one-third of the width of the swelling.

When the incident rays of light strike the sporangium and the subsporangial swelling head on and are exactly parallel to the long axis of the swelling as shown in Fig. 46 (p. 91), the spot of light rests symmetrically on the perforate protoplasmic septum at the top of the stipe, is round in outline, relatively small, and of maximum brightness (Fig. 50, C and D). Its roundness is due to the fact that the top of the swelling through which the rays are refracted is round. Its relatively small size and its brightness are due to the fact that the rays of light reach their focal point and begin to diverge just above the protoplasmic septum on which the spot of light is formed. A study of the course of the rays of light represented in Fig. 46 shows that at the level p in the particular fruit-body drawn no direct rays pass through the main central portion of the perforation of the septum, so that a spot of light formed on a transverse surface placed at the level of p would have a small dark centre. It is of interest to note that the length of the subsporangial swelling is equal to, or only just exceeds, its focal length, with the result that the rays of light diverge before they reach the

Fig. 50.—*Pilobolus Kleinii.* Variation in shape of a spot of light produced by a beam of parallel light rays in a subsporangial swelling. Light in A transverse to axis (*cf.* Fig. 51), in B oblique (*cf.* Fig. 47), and in C and D vertical (*cf.* Fig. 46). D, whole of underside of swelling, stipe represented by dotted circle. Magnification, 38.

perforate protoplasmic septum and so strike the concave surface of the septum almost perpendicularly, *i.e.* in the most effective manner for acting on the protoplasm and causing it to undergo a photochemical change. The fact that the subsporangial swelling is shaped like a pear is, therefore, from an optical point of view, highly significant.

For the further discussion of the nature of the spot of light formed at the base of the subsporangial swelling of *Pilobolus*

Kleinii, it is necessary to take into account the laws relating to the reflection of light at the surface of a transparent medium. When a ray of light strikes the surface of a transparent medium such as water, glass, or the cell-wall bounding the subsporangial swelling of Pilobolus, part of it is reflected from the medium and part of it is refracted into the medium. The percentage of light reflected varies with the refractive index from air into the medium and with the angle of incidence. The higher the refractive index, the greater the percentage of light reflected. Also the greater the angle of incidence, the greater the percentage of light reflected. The percentage of light reflected for various angles of incidence at the surface of a transparent medium when the refractive index from air into the medium is 1·55 is given in Table I.[1]

TABLE I

Light reflected at the Surface of a Transparent Medium when the Index of Refraction is 1·55

Angle of Incidence	Percentage of Light reflected	Angle of Incidence	Percentage of Light reflected
0°	4·65	60°	9·73
5°	4·65	65°	12·91
10°	4·66	70°	18·00
15°	4·66	75°	26·19
20°	4·68	80°	39·54
25°	4·73	82° 30′	49·22
30°	4·82	85°	61·77
35°	4·98	86°	67·82
40°	5·26	87°	74·56
45°	5·73	88°	82·10
50°	6·50	89°	90·54
55°	7·74	90°	100·00

[1] F. E. Fowle, *Smithsonian Physical Tables*, Ed. 5, 1910, Washington, p. 191. In the original Table the percentage of light reflected is set down as $\frac{1}{2}$ (A + B). According to Fresnel the amount of light reflected at the surface of a transparent

medium $= \frac{1}{2}$ (A + B) $= \frac{1}{2} \left\{ \frac{\sin^2 (i - r)}{\sin^2 (i + r)} + \frac{\tan^2 (i - r)}{\tan^2 (i + r)} \right\}$ where A is the amount of

light polarised in the plane of incidence, B that polarised perpendicular to this, i the angle of incidence, and r the angle of refraction.

Since the refractive index for light passing from air into the cell-wall of Pilobolus, namely, 1·5, is approximately equal to the refractive index from air into a transparent medium given in Table I, namely, 1·55, we may apply the data embodied in Table I in our study of Pilobolus.

The angle of incidence for each of the six rays, Nos. 1–6, represented as striking the subsporangial swelling in Fig. 46 (p. 91) was carefully measured, and then the percentage of light reflected from each ray at the surface of the swelling was estimated approximately from the data given in Table I, with the result shown in Table II.

TABLE II

Light reflected at the Surface of the Subsporangial Swelling of Pilobolus

Ray shown in Fig. 46	Angle of Incidence	Percentage of Light reflected
No. 1	40° 30′	5·5
No. 2	46° 45′	6
No. 3	55° 45′	8
No. 4	67° 30′	15
No. 5	75° 30′	27
No. 6	90°	100

The greater the percentage of the light reflected, the feebler will be any ray after refraction. From the data embodied in Table II, therefore, we may draw the conclusion that the rays refracted through the subsporangial swelling shown in Fig. 46 diminish in brightness in order from No. 1 to No. 6, the brightest ray being No. 1, and the dullest ray being No. 6 (theoretically this ray is not refracted at all since its angle of incidence is not less than 90°). Since, as shown in Fig. 46, the refracted rays diverge before impinging upon the basal protoplasm, it is evident that the brightest part of the spot of light produced at the base of the subsporangial swelling is centrally situated and rests on the inner edge of the protoplasmic septum ; and that the brightness of the spot of light diminishes centrifugally. The protoplasmic septum protrudes so far into the vacuole that it is just able to catch most, or

possibly all, of the strongest rays of light which come to it through the subsporangial swelling. Assuming that the pigmented proto- plasm of which the septum is composed is photochemically sensitive, this arrangement provides us with another beautiful example of the way in which structure and function are correlated.

When bright sunlight strikes a Pilobolus fruit-body head on and forms a concentrated spot of light at the base of the sub- sporangial swelling in the manner shown in Fig. 46, the protoplasmic septum, owing to its high content of carotin particles, glows with a rich orange-yellow light. The glow can be readily seen with the naked eye when a fruit-body which faces the sun is viewed from the side.

If, when a fruit-body is facing the sun as shown in Fig. 46, one looks at the top of the fruit-body so as to see it almost in face view, one observes that the fruit-body has the appearance of a *lurid red disc with a black spot in the centre*. The black spot is formed by the intensely black sporangium and the red zone which surrounds it is formed by the subsporangial swelling at the surface of which red light, emitted by the glowing carotin in the protoplasmic septum and passing upwards through the swelling's great vacuole, is refracted to the eye. At first sight such an apical view of a Pilobolus fruit- body as that just described reminds one of the pink eyes of certain albino animals.

We have seen that, when the incident rays of light strike the subsporangial swelling *transversely*, the spot of light is oval; and that, when they strike the sporangium and the subsporangial swelling *head on* and are parallel to the long axis of the swelling, the spot of light is round. It remains to add that, when the incident rays of light strike the subsporangial swelling *obliquely*, say at an angle of 19° as shown in Fig. 47 (p. 92), the spot of light formed on the back wall of the swelling is neither oval nor round but, owing to the shadow cast by the black sporangium, convexo-concave or crescentic with the two horns looking upwards toward the sporangium (Fig. 50, B, p. 101). Such a convexo-concave spot of light can be perceived when one looks down a microscope at a fruit-body which is directed obliquely downwards in the air and is illuminated from below with parallel rays coming from a plane mirror.

The subsporangial swelling is capable of producing not only a spot of light but also a more or less well-defined *image of the source of light or of an illuminated object.* Such images were observed under conditions which will now be described.

A fruit-body of *Pilobolus Kleinii* was fixed in air over a slide on the stage of a microscope, so that the long axis of the subspor-angial swelling was horizontal and so that the swelling was illuminated from below by means of parallel rays reflected upwards from a plane mirror. On looking down at the swelling, I observed on its upper wall an oval spot of light like that already described. When the microscope was placed at a distance of fourteen feet from a window, the spot of light was found to contain an image of the window-bars ; and, when a hand was held three feet in front of the microscope between the mirror and the window, the spot of light was found to contain a black image of the hand.

Subsequently, images formed by the sub-sporangial swelling of *Pilobolus longipes* were photographed, and two of the photographs are reproduced in Figs. 51 and 52.

Fig. 51.—*Pilobolus longi-pes.* Photomicro-graph of the image of a hand. *Vide* text. Magnification, 40.

Fig. 51 shows a daylight shadow-photo-graph of a hand. The microscope was pre-pared by removing the condenser and turning on the plane mirror. The fruit-body to be used to form the image, together with a little dung on which it was growing, was taken from a culture dish and set in a closed com-pressor cell (*cf.* Fig. 14, Vol. II, p. 45) so that its apical portion projected freely in the air in a horizontal direction. The moist air of the chamber prevented the fruit-body from drying up. The com-pressor cell was placed on the stage of the microscope. Daylight from a window was employed. Parallel rays were reflected upwards from the flat mirror to the subsporangial swelling of the fruit-body in the compressor cell. The hand was held one foot in front of the

mirror of the microscope and the image of its shadow as produced on the wall of the upper side of the subsporangial swelling was then focussed with the low power (ocular No. 10, objective No. 16 with the end-lens removed, Bausch and Lomb system). The exposure of the plate was for twenty seconds and the magnification of the fruit-body on the plate was forty diameters. In the photomicrograph the oval spot of light and the image of the shadow of four fingers and part of the thumb of the hand, which could be seen with the eye on the upper wall of the subsporangial swelling, are clearly visible.

In Fig. 52 is shown a photograph of a white card bearing a black printed letter A. The card was 4·1 cm. long and 3·5 cm. wide, and the letter A was 2·5 cm. long. The condenser and the mirror were removed from the microscope and the card was placed on the dark table within the horse-shoe base of the microscope, so that it was visible when the eye looked down the microscope tube. The card was illuminated by direct sunlight. The fruit-body, as before, was contained in a compressor cell set on the stage of the microscope. The exposure of the plate was for a few seconds and the magnification of the fruit-body on the plate was about thirty diameters (ocular No. 5, objective No. 16 with the end-lens removed, Bausch and Lomb system). In the photomicrograph the image of the card with its letter A, which could be seen with the eye on the upper wall of the subsporangial swelling, is clearly visible ; and, again, we have conclusive evidence that the subsporangial swelling can act like a lens.

Fig. 52.—*Pilobolus longipes.* Image of letter A. *Vide* text. Magnification, about 30.

Although, like other more or less globular lenses, the subsporangial swelling of Pilobolus is able to form images of objects—albeit rather imperfect ones—it must not be supposed that Pilobolus can see like an animal. The simple eye or ocellus of Pilobolus is specially adapted not for the perception of images but for the mechanical perception of the direction of the strongest incident rays of light by means of photochemical reactions.

According to the modern conception of heliotropism, for which Blaauw [1] is chiefly responsible, an organ, such as the sporangiophore of *Phycomyces nitens* or the coleoptile of *Avena sativa*, turns toward or turns away from a source of light because its sides are at first unequally lighted and because, as a result of this unequal illumination, one of the sides grows more rapidly than the other until the illumination of the two sides has become equalised. The turning of an organ until it points toward a source of light is therefore in itself a secondary phenomenon which results from unequal light-growth reactions.

The explanation of the heliotropism of Pilobolus which I have already given is in reality but an extension of Blaauw's general theory of heliotropism to the special case of a fungus provided with an ocellus. In *Phycomyces nitens*, so thoroughly investigated by Blaauw, the sporangiophore is a simple cylinder and therefore relatively undifferentiated, and its upper part, when unequally lighted, is the part which reacts by unequal growth. In Pilobolus, on the other hand, the sporangiophore is differentiated into a subsporangial swelling and a stipe, and there is a division of labour between these two structures of such a kind that, when the sporangiophore is bending heliotropically, the subsporangial swelling receives the rays of light and becomes unequally illuminated but does not alter in form, while the top of the stipe, which lies below the swelling and may or may not be illuminated, reacts to the unequal lighting of the subsporangial swelling by unequal growth. In the sporangiophore of *Phycomyces nitens* one and the same part both receives the light and responds to it, whereas in that of Pilobolus one part receives the light but another part responds to it.

That the decisive condition for heliotropic response in *Phycomyces nitens* is not the direction of the rays of light but unequal illumination is shown by the results of a beautiful experiment made by Buder.[2]

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[1] A. H. Blaauw, " Licht und Wachstum," I and II in *Zeitschrift für Botanik*, Jahrg. VI, 1914, and VII, 1915 ; III (" Die Erklärung des Phototropismus ") in part XV of *Mededeelingen van de Landbouwhoogeschool*, Wageningen, 1918.

[2] J. Buder, " Die Inversion des Phototropismus bei Phycomyces," *Ber. d. D. Bot. Gesellschaft*, Bd. XXXVI, 1918, pp. 104–105. Some other ingenious experiments made by Buder demonstrate that it is not the direction of the light but unequal lighting that is responsible for the heliotropism of the coleoptile of Avena. *Ibid.*, Bd. XXXVIII, 1920, pp. 14–19.

Buder placed *P. nitens* in two parallel-sided chambers with the sporangiophores directed vertically upwards. In one chamber the sporangiophores were, as usual, surrounded by air. The other chamber
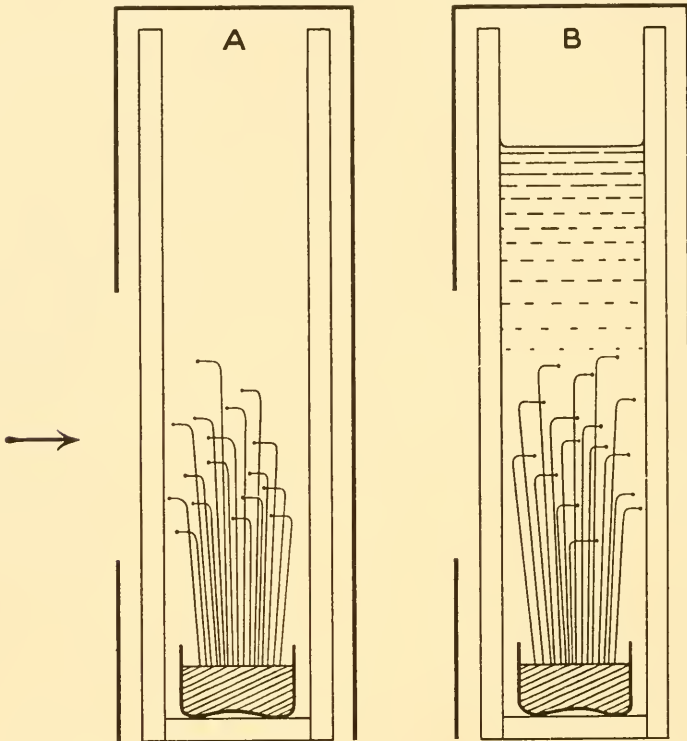
Fig. 53.—*Phycomyces nitens.* Diagrams to illustrate two comparative experiments on heliotropism. The arrow indicates the direction of the incident rays of light. In A the sporangiophores developed in the *air* and have turned *toward* the source of light. In B the sporangiophores developed in *paraffinum liquidum* (paraffin oil) and have turned *away from* the source of light. After J. Buder (*Ber. d. D. Bot. Gesell.*, Bd. XXXVI, 1918, p. 104). Re-drawn by the author.

was filled with *paraffinum liquidum* so that the sporangiophores were immersed in it. Both chambers were then illuminated on one side with equal intensity, with the result that the sporangiophores which were in the air turned *toward* the light, while those which were in the paraffin turned *away* from the light (Fig. 53). The explanation of this difference in reaction is simple. In a

sporangiophore growing in air, since air is a less dense medium than a sporangiophore and its contents, the rays of light after refraction *converge* and form a bright band of light on the side turned away from the light. Hence the back of the growing zone of the sporangiophore is lighted more intensely than the front and consequently grows faster, with the result that the end of the sporangiophore is turned toward the light. In a sporangiophore growing in *paraffinum liquidum*, since paraffin (refractive index 1·47) is a denser medium than a sporangiophore and its contents, the rays of light after refraction *diverge* from one another and, therefore, do not form a bright band on the side turned away from the light. Hence the front of the growing zone of the sporangiophore is lighted more intensely than the back and consequently grows faster, with the result that the end of the sporangiophore is turned away from the light. In a letter [1] to the author, Buder states that he has experimented with Pilobolus and has found that it reacts to unilateral light in air and in paraffin in exactly the same way as Phycomyces. We thus have evidence which supports the view that the heliotropism of Pilobolus is due to light-growth reactions and not primarily to the direction of the incident rays of light.

Assuming that a Pilobolus sporangiophore, when growing in paraffin, turns away from the light, instead of toward the light as it does when it is growing in air, the explanation of the phenomenon, presumably, is as follows. The subsporangial swelling, when immersed in paraffin, owing to the divergence of the light rays after refraction, cannot form a spot of light on its wall away from the source of light. Hence the side of the swelling toward the light is more intensely illuminated than the side away from the light. Hence the stimulus sent to the stipe from the front of the subsporangial swelling is greater than that sent from the back. Hence, therefore, the stipe grows faster in front than behind and so turns the subsporangial swelling and the sporangium away from the source of the light.

The Mechanism of Heliotropic Response in Pilobolus and in the Leaves of Certain Flowering Plants.—The mechanism of heliotropic response in Pilobolus is not necessarily unique in the plant world;

[1] Dated Sept. 12, 1921.

for, in general principle, it may be similar to that of the leaves of many flowering plants.

As is well known, there are many shade-plants of which the laminae are in a condition of heliotropic equilibrium only when they are placed at right angles to the direction of the most intense diffuse illumination. These diaheliotropic leaves assume their heliotropically fixed positions by means of appropriate curvatures or torsions of the whole petiole or of a pulvinoid portion of the petiole or of a typically developed pulvinus. It was suggested by Dutrochet [1] in 1837 that the leaf-blade of the leaves in question perceives the direction of the light and exerts a directive influence upon the petiole ; and Vöchting,[2] in 1888, by means of carefully thought-out experiments, proved that this is so for *Malva verticillata*. Haberlandt,[3] in 1905, succeeded in demonstrating a similar directive influence of the lamina in several other plants. Thus he found that : in *Begonia discolor* the petiole, even when completely darkened by a sheath of tin-foil, turns the lamina into its heliotropically fixed position ; and that in *Monstera deliciosa* the upper region of the petiole, which is developed as a pulvinus, even when completely darkened by a sheath of tin-foil, executes the curvature or the torsion necessary to restore the lamina to the position of heliotropic equilibrium with the greatest possible precision.

To explain the diaheliotropism of the leaves of shade-plants, etc., Haberlandt [4] has put forward his *ocellar theory*, according to which : (1) the power of perceiving photic stimuli is vested in the upper epidermis ; (2) the epidermis sends a stimulus to the petiole ; (3) the stimulus causes the petiole or its pulvinus to curve or twist until the lamina comes to be at right angles to the direction of the strongest incident rays of light ; and (4) each cell of the epidermis acts as a light-perceiving organ or ocellus and is only in a position

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[1] H. J. Dutrochet, *Mémoires pour servir à l'histoire anatomique et physiologique des Végétaux et des Animaux*, Paris, 1837, T. II, p. 107.

[2] H. Vöchting, " Ueber die Lichtstellung der Laubblätter," *Botanische Zeitung*, Jahrg. XLVI, 1888, pp. 519–523. Vöchting showed that the lamina, when obliquely illuminated, forces the petiole to execute movements, when the petiole is kept in the dark or, if it is lighted, in opposition to its own heliotropic tendencies.

[3] G. Haberlandt, *Die Lichtsinnesorgane der Laubblätter*, Leipzig, 1905, pp. 17–19.

[4] G. Haberlandt, *ibid.*, pp. 120–122 ; also *Physiological Plant Anatomy*, translated by M. Drummond, London, 1914, pp. 613–630.

of photic equilibrium when the protoplasm lining its inner wall, which is sensitive to photic stimuli, is symmetrically illuminated with the brightest light at its centre.   Haberlandt showed that the epidermal cells of the leaves of many plants have a more or less rounded or convex external wall and act as lenses, so that the rays of light which enter them are refracted and condensed and form a bright spot of light on their inner wall.

My own ocellar theory of the heliotropic mechanism of the sporangiophore of Pilobolus is essentially similar to Haberlandt's ocellar theory for the heliotropic mechanism of certain leaves.   The lens-like subsporangial swelling of Pilobolus corresponds in a leaf to the thousands of lens-like epidermal cells ;   and the stipe of Pilobolus, which receives a stimulus from the photically sensitive subsporangial swelling in consequence of which it bends the swelling around until the spot of light within the swelling is symmetrically situated at its base, corresponds in a leaf to the multicellular petiole, which receives a collective stimulus from the thousands of photically sensitive epidermal cells in consequence of which it bends or twists until the thousands of spots of light in the epidermal cells, on the average, have come to be symmetrically situated on their basal walls.

In the heliotropic reaction of Pilobolus, which is caused by the asymmetrical position of a spot of light in the subsporangial swelling with a consequent bending of the stipe, we have clear proof of the theory, first suggested by Haberlandt, that heliotropic reactions may take place in plants through ocellus action.

The sporangiophore of Pilobolus is the only ortho-heliotropic plant organ known which takes up its positively heliotropic position owing to the possession of a special light-perceiving cell structure.

Pilobolus may well be described as a fungus with an optical sense organ or simple eye (ocellus) ;   and, in using its eye for laying its gun, it is unique in the plant world.

The Ocellus of Pilobolus and the Eye-spots of Volvox.—As is well known, red eye-spots are present in many Protozoa, *e.g.* Euglena, and in the Volvocaceae, *e.g.* Gonium, Pandorina, Pleodorina, Eudorina, and Volvox ;   and it is therefore not without interest to enquire whether or not the ocellus of Pilobolus and the eye-spots of these other organisms work on the same principle.   According

to Mast,[1] the eye-spot in the zooid of a Gonium or a Volvox (Fig. 54) consists of three parts : a pigment-cup, a lens which is situated at the opening of the cup, and a photosensitive substance which is located within the cup. Mast [2] regards these eye-spots as " primitive eyes " and, in connexion with Volvox, explains their action as follows.[3] Volvox swims in the direction of its axis with its anterior end forwards.[4] Each of the zooids of which it is composed has an
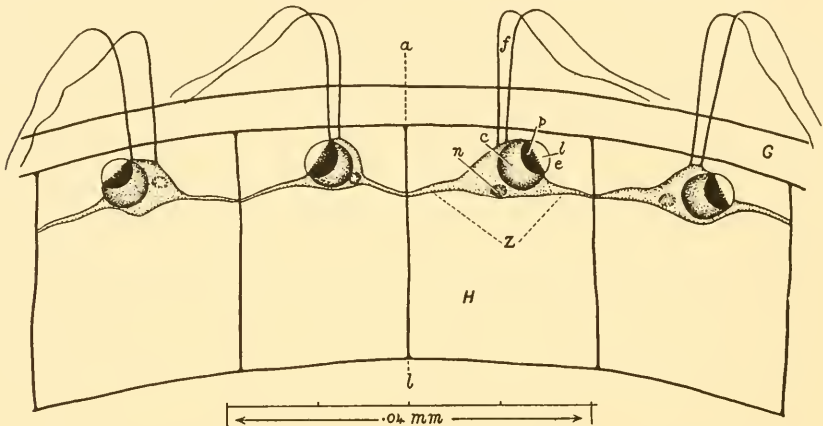
Fig. 54.—*Camera-lucida* drawing of an optical section through the longitudinal axis of a colony of Volvox : *l–a*, longitudinal axis ; G, gelatinous layer surrounding the colony ; H, hyaline portion of cell ; Z, zooid ; *n*, nucleus ; *f*, flagella ; *c*, chloroplast ; *e*, eye ; *p*, pigment-cup ; *l*, lens ; *mm*, scale. Note that the eyes on the two sides of the longitudinal axis face in opposite directions. Copied by the author from Fig. 6 of Mast's *Reactions to Light in Volvox with Special Reference to the Process of Orientation*. In the original Figure the chloroplasts are coloured green, and the pigment-cups red. Reduced to three-quarters.

eye-spot and two cilia. The eye-spots are largest at the anterior end of the colony (about 3 μ in diameter) and decrease in size from that end backwards so that, at the posterior end, they are so poorly differentiated as to be scarcely visible. In the eye-spots at the anterior end of the colony the lens is directed away from the axis of the colony and slightly forwards (Fig. 54). The cilia of all the

[1] S. O. Mast, " Reactions to Light in Volvox with Special Reference to the Process of Orientation," *Zeitschrift für vergleichende Physiologie*, Bd. IV, Heft V, 1926, pp. 648–649, 657.

[2] *Ibid.*, pp. 652, 657.   [3] *Ibid.*, pp. 643–657.

[4] This statement applies only to photopositive colonies. There are also photonegative colonies which swim away from the light.

zooids beat backwards obliquely, so that the colony progresses forwards and, at the same time, rotates in a counter-clockwise direction. When a colony which is swimming toward the light with its anterior end facing the light is suddenly subjected to lateral illumination only, in the zooids on the side of the colony facing the light the hyaline portion of each eye is fully exposed to the light, while in the zooids on the side of the colony facing away from the light the hyaline portion of each eye is well-shaded by the pigment-cup, with the result that the flagella of the zooids with shaded eyes beat backwards more directly than before while the flagella of the zooids with unshaded eyes beat backwards less directly than before (Fig. 55). Owing to this change in the direction of the stroke of the flagella on the two sides of the colony, the colony gradually turns its axis until this again becomes parallel to the incident rays of light and the anterior end of the colony faces the light directly (Fig. 55). When the colony is in photic equilibrium, the incident light falls on the eye-spots of any one lateral half of the colony in the same way and with the same intensity as upon the eye-spots of the corresponding other lateral half of the colony.

My own investigations on Pilobolus and those of Mast on Volvox thus seem to show in both these plants—the one a lowly and sedentary fungus and the other a highly organised motile alga—that photic orientation is accomplished by means of optical sense organs, and that re-orientation is due in Pilobolus to the asymmetrical illumination of a single eye and in Volvox to the combined effect of the asymmetrical illumination of many eyes.[1]

There is a red pigment in the eyes of both Pilobolus and Volvox; but, whereas that of Pilobolus is situated in the sensitive protoplasm and is relatively diffuse, that of Volvox is situated below the sensitive protoplasm and is aggregated into a dense and opaque cup-like

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[1] Pilobolus and Volvox may be compared to those mythical monsters of antiquity Cyclops and Argus: for Cyclops, like Pilobolus, looked out on the world with a single median eye; while Argus, like Volvox, had many eyes. It is said that Argus had one hundred eyes some of which were always open and on the watch, and that they were so splendid that, when he was killed, Juno transferred them to the Peacock's tail; but a *Volvox globator*, although not mentioned in the annals of Olympus, may have more than twenty thousand eyes which never close and are ever ready for service.
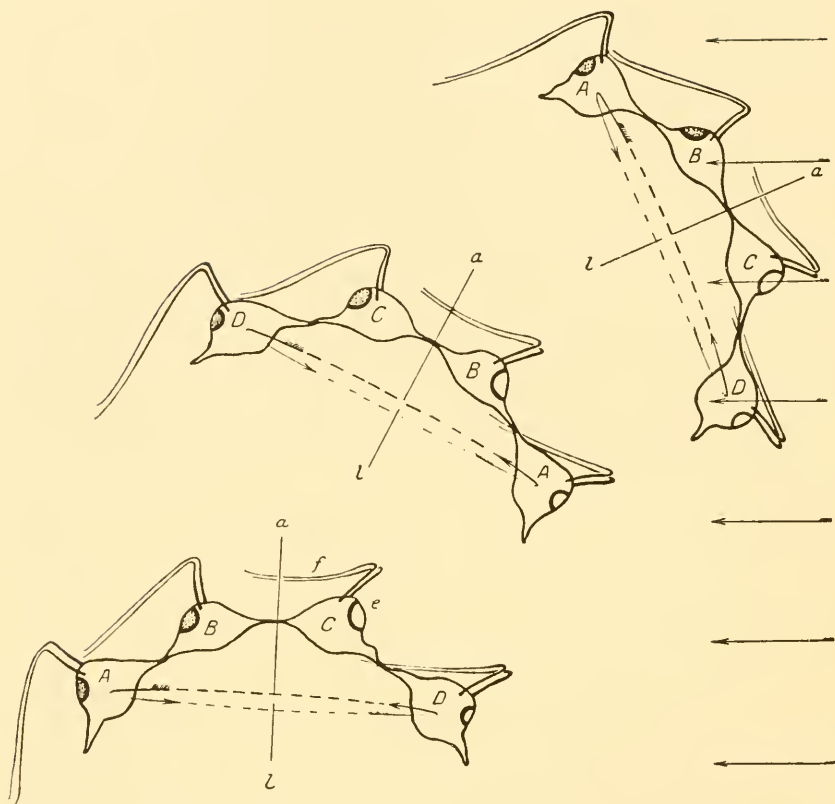
Fig. 55.—Diagrammatic representation of orientation during a positively heliotactic response in Volvox. A, B, C, and D, four zooids at the anterior end of the colony; *l–a*, longitudinal axis; large arrows, direction of illumination; curved arrows, direction of rotation; the colony is moving forward and turning so as to face the light with its anterior end; *f*, flagella; *e*, eyes, containing a pigment-cup (represented by a heavy black line) and photosensitive tissue in the concavity of the cup. Note that, when the colony is laterally illuminated, the photo-sensitive tissue in the eyes on the side facing the light is fully exposed to the light and the flagella on this side beat laterally, while the photosensitive tissue in the eyes on the opposite side is shaded by the pigment-cup and the flagella on this side beat directly backwards. The difference in the direction of the beat of the flagella on these two sides is due to alternate decrease and increase in the luminous intensity to which the photosensitive tissue in the eyes is exposed owing to the rotation of the colony on its longitudinal axis, an increase causing, in photopositive colonies, a change in the direction of the stroke of the flagella from backward or diagonal to lateral, and a decrease a change from lateral or diagonal to backward. In photonegative colonies precisely the opposite obtains. In photopositive colonies this results in turning toward and in photonegative colonies in turning from the source of light. In both the turning continues until opposite sides are equally illuminated, when changes in intensity on the photosensitive tissue are no longer produced by rotation and the orienting stimulus ceases. Reproduced from Fig. 7 of Mast's *Reactions to Light in Volvox with Special Reference to the Process of Orientation.*

mass. In Pilobolus the absorption of light by the red pigment may possibly increase the photosensitivity of the protoplasm in which it lies, while in Volvox it seems clear that the red pigment functions by shading the sensitive protoplasm of those eyes which happen temporarily to be turned away from the light.

The Ocellus of Pilobolus and the Human Eye.—The simple ocellus of a Pilobolus and the complex eye of a human being, in their heliotropic response to light, behave in essentially the same manner ; for they are both in photic equilibrium only when the spot of light formed in their interior by a luminous object, such as a candle flame, is symmetrically placed in the middle of their back wall or retina. If a man and a Pilobolus fruit-body were to be kept for some time in a dark room and then a candle flame were to be suddenly exposed in one corner of it : the man, immediately and instinctively, would turn his head until he faced the candle flame directly and, in so doing, he would unconsciously place the spot of light in each eye in the middle of the retina, *i.e.* in the region of the blind-spot ; while the Pilobolus fruit-body, very slowly (in the course of an hour or so), would turn its sporangium and sub-sporangial swelling until they faced the light directly and, in so doing, it would automatically place the spot of light in the subsporangial swelling in the middle of the red protoplasm at the back of the swelling, *i.e.* so that the centre of the spot of light would rest on the centre of the protoplasmic septum at the top of the stipe ; and the movement of the Pilobolus fruit-body, accomplished simply by the unequal growth of the stipe, and the movement of the human being, accomplished by means of a highly complex nerve-brain-nerve-muscle mechanism, would both be caused by the same physiological condition, namely, the asymmetry of a spot of light at the back of a photically sensitive optical organ or eye. Thus, in respect to their behaviour in the presence of incident rays of light, the lowly cryptogam Pilobolus and Man, the lord and master of the organic world, have much in common.

A Heliotropic Experiment made on Pilobolus longipes.—The heliotropic experiment about to be described was made on a fruit-body of *Pilobolus longipes* for the following purposes : (1) to determine how long it takes for a fruit-body which is illuminated by direct

sunlight to bend heliotropically through a right angle ; (2) to determine the length of the latent period, *i.e.* the time which elapses from the beginning of continuous heliotropic stimulation to the beginning of heliotropic reaction as indicated by the curvature of the sporangiophore ; and (3) to observe the spot of light formed on the back wall of the subsporangial swelling move down the wall of the swelling, as the stipe bends, and finally come to rest on the perforate protoplasmic septum at the top of the stipe.

The apparatus used in the experiment was a glass-ring chamber. A glass ring 3 cm. in diameter was placed on a slide and covered with a cover-glass in the manner shown in Fig. 56.   To cut off undesirable light, black paper was attached : to the outer and inner surfaces of the glass ring, except for a window-slit 2 mm. wide, as shown at A, B, and C ; to the upper and lateral sides of the slide, as shown at B and C ; and to the upper surface of the cover-glass, except for a peep-hole, as shown at B and C.

One morning, a large well-developed fruit-body of *Pilobolus longipes* which had been directed toward the source of strongest light for some hours, together with a piece of horse dung on which it was growing, was removed from a culture dish and was placed in the experimental chamber in the manner shown in Fig. 56 ; and two other pieces of horse dung were also placed in the chamber to assist in keeping its air moist ; whereupon the ring was covered with the special cover-glass which had been prepared for it.   The chamber was then set on the stage of a microscope and was attached there by clips.   The microscope was turned on its axis and its stage tilted until the upper surface of the stage and of the glass slide were parallel to the direct rays of the morning sun and a beam of sunlight entered the window-slit of the chamber and impinged on the sub-sporangial swelling, as indicated by the arrow in Fig. 56, A.   Under these conditions, the beam of sunlight struck the subsporangial swelling transversely, and the sporangiophore was so directed that, in order to make a complete heliotropic reaction, it was obliged to bend through a right angle.   After the beginning of the experiment, in order to keep the beam of sunlight on the subsporangial swelling, it was necessary (owing to the movement of the sun relatively to the earth) to revolve the microscope slightly on its axis at frequent

intervals of time. The mirror of the microscope was so turned that it could not reflect any light upwards. From the beginning to the
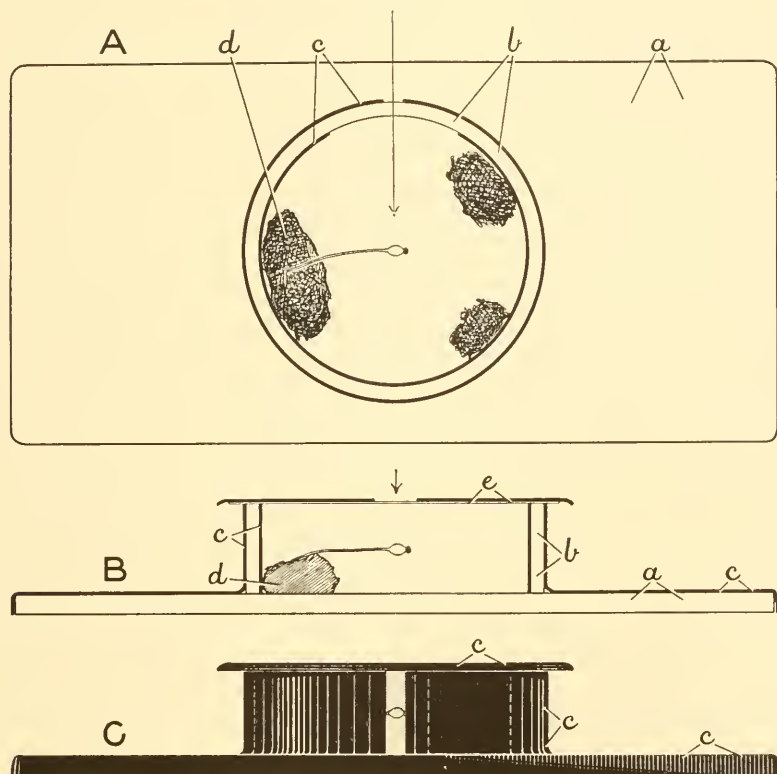
Fig. 56.—A glass-ring chamber, as used for observing the heliotropic curvature of the stipe of *Pilobolus longipes*. A, a plan of the chamber with the cover-glass removed, as seen from above : *a*, a glass slide covered with black paper ; *b*, a glass ring covered with black paper *c* within and without except for a slit in the front where, as indicated by the arrow, direct sunlight is entering the chamber ; *d*, a piece of horse dung bearing a *Pilobolus* fruit-body which has just been arranged so that the axis of the subsporangial swelling is horizontal and perpendicular to the direction of the rays of light which strike it ; the two other masses of horse dung assist in keeping the air of the chamber moist. B, a median vertical section of the chamber with the cover-glass on : *a*, the glass slide ; *b*, the glass ring ; *c c*, black paper ; *d*, the horse dung bearing the Pilobolus fruit-body ; *e*, the cover-glass, covered with black paper except at the very centre where there is a window through which, as indicated by the arrow, the observer watched with the low power of the microscope the heliotropic bending of the stipe of the fruit-body ; sufficient light came up from below to enable these observations to be made without the use of the mirror. C, the whole apparatus seen from in front where the direct rays of sunlight are entering through the slit of glass not covered by black paper : the illuminated subsporangial swelling in the middle of the chamber can readily be observed. Enlarged to 1·33 the actual size.

end of the experiment the upper part of the fruit-body, *i.e.* the sporangium, the subsporangial swelling, and the top of the stipe, was observed by looking downwards upon it through the peep-hole of the cover-glass, as indicated by the arrow shown in Fig. 56, B.

The experiment began on May 3 at 9 o'clock in the morning. For the first ten minutes, the fruit-body remained in its original position, as shown in Fig. 56 at A ; but, three minutes later, its stipe was observed to have turned very slightly toward the light, so that the presentation time was estimated to have been about ten minutes. Half an hour later, *i.e.* forty minutes after the beginning of the experiment, the stipe had bent toward the light through an angle of 45° ; and at the end of eighteen further minutes, *i.e.* approximately one hour after the beginning of the experiment, the stipe had bent through a complete right angle and had turned the subsporangial swelling and sporangium through 90° so that they now faced the sunlight head on. Immediately after the heliotropic movement had been completed, the top of the fruit-body was drawn with the *camera lucida*. A study of this drawing, which is reproduced in Fig. 57, shows that the motor region of the stipe, to which the turning movement of the sporangiophore was due, was situated almost at the top of the stipe, *i.e.* immediately under the topmost region of the stipe which is distinguished by the presence of the incomplete protoplasmic septum.

A few minutes after the drawing shown in Fig. 57 had been made, the sporangiophore shot away its sporangium, and the sporangium struck and stuck to the narrow window-slit shown in Fig. 56 at A and C.

At the beginning of the experiment, a beam of sunlight which fell on the subsporangial swelling formed a spot of colourless light on the colourless protoplasm lining the inner side of the back wall of the swelling. This spot of light could be observed with the microscope. As the subsporangial swelling was gradually being turned through a right angle by the growth-movement of the stipe, the spot of light was seen travelling slowly down the side of the swelling until at last it settled on the perforate protoplasmic septum at the top of the stipe. For the first twenty minutes or so of the turning movement of the stipe, the spot of light travelled

on colourless protoplasm.   Then it approached and travelled
on the reddish protoplasm
situated within and around
the base of the swelling; and,
finally, about five minutes before
the end of the experiment, it
could be seen leaving the red-
dish protoplasm on the interior
of the swelling and passing on
to the very red protoplasm of
the protoplasmic septum at the
top of the stipe.   Fifty-eight
minutes, or approximately one
hour, after the beginning of
the experiment when the spor-
angium and subsporangial swell-
ing had come to face the sun-
light head on, the spot of
light was seen resting sym-
metrically on the protoplasmic
septum which, in consequence,
glowed with a reddish light (*cf.*
Fig. 46, p. 91).   These obser-
vations show that, in the spor-
angiophore of Pilobolus, the
cessation of heliotropic move-
ment, or in other words the
establishment of photochemical
equilibrium, is correlated with
the passage of a spot of bright
light from one side of the
subsporangial swelling to a
radially symmetrical position
on the red protoplasm at the
base of the swelling and at
the top of the stipe; and

FIG. 57.—*Pilobolus longipes*.   The fruit-
body shown in Fig. 56, drawn with
the aid of a *camera lucida* one hour
after the beginning of the heliotropic
experiment.   The   subsporangial
swelling *s* and the sporangium with
its black outer cell-wall *c* and its
bulging basal gelatinous band *g*,
through which the spores can be seen,
have been turned through a right
angle owing to the unilateral growth
of the motor region of the stipe, *m*, in
response to the heliotropic stimulus.
The light coming from the sun (*cf.* A
in Fig. 56) is now refracted through
the subsporangial swelling and is
thereby focussed on the red bicon-
cave perforate protoplasmic septum
situated at *p*.   A few minutes after
this drawing was made the spor-
angium was shot away toward the
sun, and it struck and stuck to
the 2-mm.-wide window shown in
Fig. 56, A and C.   The size of every
part is indicated by the scale.   Mag-
nification, 37.

they may be accepted as giving strong support to the explanation

of the mechanism of the heliotropic reaction of Pilobolus already set forth in the previous Section.

A Solution of the Problem of the Reaction of the Sporangiophore of Pilobolus to Two Equal Beams of White Light.—Allen and Jolivette [1] made a study of the reactions of Pilobolus to light, in the course of which they exposed cultures of Pilobolus at one and the same time to two equal beams of light which passed through two holes (*cf.* Fig. 59, A) in the wall of a dark culture chamber. They
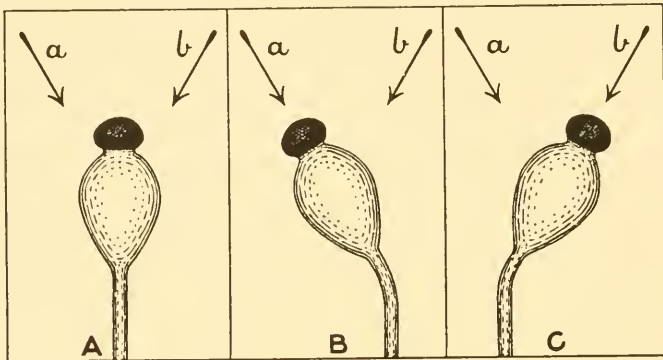
Fig. 58.—*Pilobolus Kleinii.* Diagram to show the reaction of a fruit-body to two equal beams of light making a considerable angle with one another.   The directions of the two beams of light are indicated by the arrows *a* and *b.*   A, the beginning of the experiment.   B and C, alternative end-results of the experiment : the stipe of the fruit-body has turned the sporangium and subsporangial swelling so that these have come to face *either* the beam-of-light *a* (as in B) *or* the beam-of-light *b* (as in C). Magnification, 14.

observed that, when a culture is exposed to two equal beams of white light coming from two sufficiently different directions (angle between the rays converging on the fruit-bodies greater than about 10° and up to 36° and more), the sporangium of each Pilobolus fruit-body is aimed at one or the other source of light (*cf.* Figs. 15, p. 42, and 58) and *the aim is as accurate at the source of light chosen as if the other source did not exist* ; and they [2] commented upon this curious experimental result as follows : " Light rays from both sources reach the sensitive sporangiophore.   Apparently there is nothing

[1] Ruth F. Allen and Hally D. M. Jolivette, " A Study of the Light Reactions of Pilobolus," *Trans. Wisconsin Acad.*, Vol. XVII, 1914, pp. 561–569, 593–594.

[2] *Loc. cit.*, p. 593.

to prevent each set of rays—or each individual light ray for that matter—from setting up those changes in the protoplasm which constitute the perception of a stimulus, and nothing to prevent these simultaneous stimuli from acting together to produce a resultant reaction.   But this does not occur.   The visible reaction of each sporangiophore is to one and one only of the two possible sources of stimulation."

The fact that a Pilobolus fruit-body, when illuminated by two equal beams of white light having sufficiently different directions, reacts apparently to one source of light and not the other, which so much puzzled Allen and Jolivette in 1914, can readily be explained on the theory already set forth : that the spot of light formed by the subsporangial swelling on the protoplasm on the wall of the swelling farthest away from the source of light gives the illuminated patch of protoplasm a photochemical stimulus ; that the stimulus (by diffusion of a growth-promoting substance through the protoplasm or otherwise) is conducted to the motor region of the stipe ; and that the motor region of the stipe reacts to the stimulus by growing faster on the side nearest to the source of the stimulus than on the opposite side and thus bends heliotropically.   An endeavour will now be made to apply this theory to the problem in hand.[1]

Let us suppose that a Pilobolus fruit-body, like that shown in Fig. 47 (p. 92), is pointing vertically upwards and that it is illuminated from above by two equal beams of white light which cross one another on their way to the fruit-body at an angle of $38°$ and each of which makes an angle of $19°$ with the axis of the subsporangial swelling.   One of these beams is shown in Fig. 47 striking the right-hand side of the swelling, and the other beam which strikes the left-hand side of the swelling and is like the first but reversed in direction can be readily imagined.

By reference to Fig. 47 (p. 92), it can be seen that the right-hand beam forms a spot of light *st* on the left-hand side of the subsporangial swelling at a distance of about $0 \cdot 25$ mm. above the median

[1] For an account of Van der Wey's solution of this problem and for the reason which induced me to give here my own solution, which is in conformity with his, *vide* Chapter I, pp. 44–45.

plane of the protoplasmic septum.   A similar spot of light must be
formed by the left-hand beam, also at a distance of 0·25 mm. above
the median plane of the protoplasmic septum, but on the right-hand
side of the swelling exactly opposite to the first spot.   Thus we
have two spots of light on opposite sides of the swelling and, accord-
ing to our theory, each of the spots affects the patch of protoplasm
on which it rests photochemically and causes it to send a stimulus
down to the motor region of the stipe.   The stimulus arriving from
the patch of protoplasm illuminated by the spot of light on the
left-hand side of the swelling will tend to cause the motor region of
the stipe to grow faster on its left-hand side than on its right-hand
side ;   and the stimulus arriving from the patch of protoplasm
illuminated by the spot of light on the right-hand side of the swelling
will tend to cause the motor region of the stipe to grow faster on
its right-hand side than on its left-hand side.   Now it is very un-
likely that, under natural conditions, the two stimuli given by the
two spots of light falling on opposite sides of the subsporangial
swelling will be absolutely equal.   Let us therefore suppose that
one of the stimuli, say that given by the spot of light on the left-
hand side of the swelling, is very slightly greater than the other
stimulus produced on the right-hand side of the swelling.   The
two stimuli will travel downwards on opposite sides of the swelling
to the motor region of the stipe.   Since, according to our assumption,
the left-hand stimulus is very slightly greater than the right-hand
stimulus, the left-hand side of the motor region of the stipe will be
stimulated to grow a little faster than the right-hand side, with the
result that the stipe will bend slowly to the right and, in so doing,
turn the subsporangial swelling and sporangium to the right.   As
the subsporangial swelling is being bent round to the right, the
left-hand spot of light must move *down* the side of the subsporangial
swelling, while the right-hand spot of light must move *up* the side
of the swelling ;   and, doubtless, the left-hand spot of light, as it
goes farther and farther down the left-hand side of the swelling,
gives a greater and greater stimulus to the left-hand side of the
motor region of the stipe which it is approaching, while the right-
hand spot of light, as it goes farther and farther up the right-hand
side of the swelling, gives a weaker and weaker stimulus to the

right-hand side of the motor region of the stipe from which it is retreating. Thus, in the end, the stimulus given by the descending left-hand spot of light prevails over and completely dominates the stimulus given by the ascending right-hand spot of light, with the inevitable result that the motor region of the stipe bends the sub-sporangial swelling round to the right until it comes to be directed head on to the right-hand beam of light. When this position of heliotropic equilibrium has been established, what was the left-hand spot of light now rests symmetrically on the protoplasmic septum at the top of the stipe, while the right-hand spot of light rests on the right-hand side of the swelling at a distance of about 0·5 mm. from the median plane of the protoplasmic septum which is about equal to twice its original distance from the septum. If the right-hand spot of light when in its fixed position 0·5 mm. from the septum continues to cause a stimulus to be sent down to the right-hand side of the motor region of the stipe, we must suppose that this stimulus is so feeble, relatively to the powerful stimulus sent down equally to all sides of the motor region of the stipe from the septum illuminated by the other spot of light, that its effect on the stipe, so far as causing it to bend is concerned, is negligible.

Thus, by taking into account the special structure and optical behaviour of a typical Pilobolus fruit-body, it has been possible to explain why it is that a Pilobolus fruit-body, when stimulated by two beams of light coming from sufficiently different directions, reacts by turning to one of the sources of light and pointing to it with as much precision as it would exhibit were the other source of light non-existent.

So far we have considered the heliotropic response of the Pilobolus fruit-body to two sources of light the rays of which make a relatively *large angle*, 10°–36° or more, where they meet at the fruit-body's surface. To complete our enquiry, we shall now consider the heliotropic response of the fruit-body to two sources of light the rays of which make a relatively *small angle*, say 5° or less, where they meet at the fruit-body's surface.

Let us assume that the angle between the two equal beams of white light which fall on a Pilobolus fruit-body is 5°, as shown at B in Fig. 59, and that at first the two beams fall upon one side of the
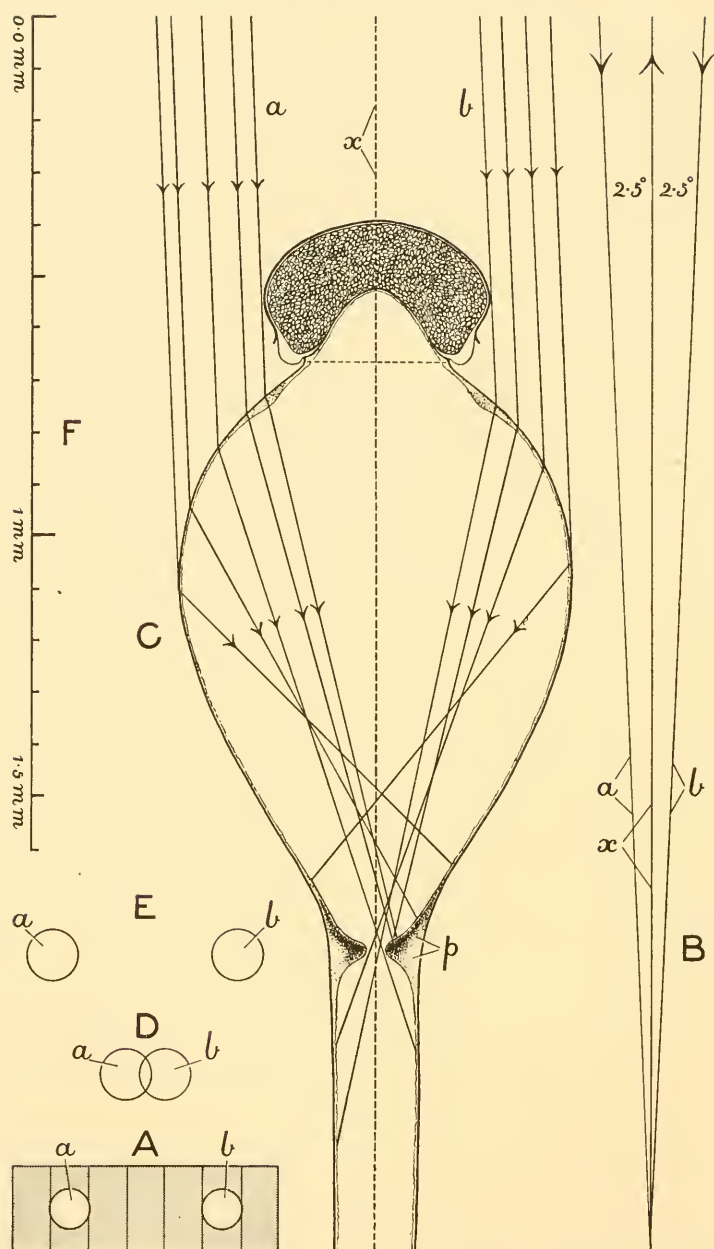
Fig. 59.—A diagram to illustrate the reaction of a fruit-body of *Pilobolus Kleinii* to light coming from two equal sources at the same time.

A, part of the wall of a dark chamber in which are two windows, *a* and *b*, with their centres 4 cm. apart. Through these windows two beams of light

fruit-body.   Each beam, after refraction of its rays at the surface
of the subsporangial swelling, will form a spot of light on the proto-
plasm lining the back wall of the swelling, and the two spots will
overlap one another to some extent, as shown diagrammatically
at D in Fig. 59.   The two heliotropic stimuli produced by the over-
lapping spots of light must travel down one and the same side of
the swelling to the motor region of the stipe and there combine in
causing the stipe to bend heliotropically in the direction of the two
sources of light.   As the bending of the stipe takes place, the two
overlapping spots of light will descend the wall of the swelling and
take up a position on the protoplasmic septum where there is room
for both of them.   There are two possibilities with respect to the
exact position of the two spots relatively to the septum : either
(1) the spot which first comes into contact with the septum takes
up an exactly symmetrical position over the septum, its centre

Fig. 59—*cont.*
of equal intensity came and struck a Pilobolus fruit-body 42 cm. distant.   The
fruit-body reacted in such a way that it pointed its gun at a spot *midway between
the two sources of light,* as indicated at B where the two outer arrows *a* and *b*
show the direction of the two beams of light and the middle arrow *x* the final direc-
tion of the axis of the subsporangial swelling and sporangium.   C shows the
final position of the fruit-body.   The rays *a* and *b* in C have all come through
the window *a* in A and are therefore drawn parallel to *a* in B.   They are refracted
into the subsporangial swelling and fall on the protoplasmic septum at *p* where
they form a spot of light (*cf. b* in D) slightly to the right of the centre of the
septum.   The corresponding rays of light coming through the window *b* in
A, and therefore in a direction parallel with *b* in B, have not been represented
in constructing C ; but, if they had, it would be seen that they would form
a spot of light slightly to the left of the centre of the septum *p,* as shown at
a in D.   The two spots of light (*cf. a* and *b* in D) overlap and they can and do
both rest on the septum at the same time, so that *the septum is symmetrically
illuminated.*   Hence, finally, the axis of the gun is directed, as shown at *x* in B,
at a spot midway between the two sources of light.
When the two windows in A are far apart, so that the beams of light striking
the Pilobolus fruit-body are inclined to one another at a *large* angle, say 40°,
instead of a small one, say 5°, as in the case already discussed, if the fruit-body
is at first pointing at a spot midway between the two sources of light, the two
spots of light formed by the two beams on the wall of the subsporangial swelling
must be far apart (*cf. a* and *b* in E) and cannot overlap, one spot being on one
side of the swelling and the other opposite to it on the other side of the swelling.
Unstable physiological equilibrium must result : one spot of light will stimulate
the motor region of the stipe more than the other with the result that the fruit-
body will turn toward the source of light which gives the stronger stimulus,
i.e. it will come to *point toward one of the windows* and not to a spot midway
between them.   At the end of the reaction, one spot of light will have moved
downwards on the wall of the swelling and will have taken up a symmetrical
position on the septum whilst the other will have moved upwards and have
become much farther removed from the septum than it was originally.   The
scale F indicates the size of each part of C, D, and E : magnification, 69.   In A
the vertical lines divide the wall into centimetres.

coinciding with the septum's centre, in consequence of which the other spot lies excentrically over the septum ; or (2) the two spots as a whole take up a symmetrical position over the septum, so that their common centre (situated in their overlapping parts, *cf*. Fig. 59, D) concides with the septum's centre. If one spot only comes to lie symmetrically on the septum, the fruit-body must point to but one of the two sources of light ; whereas, if the two spots act as one and, as a whole, lie symmetrically over the septum, the fruit-body must point in a direction which bisects the directions of the two sources of light.

A fruit-body which, in accordance with the possibility (2) as just described, is supposed to be directed between the two equal sources of light has been represented in Fig. 59. At B, the two beams of light which make an angle of 5° with one another are indicated by the lines *a* and *b* and the axis of the fruit-body, which bisects the angle, is indicated by the line *x*. At C is a construction diagram of the fruit-body having its axis *x* parallel to *x* in B and being illuminated by one of the two beams of light, namely, the one shown by *a* in B. The rays of this beam, which make an angle of 2·5° with the axis of the subsporangial swelling, are shown in C in the air at *a* and *b* and, after refraction, in the swelling where they converge and form a spot of light on the septum excentrically on its right-hand side. To avoid confusion, the other beam of light with rays parallel to *b* in B has not been represented in C, but it can be readily imagined as it would be just like the beam actually represented, but reversed in position. The spot of light formed by this unrepresented right-hand beam, like the other spot, falls on the septum excentrically, but with its centre a little to the left of the septum's centre instead of a little to the right. The construction diagram C indicates quite clearly that the two spots of light must overlap to some extent in the manner shown at D and that, as a whole, they can lie symmetrically on the septum with their common centre resting upon the centre of the septum.

If the angle between the two beams of light were to be decreased gradually from 5° to 1° the overlapping of the two spots of light in the subsporangial swelling would increase from that indicated in

Fig. 59 at D until it was very considerable indeed. If the angle were to be further decreased from 1° to 0°, the two spots finally would coincide with one another.

Allen and Jolivette [1] investigated the response of Pilobolus sporangiophores to two equal sources of light which were very close together and whose rays formed angles from about 13° to about 3° with one another and, in discussing the results of their observations, they [2] say : " When the two openings which serve as sources of illumination are close together, there are, to be sure, a small number of sporanges which land about midway between the openings " and " It is possible that the sporanges which fell between and below the openings came from sporangiophores which perceived and reacted to both lights at once, thus aiming at a point between the two openings." Evidently they suspected that with very small angles at least some of the projectiles were aimed midway between the two sources of light although, as they intimate, to admit this as a fact would conflict with their results obtained with large angles and make the explanation of the heliotropic reaction of Pilobolus very difficult. " Why," they [3] ask, " should the resultant reaction to two simultaneous stimuli appear only when the openings are close together ? "

In connexion with Fig. 59 it was shown above that, when the angles between the incident rays of light from two equal sources of light are very small, the two spots of light partly overlap and, as a whole, can take up a symmetrical position on the protoplasmic septum of the subsporangial swelling. Theoretically, therefore, when the angle between the two beams of incident light is very small, it is possible for the two stimuli produced by the two beams of light to act together and produce a resultant reaction in the motor region of the stipe. It seems to me probable that, under test conditions, such a resultant reaction would be often observed or, in other words, that with small angles between equal or almost equal beams of light the sporangiophores would be found to point more or less between the sources of light rather than at one or the

[1] Ruth F. Allen and Hally D. M. Jolivette, *loc. cit.*, pp. 566–569.
[2] *Ibid.*, p. 593.
[3] *Ibid.*, pp. 593–594.

other of them ; but whether or not this supposition is a good one can only be decided by further investigation.[1]

According to Haberlandt,[2] as we have seen, the reaction of the leaves of the higher plants to heliotropic stimuli can be explained by assuming that the epidermal cells of the lamina act as ocelli and that heliotropic equilibrium is only attained when the spot of light formed in each cell has come to lie symmetrically on the protoplasm covering the cell's inner wall. If this theory is true, a lamina when subjected to two equal beams of white light making a wide angle with one another, say of 40°, should, like the sporangiophores of Pilobolus, turn so as to face either one or the other source of light and not show a resultant reaction by turning so as to face a point midway between the two sources of light. It will be of much interest to know what happens when such a test of Haberlandt's ocellar theory is actually made.

A Model for illustrating the Pilobolus Fruit-body in its Relations with Light.—A model for illustrating the fruit-body of Pilobolus in its relations with light can be made for demonstrations to an audience as follows. Take a Florence flask or a measuring flask with a somewhat pear-shaped base and of 500 cc. capacity, fill it with water, stuff a smooth wet plug of cotton wool (free from air-bubbles) down the neck as far as the base of the neck, and then close the mouth of the neck with a cork (Fig. 60). The neck of the flask then corresponds to the stipe of Pilobolus, the bulb of the flask to the subsporangial swelling, and the cotton-wool plug to the protoplasmic septum at the top of the stipe. To represent the opaque sporangium, stick a plano-convex mass of moulding clay covered with black tissue paper over the flat surface of the flask's base, so as just to cover it. As a source of light, use direct sunlight or a beam from the arc of a projection lantern.

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[1] Since this was written, Van der Wey, in a paper referred to at the end of the last chapter (p. 43), has shown that, with small angles between the two beams of light, both beams influence the direction in which the sporangiophores point, with the result that most of the sporangia are shot in directions between the centres of the two beams. Evidence of this fact is to be seen in one of his photographs reproduced in my Fig. 16 (p. 44).

[2] G. Haberlandt, *Die Lichtsinnesorgane der Laubblätter*, Leipzig, 1905 ; also *Physiological Plant Anatomy*, translated by M. Drummond, London, 1914, p. 614 *et seq.*

(1) To imitate the condition of the Pilobolus fruit-body when in a state of heliotropic equilibrium. Hold the flask by its neck in the beam of light with its long axis parallel to the direction of the incident rays and its black basal ball facing the rays. It will now be found that the rays of light falling on that part of the flask's bulb which bulges out beyond the black ball are refracted so that they converge within the bulb and brilliantly illuminate the cotton-wool plug at the junction of the flask's bulb and neck.

(2) To imitate the condition of the Pilobolus fruit-body when not in a state of heliotropic equilibrium. Hold the flask in the beam of light with its long axis making a considerable angle with the direction of the incident rays, so that the rays fall obliquely on the flask's bulb and basal ball. It will now be found that the cotton-wool plug is no longer brilliantly illuminated, but that a spot of light is formed by the refracted light rays upon the side of the bulb. The spot of light can be made evident by placing a sheet of white paper against it or near it.

(3) To imitate the movement of the spot of light down the side of the sub-sporangial swelling of the Pilobolus fruit-body when the stipe, responding to a heliotropic stimulus, is turning the sub-sporangial swelling and sporangium through an angle. Hold the flask as just described in (2), so that the spot of light is upon one side of the bulb. Now turn the flask so that its axis gradually assumes a direction parallel
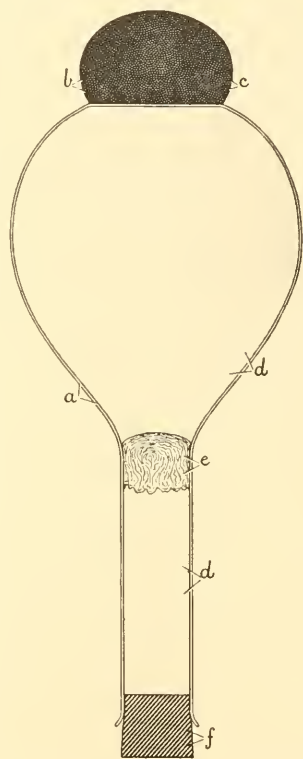
Fig. 60. — Diagrammatic longitudinal median section through a model used to illustrate the ocellus function of the subsporangial swelling of *Pilobolus Kleinii* : *a*, a Florence flask ; *b*, moulding clay adhering to the base of the flask ; *c*, black tissue paper covering the ball of clay ; *d d*, water ; *e*, cotton wool ; *f*, a cork. When the model is pointed toward the sun or toward an artificial source of light a few feet away, the rays of light are refracted through the bulb of the flask on to the plug of cotton wool, so that this becomes brightly lighted. About one-half the natural size.

to, and its basal ball comes to face, the incident rays.   During the progress of the turning movement, one can observe that the spot of light gradually moves down the side of the bulb (this can be made evident by applying a sheet of white paper to it) until finally it takes up a perfectly symmetrical position on the cotton-wool plug which, in consequence, becomes brilliantly lighted.

(4) To show that, if the cell-sap in the subsporangial swelling were replaced by air, the swelling would not act as a lens.   Hold the flask in the beam of light so that its basal ball faces the light, as in the first experiment.   The light is refracted on to the cotton-wool plug.   Remove the water from the flask without removing the plug. Now hold the flask in the beam of light in the same position as before.   The light is no longer refracted on to the plug.

The Periodicity in the Development of Pilobolus Fruit-bodies.— As we have seen, in a good natural horse-dung culture of Pilobolus, a crop of fruit-bodies is produced daily for several days in succession, and each crop takes about 24 hours to complete its development. An individual fruit-body consists : at *mid-day*, of a tuber (tropho-cyst) or swollen plasma-filled cell which becomes the basal swelling (Fig. 24, A, p. 60) ; in the *afternoon*, of a basal swelling and stipe which is growing in length (B and C) ; in the *late evening*, *i.e.* 11–12 P.M., of a basal swelling. a stipe, and a sporangium (D) ; *early next morning*, *i.e.* about 6 A.M., of a basal swelling, a stipe, a subsporangial swelling and a sporangium (G) ; and, finally, *at or before noon*, of a collapsed sporangiophore and a discharged sporangium (H).   Normally the discharge of the sporangium, which marks the climax in the development of the fruit-body, takes place between 10 A.M. and early afternoon.

The naked stipe, whilst growing in length during the afternoon, is positively heliotropic and always points with great precision to the source of the strongest incident rays of light (Fig. 24, B and C, p. 60).   It contains a mass of colourless protoplasm at its apex, dense red protoplasm just below its apex, and less red protoplasm farther down its shaft.   Doubtless, when the stipe is growing toward the light, the rays are refracted at its apical surface, converge, and concentrate themselves in the sub-apical dense red protoplasm ; and it may well be that this red protoplasm is

photically sensitive and directs the growth of the stipe towards the light.

The stipe is positively heliotropic so long as it is growing in length ; but, as soon as it ceases to elongate and begins to develop a sporangium at its free end, it ceases to respond to unilateral illumination. Even when it is exposed to the direct rays of the sinking sun, so that its axis is at right angles to the rays, it shows no heliotropic reaction whatever. The inability of the stipe to make heliotropic curvatures coincides roughly with the hours of darkness and lasts from about 7 o'clock in the evening until early next morning, by which time the subsporangial swelling has been developed.

What advantage to Pilobolus, if any, is its periodical development which results in the production of diurnal crops of fruit-bodies and in the discharge of the sporangia between about 10 A.M. and early afternoon ? The answer to this question is not far to seek. Pilobolus uses light to direct its guns toward open spaces, with the result that its projectiles are shot away from dung-plats in pastures on to the surrounding herbage where they remain until they are swallowed by herbivorous animals. If the guns were to be developed and discharged during the night, light could not be used to lay them and the sporangia would not be scattered nearly as effectively as they actually are ; but the periodicity in the development of a single fruit-body is such that the diminishing afternoon light can be employed for directing the growth of the naked stipe and the strong late-morning light for the precise orientation of the mature gun just before it shoots away its sporangium. We may conclude, therefore, that the periodicity in the development of the fruit-body of a Pilobolus indirectly favours the dispersion of its sporangia and thus assists the species in maintaining its place in nature.

The Subsporangial Swelling and the Discharge of the Pilobolus Gun.—The first special function of the subsporangial swelling—that of acting as an ocellus for perceiving the direction of the strongest incident rays of light and for assisting in the heliotropic laying of the Pilobolus gun—has been fully treated of in previous pages. The second special function of the subsporangial swelling—that of acting as part of a squirting mechanism for the discharge of the sporangium—will be discussed in what follows.

The Pilobolus gun, as pointed out in an earlier Section,[1] is discharged explosively : the wall of the sporangiophore breaks across transversely just beneath the sporangium and columella (*vide* the dotted line *a* in Fig. 28, p. 70) ; the elastic wall of the subsporangial swelling, stipe, and basal swelling, which has been distended by the pressure of the cell-sap in the great vacuole, suddenly contracts ; the cell-sap is violently squirted out of the open mouth of the subsporangial swelling ; and a spherical drop of cell-sap carries away the sporangium at a speed [2] of 10–20 feet per second through the air. A contracted sporangiophore seen in water immediately after the sporangium has been discharged is shown in Fig. 61.

In the process of squirting away the sporangium, the subsporangial swelling, on account of its position just beneath the sporangium, its large size, and the great elasticity of its cell-wall, plays a chief part. Its external appearance just before the discharge of the sporangium, immediately after, and during the next few minutes, is illustrated in Fig. 62.

The subsporangial swelling, as may be realised by a glance at Fig. 28 (p. 70), contains a large amount of cell-sap.
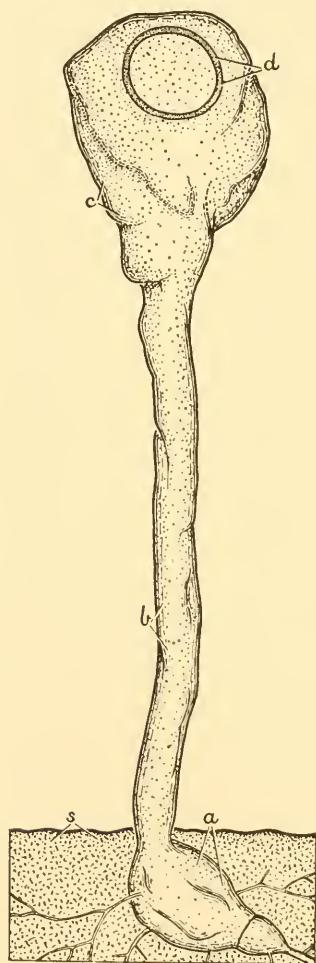
FIG. 61.—*Pilobolus Kleinii.* The sporangiophore after the discharge of the sporangium ; seen in water with the basal swelling embedded in part of the substratum : *s*, the substratum ; *a b c*, the sporangiophore, shrunken and collapsed ; *a*, the basal swelling with a turgid apophysis below ; *b*, the stipe ; *c*, the subsporangial swelling with its open mouth towards the observer ; and *d*, the contracted collar of the subsporangial swelling now forming a lip to the mouth of the swelling. Upper half drawn with the help of the *camera lucida*, the lower half somewhat diagrammatic. Magnification, 53.

[1] *Vide* p. 62.   [2] *Vide* p. 67.

When the sporangiophore contracts during the discharge of the sporangium, the volume of the sporangiophore decreases to at least one-half of what it was originally (*cf.* A and B in Fig. 62), with the result
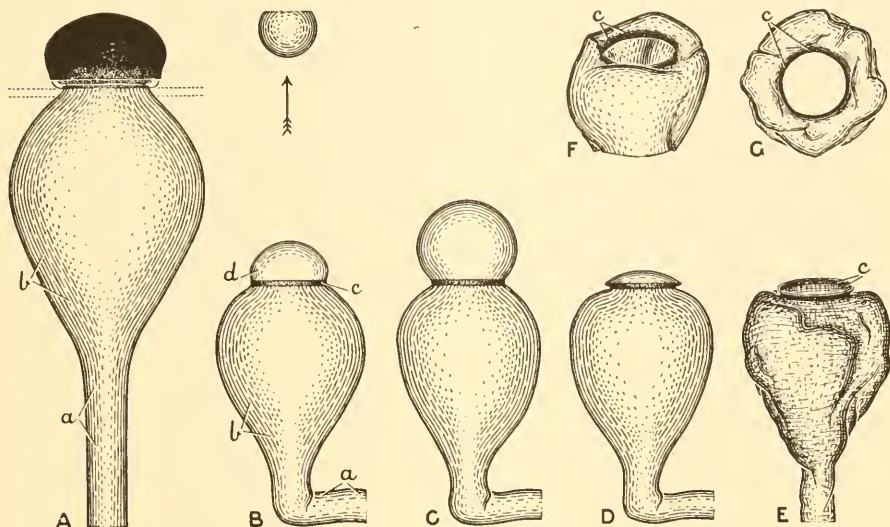
Fig. 62.—*Pilobolus Kleinii.* Changes in the upper part of the sporangiophore immediately after the sporangium has been shot away, represented somewhat diagrammatically. A, a fruit-body just before discharge : *a*, the stipe ; *b*, the subsporangial swelling, crowned above the upper broken line by a dehisced sporangium in which, between the two parts of the sporangium-wall, can be seen the swollen gelatinous ring and some of the spores ; the top part of the wall of the subsporangial swelling between the two broken lines contracts at the moment of discharge and becomes the lip or collar of the swelling shown at *c* in B. B, the contracted sporangiophore a fraction of a second after the sporangium and a jet of cell-sap have been shot away ; the jet broke up into droplets of which the last is shown above the arrow ; *a*, the stipe, now contracted and bent ; *b*, the subsporangial swelling, contracted to about one-half its original volume and now showing the lip or collar *c* which appears dark owing to crystals being present on its exterior ; at the mouth of the swelling is seen a drop of cell-sap *d* which is growing in size owing to continued contraction of the wall of the swelling and stipe. C, about half a minute after B ; the drop has become much larger and has attained its maximum size. D, a minute or two after C ; the drop is evaporating and has almost disappeared. E, a minute or two after D ; the drop has disappeared and the stipe and swelling have collapsed irregularly ; *c*, the lip or collar now slightly tilted. F and G, respectively, an oblique and a top view of the upper half of a collapsed swelling ; *c*, the lip or collar still rounded and comparatively rigid. Magnification, 38.

that most of, or perhaps all of, the cell-sap held in the subsporangial swelling is shot out into the air. At the same time, something like one-half of the cell-sap held in the basal swelling and stipe is forced upwards into the subsporangial swelling, so that the subsporangial

swelling, after its contraction, is filled with sap and not with air. Even after the sporangium has been shot away and the sporangiophore has been thrown backwards on to the substratum, the wall of the sporangiophore continues to contract, although slightly, with the result that cell-sap slowly issues from the open mouth of the sporangiophore and there forms a rounded globule (Fig. 62, B and C). As soon as the contraction ceases, the globule, owing to evaporation, begins to diminish in size (D), and soon it disappears completely. As more and more of the water of the cell-sap in the contracted subsporangial chamber evaporates, the sides of the chamber fall inwards and become irregularly folded (Fig. 62, E, F, and G).

The length and breadth of a subsporangial swelling of *Pilobolus longipes* were measured (1) when the swelling was fully turgid and (2) after the swelling had been made to contract symmetrically by rupturing the stipe below it ; and it was found that, as a result of contraction, the length of the swelling had decreased from $1\cdot17$ mm. to $0\cdot8$ mm. or by approximately 30 per cent. and that the breadth of the swelling had decreased from $0\cdot875$ mm. to $0\cdot65$ mm. or by approximately 26 per cent. These figures indicate that the cell-wall of the subsporangial swelling is highly elastic and contracts very considerably when the sporangium is discharged.

The cell-wall of the subsporangial swelling, when distended by the pressure of the cell-sap, is uniformly thin right up to the columella. As it contracts and drives the cell-sap away at the moment when the sporangium is discharged, it thickens, but not uniformly : it thickens most just around the mouth of the swelling and in a zone which stretches a short way below the mouth (*cf.* B with A in Fig. 63). From this differential thickening of the cell-wall on contracting and also from the external appearance of the top of a contracted swelling (Fig. 62, B-G), it may be safely inferred that the most contractile part of the wall of the swelling is that which lies immediately below the sporangium and eventually forms the lip or *collar* of its open mouth.

The collar of the contracted subsporangial swelling is shown externally in Fig. 62 and in vertical section in Fig. 63. It is covered externally by numerous small crystals of calcium oxalate which, when it is immersed in water, give it a dark appearance, apparently

by imprisoning air and thus preventing the water from coming directly into contact with its surface.

The level at which the wall of the subsporangial swelling splits across when the sporangium is discharged (called by de Bary the *Riss-stelle*) is indicated by a dotted line in Figs. 28 (p. 70) and 32 (p. 76), and by the upper dotted line in Fig. 62. The splitting is perfectly accomplished, so that the edge of the mouth of the contracted subsporangial swelling is always smooth and circular (Fig. 62, B–G). Doubtless, therefore, the wall at the abscission level is
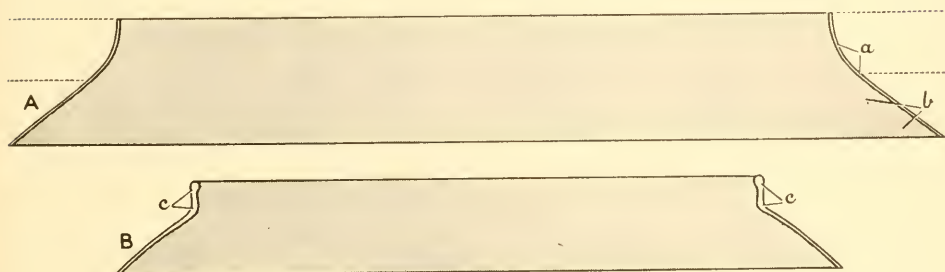
Fig. 63.—*Pilobolus Kleinii.* Vertical median section through the top part of a subsporangial swelling : A, just before the discharge of the sporangium ; and B, a fraction of a second after discharge when the cell-wall has contracted. A : the upper broken line indicates the level of abscission just beneath the sporangium (*cf.* Fig. 32) ; *a*, the cell-wall stretched by the pressure of the cell-contents *b*. B, the wall has contracted and that part between the two broken lines in A has thickened and formed the lip or collar *c c* (*cf.* c in Fig. 62). Magnification, 300.

prepared for breaking whilst the wall is being formed and long before the discharge of the sporangium. Some evidence in support of this view will now be given. If one strokes a ripe sporangium off its columella (*cf.* Fig. 31, p. 75) and examines the wall of the turgid subsporangial swelling and columella with the microscope, the wall appears everywhere about equally thin (*cf.* Fig. 30, B, p. 73). If now one ruptures the stipe, so that the turgidity of the whole sporangiophore is lost, the wall of the subsporangial swelling and columella contract. It is then seen that the wall is fully twice as thick immediately under the abscission level as it is immediately above. Also, when a subsporangial swelling and attached columella are treated with chlor-zinc iodine, the upper part of the wall of the subsporangial swelling swells greatly except at the abscission level.

Here, as shown at *e* in Fig. 64, the wall remains relatively thin. It seems not unlikely that, just before abscission actually takes place,
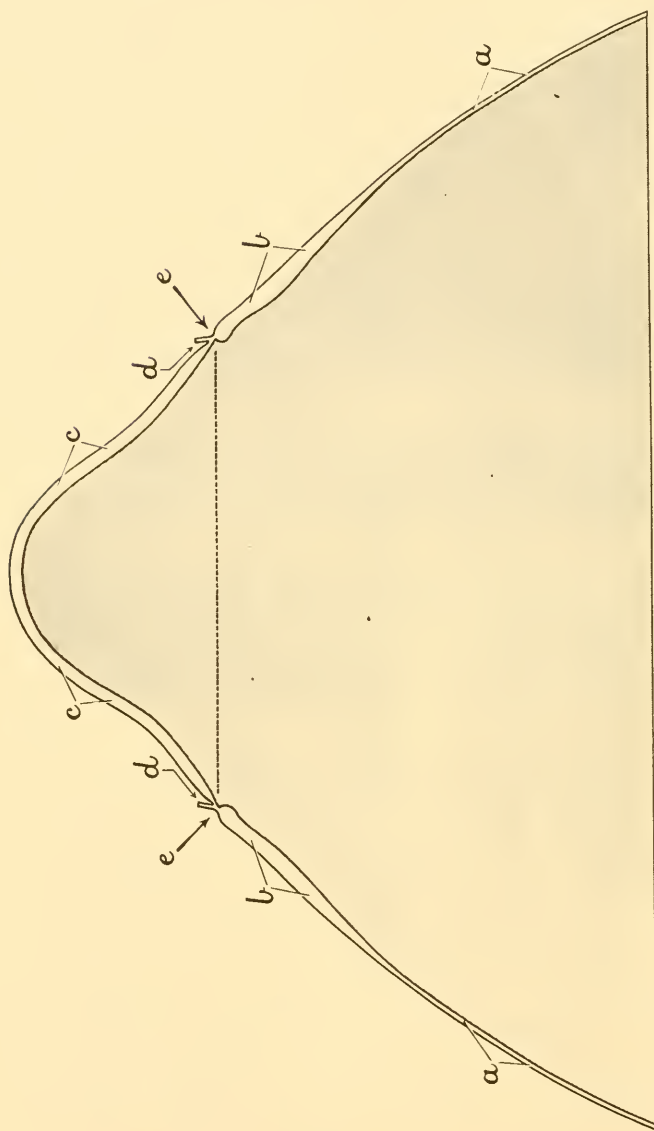
FIG. 64.—*Pilobolus longipes.* Vertical optical section through the apical part of a fruit-body from which the sporangium has been stroked off (*cf.* Fig. 29, C) and which has been treated with chlor-zinc iodine ; to show the place of rupture (*Riss-stelle*) in the cell-wall and the level of abscission when the sporangium and columella are shot away. The reagent killed the fruit-body, so that the wall at once contracted in area, and then it caused the wall to swell and thicken differentially : *a–b*, the upper part of the subsporangial swelling ; *c*, the columella ; *d*, the basal band of sporangium-wall still attached to the columella ; and *e e*, arrows which point to the place of weakness in the cell-wall just beneath the junction of the sporangium-wall and the columella and at the very top of the subsporangial swelling. In the subsporangial swelling, the wall at *a a* is but little swollen and at *b b* much swollen. The part of the wall *b b* becomes a thickened more or less rigid lip or collar when the subsporangial swelling is squirting out cell-sap against the sporangium at the moment of discharge. The wall of the columella *c c* is also much swollen. The thinnest part of the cell-wall is at *e e* where abscission eventually takes place. The broken line between the columella and the subsporangial swelling indicates the abscission level. Magnification, 200.

the wall at the level of abscission is weakened by the action of an enzyme applied to it through the activity of the adjacent

protoplasm. Since, when maturing fruit-bodies are placed in the dark, the discharge of the sporangia is delayed for several hours, it may well be that the weakening of the wall along the splitting line is controlled by light, and it is possible that the shallow heap of red protoplasm which lies in the subsporangial swelling just below the sporangium and is fully exposed to the light (*f* in Fig. 28, p. 70) is concerned with the control mechanism.

The columella, which is carried away with the sporangium when this is discharged, has a wall which, except for gelatinous swelling at the peak,[1] remains very thin even after the sporangium has been discharged and it is no longer pressed against by the cell-sap of the subsporangial swelling. Evidently, the wall of the columella differs from that
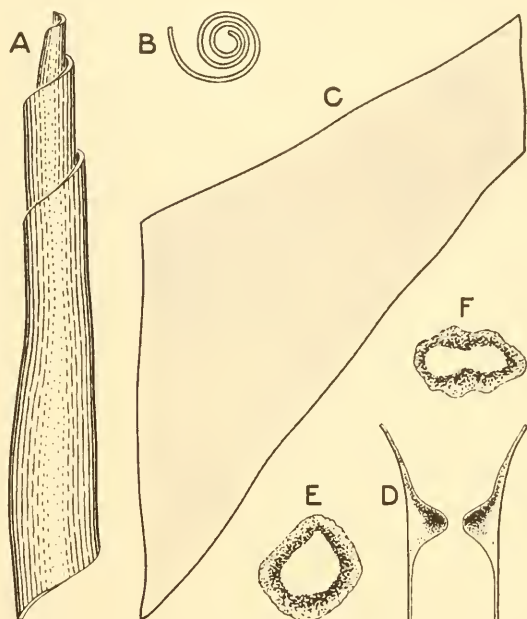
FIG. 65.—*Pilobolus Kleinii.* A, one of the fragments of a subsporangial wall which burst when the fruit-body was placed in water : when set free it at once became spirally rolled. B, the same, represented in transverse section. C, the same, represented as flattened out. D, vertical section of a fruit-body at the junction of the stipe and subsporangial swelling, to show the perforate protoplasmic septum *in situ.* E, a protoplasmic septum which was expelled from the sporangiophore as the sporangiophore contracted and discharged its sporangium under water. F, a similar septum. Magnification, A–C, 293 ; D–F, 67.

of the subsporangial swelling in being but slightly contractile.

Occasionally, when a fruit-body of Pilobolus is placed in water on a slide under the microscope, it explodes in such a way that the wall of the subsporangial swelling is torn into several fragments some of which at once become curled up spirally like a roll of paper (Fig. 65, A–C).

[1] *Cf.* p. 81.

This phenomenon is due in part to the enormous pressure to which the cell-wall is subjected by the cell-sap and in part to the cell-wall's wonderful elasticity.

The cell-sap which is expelled from the sporangiophore, when this contracts, issues from the circular mouth of the subsporangial swelling as a continuous jet which immediately breaks up into several separate drops. All these drops, which are shot some distance, can be readily caught on a sheet of glass placed a few inches in front of a fruit-body just before it discharges its sporangium (Fig. 66). The largest drop is formed at the top of the jet, has the greatest momentum, travels farthest, from the first adheres to the under wettable gelatinous side of the sporangium, and carries off the sporangium through the air in the manner shown in Fig. 74, A (p. 151). The other smaller drops are formed from the jet in succession under the first drop, are not so violently expelled as the first drop, have less momentum, and fall much nearer to the sporangiophore which has discharged them. Direct evidence that the range for the smaller drops is not nearly so great as for the larger drop is afforded by the fact that, when fruit-bodies look upwards and a glass plate is placed above them at greater and greater heights as in a set of experiments already recorded,[1] after a certain height the only drops which strike the plate are those which bear sporangia.

FIG. 66.—*Pilobolus longipes.* A–E, five sporangia and the cell-sap shot away from five fruit-bodies, after they had struck a sheet of glass held obliquely a few inches in front of the fruit-bodies and after the sap had dried up. The jet of cell-sap, on being squirted out from the top of the subsporangial swelling, broke up under the influence of surface tension into a series of drops. The largest drop travelled with the sporangium to the greatest distance. The smaller drops trailed behind the drop attached to the sporangium. Magnification, 2·6.

[1] *Vide* pp. 63–66.

The contracting sporangiophore, in addition to expelling from its interior a large quantity of cell-sap, sometimes also expels the protoplasmic septum from the top of the stipe ; for, immediately after observing the discharge of a ripe fruit-body in water on a slide under the microscope, I have occasionally found the septum, which is easily recognised by its annular shape and its red colour, lying in the water at some distance from the mouth of the collapsed sub-
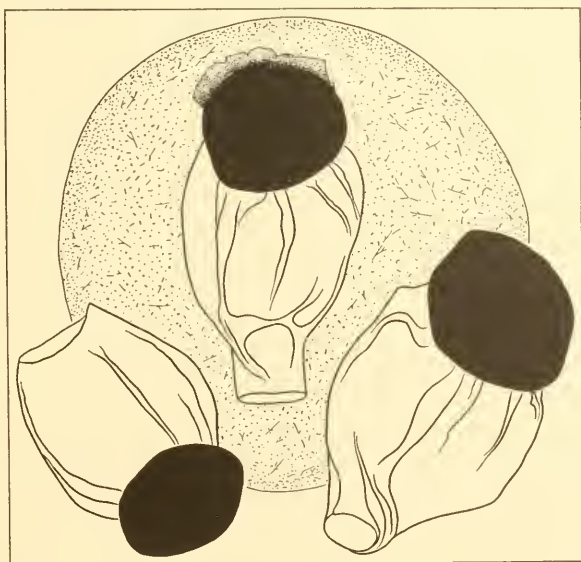
Fig. 67.—*Pilobolus Kleinii*. Three sporangia which were shot away with the subsporangial swelling attached to them. Magnification, 50.

sporangial swelling (Fig. 65, D–F). Furthermore, on examining the drops which have carried away sporangia through the air, immediately after they have settled on white paper placed on a table to receive them, I have sometimes observed that the drops contain a little mass of red protoplasm. There is therefore reason to suppose that, when the Pilobolus gun is discharged, the sap rushes up the stipe with such violence that it is apt to tear the protoplasmic septum away from the cell-wall, thus making it possible for the septum to be included in the large drop of sap which is expelled from the subsporangial swelling and carries the sporangium through the air.

In a culture of *Pilobolus Kleinii* about twenty among several hundred fruit-bodies that came up on fresh horse-dung balls discharged their sporangia abnormally ; for, as was inferred from an examination of their projectiles (Fig. 67), abscission in the sporangiophores took place not immediately under the sporangium but at the base of the subsporangial swelling or across the top of the stipe. The sporangia with the subsporangial swellings attached were found sticking to the under side of a glass cover situated several inches above the horse dung on which the fruit-bodies had been growing. Spores derived from the abnormal projectiles were sown and a new generation of fruit-bodies was obtained, but none of the new fruit-bodies in their mode of discharging their sporangia exhibited the abnormality of their parents.

The Osmotic Pressure of the Cell-sap of Pilobolus.—The force which expands the highly elastic cell-wall of the Pilobolus gun and is primarily responsible for the explosion of the gun and for the discharge of the projectile is the osmotic pressure (turgor pressure) of the cell-sap of the sporangiophore.   It is therefore of considerable interest to determine what this pressure is and how it compares with osmotic pressures known to exist in the cells of higher plants.

In an endeavour to determine the osmotic pressure of the sap of Pilobolus, three methods were tried : (1) the classical plasmolytic method of Nägeli, Pfeffer, and de Vries ; (2) extracting the sap of Pilobolus and using it in an attempt to plasmolyse cells having a known or determinable osmotic pressure ; and (3) Barger's capillary-tube method.   The freezing-point method was not employed owing to the difficulty of collecting an amount of cell-sap sufficient for the requirements of the Beckmann apparatus.

(1) The plasmolytic method for determining osmotic pressures was found unsuitable in its application to Pilobolus : firstly, because the sporangiophore is large and has a peculiar rounded form ; secondly, because the sporangiophore wall is highly elastic and contracts much more than the cell-walls of higher plants ; and, thirdly, because there is an optical difficulty in observing the primordial utricle just beginning to separate itself from the cell-wall.

(2) Cell-sap was extracted from a number of Pilobolus fruit-bodies and then small strips of a leaf of *Elodea canadensis*, cut with

a pair of scissors, were immersed in it. No plasmolysis took place. The Elodea cells could be plasmolysed slightly with a solution of sucrose having an osmotic pressure of 10 atmospheres but not with one having an osmotic pressure of 8 atmospheres. It therefore seemed reasonable to suppose that the osmotic pressure of the Pilobolus sap is less than 10 atmospheres, a supposition confirmed later by results obtained with the Barger method. No further work with method (2) was attempted.

(3) Barger's capillary-tube method, which was described in 1904, has the merit of being applicable to cell-sap which can be
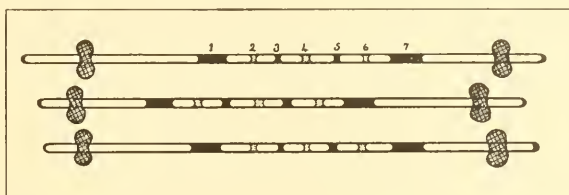
Fig. 68.—Part of the apparatus used for determining the osmotic pressure of the cell-sap of *Pilobolus longipes* by the Barger method. Three capillary tubes attached to a glass slide by Canada balsam. Each tube contains 7 drops : those shown in black, Nos. 1, 3, 5, and 7, are drops of sugar solution of known osmotic strength ; those lightly shaded, Nos. 2, 4, and 6, are drops of Pilobolus cell-sap. The tubes are closed at both ends. The whole was kept immersed in water in a Petri dish. Natural size.

obtained only in small quantities.[1] Its essence is the comparison of a solution of unknown osmotic pressure with standard solutions of known osmotic pressures made from a substance of known molecular weight, a series of drops taken alternately from the solution of unknown osmotic pressure and from a solution of known osmotic pressure being introduced into a capillary tube. The vapour pressure of the drops with the lower osmotic pressure is greater than that of the drops with the higher osmotic pressure, in consequence of which water vapour passes from the drops with the lower osmotic pressure and condenses on the drops with the higher osmotic pressure, with the result that the drops with the higher

[1] G. Barger, " A Microscopical Method of Determining Molecular Weights," *Journal of the Chemical Society, Transactions,* Vol. LXXXV, 1904, pp. 286–324.

osmotic pressure increase in length while those with the lower osmotic pressure decrease in length.

In accordance with the details of Barger's technique : the capillary tubes were made about 6–8 cm. long and with a bore about 1·0 mm. in diameter ; seven alternating drops separated by short air-spaces were introduced into each capillary tube (Fig. 68) ; the ends of the filled tubes were sealed up in a flame ; two or three tubes with like contents were attached with Canada balsam to a glass slide ; the slide was immersed in water at room temperature, the water being contained in a Petri dish ; the lengths of the five central
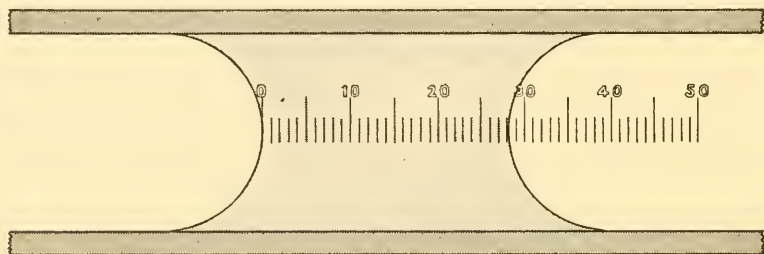
Fig. 69.—Optical section through part of a capillary tube used for determining the osmotic pressure of the cell-sap of *Pilobolus longipes* by the Barger method, showing the appearance of a drop when its length is being measured with the scale of the eye-piece micrometer of the microscope. The length of the drop here shown is 28·2 units of the scale.   Magnification, 27.

shorter drops (two of the standard solution, sucrose, and three of the cell-sap of Pilobolus) were measured (Fig. 69) under the microscope with the help of an eye-piece micrometer shortly after the tubes had been placed in the water ; and the drops were measured again next day after a lapse of 30–36 hours.

The sap was obtained as follows.   Fresh dung from a stable was spread out on the floor of a large glass case.   On the fourth day thereafter, many thousands of fruit-bodies of *Pilobolus longipes* (Fig. 70) came to maturity without any admixture of Mucor, etc. When the fruit-bodies were beginning to discharge their sporangia, they were caused to burst by pressing their subsporangial swellings (*cf*. Fig. 18, p. 50) against a glass slide.   As soon as the sap thereby set free amounted to about two drops, it was allowed to run off the slide into a test-tube.   When a sufficient quantity of the sap had

been harvested in this way, the sap was poured into a watch-glass
and covered with a sheet of glass to check evaporation.

The microscope employed had a mechanical and revolving stage
and a micrometer in its eye-piece;
and it was fitted with lenses which
gave a magnification of 40. The Petri
dishes containing slides, tubes, and
water were set on the stage of the
microscope and the lengths of the
five central drops in each tube were
read and recorded in succession. With
very little practice it was found possible
to measure the lengths of the drops
with accuracy and rapidity. The appear-
ance of the scale of the micrometer,
when applied to a drop for its measure-
ment, is shown in Fig. 69.

In carrying out the experiment
with the Pilobolus cell-sap, I received,
and here wish to acknowledge with
my best thanks, valuable assistance
from my friend and former pupil,
Dr. W. F. Hanna. He prepared the
standard solutions of sugar and filled
the capillary tubes, etc.; I harvested
the cell-sap just before it was re-
quired for use and kept the watch-
glass which contained it covered
except when a drop was being drawn
from it; and we both took part in
measuring and recording the lengths
of the drops after the tubes had been
sealed and placed in water.

Fig. 70.—*Pilobolus longipes* on
horse dung. The dung,
taken fresh from a stable,
was placed in a covered
crystallising dish. The
photograph shows part of
the culture four days later.
There are about 500 fruit-
bodies in view. Magnifica-
tion, 1·3.

The standard solution employed for comparing with the sap
was sucrose. Of this substance a stock solution of 34·2 grams
in 100 cc. distilled water, *i.e.* a weight-normal solution, was
prepared. This solution, at a temperature of 20° C., has an

osmotic pressure of approximately 24 atmospheres,[1] and from it a series of other solutions was made, these solutions having osmotic pressures of approximately 12, 10, 9, 8, 7, 6, 5, 4. and 3 atmospheres.

As a result of making five sets of experiments on five different occasions, the sugar solutions having been freshly made and the sap freshly collected on each occasion, it was found that the osmotic pressure of the cell-sap of *Pilobolus longipes* is less than 24, 12, 10, 9, 8, 7, and 6 atmospheres and greater than 3, 4, and 5 atmospheres. We may therefore take it that the osmotic pressure of the cell-sap of *P. longipes* is approximately equivalent to 5·5 atmospheres.

The collective work of various investigators has shown that the osmotic pressure of the cell-sap of the higher plants varies from about 3·5 atmospheres in the leaves of *Aloe americana* grown in a greenhouse to upwards of 100 atmospheres in the leaves of plants growing in the rocky desert of the Sahara [2] and to a maximum of about 170 atmospheres in the leaves of *Atriplex nuttallii* growing in the desert areas of Utah (U.S.A.).[3] For the sake of comparison with Pilobolus, a series of osmotic pressures determined by Dixon and Atkins will now be cited. Dixon and Atkins [4] treated the leaves of a number of different kinds of plants with liquid air, pressed out the sap, measured the freezing point of the sap, and then calculated the osmotic pressure of the sap. Their findings, showing the range of the osmotic pressures, have been embodied in the adjoining Table.

[1] This pressure was calculated from the equation : $P = \dfrac{RTg}{M}$, where P is the osmotic pressure in atmospheres, R the gas constant (0·082), T the absolute temperature (293° C.), g the grams of sucrose per litre of solvent (342), and M the molecular weight of sucrose (342). *Vide* J. C. Phillip, *Physical Chemistry, Its Bearing on Biology and Medicine*, London, 1925, p. 55.

[2] Hans Fitting, " Die Wasserversorgung und die osmotischen Druckverhältnisse der Wüstenpflanzen," *Zeitschrift für Botanik*, Bd. III, 1911, pp. 270–271.

[3] J. A. Harris, R. A. Gortner, W. F. Hoffman, J. V. Lawrence and A. T. Valentine, "The Osmotic Concentration, Specific Electrical Conductivity, and Chloride Content of the Tissue Fluids of the Indicator Plants of Tooele Valley, Utah," *Journal of Agricultural Research*, Vol. XXVII, 1924, p. 909.

[4] W. R. G. Atkins, *Some Recent Researches in Plant Physiology*, London, 1916, pp. 154–155.

Osmotic Pressure of Leaves as determined by Dixon and Atkins

Species and Dates	O.P.	Species and Dates	O.P.
*Aloe americana,** Jan. 11 .	3·52	*Ilex aquifolium*, new, ultimate,	
*Saccharum officinale,** Dec. 10	5·83	Dec. 4	14·65
*Monstera deliciosa,** Dec. 10 .	6·64	*Ulmus campestris*, Oct. 2 .	14·88
*Anthurium andreanum,** Jan.		*Fraxinus oxyphylla*, Oct. 3 .	15·06
27	7·49	*Ilex aquifolium*, antepenulti-	
*Platycerium alcicorne,** Jan. 27	7·51	mate, Dec. 4 . . .	15·14
Wistaria sinensis, Sept. 30 .	8·52	*Pinus laricio*, leaves one year	
*Anthurium crystallinum,**Jan.		old, Nov. 30 . .	15·50
27	8·73	*Apium graveolens* (etiolated	
Vitis Veitchii, Oct. 2 . .	9·18	bases), Dec. 5 . . .	15·66
Helianthus multiflorus, Oct. 2 .	9·18	*Populus alba*, spring leaves,	
Equisetum telmateia, lateral		Aug. 28	15·95
branch, Aug. 14 . .	9·28	*Chamaerops humilis*, mature,	
*Musa sapientum,** Dec. 10 .	9·44	Nov. 28	17·13
Equisetum telmateia, main stem,		*Hedera Helix*, Nov. 29 . .	17·29
Aug. 14	9·64	*Populus alba*, summer leaves,	
*Agave americana,** Jan. 11 .	10·11	Aug. 28	17·88
*Selaginella mertensii,** leaves		*Magnolia acuminata*, Sept. 30.	18·07
and aerial stems, Jan. 27 .	10·16	*Cerasus laurocerasus*, Nov. 28 .	18·31
*Polypodium iriodes,** Jan. 27	10·65	*Chamaerops humilis*, just ex-	
Eucalyptus globulus, Nov. 29 .	11·68	panded, Nov. 29 . .	19·22
Cordyline australis, Nov. 28 .	13·43	*Fraxinus excelsior*, Sept. 30 .	19·52
Passiflora quadrangularis, Dec.		*Syringa vulgaris*, Aug. 13 .	24.10
10	13·98	*Syringa vulgaris*, Aug. 22 .	25·50

* Growing in greenhouses.

By reference to the Table it will be seen that in only one species, namely, *Aloe americana*, was the leaf sap found to have an osmotic pressure less than 5·5, *i.e.* less than that of Pilobolus. It is therefore evident that the osmotic pressure of the cell-sap of Pilobolus, relatively to the osmotic pressure of the cell-sap of the leaves of most of the higher plants, is low.

Factors in the Efficient Working of the Pilobolus Gun.—It may be asked: how is it that the Pilobolus gun, notwithstanding that its cell-sap has a relatively low osmotic (turgor) pressure, can shoot its projectile to a maximum measured height of six feet and a maximum measured horizontal distance of eight and a half feet? The answer is: firstly, an osmotic pressure of 5·5 atmospheres is, after all, a strong pressure, for it is equal to about 82 lbs. to the square inch; and, secondly, the action of the Pilobolus gun is

dependent on several different factors of which osmotic pressure is only one, and among which we may recognise (1) the osmotic pressure of the cell-sap, (2) the great elasticity of the cell-wall, (3) the suddenness with which the aperture in the end of the sporangiophore is formed when the explosion takes place, (4) the control of the explosion, in that the wall of the sporangiophore breaks open not irregularly but at a single prepared place— a circular subterminal line or ring, and (5) the opening at the end of the sporangiophore having a smooth rim and being just of the right size (not too large and not too small) for permitting the cell-sap to be forced through it in sufficient quantity and with a sufficiently high initial velocity to carry off the projectile advantageously. It is the combination of all these factors which makes the Pilobolus gun so wonderfully effective.

Fig. 71.— *Pilobolus longipes.* Photomicrograph of the upper side of a discharged sporangium and of the drop of cell-sap (now dried) which accompanied it ; No. 1, the precipitate of the cell-sap, consisting of both organic and inorganic matter, in part in the form of branched crystals ; No. 2, a broad clear ring-layer of jelly in contact with the glass on which the projectile came to rest ; No. 3, the convex, very black, sporangium-wall covering many thousands of spores ; No. 4, a few spores lying under the jelly, they were forced out of the sporangium when this struck the glass. Magnification, 51.

An Analysis of the Cell-sap of Pilobolus longipes.—An inspection of a vertical section through a fruit-body of *Pilobolus longipes* (*cf.* Figs. 27 and 28, pp. 69 and 70) suggests that at least nine-tenths of the fruit-body consists of cell-sap. When the sap is *in situ* in the subsporangial swelling and stipe, it is a very clear fluid. Dissolved within it, however, are various crystalloidal substances ; for, when a few drops of the sap are extracted from a number of fruit-bodies

and are allowed to dry up in a watch-glass, a moderately heavy white deposit is left behind. This deposit, when seen with the microscope, is found to consist of a fine flocculent precipitate through which run numerous, long, fairly thick, branched needles. A similar deposit forms the halo around a discharged sporangium (Figs. 71, 72, and 73). It is, of course, the substances dissolved in
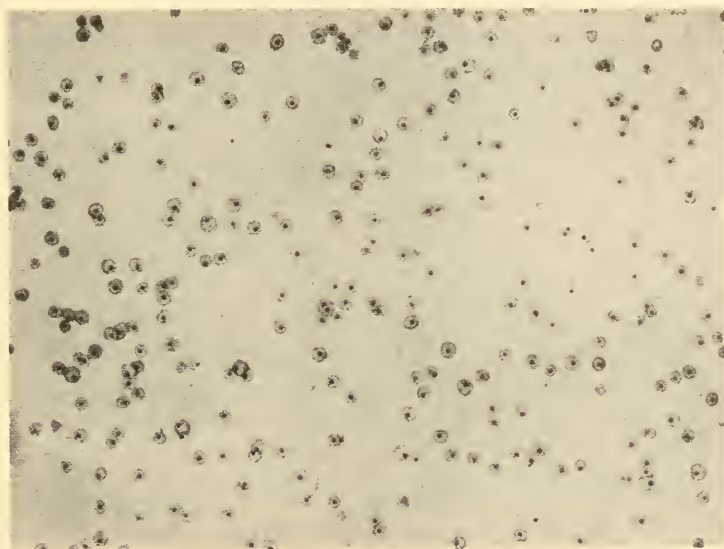
Fig. 72.—*Pilobolus longipes*. Sporangia which were shot vertically upwards for a distance of 4 feet 7 inches on to the under surface of a horizontal glass plate. Each sporangium is surrounded by a halo made up of a precipitate of salts, etc., which were dissolved in the large drop of cell-sap which accompanied the sporangium in its flight. Photographed dry in transmitted light.   Natural size.

the cell-sap which enable the sap to exercise its osmotic (turgor) pressure.

Since the discharge of the projectile is in large measure due to the osmotic (turgor) pressure of the cell-sap, and since this pressure is due to the sap's content of dissolved substances, it is of interest to determine what these substances are.

Employing the slide-crusher method already described, and working on four cultures of *Pilobolus longipes* which came to maximum fruition on four different mornings, I succeeded in harvesting

about 8 cc. of cell-sap ; [1] and I gave the sap to my colleague Dr. H. P. Armes, who very kindly undertook the task of analysing it chemically in so far as that was possible with the limited amount supplied.

Dr. Armes' analysis of the cell-sap of *Pilobolus longipes* yielded the following results :

Total solids (dried at 150° C.) . . . 1·84 grams in 100 cc.
Inorganic matter after ignition . . 1·14 grams per 100 cc.
The cell-sap contains : *potassium, sodium, chloride, sulphate, phosphate,* and *oxalate.*
100 cc. contains 0·26 grams [2] *phosphate* ion (PO_4).
100 cc. contains 0·14 grams [3] *oxalate* ion (C_2O_4).
There are indications of a carbohydrate, as shown by the Molisch test. This carbohydrate is not a reducing sugar. Seliwanoff's test is not given, so that sucrose is not present. Boiling with dilute hydrochloric acid gives a solution which reduces Fehling's solution. This might indicate a non-reducing sugar (? trehalose).

From this analysis we may conclude that the osmotic (turgor) pressure of the cell-sap of *Pilobolus longipes* is largely due to phosphate and oxalate ions, but is also due in part to potassium, sodium, chloride, and sulphate ions, and in part to some as yet unrecognised carbohydrate, possibly a non-reducing sugar such as trehalose.[4]

The Landing of the Pilobolus Projectile and the Attachment of the Sporangium to Herbage.—A sporangium, immediately after being squirted off the top of its sporangiophore, is in reality a concavo-convex body which in side view (Fig. 74) has the appearance of a plano-convex disc. As may be seen by reference to Fig. 30, C (p. 73), it is bounded : (1) on its rounded upper and lateral sides by the convex cap-like portion of the sporangium-wall, which is intensely black except near its free margin where it is paler ; and (2) on its under side, in part by the annular mass of transparent jelly which on swelling split the sporangium-wall into two parts and thus forced its way to the exterior of the sporangium, and in part by the obtusely conical wall of the columella. To the lower margin of the

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[1] The harvesting process is a tedious one ; only about 1 cc. of sap was collected per hour.

[2] and [3] These amounts can be regarded as approximate only.

[4] When this Section was written Lepeschkin's analysis of the cell-sap of *P. longipes*, recorded in Chapter I, p. 25, was unknown to me.

wall of the columella is attached, as shown in Figs. 35 (p. 79), 36 (p. 80), and 37 (p. 81), a very narrow circular collar-like band of the sporangium-wall, which was separated from the convex cap-like portion of the sporangium-wall when the pressure of the annular mass of jelly caused the sporangium to dehisce. Within the sporangium are enclosed many thousands of orange-yellow spores
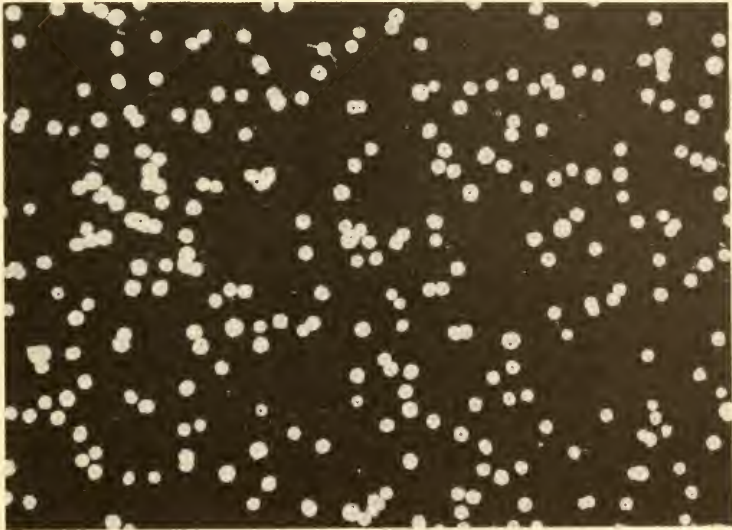
FIG. 73.—*Pilobolus longipes.* The projectiles, each consisting of a sporangium and a large drop of cell-sap, were shot vertically upwards for a distance of 4 feet 7 inches on to the under surface of a horizontal glass plate. The drops then dried up and thus formed around each sporangium a white halo of precipitated salts, etc. The photograph is similar to that shown in Fig. 72, except that it was taken, not in transmitted light, but against a black background in reflected light. Natural size.

(Fig. 30, C). As we shall see shortly, the fate of the spores is in a high degree dependent on the peculiar physical properties of the membranes which bound the sporangium at the moment of its discharge.

A Pilobolus projectile, as may be ascertained by examining it immediately after it has struck a sheet of glass or paper, consists of a sporangium and a large drop of cell-sap (Fig. 33, p. 77). When the projectile strikes any object, *e.g.* a blade of grass, the drop of cell-sap, owing to the force of impact, flattens out, and the sporan-

gium takes up a position at or near the centre of the drop *with its gelatinous side turned toward the object struck* as shown in Fig. 74, B. The water in the drop and in the sporangium then evaporates, in consequence of which the sporangium becomes fixed to its substratum by its gelatinous band and surrounded by a halo of tiny particles precipitated from the drop of cell-sap (Figs. 72 and 73). As the sporangium dries up, its content of spores and its band of jelly shrink to such an extent that the free pale edge of the black sporangial wall comes into contact with the surface of the substratum. Therefore, after a sporangium has landed on a blade of grass or other herbage and has dried up, it is very firmly attached to its substratum and, at the same time, its spores are all protected from the light and from mechanical displacement by its tough black wall.

The rule for the mode of landing of a sporangium, already briefly stated, may be re-stated more precisely as follows : a sporangium lands on any object which it happens to strike—no matter what may be the velocity of the sporangium when it strikes and no matter whether the surface of the object looks upwards or downwards or is vertical—so that, with rare exceptions, *its under gelatinous side is turned toward the surface of the object* and *its upper black convex side away from the surface of the object.* This remarkable rule has long been known, for it was enunciated by Coemans[1] in 1861 and discussed by Grove[2] in 1884. Coemans examined 413 sporangia which had fallen on to a sheet of white paper set out to receive them and he found that all but three of them had their gelatinous sides turned downwards toward the paper. The three exceptional sporangia were upside down and rested on their black sporangial walls. Some observations of my own on the landing of sporangia on the under side of sheets of glass, when the sporangia were travelling at various speeds, will now be recorded.

Some fruit-bodies of *Pilobolus longipes,* attached to a small mass of dung on which they were growing, were placed upright in a compressor cell, so that their sporangia were only about 5 mm.

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[1] E. Coemans, " Monographie du genre Pilobolus," *Mém. cour. et des Sav. étrang. Acad. roy. de Belgique*, T. XXX, 1861, p. 59.

[2] W. B. Grove, *loc. cit.,* p. 17.
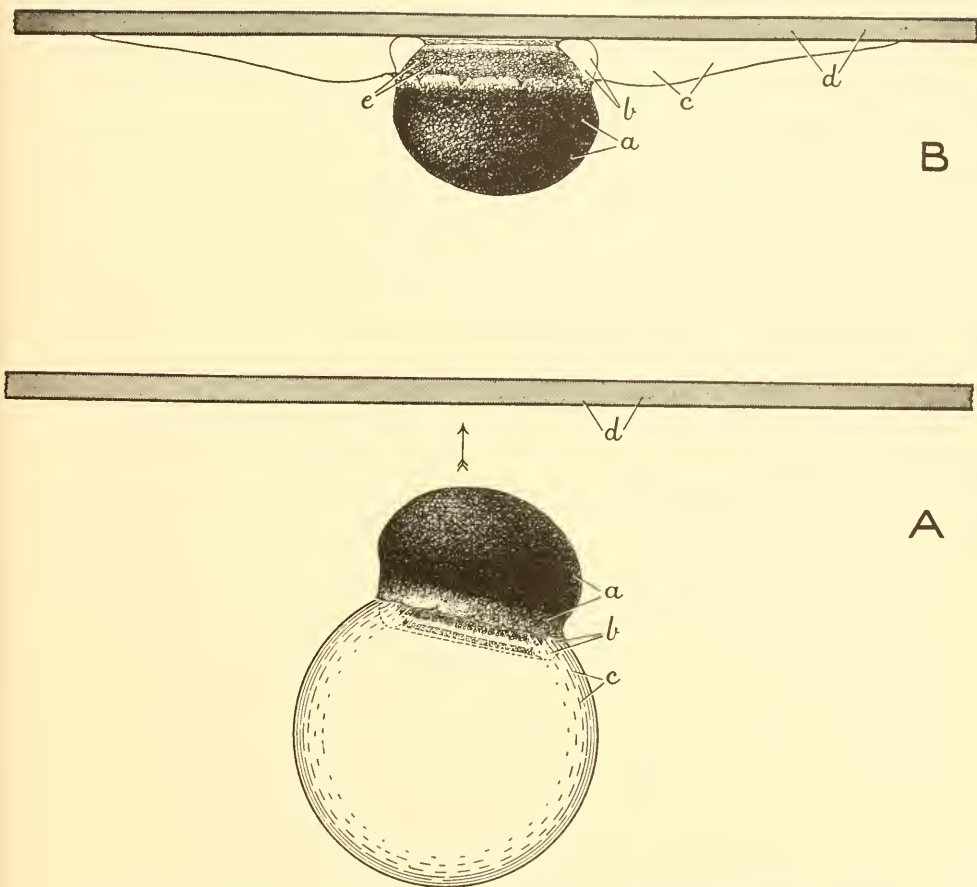
Fig. 74.—Diagram to illustrate the manner in which a projectile of *Pilobolus Kleinii* strikes and adheres to an object in the path of its trajectory. A, a projectile which is being shot upwards in the direction shown by the arrow and is about to strike the cover-glass *d*. The projectile consists of : (1) a sporangium, filled with spores, covered in part by a black unwettable sporangium-wall *a* and in part by a basal gelatinous ring *b* and the wall of the columella (not here shown), both of which are readily wetted ; and (2) a large drop of cell-sap *c* which has been shot out of the subsporangial swelling of the Pilobolus gun concerned and which adheres to the wettable structures just mentioned. A point on the left side of the sporangium just below the arrow will strike the cover-glass first. There will be a moment of momentum about this point and the drop of cell-sap will quickly move upwards about the point, strike the cover-glass, and flatten out upon it. As the drop flattens, it must inevitably push the unwettable black wall of the sporangium away from itself and the cover-glass and drag the wettable gelatinous cell-wall and the wall of the columella toward itself and the cover-glass. The result of all this, when the projectile has come to rest, is represented at B. In B there are shown : *a*, the unwettable black sporangium-wall ; *b*, the wettable gelatinous ring surrounding the spores *e* ; and the drop of cell-sap *c*, which is now flattened out on the cover-glass *d*. As the whole dries up, the sporangium shrinks until the broken edge of the black wall touches the cover-glass, the drop *c* becomes a halo of precipitated particles, and the gelatinous layer causes the sporangium to adhere very firmly to the cover-glass. Magnification, 63.

away from the under surface of the cover-glass. The sporangia in due course were discharged. Although their initial velocity must have been 10–20 feet per second [1] and they must have struck the cover-glass within less than one five-hundredth of a second after leaving their sporangiophores, yet, when they landed on the cover-glass, they settled there with their gelatinous lower surfaces turned upwards and in contact with the under surface of the glass (*cf.* Fig. 74, B). The results of this experiment and of others [2] made when determining the vertical range of the Pilobolus gun prove that, whatever may be the speed at which it is travelling, a sporangium normally lands on any object it may strike so that its basal gelatinous surface is next to the surface of the object.

An attempt to explain why a sporangium lands with its gelatinous side toward the surface of the object struck was made by Grove [3] who says : " The upper surface of the sporangium is round and practically smooth (though not actually so), and the lower edge and face are occupied by the gelatinous substance. Now, when a sporange is thrown upwards it will certainly rotate as it flies ; if the smooth top only comes in contact with the glass (or other vertical surface) it will not adhere, and the sporange will fall down again. But, if any portion of the gelatinous substance touches the glass, the force of progressive attraction between it and the thin film of moisture which will usually cover the glass [4] must invariably bring the lower, somewhat plane, surface of the sporangium in close contact with the glass. In the case of the paper, the sporangia would naturally roll over, if they fell on the convex surface, and settle on their lower face." This explanation is unsatisfactory because it fails to take into account the fact that a discharged sporangium is accompanied by a large drop of cell-sap and also involves two unsound assumptions : (1) that, if a sporangium strikes a vertical surface by its smooth rounded black surface only, it will fall to the ground ; and (2) that a vertical surface must be covered by a film of moisture to enable the sporangium to stick to

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[1] *Vide supra*, p. 67.   [2] *Vide supra*, pp. 65–66.

[3] W. B. Grove, *loc. cit.*, p. 17.

[4] Grove covered his culture with a bell-jar, on the inner surface of which moisture was condensed.

it. The assumptions are not justified : for there is no evidence to show that a sporangium ever strikes an object without sticking to it, and there is plenty of evidence to show that a sporangium will stick to the vertical surface of any object when the surface is perfectly dry.

Ingold [1] has supposed that, from the moment the sporangium is discharged, it trails behind the drop which carries it forward, so that the drop always strikes the obstacle first and causes the sporangium to settle with its gelatinous side toward the obstacle. As already pointed out in Chapter I,[2] it seems most unlikely that the projectile, on leaving the sporangiophore, should rotate through exactly 180° and no further.

A satisfactory explanation of the mode of landing of a sporangium, as we shall see, can be made if one takes into account the fact—hitherto noticed only by Ingold—that water can adhere to the under side but not to the upper side of the sporangium or, in other words, that *the lower gelatinous side of the sporangium can be wetted by water*, whereas *the upper convex side covered by the sporangial wall is unwettable*. The resistance to being wetted offered by the sporangial wall may be due in part to the crystals of calcium oxalate which protrude in such large numbers from its surface. Some evidence to show that the sporangial wall actually is unwettable will now be brought forward.

If one brings some freshly-discharged sporangia into contact with water contained in a crystallising dish, they at once float (*cf.* Fig. 76, B) at the surface with their lower gelatinous side immersed in the water and their upper black convex side standing out of the water, looking upwards, and appearing to be perfectly dry. The sporangia will float in this way for several days. If one tries to submerge the floating sporangia with a glass rod, it is difficult to drive any of them below the surface of the water, and one perceives that this is due to the resistance of the black sporangial wall to being wetted and the consequent action of the force of surface tension. When a sporangium has been submerged, one can see

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[1] C. T. Ingold, "The Sporangiophore of Pilobolus," *The New Phytologist*, Vol. XXXI, 1932, pp. 58–63.

[2] This volume, p. 46.

that the surface of the black sporangial wall shines with a white light owing to its being covered by a thin film of air.[1]

A wide test-tube was almost filled with water and then covered

with a dung-ball which bore a number of Pilobolus fruit-bodies in such a way that the fruit-bodies pointed directly downwards to the water. After a time, a number of the fruit-bodies discharged their sporangia which were shot downwards at the surface of the water which was only one centimetre or so distant (*cf.* Fig. 75 in which the water is 3 cm. from the sporangia). The velocity of the sporangia was probably not less than 10–20 feet per second. Most of the sporangia were shot through the surface film of the water, descended through the water for a short distance, and then slowly rose until they again reached the surface film under which they remained without breaking through into the air. However, some of the sporangia, when shot downwards, *failed to penetrate through the surface film of water.* This failure was evidently due to the sporangium-wall being unwettable

Fig. 75.—The discharge of Pilobolus projectiles into water. A wide test-tube was half filled with water. A piece of horse dung bearing fruit-bodies of *Pilobolus longipes* was then removed from a culture dish and placed in the mouth of the tube so that the sporangia looked downwards. When the sporangia were shot away, many of them failed to penetrate the surface film of the water, owing to the sporangial wall being unwettable. Natural size.

[1] A submerged sporangium may sink to the bottom of the water or slowly rise and come to the surface, where it may either break through the surface film and resume its old position with its black wall protruding clear above the water or it may fail to break through the surface film and lie just beneath it.

and to the resistance consequently offered to the passage of the sporangia by surface tension.

A number of sporangia were floating at the surface of some water contained in a crystallising dish. With a pipette some of the water and some of the sporangia were removed from the dish, and a drop of the water bearing a sporangium was caused to hang at the end of the pipette, as shown in Fig. 76, A. The sporangium remained at the surface of the hanging drop in an inverted position, with
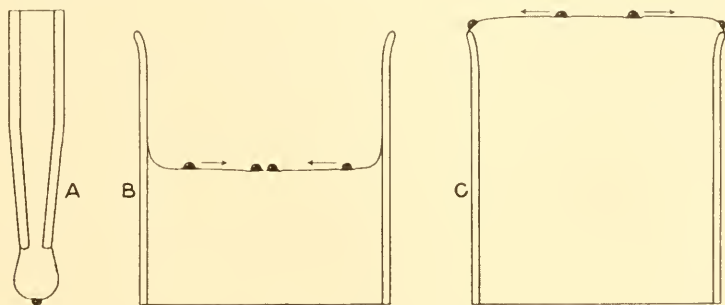
FIG. 76.— *Pilobolus longipes*. Diagrams showing sporangia in water. The wettable annular mass of jelly of each sporangium is immersed in the surface film of water, while the black unwettable sporangial wall protrudes into the air. A, a sporangium attached to a drop of water hanging from a pipette. B, a test-tube partly filled with water, and C another test-tube overfilled with water. Some sporangia have been placed in the water film. As indicated by the arrows, the sporangia in B move toward the centre of the surface film of water, while those in C move toward the edge. If the black sporangial wall were wettable instead of unwettable, the movements would be in directions opposite to those indicated. Somewhat enlarged.

its gelatinous side submerged in the surface film of water and its unwettable black convex side protruding into the air.

As is well known, glass is wettable by water and, when water is set in a glass dish, the water film bends upwards against the glass. If now tiny wettable objects which float, such as hollow glass beads, are set in a small glass vessel containing water, they move to the sides ; whereas, if similar objects but unwettable, such as hollow glass beads coated with paraffin wax, are set in the water, they move away from the sides of the vessel toward the middle of the film of water.[1] Some sporangia of Pilobolus were placed in water contained

[1] C. V. Boys, *Soap Bubbles and the Forces which Mould them*, The Romance of Science Series, S.P.C.K., London, 1895, pp. 33–34.

in a test-tube one inch in diameter. They behaved like paraffined beads, *i.e.* they at once moved away from the sides of the test-tube and collected in the middle of the surface film of water (Fig. 76, B).

If a small glass vessel is over-filled with water so that the surface film of water bends downwards to the glass rim, tiny wettable floating objects, such as unparaffined hollow glass beads, move from the sides of the vessel to the centre of the film of water, whereas tiny unwettable floating objects, such as paraffined hollow glass beads, move from the centre of the water film to the sides of the vessel.[1] Some sporangia of Pilobolus were placed in water which overfilled a wide test-tube one inch in diameter. They behaved like paraffined glass beads, *i.e.* they at once moved away from the centre of the surface film of water and came into contact with the rim of the test-tube (Fig. 76, C).

The two physical experiments just described afford further evidence that the black convex sporangial wall of Pilobolus is unwettable.

In the light of the observations just recorded, an attempt will now be made to explain how it is that a discharged sporangium always settles on any object it strikes so that its basal gelatinous side is turned toward the surface of the object.

The Pilobolus projectile consists not of a sporangium only as Grove and others have supposed but, as we have seen, of a sporangium and a large drop of cell-sap. Owing to the fact that the gelatinous under side of the sporangium is wettable while the black convex upper side is unwettable, it is clear that, as the sporangium is travelling through the air, the drop of sap must be attached to the gelatinous side of the drop, as shown in Fig. 74, A (p. 151) ; and this view is supported by the pipette-drop observations described above. It may well be, as Grove supposed, that the projectile rotates as it travels forward, but whether it rotates or not does not make any difference to the explanation of the mode of settling of the sporangium, which is now about to be given.

Let us suppose that a Pilobolus projectile is travelling vertically upwards toward a sheet of glass. As it is about to strike the surface

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[1] C. V. Boys, *Soap Bubbles and the Forces which Mould them*, The Romance of Science Series, S.P.C.K., London, 1895, pp. 33–34.

of the glass there are five possible ways in which its two elements may be arranged : (1) the drop of cell-sap may be directly in front of the sporangium, so that the axis of the projectile is perpendicular to the surface of the glass ; (2) the drop of cell-sap may be more or less in front of the sporangium, so that the axis of the projectile is inclined to the surface of the glass ; (3) the sporangium and the drop of cell-sap may be side by side, so that they strike the glass simultaneously ; (4) the sporangium may be more or less in front of the drop of cell-sap, so that the axis of the projectile is inclined to the surface of the glass ; and (5) the sporangium may be directly in front of the drop of cell-sap, so that the axis of the projectile is perpendicular to the surface of the glass. All these possible arrangements must now be considered and, for the sake of convenience, they will be considered in the following order : (1) and (2), (4), (3), and (5).

(1) and (2). If *the drop of cell-sap is either directly or more or less in front of the sporangium*, the drop will strike the surface of the glass first, flatten out there, and so cause the sporangium, which owing to adhesion cannot leave its surface, to take up a position at or near its centre, so that the gelatinous side of the sporangium will be turned toward the surface of the glass and the unwettable convex side will be turned away from the surface of the glass and will project freely into the air, as shown in Fig. 74, B.

(4). If *the sporangium is more or less in front of the drop of the cell-sap, but not directly in front* as in case (5), as shown in Fig. 74, A, the sporangium will strike the surface of the glass first and a moment of momentum about the point of first contact of the sporangium with the glass (to which the arrow in Fig. 74, A, points) will rotate the projectile (to the left in Fig. 74, A), with the result that the drop of cell-sap will strike the surface of the glass, spread out owing to the force of impact and, in spreading out, drag the sporangium round by its adhesive wettable side at the same time pushing away its non-adhesive unwettable side, thus forcing the sporangium to take up a position at or near the centre of the drop with its gelatinous under side toward the glass and still in contact with the water and with its black convex side projecting into the air.

(3). If *the sporangium and the drop of cell-sap strike the surface*

of the glass simultaneously, the drop will flatten out on the surface of the glass and force the sporangium to take up its characteristic position on the glass by the same means as described under (4).

(5). If *the sporangium is directly in front of the drop of cell-sap* so that the axis of the projectile is perpendicular to the surface of the glass, the sporangium will strike the surface of the glass first and be momentarily overwhelmed and imprisoned between the glass and the drop ; but, immediately thereafter, owing to the lateral spreading of the drop and to the unwettability of the sporangium-wall, it is probable that the thin water-film covering the sporangium will break in such a way as to cause the drop to pull
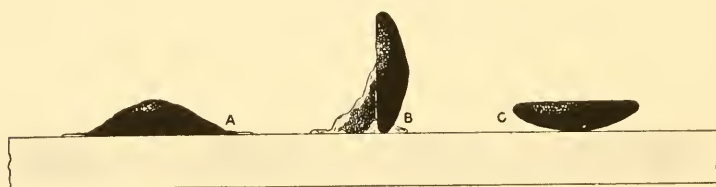
Fig. 77.—*Pilobolus longipes.* Diagram showing in lateral view the position of a discharged sporangium after landing on a substratum. A, normal position ; sporangium attached to the substratum by its gelatinous ring. B and C, abnormal positions. B, sporangium resting on its side, gelatinous ring and spores partly displaced from their normal position. C, sporangium upside down ; it has no gelatinous ring. Magnification, about 44.

the sporangium round through an angle of 180° and so cause the sporangium to reverse its former position, and take up the normal position for a discharged sporangium, namely, that shown in Fig. 74, B.

Summing up the explanation given above, it may be said in general terms that a sporangium settles on any object it may strike by its gelatinous side because its gelatinous side is wettable and its convex side covered by the black sporangial wall is unwettable, and because the sporangium is accompanied to its destination by a large drop of cell-sap.

Very occasionally, a sporangium lands on the side of a glass plate or other object so that it comes to rest in an abnormal manner, *i.e.* so that its gelatinous side is not flattened out against the object struck and so that it rests on its side (Figs. 77, B, and 78, A) or on

its apex (Figs. 77, C, and 78, B) or in some intermediate position. I have observed that two sporangia which had landed upside-down had lost their gelatinous ring (Figs. 77, C, and 78, B). It seems likely that the loss of this wettable substance was the cause of the sporangia coming to rest upside-down. The sporangium which had landed in such a way as to rest on its side (Figs. 77, B, and 78, A)
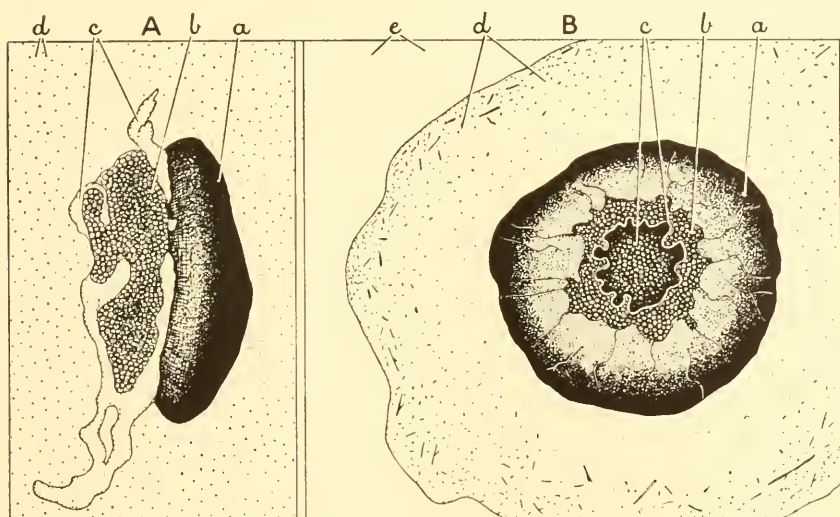
FIG. 78.—*Pilobolus longipes*. Two projectiles which landed on a glass slide abnormally; seen from above under the low power of the microscope. A, the sporangium is resting on its side: *a*, the sporangium-wall; *b*, spores, outside the sporangium; *c*, the gelatinous ring, abnormally torn and displaced; *d*, precipitate from the drop of sap which accompanied the sporangium. B, the sporangium is resting upside down; it lacks its gelatinous ring: *a*, the sporangium-wall incurved over the spores; *b*, spores inside the sporangium; *c*, the columella through which spores can be seen; *d*, precipitate of crystals and amorphous particles from the drop of sap which accompanied the sporangium; *e*, the surface of the glass slide. Magnification, 100.

had its gelatinous ring broken and displaced. Here, again, the abnormal position taken up by the sporangium appears to have been caused by an accident to the gelatinous ring, in this case its displacement from its usual position.

It has been shown (1) that the sporangium alights on any object it strikes so that its gelatinous side is turned toward the object struck and (2) that the ring of jelly, on drying up, attaches the sporangium to its substratum. We may now enquire into (3) the effectiveness of the attachment after it has once been accomplished.

The gelatinous ring of a sporangium of *Pilobolus Kleinii* which has dried up on a sheet of glass is so strongly attached by its lower surface to the glass and by its upper surface to the lowest layer of the spores which make up the spore-mass that, if one scrapes the sporangium off the glass with a knife, the dried-up gelatinous ring and the adherent lower halves of the walls of the lowest layer of spores are left behind together *in situ*. The broken walls of the spores are colourless and, in the mass, form a hexagonal pattern reminding one of honey-comb. When a drop of water is added, the jelly swells up and each hexagonal half-spore wall separates from its neighbours and comes to have an oval outline.

Under natural conditions in the open, *Pilobolus Kleinii*, *P. longipes*, etc., grow in pastures on the dung-plats of horses, cows, and other herbivorous animals, and the sporangia which their guns shoot away strike and stick to the surface of leaves, stems, and inflorescences of grasses and other plants making up the surrounding herbage (Fig. 80, p. 164) ; and the sporangia, on drying up, become very firmly attached to their substrata, so firmly indeed that the wind, however strongly it may blow, cannot dislodge them.

Sporangia which have dried up are also not easily dislodged from their places of attachment by falling drops or moving films of water. Evidence of this fact was obtained from some experiments which will now be described.

A plate of glass to which a number of sporangia were attached was set obliquely under a tap in such a way that six of the sporangia were struck by a rapid stream of large water drops which fell from the nozzle of the tap for a distance of about one foot. All of the six sporangia withstood the hammering of the drops unmoved for half an hour. Then one of them was washed away. The other five were washed away in the course of the next two and a half hours. A number of other sporangia which were on the plate and were washed by the stream of water running down the plate for three hours were, at the end of this time, still attached to the plate in their original positions.

In another experiment, a postage stamp was stuck on a glass plate to which some sporangia were already attached and, as before,

drops of water were allowed to fall from a tap on to some of the sporangia ; and the plate was so arranged that the stream of water, after leaving the sporangia, ran over the postage stamp. The postage stamp was washed off the plate in 3·5 minutes, whereas the sporangia withstood the impact of the drops for half an hour.

The effectiveness of the gelatinous ring in enabling the sporangium to cling to its substratum even when battered by large drops of water as in the experiments just described appears to be due to the fact that the jelly, after being dried and placed in water again, swells up by the absorption of water but only to *about its original volume*, so that, unlike the mucilage on a postage stamp, it does not dissolve in water.

From the observations just recorded we may conclude that, under natural conditions in pastures, the sporangia are so effectively attached by their gelatinous bases to the herbage on which they have alighted that, in dry weather, they cannot be detached from their substrata by the strongest winds and, in wet weather, they cannot easily be detached by prolonged rain or even by violent thunderstorms.

The Relations of Pilobolus with Flowering Plants and with Herbivorous Animals.—Pilobolus is a highly specialised coprophilous fungus which is dependent for its existence : firstly, on *flowering plants* which provide its sporangia with a temporary but prospectively favourable lodging-place and, secondly, on *herbivorous animals* which swallow the sporangia and herbage together, break open the sporangia and disperse the spores within their alimentary canals, and finally extrude the spores undamaged in their solid faeces. The spores, thus sown in dung-plats in pastures, rapidly germinate, and the mycelia to which they give rise soon develop new fruit-bodies which, in their turn, shoot away their sporangia on to the surrounding herbage (Fig. 79).

The smaller sporangia of *Pilobolus Kleinii* and of *P. longipes* are often shot to a horizontal distance of 3–5 feet whilst the largest ones, as we have seen, are sometimes shot a horizontal distance of about 8 feet. Therefore, when fruit-bodies of these species are growing on a dung-plat in a pasture, hundreds of sporangia may be, and often actually are, shot away so as to dot the leaves and stems of grasses
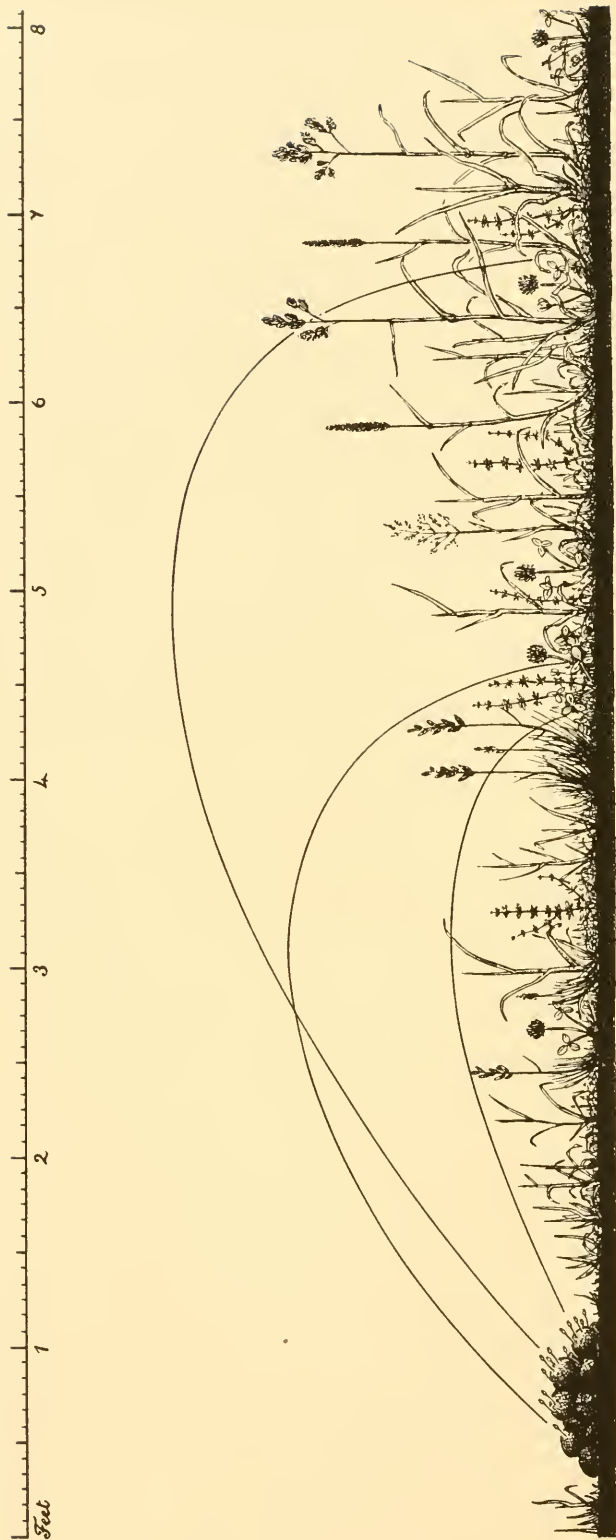
Fig. 79.—*Pilobolus longipes*. Diagrammatic representation of the discharge of sporangia in a meadow. To the left is a horse-dung plat, deposited about five days ago and now bearing fruit-bodies. It is noontide and the fruit-bodies are beginning to discharge their sporangia. The trajectories of three discharged sporangia are indicated. Owing to the resistance of the air, the sporangia, like spent bullets, have descended more steeply than they rose. As may be seen by reference to the scale of feet, the sporangium shot farthest travelled horizontally about six feet.

and other plants growing on many square feet in the neighbourhood of the dung-plat.

The discharge of the Pilobolus guns brings about the dispersion of the sporangia but not of the spores ; for each sporangium, on its flight through the air, carries all its spores with it and, after it has become fixed to the leaf or the stem of a flowering plant (Fig. 80), its tough black indissoluble sporangial wall prevents the spores from escaping from its interior.   When a sporangium has once become attached to a flowering plant, neither wind nor rain can dislodge it or open it, so that clearly wind and rain can have nothing to do with the dispersion of the spores.   The dispersion of the spores can be effected only through the agency of herbivorous animals.

The greater the horizontal range of the Pilobolus gun, the greater the number of square feet of herbage surrounding dung-plats which will become sprinkled with sporangia, and the greater the chance of the sporangia being swallowed by horses, cows, and other herbivora.   Hence the great violence of discharge of the sporangia is a factor which, in the end, favours the dispersion of the spores and the persistence of Pilobolus species.

The heliotropic response of the fruit-bodies of Pilobolus results in each sporangium being shot toward the source of the strongest incident rays of light and, therefore, *in the direction in which there are fewest obstacles to its flight through the air*.   In laying the Pilobolus gun, light is used in the most efficient manner as a directive agent.   The discharge of the sporangia in the direction of least resistance to their flight favours the scattering of the sporangia on surrounding herbage and, therefore, ultimately increases the chance of the sporangia being swallowed by herbivorous animals.

As a rule, only the spores of sporangia which are swallowed by a grazing animal find their way into a dung-plat.   Hence the importance of the sporangia becoming firmly fixed to the flowering plants which they happen to strike and being thereby prevented from falling to the earth where they would be wasted.   Hence therefore, also, the advantage of the discharged sporangium being provided with a strongly adhesive gelatinous ring, and of the sporangium always landing with the ring toward the surface of the flowering plant.   It is the resistance to being wetted offered by the wall of

Fig. 80.—Living shoots of a wild grass, bearing numerous, firmly adherent sporangia of *Pilobolus longipes*. The sporangia were shot on to the leaf-blades, leaf-sheaths, and stem from fruit-bodies growing on near-by horse-dung balls contained in a large culture chamber in the laboratory. Photographed through a green screen. Natural size.

the sporangium which enables the drop of cell-sap accompanying the discharged sporangium to turn the sporangium round at the moment of landing so that the gelatinous ring comes into contact with the surface of the object struck. The unwettability of the sporangium-wall, in promoting the fixation of the sporangium, indirectly promotes the dispersion of the spores.

After a sporangium has become attached to the leaf or stem of a grass or other flowering plant, many weeks or even months must often go by before it is swallowed by a herbivorous animal. During this time it may be exposed for a great many hours to brilliant sunshine and yet (there is every reason to suppose) the spores retain their vitality unimpaired. The spores of Pilobolus have colourless spore-walls and therefore, like the colourless spores of *Schizophyllum commune* and *Daedalea unicolor*,[1] they might be killed by prolonged exposure to the sun's direct rays ; but they are protected from any possibly injurious rays of light by the sporangium-wall which is so intensely black that it must absorb practically all the light which it does not reflect or diffuse.[2]

[1] These *Researches*, Vol. I, 1909, pp. 24–26.

[2] Coprinus, Panaeolus, Anellaria, Sordaria, and certain other genera of coprophilous fungi have black spores. These spores, like the black sporangia of Pilobolus, settle on and become firmly fixed to the stems and leaves of grasses and other flowering plants in pastures, and they must often wait through long periods of sunny weather before they are swallowed by herbivorous animals (*cf.* these *Researches*, Vol. III, pp. 229–230). While exposed to sunlight, these spores are protected from possibly injurious rays of light by their own light-screens, namely, their rather thick black walls. In Pilobolus where the spores in a sporangium are protected by a common light-screen, namely, the black sporangial wall, and the spore-walls therefore cannot function as light-screens, the spore-walls are colourless. The presence of a blackish pigment in the wall of the sporangium and the absence of such a pigment from the walls of the spores are just what might be expected if, as I think probable, the sporangial wall does actually protect the spores from injurious rays of light.

While in Coprinus, Panaeolus, etc., as we may suppose, the pigment in the spore-wall enables the wall to act as a light-screen and is therefore functionally useful, in other equally coprophilous fungi, e.g. *Aleuria vesiculosa*, *Lachnea stercorea*, and *Humaria granulata*, the spore-walls are colourless. It is possible that, in these species, owing to the fact that the spore-walls are colourless and therefore cannot function as a light-screen, the spores, when exposed to sunlight, are more readily killed than the black spores of Coprinus, etc. An experimental test of this suggestion is desirable.

The spores of Hypoxylon, Xylaria, Rosellinia, Bulgaria, and certain other fungi which are not normally coprophilous but live on dead wood or other plant

That the spores contained in sporangia attached to grasses, etc., do actually retain their vitality for at least nine months may be inferred from the fact that at Winnipeg, in March and April, *i.e.* toward the end of the long winter, horses fed in stables on hay which has been gathered in the previous summer yield dung-balls which often produce fruit-bodies of *Pilobolus Kleinii* and *P. longipes* in great abundance.

The sporangial wall becomes impregnated with its black pigment long before the sporangium is shot away ; and, therefore, if there were no subsporangial swelling, the sporangium of an intact fruit-body would, when the fruit-body was turning toward the light, cut off light from the stipe and render an accurate heliotropic reaction to the strongest incident rays impossible. The intercalation of the subsporangial swelling, which acts as an ocellus, between the black sporangium and the stipe is an admirable arrangement which neutralises the shadowing effect of the sporangium, enables the stipe to turn the subsporangial swelling and sporangium toward the source of strongest light with a considerable amount of precision and, therefore, increases the chance of the sporangium being shot to a distance on to herbage.

substances are as black as those of Coprinus, Panaeolus, etc., although they are not of necessity exposed to sunlight for long periods of time. Their black walls must act as a light-screen to the protoplasm contained in their interior, but it is possible that the pigment is a mere by-product of the metabolism concerned with the development of the wall and, for the spores in question, is of little or no ecological significance. The black pigment in the walls of the outer cells of the shoe-string-like *rhizomorpha subterranea* of *Armillaria mellea* and of the cells making up the blocking layer of the mycelium of *Polyporus squamosus*, *Fomes applanatus*, *Armillaria mellea*, and many other wood-destroying fungi (*vide infra*, Vol. VII), so far as the absorption of light is concerned, can be of no possible ecological significance since the pigment is present in structures which normally are developed only in the dark.

In support of the view that the black pigment of the sporangium-wall of Pilobolus and the black pigment of the spore-walls of Coprinus, etc., do actually protect the spores from injurious radiation may be cited the recent work of Rabinovitz-Sereni ("Il grado di resistenza di alcuni funghi all'azione dei raggi ultravioletti," *Boll. R. Staz. Pat. Veg.*, N.S., XII, 1932, pp. 115-144 ; cited from *Review of Applied Mycology*, XI, 1932, pp. 737-738) who found that " dark thick-walled conidia such as those of *Helminthosporium gibberosporum*, *Coniosporium bambusae*, and *Epicoccum purpurascens* resisted the exposure to ultraviolet light for 180 minutes ; slightly olivaceous conidia, such as those of *Microascus stysanus* and *Penicillium crustaceum* withstood exposure for 25 minutes, while hyaline conidia, such as those of *Clonostachys araucaria*, *Fusarium martii*, and the pycnospores of *Deuterophoma tracheiphila* withstood exposure for only 10 minutes."

Mucor and Pilobolus are closely related genera and it may well be that Pilobolus, through Pilaira, was evolved from a Mucor.   It is therefore not without interest to compare the sporangial walls of these two genera.   The sporangial wall of Mucor is *wettable, non-persistent* in that it is diffluent in water, and more or less *colourless* ; whereas the sporangial wall of Pilobolus is *unwettable, persistent* and not diffluent in water, and *intensely black*.   The unwettability, persistency, and high pigmentation of the sporangial wall of Pilobolus can all be regarded as special adaptations which contribute to the success of Pilobolus as a coprophilous fungus ; for, as we have seen : (1) the unwettability of the wall is a prime factor in causing the sporangium to alight on any object with its gelatinous side turned toward the object : (2) the persistency of the wall enables the wall, when the sporangium is attached for weeks or months to a flowering plant, to prevent the spores from escaping from the sporangium even during rainy weather ; and (3) the intensely black pigment in the wall enables the wall to absorb sunlight and thus to act as a light-screen in cutting off injurious rays of light from the spores which lie beneath it.

After swallowing a sporangium attached to a blade of grass, a herbivorous animal must often travel many miles before extruding the faeces in which the spores have become embedded.   Hence it is clear that herbivorous animals are responsible for the geographical distribution of Pilobolus under natural conditions.   It may also be remarked that, since the sporangia of Pilobolus cling so tenaciously to dry grass, the commercial transportation of hay must often involve the spread of Pilobolus species from one country to another.

Often, on a cow dung-plat or a horse dung-plat in a field, one may observe several hundred fruit-bodies of Pilobolus producing sporangia at one and the same time.   Let us suppose that the fruit-bodies of *Pilobolus Kleinii* produced on a single dung-plat in the course of several days have shot away 1000 sporangia on to the surrounding grass and that, on the average, each sporangium contains 45,000 spores.   Then the total number of spores contained in the sporangia on the grass will be 45,000,000.   When a horse or a cow comes and grazes near such a dung-plat as the one under consideration, in a few minutes it may take hundreds of sporangia into

its alimentary canal so that, in the end, its faeces—deposited several days later in various places in the pasture—will be thickly sown with hundreds of thousands, or even millions, of Pilobolus spores. Owing to (1) the very efficient way in which the sporangia are discharged and fixed to herbage, (2) the large number of the sporangia and still larger number of the spores often produced on a single dung-plat, and (3) the high probability that sporangia attached to herbage will be swallowed sooner or later by a herbivorous animal, it is not surprising that Pilobolus is so successful in maintaining its existence in pastures and often flourishes there in such great abundance.

Pilobolus gives to flowering plants and herbivorous animals nothing in return for their services in dispersing its spores. However, although Pilobolus does not pay for what it receives, it imposes on the organisms which assist it a burden which is so light as to be practically negligible.

CHAPTER III

Introduction—General Description—Taxonomic Description and Latin Diagnosis—
Remarks on the Pilobolidae.

Introduction.—In the winter of 1931–1932, two species of Pilobolus, *P. longipes* and *P. Kleinii*, were being cultivated and studied in the botanical laboratory of the University of Manitoba. These fungi commonly appeared on fresh horse dung, cow dung, etc., brought into the laboratory. Hans Ritter, a boy eleven years old, became interested in these cultures and made many similar ones for himself. His microscope was provided with a low-power objective only. With the help of this instrument he became well acquainted with *P. longipes* and *P. Kleinii*. One day he observed on a horse-dung culture a Pilobolus which he had not previously seen (Fig. 105, A, B, C, and D, p. 210), and he at once brought it to my laboratory, where an examination showed that it was a new species not yet described by any mycologist.

Fig. 81.—*Pilobolus umbonatus.* Wild, small fruit-body, in air. Magnification, 61.

The new species is distinguished from all other species of the genus by its decidedly umbonate sporangium (Fig. 81) and it is on this account that I have named it *umbonatus.*

Shortly after becoming acquainted with *Pilobolus umbonatus,*

I happened to be writing to the late Dr. Roland Thaxter of Harvard University and, in the course of my letter, hoping to receive from him some comment on the matter, I mentioned the new Pilobolus and gave a brief description of it.   In his reply he informed me that he had known my *P. umbonatus* for forty years and that its chief substratum was sheep dung.[1]   Thus the species occurs not only in central Canada but also in the eastern part of the United States of America.

General Description.—*Pilobolus umbonatus* was obtained upon horse-dung balls taken from the streets of Winnipeg in mid-
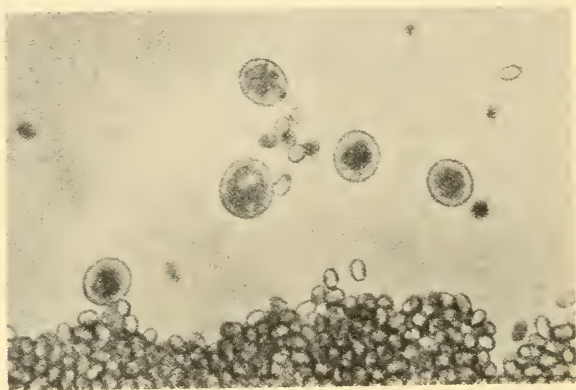
Fig. 82.— Photomicrograph of a mass of spores of *Pilobolus umbonatus* (small, ellipsoid) together with five spores of *P. longipes* (large, rounded-oval). Magnification, 510.

winter of the years 1931–1932 and 1932–1933.   The balls were brought into the laboratory and were placed in a crystallising dish covered with a glass plate.   Soon they thawed and, after a few days, the fruit-bodies of the new species began to appear upon them. New crops of fruit-bodies came up each day for four or more days in succession.   Well-grown fruit-bodies are 7–9 mm. in length but much shorter fruit-bodies have often been seen (*cf.* A and B in Fig. 105, p. 210).   It was observed on one dung-ball that the length of the fruit-bodies diminished as successive crops appeared : on the

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[1] Roland Thaxter : " I have known that ' umbonate ' Pilobolus for many (40) years.   You will find that on its natural substratum, which is more commonly sheep dung, it varies from a sharply pointed type to one which is quite bluntly rounded."   *In litt.*, Feb. 12, 1932.

first day the length was about 7 mm., on the second day about
5 mm., on each of the next five days 2–3 mm., and on the next day
only about 1·5 mm. The reduction in length of the fruit-bodies
was chiefly due to a shortening of the stipe and, no doubt, was
associated with a gradual diminution of the food supply. The
sporangia were shot away in the usual manner, and sporangium-
deposits were obtained upon glass slides.

As compared with *Pilobolus longipes* and *P. Kleinii*, *P. umbonatus*
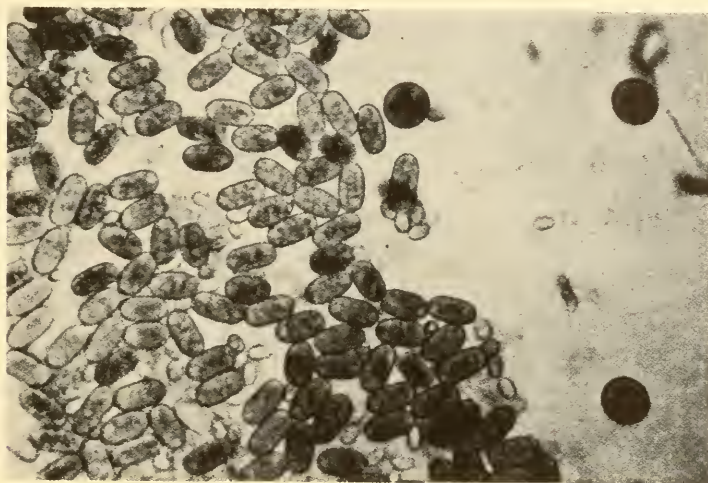
FIG. 83.—Photomicrograph of spores of three species of Pilobolus;
P. umbonatus (very small, pale, ellipsoid); *P. Kleinii* (much
larger, darker, ellipsoid); and *P. longipes* (three only to the
right, rounded-oval, very large and dark). Magnification, 510.

is to be regarded as a smaller and more delicate species. This may per-
haps be realized by comparing Figs. 81, 84, and 88, which respectively
show a rather small, a very large, and a medium-size fruit-body of
P. umbonatus, with Fig. 85, which shows a medium-size fruit-body of
P. longipes, and with Fig. 86, which shows two medium-size fruit-
bodies of *P. Kleinii*. All these Figures have the same magnification.

The spores are ellipsoid and very small, their dimensions being
5·0–6·0 × 3·0–3·8 μ. Singly they appear quite colourless, but in
the mass they are yellow.

The photomicrograph reproduced in Fig. 82 shows a mass of
Pilobolus umbonatus spores together with five of the rounded-oval,

much larger, *P. longipes* spores ; while the photomicrograph repro-
duced in Fig. 83, with the same magnification as for Fig. 82, shows
spores of *P. umbonatus*, *P. Kleinii*, and *P. longipes* in a single
field of view. In Fig. 83 one can readily distinguish the spores
of *P. umbonatus* by their ellipsoid form, paleness, and very
small size from those of *P. Kleinii* which,
although also ellipsoid in form, are very
much larger and darker, and from those
of *P. longipes* (three only, to the right)
which are rounded-oval in form, very
much larger, and darker. Thus Figs. 82
and 83 serve to demonstrate, in respect
to the nature of their spores, how very
distinct from one another are *P. umbo-
natus*, *P. Kleinii*, and *P. longipes*.

At room temperatures, in hanging drops
of cleared dung-agar, in the course of two
days, the spores of *Pilobolus umbonatus*
swell up greatly and put out germ-tubes
(Fig. 89, A, p. 181). Thereafter the germ-
tubes branch and develop into mycelia
(Fig. 89, B–D) with the usual two kinds of
hyphae : (1) *main* hyphae which become
very thick, contain much orange-yellow
protoplasm, and give rise to basal swellings
(trophocysts) of fruit-bodies ; and (2) much
smaller lateral hyphae which doubtless
send protoplasm and other materials into
the main hyphae.

FIG. 84. — *Pilobolus umbo-
natus*. Large fruit-body
from pure culture. Mag-
nification, 61.

The basal swellings (trophocysts) of fruit-bodies arise as rounded
or oval swellings in the coarse stolon-like hyphae (Fig. 89, B–D,
p. 181). They may be apparently terminal at the end of a hypha
(Fig. 90, C–E, p. 182) or be obviously intercalary (Figs. 89, D, and
90, A, B). Sometimes they are dispersed at intervals along a single
longer hypha (Fig. 90, G). Each basal swelling gives rise to a stipe
and, as the stipe grows out from it, it usually becomes more or less
turnip-shaped (Figs. 90, C–E, and 105, C, D, and H, pp. 182 and 210).

The stipe at its base where it adjoins the basal swelling is about 0·065 mm. thick. It increases slightly in thickness upwards until, just beneath the subsporangial swelling, it is about 0·1 mm. thick.

At the top of the stipe, just beneath the subsporangial swelling, there is an orange band of protoplasm.

The subsporangial swelling (Figs. 81, 84, 88, and 105, C–E) is ellipsoid, its greatest diameter being about the middle of its length. In well-grown fruit-bodies it is about 0·65 mm. long and 0·46 mm. wide. Like the stipe, it exudes numerous colourless mucilaginous drops (Fig. 84). In lateral view in air, when seen with a hand-lens, its base appears very pale, thus differing from the subsporangial swellings of *P. longipes* and *P. Kleinii* in which an orange tint is readily observable.

Fig. 85.—*Pilobolus longipes.* Upper part of a wild fruit-body of medium size. Drops on subsporangial swelling drying up, those on sporangium, already dry. Transparent jelly around base of dehisced sporangium not visible in the photograph. Magnification, 61.

The subsporangial swelling of *Pilobolus umbonatus* resembles that of *P. longipes* in being ellipsoidal and having its maximum diameter about the middle of its length, but differs from that of *P. Kleinii* which is distinctly pyriform (*cf.* Figs. 81, 84, and 88 with Figs. 85 and 86).

Pilobolus umbonatus is unique among Piloboli in having a sporangium which is more or less conical and decidedly umbonate (Figs. 81, 84, and 105, C–F). This characteristic enables one to
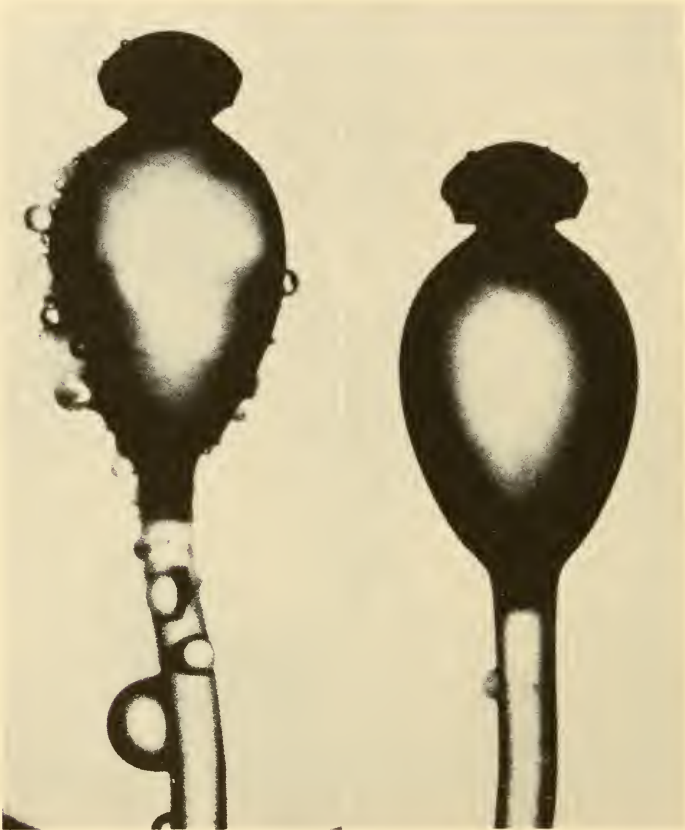
FIG. 86.—*Pilobolus Kleinii.* Two wild fruit-bodies of medium size, left with drops, right with drops removed or dried up. Transparent jelly around base of each dehisced sporangium invisible in the photograph. Subsporangial swellings pyriform. Magnification, 61.

recognise the species with certainty even with a hand-lens. Hundreds of sporangia have been observed and in every one a more sharply-rounded or less sharply-rounded umbo was present. The width of the sporangium in well-grown fruit-bodies varies from about 0·2 to 0·26 mm., and the height of the sporangium from about 0·14 mm. to 0·19 mm.

The sporangium-wall is intensely black except below, where it is very pale-grey or colourless. After a sporangium has dehisced and just before it is discharged, one can observe the colourless lower part or fringe of the sporangium-wall overlying the protruding jelly.

The ratio of the width of the sporangium to the width of the subsporangial swelling in *Pilobolus umbonatus* is almost exactly $\frac{1}{2}$, whereas this ratio in *P. longipes* is about $\frac{3}{5}$ and in *P. Kleinii* about $\frac{3}{4}$. When one looks at a living fruit-body of *P. umbonatus* in side view with a hand-lens, one perceives at a glance that the width of the sporangium relatively to the width of the subsporangial swelling is much less in this species than in *P. longipes* and *P. Kleinii*. An apical view of a fruit-body showing both sporangium and subsporangial swelling is reproduced in Fig. 105, F (p. 210).

A sporangium, after being discharged, dries and shrinks and becomes

Fig. 87.—*Pilobolus umbonatus.* Photomicrograph of the upper side of a dried discharged sporangium on a glass slide. No. 1, the dried cell-sap ; No. 2, the broad flat clear ring-layer of jelly ; No. 3, the narrow transparent fringe of the sporangium-wall overlying the jelly ; and No. 4, the convex very black main portion of the sporangium-wall covering the spores. Magnification, 120.

acutely pointed (Fig. 105, K). When sporangia of *P. umbonatus*, *P. longipes*, and *P. Kleinii* have been discharged on to the side of a glass dish and lie mixed near the top and one examines them in lateral view with a hand-lens, one can readily distinguish those of *P. umbonatus* from the two other species by the fact that the former are conical in shape, whereas the latter are rounded.

When a sporangium which has just been discharged on to a glass slide and has dried (Fig. 87) is examined from above with the microscope, it can be seen : (1) that the main convex portion of the sporangium-wall, which covers the spores, is intensely black and is

ornamented with minute crystals of calcium oxalate ; (2) that the fringe of the sporangium-wall, which overlies part of the gelatinous ring, is more or less *circular* at its free margin (not extended radially into broad rays),[1] is *transparent*, is *largely or quite free from crystals* of calcium oxalate, and is *colourless* or tinged very faintly with bluish-grey ; and (3) that the gelatinous ring extends radially from the black portion of the sporangium more than twice the distance of the fringe of the sporangium-wall.

The fringe of the sporangium-wall of the dried discharged

Fig. 88. — *Pilobolus umbonatus*, after removal of sporangium. The dark, bluntly rounded columella crowns the subsporangial swelling. Magnification, 61.

sporangium of *Pilobolus umbonatus* is so characteristic for the species that by this character alone one can readily distinguish *P. umbonatus* from *P. longipes* and *P. Kleinii*. In these two last-named species the fringe : (1) instead of being circular at the free margin, is *split into* broad radially-extended *rays* (Figs. 33, 34, and 39 ; pp. 77, 78, and 83) ; (2) instead of being largely or quite free from crystals, is *finely punctate with evenly-spaced crystals*, larger and smaller crystals usually forming a sort of pattern (Fig. 44, A, p. 88) ; and (3), instead of being colourless, is distinctly *brownish*.

As a sporangium which has been discharged on to a glass slide dries up, the umbo becomes more prominent (Fig. 105, K) and at the same time the black part of the sporangium-wall which covers the spores contracts considerably. In some sporangia the contraction is uniform or almost so, so that the dark wall remains smooth and devoid of depressions (Fig. 105, I) ; whilst in other sporangia it is uneven, so that the wall becomes dimpled or wrinkled. The wrinkles tend to take a radial direction, so that the depressions between them are often more or less triangular in outline (Fig. 105, J).

[1] It is possible that the part of the fringe corresponding to the rays of *P. longipes* and *P. Kleinii* is broken away from the fringe at the time the sporangium strikes an obstacle.

When with the help of the microscope one looks down upon dried discharged sporangia of *Pilobolus umbonatus, P. longipes*, and *P. Kleinii* attached to a glass slide, one can at once distinguish those of *P. umbonatus* from those of the other two species : (1) by the presence of the umbo which in strong lateral illumination reflects the light on one side ; (2) by the smoothness or radial wrinkling of the black sporangium-wall below the umbo ; and (3) by the fringe of the sporangium-wall which, as already mentioned, is transparent, practically colourless, circular at its margin, and largely or quite devoid of crystals (Fig. 105, I and J).

To observe the columella whilst this is still attached to the subsporangial swelling, one places a fruit-body in a drop of water on a slide and one then strokes away the sporangium with a needle. A columella exposed to view in this way (Fig. 88) is very bluntly conical or rounded in shape, distinctly grey or bluish-grey in colour, and connected at its base with the usual thin lower band of sporangium-wall that separated from the rest of the sporangium-wall when dehiscence of the sporangium took place.

The columella in a dried discharged sporangium attached to a glass slide can be brought into view by adding water and a cover-glass, and by then pushing the cover-glass laterally. Columellae brought into view in this way reveal their grey or bluish-grey colour and also the narrow band of sporangium-wall attached to their free margin.

Taxonomic Description and Latin Diagnosis.—An attempt will now be made to describe *Pilobolus umbonatus* for taxonomic purposes.

Pilobolus umbonatus, sp. nov. *Fruit-body* 3–9 mm. high, arising from an oval to turnip-shaped basal swelling or trophocyst which may be terminal or intercalary, single or dispersed at intervals along a coarse stolon-like main hypha. *Stipe* increasing slightly in diameter from below upwards, until just beneath the subsporangial swelling it is about 0·1 mm. in diameter. *Subsporangial swelling* ellipsoid, in well-grown fruit-bodies about 0·65 mm. long and 0·46 mm. broad ; a pale orange-red band of protoplasm at the junction of the stipe and the subsporangial swelling. *Sporangium* decidedly umbonate and more or less conical, 0·21–0·23 mm. in

diameter or about one-half the diameter of the subsporangial swelling, shrinking on drying after discharge and becoming acutely pointed ; columella very bluntly conical or rounded (when removed from a discharged sporangium its edge is turned inwards toward the axis), greyish, distinctly darker than the subsporangial swelling. *Spores* ellipsoid, singly almost colourless but yellow in mass, 5·0–6·0 × 3·0–3·8 μ.

On horse dung, Winnipeg, Canada, and, according to a communication from the late Dr. Roland Thaxter (who observed the species forty years ago but did not describe it), more frequently on sheep dung, at Boston, U.S.A.

Easily distinguished from all other species of Pilobolus by its decidedly umbonate sporangium and its minute ellipsoidal spores. With a hand-lens one can readily make out the acutely-pointed umbonate shape of the dried discharged sporangia when these are seen in lateral view.

LATIN DIAGNOSIS

Pilobolus umbonatus, sp. nov. *Hyphae sporangiiferae* 3–9 mm. altae, e trophocystide terminali v. intercalari ovali v. napiformi solitaria v. in hypha crassa repenti sparsa oriundae. *Stipes* filiformis, circa 0·1 mm. diam., sursum sensim latior. *Vesiculum subsporangiale* ellipsoideum, 0·65 mm. longum, 0·46 mm. latum, circa medianam partem latissimum. *Sporangium* eximie umbonatum, plus minusve conicum, 0·23 mm. diametro, arescendo acutius evadens, columellâ obtusissime conicâ v. convexâ, cinereâ, quam vesiculum obscuriore. *Sporae* minutae, ellipsoideae, dilutissime luteolae, ferme achroae, 5·0–6·0 × 3·0–3·8 μ.

Hab. in stercore equino, Winnipeg, Canada, atque (sec. litt. Thaxteri) in stercore ovino apud Boston Americae borealis.

Remarks on the Pilobolidae.—At my request Mr. W. B. Grove has prepared for this Volume a systematic account and an arrangement of the Pilobolidae. I have sought to assist him by providing the necessary illustrations and by placing at his disposal the results of my studies of *Pilobolus longipes, P. Kleinii, P. umbonatus*, found

wild at Winnipeg, and of *Pilaira anomala* and *P. Moreaui* sent to me from Baarn.

Piloboli commonly occur on horse dung, cow dung, etc., and are so different in appearance from all other fungi and discharge their black sporangia with such vigour that most mycologists have seen one or more of them and have recognised them for what they are. On the other hand, Pilairae are comparatively rare, may easily be mistaken by the uninitiated for Mucors, and have been less often found and studied. Hence perhaps it is that Fitzpatrick [1] in his recent book on the Phycomycetes, after remarking that " at least four species of Pilaira, all of them from dung, have been described " adds : " The possibility that they were based on abnormal material of Pilobolus leads the writer to regard the genus as somewhat doubtful." From my own comparative studies of Piloboli and Pilairae, I have become convinced that the genus Pilaira is a good one. In the next Chapter the characteristics of Pilobolus and Pilaira have been set forth in detail.

Anderson [2] has recently taken the trouble to demonstrate the stability of the genus Pilaira by means of a special investigation. He made monosporous cultures of *Pilaira anomala* and found that they remained true and did not give rise to a Pilobolus form. Further, he fed two rabbits, one with lettuce bearing Pilaira sporangia and the other with lettuce bearing Pilobolus sporangia. The dung of each rabbit produced the respective fungus only. As a result of these experiments Anderson concluded that the genus Pilaira van T. is a valid one.

Our knowledge of the Pilobolidae is not as satisfactory as it should be. A number of species, more especially of Pilobolus, have been imperfectly described and illustrated. What is now required is that some systematist should take up the study of the Pilobolidae with a view to preparing a monograph upon them, based on a comparison of as many species as could be brought together in a long term of years. The resources of photomicrography, unknown to

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[1] H. M. Fitzpatrick, *The Lower Fungi, Phycomycetes*, New York, 1930, p. 253.

[2] R. S. Anderson, " The Validity of the genus Pilaira," *University of Iowa Studies*, Vol. XV, 1933, pp. 3-5. Cited from *Journ. Roy. Micros. Soc.*, Ser. III, Vol. LIII, 1933, p. 284.

the older mycologists, are now at our disposal and would facilitate the task.

To the criteria so far employed by taxonomists for distinguishing species of Pilobolus should be added : (1) the exact shape of the subsporangial swelling, whether ellipsoidal or pyriform, etc. ; (2) the ratio of the width of the sporangium to the width of the sub-sporangial swelling ; (3) the nature of the depressions or wrinkles on dried discharged sporangia when seen in strong reflected uni-lateral light ; and (4) the nature of the fringe of the sporangium-wall of dried discharged sporangia, in respect to form, colour, and disposition of crystals.

The spores of all the species of Pilobolus and Pilaira contain a carotinoid pigment which colours oil-drops held within the proto-plasm. Large spores which contain much of the pigment, *e.g.* those of *Pilobolus longipes* and *P. Kleinii*, when seen singly in water in transmitted light, are orange-yellow, while small spores which contain very little of the pigment, *e.g.* those of *P. umbonatus*, when seen singly are almost colourless and when seen many together are yellowish.

Van Tieghem [1] remarked that the spores of *Pilobolus longipes*, when seen in the mass, appear dull green, and he regarded this as being due to the slaty-blue colour of the spore-wall combining with the golden-yellow colour of the spore-protoplasm. When spores of *P. longipes* are seen in the mass, they are seen by reflected and not by transmitted light. I have observed that, if Pilobolus spores are spread out in a drop of water under a cover-glass and are looked at with the low-power objective of the microscope, they exhibit dichroism, in that they are orange-yellow or yellowish in transmitted light and green in reflected light. This applies to the spores not only of *P. longipes*, but also of *P. Kleinii* [2] and *P. umbonatus*. It is therefore clear that a dull green colour for spores in the mass cannot be regarded as a distinctive character of *P. longipes*.

The basal swelling (trophocyst) of *Pilobolus longipes*, as com-pared with that of other Piloboli, is so much elongated (Fig. 100,

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[1] P. van Tieghem, "Troisième mémoire sur les Mucorinées," *Ann. Sci. Nat.*, 6 sér., T. IV, 1876, p. 339.

[2] *Cf. supra*, p. 72.

p. 203) that its form is a valuable aid in identifying this species. In the other Piloboli, the basal swelling as a rule is rounded, turnip-shaped, or oval.

In all the species of Pilobolus, the basal swelling together with the fruit-body which grows out of it may be and usually is solitary ; but in some species, *e.g. P. roridus* (Fig. 101, F, p. 204) and *P. nanus*
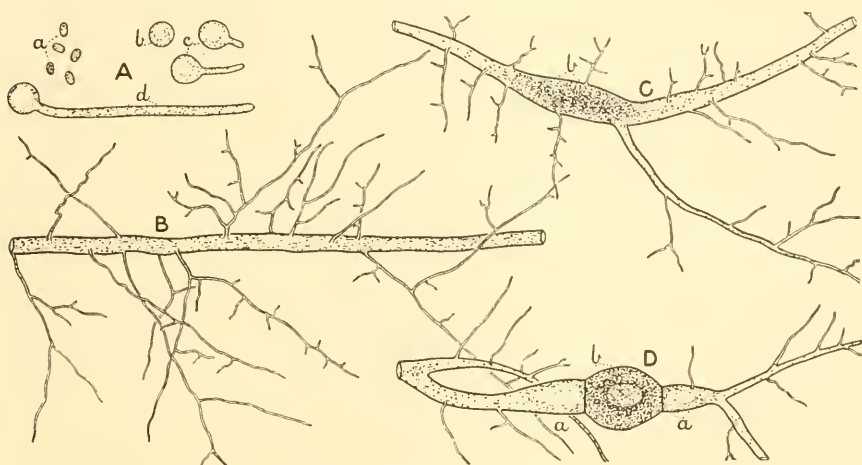
FIG. 89.—*Pilobolus umbonatus.* Germination of spores and development of a trophocyst (which becomes the basal swelling of a fruit-body). Culture medium, cleared horse-dung agar. A : *a*, spores placed in culture medium ; *b, c,* and *d,* two days later ; *b,* a spore swelling ; *c,* two spores putting out germ-tubes ; *d,* a spore with a long germ-tube. B : a main hypha of a mycelium, with numerous slender branches (secondary hyphae). C : a main hypha which has become swollen at *b* as a step toward the formation of a trophocyst. D : a main hypha in which a local swelling has become divided by two septa, so as to form a trophocyst *b* and two apophyses *a a.* Drawn by A. H. R. Buller and E. S. Dowding. Magnification : A, 350 ; B–D, 80.

(Fig. 106, C, p. 212), two or three or possibly more basal swellings, which become extended into fruit-bodies, may occur in short chains.

A single basal swelling in any Pilobolus species usually arises in the middle of one of the stouter hyphae in an intercalary manner and becomes cut off from the rest of the hypha in which it has originated by two septa (Fig. 89, also Figs. 21 and 27, pp. 52 and 69). One or both of the adjacent portions of the hypha may then become swollen to form one or two so-called apophyses (Figs. 89 and 90). When one examines a mature fruit-body, if only one apophysis has been developed (Fig. 90, C–E ; also Figs. 27 and 101,

B, pp. 69 and 204), one gains the impression that the basal swelling is terminal, whereas, if two apophyses have been developed (Figs. 89,
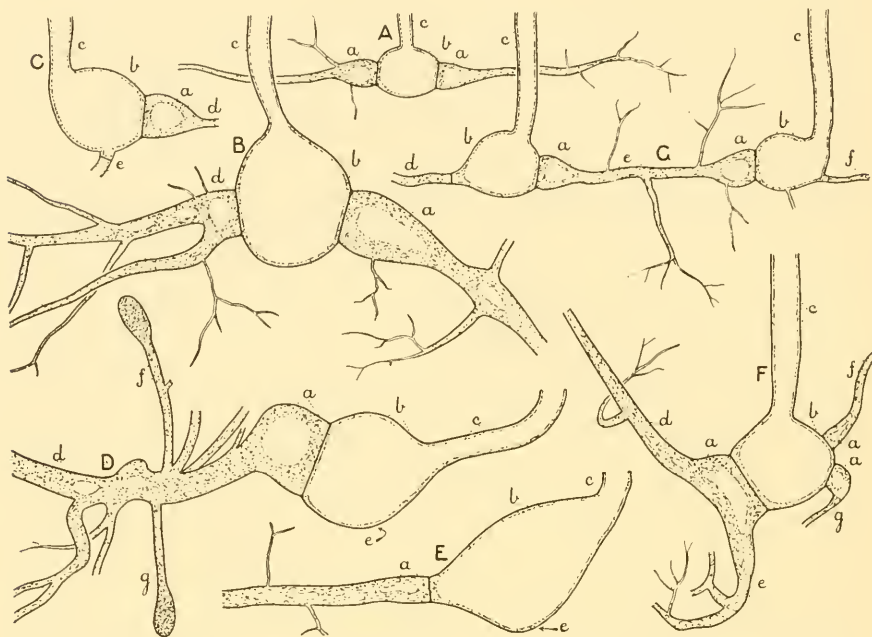
Fig. 90.—*Pilobolus umbonatus.* Variations in the basal swelling and its apophyses. Fruit-bodies removed from a horse-dung culture and examined in water. Outlines made with the help of the *camera lucida* ; protoplasm added semi-diagrammatically. In all the drawings : *a* is an apophysis ; *b*, a basal swelling ; and *c*, part of the stipe of a fruit-body. A : the basal swelling is evidently intercalary ; there are two apophyses. B : a much larger basal swelling, again evidently intercalary ; the apophysis at *d* is smaller and less typical than that at *a*. C : the basal swelling has only one apophysis, *a* ; it is not terminal but intercalary, as it and its apophysis were developed from a local enlargement on the hypha *d e*. D : a basal swelling with one apophysis ; it is apparently terminal, but at *e* a slender continuation of the hypha *d* may have been torn away in removing the basal swelling from the substratum ; if such a continuation existed, the basal swelling must have developed in an intercalary manner ; at *f* and *g* are two hyphae with club-shaped ends which possibly might have given rise to truly terminal basal swellings. E : a slightly elongated basal swelling without any typical apophyses ; it is possible that a continuation of the hypha *a* was broken away at *e* when the fruit-body was removed from the substratum ; if such a continuation existed, the basal swelling was intercalary and not terminal in its mode of origin. F : a basal swelling with three apophyses. G : two basal swellings which have arisen on the same main hypha, *d e f* ; each has a single apophysis on one side of it and an unswollen part of the main hypha on the other side. Drawn by A. H. R. Buller and E. S. Dowding. Magnification, 62.

D, and 90, A and B ; also Fig. 101, G), one readily perceives that the basal swelling at its origin was intercalary. Although most

apparently terminal basal swellings in reality have originated in an intercalary manner (Fig. 90, C ; also Fig. 21, E–G, p. 52), yet it may well be that now and again some basal swellings originate in a truly terminal manner, for Cohn [1] (1851) observed in *Pilobolus oedipus* certain club-shaped hyphae coming off from stout hyphae, which he considered to be the possible beginnings of new fruit-bodies, and I have observed exactly similar club-shaped hyphae in the mycelium of *P. umbonatus* (Fig. 90, D, *f* and *g*).

The ratio of the width of the sporangium to the width of the subsporangial swelling in the fruit-body of a Pilobolus may be called the *width-ratio*. To obtain the data for this ratio, all that one needs to do is to remove a fruit-body from a culture, to lay it horizontally in a drop of water on a glass slide, and then to measure in succession the width of the sporangium and the width of the subsporangial swelling.

A preliminary study of the width-ratio of three species of Pilobolus was made under my direction by Dr. Dowding, and the results of it are embodied in the diagram reproduced in Fig. 91. The upper five drawings at A show in top view the sporangia of five wild fruit-bodies of *Pilobolus longipes* ; their average width-ratio was found to be approximately $\frac{3}{5}$. The lower five drawings at B show five sporangia obtained from a pure culture of *P. Kleinii* ; their average width-ratio was found to be approximately $\frac{4}{5}$. The drawing C shows a single sporangium of a wild fruit-body of *P. umbonatus* ; its ratio was found to be—as in other fruit-bodies of this species— approximately $\frac{1}{2}$. Thus the average ratio of the width of the sporangium to the width of the subsporangial swelling appears to differ appreciably in the three species of Pilobolus which have been investigated.

Lepeschkin [2] found that the width of the subsporangial swelling in a Pilobolus is determined in part by the osmotic value of the substances dissolved in the water permeating the substratum. On

[1] F. Cohn, " Die Entwicklungsgeschichte des Pilobolus crystallinus," *Nova Acta Acad. Caes. Leop.*, Bd. XXIII, 1851, Plate LII, Figs. 14 and 16. His *P. crystallinus* was in reality *P. oedipus*.

[2] W. W. Lepeschkin, " Zur Kenntnis des Mechanismus der aktiven Wasseraus-scheidung der Pflanzen," *Beihefte z. Bot. Centralb.*, Bd. XIX, 1906, p. 423. Also *vide supra*, p. 34.

this account, in determining the width-ratio of fruit-bodies of any species of Pilobolus, it is advisable to use wild fruit-bodies obtained from their natural substratum. Unfortunately, the sporangia of *Pilobolus Kleinii* shown at B in Fig. 91 were obtained from a pure culture. Had they been obtained from a wild culture, it is possible that their average width-ratio would have been slightly smaller, perhaps $\frac{3}{4}$ instead of $\frac{4}{5}$, as wild fruit-bodies of *P. Kleinii* with a width-ratio of about $\frac{3}{4}$ have been observed.

In concluding this Section, we may enquire to what extent, if
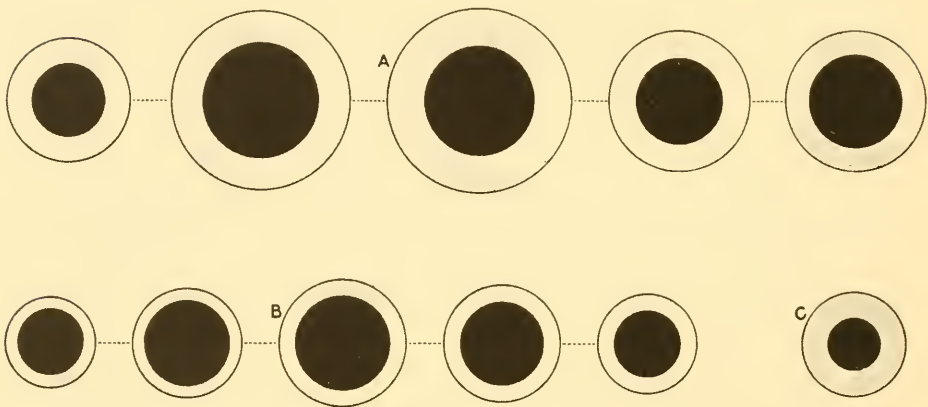
FIG. 91.—Diagram illustrating a study of the ratio of the width of the sporangium to the width of the subsporangial swelling in three species of Pilobolus. Each fruit-body is shown in top view ; the black area represents the sporangium, the pale area the subsporangial swelling. A, five wild fruit-bodies of *Pilobolus longipes* (average width-ratio approx. $\frac{3}{5}$). B, five fruit-bodies from a pure culture of *P. Kleinii* (average width-ratio approx. $\frac{4}{5}$). C, a wild fruit-body of *P. umbonatus* (width-ratio $\frac{1}{2}$). Magnification, 30.

any, the patterns that can be found on the dried discharged sporangia of certain Piloboli are of diagnostic value.

Coemans,[1] in 1861, observed some curious markings on the sporangium of *Pilobolus crystallinus* (Fig. 92). He says : " In *Pilobolus oedipus* the tint of the coloration is uniform, but in *P. crystallinus* it sometimes exhibits beautiful hexagonal patterns which have a close analogy with the hexagonal cells of the choroid

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[1] E. Coemans, " Monographie du genre Pilobolus Tode, spécialement étudié au point de vue anatomique et physiologique," *Mém. cour. et des Sav. étrang. Acad. roy. de Belgique*, T. XXX, 1861, pp. 23–24.

of the higher animals. These patterns are very regular ; a chief alveolus occupies the centre at the top of the globule (sporangium) and six other exactly similar cells are placed around the sides of the principal polyhedron. These alveoli have shades of coloration, their centre is usually pale, and a colourless or paler streak separates them from one another. I have noticed similar patterns, but ovoid in form, on the sporangium of *Ascophora Cesatii* " (= *Pilaira anomala*).

" It is remarkable that these patterns are not produced regularly each year. In 1859, in a hot summer, they ornamented all the globules of *Pilobolus crystallinus* that I observed ; in 1860, the summer being cold and wet, I found them very rarely and always faintly indicated. The cause of these variations is probably connected with the effects of light and heat. It may also be noted that, the irregularity of the appearance of these patterns being proved, they cannot have any diagnostic value."
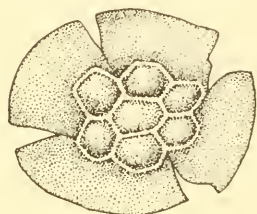
Fig. 92.—*Pilobolus crystallinus*. Sporangium flattened out and seen from above. Copied by the author from Coemans' *Monographie* (1861). Magnification increased from 220 to 293.

Coemans in his illustrations shows the pattern on a discharged sporangium (Fig. 92), but not on sporangia still seated on subsporangial swellings. There is good reason to suppose, therefore, that Coemans saw the pattern on discharged sporangia only.

Van Tieghem,[1] in his description of *Pilobolus crystallinus*, says : " A white network with the meshes most often hexagonal ornaments the upper surface of the cuticularised hemisphere (sporangium) ; there is a hexagon at the top and six other hexagons arranged in a circle around the first with their free sides rounded below. Sometimes the central polygon has four, five, seven, or eight sides. This regular system of white lines, left untouched by the coloration which affects all the rest of the cuticularised hemisphere, is quite characteristic of this species." Van Tieghem, in a foot-note, adds : that he has found the network in all the sporangia of *P. crystallinus*

[1] P. van Tieghem, " Troisième mémoire sur les Mucorinées," *Ann. Sci. Nat.*, 6 sér., T. III, 1876, p. 336.

that he has seen both in winter and summer in numerous generations obtained in cultures ; and that the reason why Coemans did not always find them in this species was that he, Coemans, had sometimes had *P. Kleinii*, which has no network, under his eyes instead of *P. crystallinus*.   Van Tieghem, in a diagrammatic illustration of the network (Fig. 93), shows the top of a discharged sporangium in the form of a black disc marked out with white lines.

Still later Zopf,[1] in a study of the parasites of Pilobolus, illustrated the supposedly normal fruit-bodies of *P. crystallinus*.   He shows not only a discharged sporangium with polygonal markings on its upper surface, but also intact fruit-bodies bearing undischarged sporangia with similar markings.   Subsequently, he reproduced two of these illustrations in the form of wood-cuts in his text-book of mycology.[2]

Fig. 93. — *Pilobolus crystallinus*. A, upper view of sporangium showing a white network.   B, spores.   Copied by the author from van Tieghem's *Troisième Mémoire* (1876).   Magnification, not given.

My own studies of the patterns on the sporangia of *P. Kleinii*, *P. longipes*, and *P. umbonatus* have led me to the following conclusions : (1) the patterns are never present on sporangia seated on their sporangiophores, but only on discharged sporangia ; and (2) the patterns develop on discharged sporangia as these dry up and flatten down.   I am therefore of the opinion that Zopf was in error in representing a pattern on the undischarged sporangia of *P. crystallinus*.

The netted pattern on a dried sporangium is due merely to the wrinkling of the hemispherical sporangium-wall as this settles down on the drying mass of spores.   This mass, as it loses water by evaporation, shrinks to less than one-half of its original volume. The white lines represented by Coemans and van Tieghem in their drawings of *Pilobolus crystallinus* (Figs. 92 and 93) were doubtless ridges around depressions.   Sometimes, as I have observed in a large flattened sporangium of *P. Kleinii*, the sporangium-wall

[1] W. Zopf, " Zur Kenntniss der Infectionskrankheiten niederer Thiere und Pflanzen.   No. IV.   Einfluss von Parasitismus auf Zygosporenbildung bei *Pilobolus crystallinus*," *Nova Acta Acad. Caes. Leop. Nat. Cur.*, Bd. LII, 1888, Plate XXII, Figs. 1–3.

[2] W. Zopf, *Die Pilze*, Breslau, 1890, Fig. 54, Nos. 2 and 3, p. 84.

becomes cracked along these ridges and then the cracks appear
as lines which are whitish when contrasted with the dark sporangium-
wall.

The formation of a pattern on a discharged sporangium, con-
trary to the view expressed by van Tieghem, is not limited to
Pilobolus crystallinus, for I have observed patterns on the discharged
sporangia of *P. Kleinii* (Fig. 39, p. 83), *P. longipes* (Fig. 40, p. 84),
and *P. umbonatus* (Fig. 105, J, p. 210).

While the existence of a pattern on a discharged and dried
sporangium cannot be held to be a diagnostic character for *Pilobolus
crystallinus*, yet differences in pattern are displayed by different
species. Thus a well-developed, discharged, and dried sporangium
exhibits : in *P. Kleinii* a few rounded regular dimples, one of which
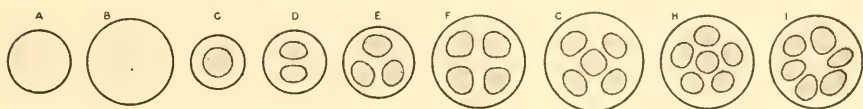
FIG. 94.—*Pilobolus Kleinii*. Diagram showing variations in the pattern on dried
discharged sporangia obtained from a pure culture. A and B, sporangia with
a smooth wall. C–G, sporangia with one dimple, two, three, four, and five
dimples respectively. H, a sporangium with six dimples, one central. I, a
sporangium with six dimples in a circle. Magnification, 47.

often holds a central position (Fig. 39) ; in *P. longipes* numerous
irregular somewhat gyrose depressions (Fig. 40) ; and in *P. um-
bonatus*, a central umbo with lateral more or less radial depressions
(Fig. 105, J). These differences in sporangial pattern are un-
doubtedly of diagnostic value and I have often used them in the
laboratory for distinguishing *P. Kleinii*, *P. longipes* and *P. umbonatus*
from one another.

The pattern made by the dimples in the sporangial wall of dried
discharged sporangia of *Pilobolus Kleinii* varies considerably in
detail, as will appear in what follows. A pure culture of *P. Kleinii*
was obtained by inoculating sterilised horse dung with a single
sporangium. As soon as new sporangia began to be discharged,
they were caught on glass slides and examined under the microscope.
Whilst still wet, their sporangial walls were perfectly smooth ; but,
as they dried up, in the course of about two minutes, it was observed
that certain circular areas of the walls sank inwards and so formed

crater-like depressions. Fifty dried discharged sporangia were selected at random and were observed under the low power of the microscope by reflected light, and it was found that the number of depressions in the sporangial wall varied from none at all to six (Fig. 94), as indicated in the accompanying Table.

Fifty dried discharged sporangia of Pilobolus Kleinii

No depressions or faint traces	12 sporangia
One central depression	8 sporangia
Two depressions	5 sporangia
Three depressions	7 sporangia
Four depressions	11 sporangia
Five depressions	4 sporangia
Six depressions, one central	2 sporangia
Six depressions, irregularly arranged	1 sporangium

Further observation showed that in dried discharged sporangia of *Pilobolus Kleinii*, as a general rule, the number of depressions

FIG. 95.—*Pilobolus Kleinii.* The upper convex surfaces of two large dried discharged sporangia produced by wild fruit-bodies, each with a larger central dimple surrounded by smaller dimples. In A there are ten smaller dimples and in B nine. Magnification, 100.

in the sporangial wall varies directly with the diameter of the sporangium, the smallest sporangia having the fewest depressions and the largest sporangia the greatest number of depressions. In wild cultures where the sporangia were larger than those in the artificial pure culture from which the fifty sporangia were obtained, it was observed that the number of depressions in a sporangial wall was often nine or ten and that, as a rule, there was one larger central polygonal depression surrounded by a ring of small depressions (Fig. 95).

An investigation similar to that just described was made on

Pilobolus longipes. Of sixty-two sporangia twelve showed no depressions, whilst in the other fifty the depressions in the form of elongated, meandering, irregularly arranged furrows (Fig. 96) varied from ten to twenty in number. Of seventy-five sporangia derived from a pure culture only three or four small sporangia
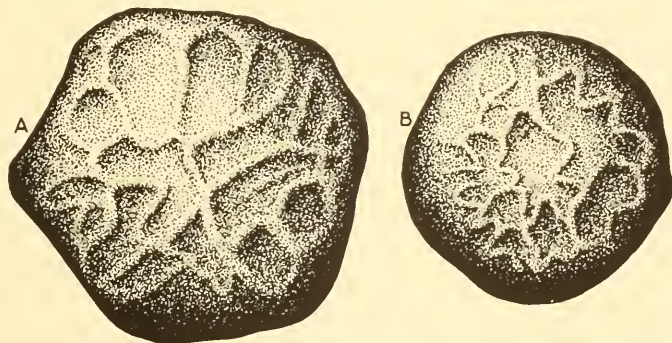
FIG. 96.—*Pilobolus longipes.* A and B, the upper convex surfaces of two large dried discharged sporangia, produced by wild fruit-bodies, each showing a pattern of irregular depressions or furrows. Magnification, 100.

could be found which possessed in their walls depressions that more or less resembled the rounded depressions of *P. Kleinii.*

From the special investigations on *Pilobolus Kleinii* and *P. longipes* that have just been recorded it is clear that the pattern on dried discharged sporangia, while fairly constant in its general aspect for each species, is subject within each species to a large amount of variation in detail. The typical pattern for any particular species ought to be sought for in the larger sporangia rather than in the smaller ones.

CHAPTER IV

A SYSTEMATIC ACCOUNT AND ARRANGEMENT OF THE PILOBOLIDAE

CONTRIBUTED BY W. B. GROVE

Introductory Remarks—Historical Account—Systematic Arrangement—
Bibliography

Introductory Remarks.—The section of the Mucorini entitled Pilobolidae includes only two genera, Pilobolus and Pilaira. In 1884 the writer of this chapter published a *Monograph of the Pilobolidae*, in which he gave a systematic arrangement of the ten species which had been up to that time described. This arrangement was followed by Saccardo in the seventh volume of his Sylloge Fungorum, pp. 184–189, and has since been revised by Palla and Morini.[1]

The writer's personal knowledge of the Pilobolidae in England is based upon the forms called *Pilobolus crystallinus, P. Kleinii, P. sphaerosporus, P. oedipus,* and *Pilaira anomala,* and in addition he has seen *Pilobolus longipes* and *P. umbonatus,* which have been found in Canada as shown by Professor Buller in preceding chapters of this volume. The six forms of Pilobolus just mentioned, as well as *Pilaira anomala, P. nigrescens, P. Saccardiana,* and *P. Moreaui,* may probably be regarded as definitely established species ; in regard to most of the others there remains a certain doubt which can only be removed when some one has devoted to the group

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[1] This account of the Pilobolidae, written at the request of my friend Professor Buller, may be considered to be a revision of the systematic part of the *Monograph of the Pilobolidae* which I published in the Midland Naturalist exactly fifty years ago. Professor Buller has been good enough to give me the benefit of his assistance in defining the group and the genera, in revising the descriptions of certain species, and in preparing the illustrations.—W. B. G.

a large amount of time and patience. The account of those species which I have not seen is of course compiled from the published sources.

The drawings of the earlier authors were but free-hand sketches, inaccurate in certain details. For instance, Bolton's figure of *Pilobolus roridus* (Fig. 98) makes the subsporangial swelling appear nearly twice as broad as it is high (seemingly in an attempt at perspective) ; Zopf and Klein represent the subsporangial swelling of *P. Kleinii* (their *P. crystallinus*) as hardly or not at all wider than the sporangium ; van Tieghem makes the same error in his figure of *Pilobolus nanus* (Fig. 106, p. 212), while in *Pilobolus longipes* (Fig. 100, p. 203) he draws the spores in the sporangium out of all proportion to the sporangium in which they are enclosed. By future workers pure cultures of each so-called species should be obtained and their special characteristics should be fully illustrated by photographs and *camera-lucida* drawings.

The Historical Account which follows is founded, with many emendations and the necessary later additions, on that given in the Monograph of 1884.

Historical Account.—The earliest record I have been able to find of a species belonging to Pilobolus is in the works of the famous British botanist, John Ray. In his Historia Plantarum (1688) occurs the following passage which, on account of its importance, shall be quoted in full :—

" E Catalogo huc tranſmiſſo *Anno* 1680, quem compoſuit eruditiſſimus Vir et conſummatiſſimus Botanicus D. *Johannes Baniſter* Plantarum à ſeipſo in Virginia obſervatarum.

" Fungus (ex ſtercore equino) capillaceus capitulo rorido, nigro punctulo in ſummitate notato. Ex recenti fimo noctu exoritur cauliculis erectis, vix digitum longis, capillorum inſtar tenuibus nec minùs denſis ſeu confertis. Singuli Cauliculi parvulo globulo aqueo coronantur, qui in ſumma ſui macula parva nigra Limacis oculi ſimili inſignitur."

The same species of Pilobolus was then mentioned and figured by Plukenet (1691) as " *Fungus Virginianus* ex ſtercore equino capillaceus canus capitulo rorido, nigro punctulo in ſummitate notato, D. Baniſter." Plukenet did not merely copy what Ray had

published, for he adds the correct descriptive word "canus," of which Ray says nothing, as well as a small but characteristic figure (Fig. 97). Both these additions he obtained from Banister's MS. From Ray's description and Plukenet's figure, it is evident that the species they had in view was similar to that which was afterwards called *Mucor roridus*.

These two notices stimulated observation, for a few years later the first British record was published in Ray's Synopsis (1696), in a list of plants communicated by Mr. James Petiver, who remarks " *This I have observed on Horse-dung about* London," and refers to Plukenet's figure. This record, therefore, may be considered to belong to *Pilobolus roridus*. It is repeated by Ray in his Historia Plantarum in 1704, and again in his Synopsis in 1724, and by Petiver in his Gazophylacium (1711), where he gives a figure similar to that of Plukenet.
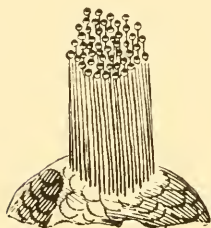
Fig. 97.—The first illustration of a Pilobolus. Banister's drawing, reproduced by Plukenet (1691).

Another mention of a fungus belonging to this genus (the earliest known to Coemans in his review, 1861, of the literature of the subject up to his time) is due to Henry Baker, who, in his Natural History of the Polype Insect (1744), described a number of small vase-like plants, filled with a clear liquid and crowned by a black ball ; these, which he had found on mud brought from the river Thames, were undoubtedly a species of Pilobolus, presumably *Pilobolus oedipus*.

In 1764 Otto Müller discovered and afterwards (1782) described and figured a Pilobolus under the name of " Kristallschwämmchen " ; he imagined it was in part an animal, in part a plant, and even in part a crystal, thus partaking of all the three kingdoms of nature. He thought he saw a slender worm-like body residing within the organism, which, he says, " crawled round in the crystal globe and seemed to swim at its ease in a tiny ocean." This was no doubt a species of Anguillulidae, but outside, not within, the subsporangial swelling. The singularity of this view accounted for the widespread attention which was given to his discovery.

It was not till 1772 that Scopoli in his Flora Carniolica first

gave to the plant a name which showed a recognition of its true affinities. He called it *Mucor obliquus*, from the oblique manner in which the stipe frequently sprang from the side of the basal reservoir, but his description, though very interesting, is insufficient to enable us to identify the species.

Withering, in his Botanical Arrangement (1776), quoted Petiver's plant from Ray's Synopsis and bestowed upon it the name *Mucor roridulus*.

In Wiggers' Primitiae Florae Holsaticae (1780) Scopoli's plant was placed in a new genus, under the name *Hydrogera crystallina* suggested by his tutor Weber.

But the first good description of the genus was given by Tode, who imposed upon his species the name of *Pilobolus crystallinus* by which it is now known. The generic name is a translation of the title " Hutwerfer," which he used in the Schrifte der Berlinsche Gesellschaft naturforschenden Freunde (1784) ; his account was repeated in his Fungi Mecklenburgenses selecti in 1790.

Species of Pilobolus were then mentioned successively : by Dickson (1785), who figured one under the name *Mucor urceolatus* ; by Bulliard, under the same name, in his Herbier de la France (1784), with a figure added in 1789 ; by Bolton (1789), who, besides figuring under that name a form resembling a badly grown *Pilobolus Kleinii*, added another supposed to be identical with Petiver's as *Mucor roridus* (Fig. 98) ; and by Vahl, in the Flora Danica (1792), who figured one as *Pilobolus crystallinus*.

FIG. 98.—*Pilobolus roridus* (Bolton) Pers. A cluster of fruit-bodies on horse dung, about natural size, and two fruit-bodies much enlarged. Reproduced by photography from Bolton's *History of Fungusses* (1789).

Persoon gives an excellent description of *P. crystallinus* in his Observationes Mycologicae (1796), accompanied by an imperfect figure ; and in his Synopsis methodica Fungorum (1801) he mentions both that and *P. roridus*, but considers the latter as doubtfully distinct.

Sowerby, in his English Fungi (1803), gives a figure of *Mucor urceolatus* which seems to represent *P. crystallinus*.

Link, in his First Dissertation (1809), attributed for the first time the projection of the sporangium to its true cause, namely the tension of the swelling below the sporangium. His words are : " Explosio fieri mihi videtur, dum suprema pars stipitis bullata, sporangium inferne ambiens, contrahitur."

Relhan, in his Flora Cantabrigiensis (1820), maintained that *Pilobolus roridus* was distinct from *P. crystallinus* ; but Purton, in his Midland Flora (1821), recorded both of them under one name *Pilobolus urceolatus*, giving detailed reasons from experiment to show that they are not distinct, and accompanying his account with " a very beautiful and accurate drawing " by his niece, " taken from the fresh plant."

In 1823 Ehrenberg published in Kunze u. Schmidt's Mykologische Hefte an account of some observations he had made upon *P. crystallinus*, in which, while searching for Otto Müller's " worm," he noticed a curious movement of yellowish particles arranged in a snake-like form in a drop of water which occupied the summit of the sporangium. This, he thought, might be the " worm," because it moved with a " slow, steady, circling motion " which excited his wonder.

All the authors mentioned so far correctly placed the genus in the immediate vicinity of Mucor. Fries, however, in 1823, considered it as nearly allied to Sphaerobolus and placed it in the Gasteromycetes in the section Carpoboli, but in 1829 he discovered his mistake and restored it again to the Mucorini. Nevertheless Berkeley, in Smith's English Flora (1836), incautiously repeated Fries' error, and in his Outlines of British Fungology (1860), perhaps by reason of this confusion, he omitted Pilobolus altogether.

Up to this time only the two species already mentioned, called *P. crystallinus* and *P. roridus*, were generally known, but in 1828 Montagne had already described a third, to which he gave the name of *P. oedipus* (*cf.* Fig. 104, p. 209) on account of the swollen basal reservoir which is so conspicuous a feature in that species. He repeated this in his Sylloge generum specierumque cryptogamarum in 1856.

In 1837 Corda instituted in his Icones Fungorum the group Pilobolidae to contain Pilobolus and Chordostylum ; in 1842 he

added to the group Pycnopodium and Caulogaster, placing in the former genus, as *Pycnopodium lentigerum*, a species which he had formerly included in Pilobolus and which would seem to be merely an abnormal state of *P. Kleinii*. After Corda's lamented early death, Zobel published (1854) from his friend's manuscript notes a sixth volume of the Icones, in which he gives a long account of *Pilobolus crystallinus* containing numerous errors. In his drawing (f. 32) he represents the interior of the subsporangial swelling as lined with reticulations of orange-coloured granules such as no other author has seen, and which are probably only the meridional streams, occasionally met with but rarely figured,[1] disturbed by the pressure to which the preparation had been subjected.

When Cohn published, in 1851, his celebrated monograph " Die Entwicklungsgeschichte des Pilobolus crystallinus," he had before him, not that species with which he was really unacquainted, but the species of Montagne. He figures the characteristic yellow, spherical, thick-walled spores of *Pilobolus oedipus*, and then remarks with naïve surprise that Corda had represented the spores of *P. crystallinus* as elliptic and colourless " in contradiction to nature."

Cesati discovered, in 1850, a species which he published the next year in Klotzsch's Herbarium vivum mycologicum under the name *Pilobolus anomalus*, now known as *Pilaira anomala* Schröt.

Bonorden, in his Handbuch (1851), described a species under the name of *P. crystallinus*, which on account of its round spores Coemans referred to *P. oedipus*, but which I think there is greater reason for considering as *P. Kleinii*, forma *sphaerospora*.

In 1857 Currey wrote a note " On a species of Pilobolus " which he thought to be *P. roridus*, but his plate and description clearly show that the species he had in view was *P. Kleinii* ; according to van Tieghem, Léveillé in 1826 had fallen into the same error, giving the name of *P. roridus* to a form of *P. Kleinii*. Currey also attributed the projection of the sporangium to the eversion and upward pressure of the columella, which he believed not to be thrown off with the sporangium.

In 1861 Coemans issued his " Monographie du genre Pilobolus," in which he summarised what he had read about this subject, and

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[1] I figured them in my Monograph (1884), pl. 4, f. 12.

gave a list of all the species referred by other authors to this genus. He considered *P. crystallinus* and *P. oedipus* (Fig. 104, p. 209) to be the only certain species ; *P. roridus* he regarded as doubtful, *P. lentiger* he refers, wrongly, to *P. oedipus*, and *P. anomalus* he places in the genus Ascophora by the name of *A. Cesatii*.

In 1871 Cooke published the Handbook of British Fungi, but, though he mentions the two conventional species, *P. crystallinus* and *P. roridus*, it is impossible to recognise exactly what he means by the names.

It was in 1872 that Klein (after a short note in 1870) gave to the world his monograph " Zur Kenntniss des Pilobolus," a monument of patient and minute investigation. In this he describes two species, " *P. crystallinus* " and " *P. microsporus* " ; under the former name he says that he unites the *P. crystallinus* and *P. oedipus* of former authors. But, though he records his painstaking observations with great accuracy, in respect of the identification of his specimens Klein was peculiarly unfortunate. His *P. microsporus* is identical with *P. roridus*, and he was unacquainted with either the true *crystallinus* or the true *oedipus*. He had before his eyes, without knowing it, another species hitherto undistinguished, to which van Tieghem afterwards gave in his honour the name of *Pilobolus Kleinii*. The spores of *P. oedipus* are yellow, nearly spherical, and surrounded by a thickened epispore ; those of *P. crystallinus* are ellipsoidal and nearly colourless. Now those of *P. Kleinii* are also ellipsoidal, but of an orange-yellow colour and twice as long as those of *P. crystallinus* ; in certain circumstances, however, *P. Kleinii* bears sporangia containing nearly spherical spores of the same colour but without a thickened epispore. It was this abnormal state, to which I gave in my monograph of the Pilobolidae the name *forma sphaerospora*, that led Klein erroneously to imagine that he had met with forms intermediate between *oedipus* and *crystallinus*. The credit of clearing up this mistake was due to van Tieghem (1876).

In my own cultures, on many occasions, I found that the first two or three days' crops of *P. Kleinii* bore small sporangia, containing roundish spores, of unequal size in the same sporangium. These, however, could be distinguished at once by the want of the thickened

epispore from those of *P. oedipus*, and. moreover, the fungus agreed in all other respects but its minuteness with the true *Kleinii* into which it gradually passed on the following days. It would seem likely that the inequality of the spores in the same sporangium, together with the dwarfed size, was due to the fact that the fungus had not yet established itself, and was of weak and uncertain growth, like some of the dwarf forms of Coprinus.

Brefeld, in 1872, in his Untersuchungen über Pilze mentions and figures a species which he assigns to the genus Pilobolus, under the name *P. Mucedo*, but afterwards (1881) he discovered this to be, in part at least, the same as that previously called by Cesati *P. anomalus*. In the same work (1881) he gives a short account of the other species assigned by him to Pilobolus, but not one of the names he uses is that to which the species is entitled. as will be seen by the following list : his

P. crystallinus (Fig. 99, A) = *P. Kleinii* van Tiegh.
P. oedipus (Fig. 102)   = *P. Kleinii*, forma *sphaerospora* Gr.
P. microsporus (Fig. 99, B) = *P. crystallinus* Tode.
P. roridus   = *P. longipes* van Tiegh. (Fig. 100).

Brefeld, however, like van Tieghem, observed zygospores in *Pilobolus anomalus* (= *Pilaira anomala*). He found them on horse dung.

It will be seen that in " this strange eventful history " nearly every author seemed to be fated to misunderstand in some degree the opinions of those who had preceded him. It was not until 1875 that van Tieghem succeeded in clearing up the confusion in which the subject had been plunged, especially in relation to the *Mucor roridus* of Bolton. Bolton expressly describes his species (Fig. 98, p. 193), which he had found in the neighbourhood of Halifax, as " four lines high, pellucid and white, sustaining a small globular head, like a minute pearly drop, with a black spot on its upper part, which gives to the globe the resemblance of an eye in miniature." No other author but Klein had been able, up to this time, to meet with a species answering to this description, and hence it was doubted by some, as by Persoon, Coemans, Greville, and Purton, whether it was really distinct ; Klein, as has been said, failed to recognise it in his *microsporus*, and it was reserved for van Tieghem

to describe and figure (Fig. 101, p. 204) a form which possibly is that which Bolton had found, and which is as similar as may be to Klein's *microsporus*. The long slender stem, the rounded subsporangial swelling, the minute sporangia, and the want of colour of van Tieghem's species, all point in this direction and agree pretty well with Bolton's figure. But it must be admitted that, since Bolton does not describe the spores, a certain amount of doubt must always attend any identification of his species; it would, perhaps, be better to quote *P. roridus* van T. rather than *P. roridus* (Bolt.).

In the same memoir (1875) van Tieghem also instituted the new genus Pilaira for the reception of the old *Pilobolus anomalus* of Cesati, and added a new species *Pilaira nigrescens* (Fig. 108, p. 218). He also discovered the zygospores of *P. anomala* (Fig. 107, p. 217).

In 1876 van Tieghem completed his work by publishing the descriptions of two new species, *P. longipes* (Fig. 100, p. 203) and *P. nanus* (Fig. 106, p. 212), while at the same time he pointed out the error which Klein had made and bestowed the name of *P. Kleinii* on the species with which Klein had worked. He also described the chlamydospores of *P. nanus* (Fig. 106, p. 212), a mode of reproduction which had already been signalised by Roze and Cornu (1871) in *P. crystallinus*.

Bainier, in 1882, published his " Étude sur les Mucorinées," in which he described specimens he had met with of *P. longipes*, *P. oedipus*, *P. Kleinii*, *P. roridus*, as well as a supposed new species, *P. exiguus* ; he also confirmed van Tieghem's account of *Pilaira nigrescens*.

In 1884 my " Monograph of the Pilobolidae " was published in the Midland Naturalist, Birmingham, in which there was added to the hitherto known species the curious form of *Pilobolus Kleinii* to which, as already mentioned, the name forma *sphaerospora* was affixed. A new species of Pilaira was also described, to which the name *P. dimidiata* (Fig. 109, p. 218) was given on account of its possessing an apophysis nearly as large as its sporangium ; unfortunately this has never been met with since, although at the time of publication such a sequel was not imagined possible.

Dewèvre, in 1894, included a good account of the species of Pilobolus in his " Contribution à l'Étude des Mucorinées." He

emphasised the distinction of *P. crystallinus* from *P. Kleinii*, with both of which he was personally acquainted.

Palla, after that date, issued a long account " Zur Kenntniss der Pilobolus-Arten " (1900), in which he classified the known species in a novel way, but without adding much to what was previously discovered ; he also invented a new name, *Pilobolus heterosporus* (Fig. 103, p. 208), which was my *P. Kleinii*, forma *sphaerospora*, under a different designation.

In the same year (1900), Morini gave a short description, without a name, of a new species of Pilobolus, to which afterwards (1905) Saccardo added the name *P. Morinii*. In 1906, Morini described another species of Pilobolus, *P. Borzianus*, of which he also discovered the zygospores, and in 1904 he had figured a new species of Pilaira, *P. Saccardiana* (Fig. 110, p. 219). But *Pilobolus Morinii* and *P. Borzianus* seem to be involved in a cloud of doubt, as do many of the others, like those of Spegazzini and Massee, which are placed towards the end of the following systematic lists.

In 1926 Ling Yong, working in France, described a new species of Pilaira, which he named *P. Moreaui* (Fig. 111, p. 220), distinguished from *P. anomala* by its larger spores.

The latest addition to our knowledge of the species of Pilobolus has been made by Buller who, in the preceding chapter of this volume, has described *P. umbonatus*, a new species distinguished by the possession of a remarkably umbonate sporangium (Fig. 105. p. 210).

This long story of the observations made on the systematics of Pilobolus during nearly two hundred and fifty years is deeply interesting to the mycologist ; it shows how slowly and painfully a little accurate knowledge has been accumulated. From now on, with the modern technique of pure cultures and the art of photography at our disposal, progress should be more rapid. It is to be hoped that some younger mycologist may be stimulated by this presentation of the subject to make a life-study of the genus and give us a comparative description of all the species that can be gathered together from different parts of the world.

Systematic Arrangement.—An attempt will now be made to arrange systematically all the species of the Pilobolidae and to give

to the group itself and to the two genera Pilobolus and Pilaira included within it more precise definitions than have yet appeared.

PILOBOLIDAE

A family of the Mucoraceae having a sporangium which contains a mass of dense jelly around its base between the sporangium-wall and the spore-mass. At maturity the mass of jelly swells up and causes the sporangium to dehisce near its base, so that the jelly protrudes between the sporangium-wall and the columella. The sporangium-wall is usually intensely black and is persistent when immersed in water.

Pilobolus Tode, in Schrift. d. Berl. Gesell. naturf. Fr., vol. v, p. 46 (1784).

Fruit-body consisting of a sporangiophore and a sporangium. Sporangiophore seated on a main mycelial hypha, from which it is separated by one or two septa. From below upwards, it is composed of a basal swelling or trophocyst, a cylindrical stipe, and a more or less ovoid subsporangial swelling, and when young it is generally ornamented by beads of moisture. Sporangium usually jet-black, seated on the apex of the subsporangial swelling, separated from it by a columella, and having its wall highly cutinised and persistent when immersed in water. When mature the sporangium and columella are violently projected. The species usually occur on the dung of herbivorous animals.

A. *Sporangium black when mature ; no apophysis.*

1. P. crystallinus.
2. P. longipes.
3. P. roridus.
4. P. Kleinii

5. P. sphaerosporus.
6. P. heterosporus.
7. P. oedipus.
8. P. umbonatus.

B. *Sporangium yellow when projected ; provided with an apophysis.*

9. P. nanus.

All these species have been adequately described and figured, and (whether they are truly distinct from one another or not) may

be recognised with certainty. After them are placed others which have been insufficiently or negligently described, and which are possibly nothing but variants of some of the preceding species :

DOUBTFUL SPECIES

10. P. Morinii.	14. P. minutus.
11. P. Borzianus.	15. P. pullus.
12. P. argentinus.	16. P. Schmidtii.
13. P. roseus.	

KEY TO THE SPECIES OF PILOBOLUS

{Sporangium decidedly umbonate .   .   .   *umbonatus*
{Sporangium rounded above   1

1 {Trophocyst much elongated   .   .   .   *longipes*
  {Trophocyst ovoid or turnip-shaped   .   .   2

2 {Spores globose and ellipsoid in the same sporan-
  {   gium   .   .   .   .   *heterosporus*
  {Spores globose or subglobose only   .   .   3
  {Spores ellipsoid only   5

3 {Spores with two coats .   .   .   .   .   *oedipus*
  {Spores with only one coat   .   .   .   4

4 {Sporangium yellow when projected   .   .   *nanus*
  {Sporangium black when projected.   .   .   *sphaerosporus*

5 {Spores from 10 μ upwards in length   .   .   *Kleinii*
  {Spores averaging less than 10 μ in length   .   6

6 {Spores 5–10 × 4–6 μ ; sporangium large   .   *crystallinus*
  {Spores 6–8 × 3–4 μ ; sporangium small   .   *roridus*

(Probably *crystallinus* and *roridus* are merely forms of one species.)

A. *Sporangium black when mature ; no apophysis.*

1. **Pilobolus crystallinus** Tode, *l.c.* p. 46, pl. 1 (1784). Van Tieghem, *Trois. Mém.* in Ann. Sci. Nat. ser. 6, vol. iv, pp. 335–8, pl. 10, f. 4, 5, repeated from Bull. Soc. Bot. Fr. vol. xxii, pp. 283–4

(1875). Coemans, *Monographie*, pp. 57–8, pl. 2, f. 1–20. Sacc. Syll. vii. 185. Grove, *Pilobolidae*, p. 333 (p. 33 of Reprint), pl. 4, f. 16. Palla, *Zur Kenntniss der Pilobolus-Arten*, p. 399.

> *Mucor urceolatus* Dickson, *Fasc. Pl. Crypt.* part 1, p. 25, pl. 3, f. 6 (1785). Sowerby, *English Fungi*, pl. 300. Bolton's figure under this name (*Fung. Halif.* pl. 133, f. 1) is doubtful.
>
> *Pilobolus urceolatus* Purton, *Midland Flora*, vol. iii, p. 323, pl. 31 (1821).
>
> *P. microsporus* Brefeld, *Botan. Untersuch.*, part 4, p. 70, pl. 4, f. 16, 19–22 (1881).
>
> Not *P. crystallinus* of Bonorden, or of Cohn, or of Klein, or of Brefeld, or of many others.

Sporangiophore 5–12 mm. high, or even up to 2 cm., rising usually from a single, more or less erect, terminal, ovate trophocyst,
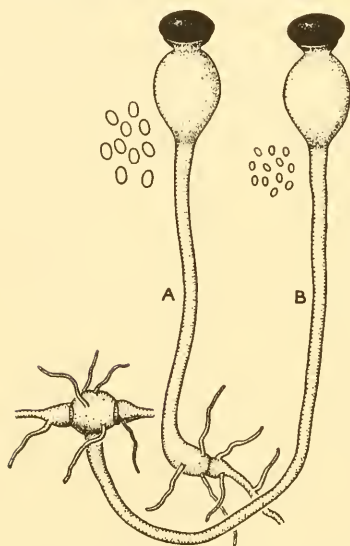
Fig. 99.—Ripe fruit-body and spores : A, *Pilobolus Kleinii* (= Brefeld's *P. crystallinus*); B, *Pilobolus crystallinus* (= Brefeld's *P. microsporus*). Copied by A. H. R. Buller from Brefeld's *Untersuchungen*. Magnification: fruit-bodies, 20 ; spores, 200.

which is often concealed in the substratum ; occasionally the trophocyst may be intercalary. Subsporangial swelling ovoid or elliptic-ovoid, about 1 mm. high, 600–800 μ broad. Sporangium convex above, from half as wide to nearly as wide as the subsporangial swelling, usually 350–500 μ in width ; columella conical, faintly blackish-blue ; spores ellipsoid, pale yellowish or quite colourless, 5–10 μ long (8–10 × 5–6 μ, van T. ; 5–12 × 3–6 μ, Palla ; 6 × 4·7 μ, Brefeld ; 7–11 × 4·5–7, Dewèvre).

Zygospores subglobose, 140–200 μ in diameter, nearly smooth, faintly coloured, filled with oil-drops (Krafczyk. in Ber. d. D. Bot. Gesell. 1931, p. 145, f. 2). Apparently not quite mature.

On dung of cows, horses, and the like. Reputed common. Europe, North and South America, Porto Rico, etc. Not seen by Buller at Winnipeg.

The upper cutinised part of the sporangium, when it is dried up, is sometimes marked with a few polygonal reticulations (one central) ;

but the reticulations are not always so geometrical as van Tieghem represents them, and they can be found also in other species.

Illustration : 99, B.

2. **Pilobolus longipes** van Tiegh. *Trois. Mém.* in Ann. Sci. Nat. ser. 6, vol. iv, pp. 338–340, pl. 10, f. 11–15 (1876), repeated from Bull. Soc. Bot. Fr. vol. xxii. pp. 283–4 (1875). Grove, *Pilobolidae*, p. 335, pl. 6, f. 1. Sacc. Syll. vii. 185. Palla, *l.c.* p. 399.

> *P. roridus* Brefeld, in Bot. Zeit. 1875, p. 852 ; *Botan. Untersuch.* part 4, p. 70, pl. 4, f. 17.

Sporangiophore 2–3 cm. high, sometimes 4–5 cm. or even 6–7 cm. ; trophocyst usually external to the substratum, elongated horizontally, 1·5–2 mm. long, golden-yellow, almost cylindrical or slightly tapering, giving rise to the stipe at one end. Subsporangial swelling oval, rather less than 1 mm. broad. Sporangium globose, black, about 500 μ across ; columella broadly conical, tinged with bluish-black ; spores globose or ovoid, 12–15 × 10–12 μ, the wall rather thick and tinged (often very faintly) with bluish-black, contents yellow-orange.
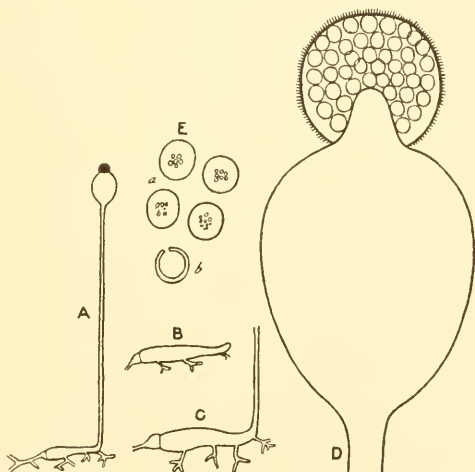
FIG. 100.—*Pilobolus longipes* v. Tiegh. A, whole fruit-body. B, trophocyst. C, basal swelling and part of stipe. D, upper part of fruit-body enlarged. E, spores: *a*, intact; *b*, broken open. Reproduced by photography from van Tieghem's *Troisième Mémoire*.

On dung of horses. Europe, Canada, U.S.A.

Distinguished by its large roundish spores, its elongated trophocyst, and its height.

Illustration : Fig. 100. Other illustrations in this volume : Figs. 18, 24, 40, 57, 70, 82, 83, 85, 91, and 96.

3. **Pilobolus roridus** (Bolt.) Pers. *Syn. meth.* p. 118 (1801). Coemans. *Monographie*, pp. 61–2, pl. 2, f. B. (copied from Bolton). Van Tiegh. *Nouv. Rech.* Nat. in Ann. Sci. ser. 6, vol. i, pp. 46–50, pl. 1, f. 7–13. Grove, *Pilobolidae*, p. 336. Sacc. Syll. vii. 185. Palla, *l.c.* p. 398.

> *Mucor roridus* Bolton, *Fung. Halif.* vol. iii, pl. 132, f. 4 (1789).
>
> *Pilobolus microsporus* Klein, *Zur Kenntniss des Pilob.* in Jahrb. f. wiss. Bot. vol. viii, p. 360, pl. 27, 28, f. 53–67 (1872).
>
> Not *P. roridus* of Brefeld, or of Currey, or of many others.

Sporangiophore 1–2 cm. high, nearly colourless, the trophocyst often intercalary between two (or even three) mycelial swellings. Subsporangial swelling oval or almost globose, up to 600 or 700 μ diam. Sporangium very much less in diameter, even as little as 200 μ; columella flatly convex or conical, tinged with bluish-black; spores ellipsoid, nearly colourless, 6–8 × 3–4 μ (van T.).
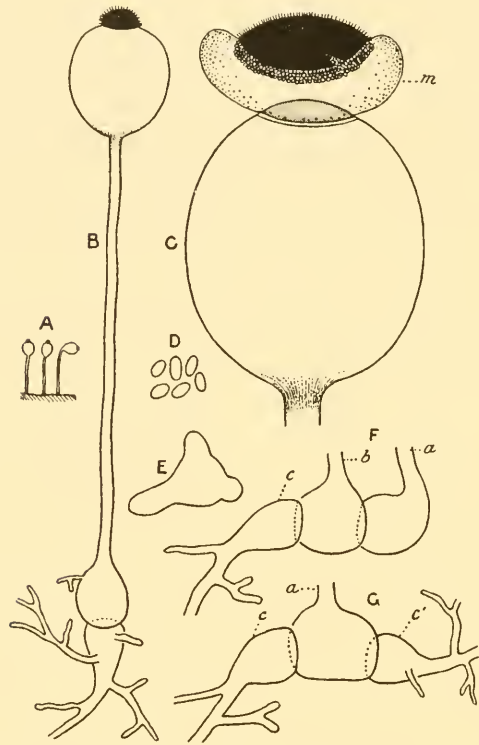
FIG. 101.—*Pilobolus roridus* (Bolt.) Pers. A, three fruit-bodies. B, whole fruit-body, shortened. C, upper part of fruit-body after sporangium has dehisced in water; *m*, mucilage. D, spores. E, germinating spore. F, two basal swellings, *a* and *b*, inserted successively on the same apophysis *c*. G, an intercalary basal swelling *a* formed between two apophyses, *c* and *c'*. Copied by A. H. R. Buller from van Tieghem's *Nouvelles Recherches*. Magnification : A, natural size; B, 25; C, 65; D and E, 380; F and G, 50.

On dung of herbivorous animals, including rabbits. Not common. Distribution uncertain.

This species shows generally much less of the yellow colour

(carotin) than its allies ; but there has been in most cases considerable doubt whether the plants entered under this name were anything but forms of *P. crystallinus*. The chief points which are supposed to characterise the species are the smallness of the spores and of the sporangium. The " dew-drops " which are implied in the name *roridus* are to be found in suitable circumstances in equal abundance on other species. It tends to excite our suspicions when we find authors recording the occurrence of *P. roridus* in company with *P. crystallinus*.

Illustration : Fig. 101.

4. **Pilobolus Kleinii** van Tiegh. *Trois. Mém.* in Ann. Sci. Nat. ser. 6, vol. iv, pp. 337–8, pl. 10, f. 6–10 (1876), repeated from Bull. Soc. Bot. Fr. vol. xxii, pp. 282–3 (1875).   Grove, *Pilobolidae*, p. 335, pl. 4, f. 1–8, 10–13, and in Journ. Bot. 1884, p. 131, pl. 245, f. 4. Sacc. Syll. vii. 185.   Bainier, *Étude*, p. 43, pl. 2, f. 14, 15.   Palla, *l.c.* p. 399.

> *P. roridus* Currey, in Journ. Linn. Soc. Lond. Bot. vol. i, pp. 162–7, pl. 2, f. 1–10 (1857).   Not *M. roridus* Bolton.
> *P. crystallinus* Klein, *Zur Kenntniss des Pilobol.* in Jahrb. f. wiss. Bot. vol. viii, p. 360, pl. 23–7, f. 1–52.   Brefeld, *Botan. Untersuch.* part 4, p. 70, pl. 4, f. 15.   Zopf, *Zur Kenntniss der Infectionskrankheiten*, p. 354.   Not of Tode.
> *P. Kleinii* var. *minor* Dewèvre, in Grevillea, vol. xxii, p. 74 (only 1 mm. high).

Sporangiophore 2–5 mm. high, varying up to 10–12 mm. high, rising singly from a turnip-shaped trophocyst which is often buried in the substratum.   Subsporangial swelling obovoid or subellipsoid, 400–800 μ high.   Sporangium black, more or less depressed or subglobose, about two-thirds as wide as the swelling to nearly as wide ; columella sometimes with a faint blackish tinge, generally colourless, broadly conical below, but occasionally narrowed in the middle so that the apex resembles a papilla ; spores in varying shades of orange-yellow, ellipsoid, 11–20 $\times$ 6–10 μ, with a thin smooth colourless cell-wall.

Zygospores thick-walled, spherical, nearly smooth, about 200 μ diam.   (Zopf, *l.c.* pl. 6, f. 8–19.)

On all kinds of dung. Very common. Europe, E. Africa, Canada, U.S.A., etc.

This appears to be the commonest species in Europe, and perhaps in most countries where Pilobolus occurs. It has often been confounded with *P. crystallinus*, but is not so high, is more tinged with yellow, the thickened band of protoplasm at the top of the stipe is more brightly orange, and its spores, although of the same shape, are perceptibly larger. Trophocysts usually with a single apophysis, rarely with two.

Illustration : Fig. 99, A. Other illustrations in this volume : Figs. 2, 12, 13, 22, 27, 28, 29, 32, 39, 42, 46, 61, 62, 83, 86, 91, 94, and 95.

5. **Pilobolus sphaerosporus** Palla, *Zur Kenntniss der Pilobolus-Arten*, in Oesterr. Bot. Zeitschr. vol. 50, p. 400, pl. 10 (1900).

> *Pilobolus lentiger* Corda, *Icon. Fung.* vol. i, p. 22, pl. 6, f. 286 (1837), including var. *macrosporus* Berl. & de Toni, in Sacc. Syll. vii. 188 (1888).
>
> *Pycnopodium lentigerum* Corda, *ibid.* vol. v, p. 18 (1842), an ill-nourished form.
>
> *Pilobolus crystallinus* Bonord. *Handb.* p. 128, pl. 10, f. 203 (1851).
>
> *P. oedipus*, var. *intermedia*, Coemans, *Spic. Mycol.* in Bull. Acad. Belg. ser. 2, vol. xvi, p. 71 (1863).
>
> *P. crystallinus* (*P. oedipus*, forms *b* and *c*) Klein, *Zur Kenntniss des Pilobol.*, p. 360, pl. 26, f. 40 *b*, 46–8 ; pl. 27, f. 49, 50 (1872).
>
> *P. intermedius* (Coem.) Karst. *Myc. Fenn.* part 4, p. 71 (1879).
>
> *P. oedipus* Brefeld, *Botan. Untersuch.* part 4, p. 69, pl. 3, f. 1–10 ; pl. 4, f. 11–14 (1881).
>
> *P. exiguus* Bainier, *Étude*, p. 47, pl. 2, f. 17 (1882).
>
> *P. Kleinii*, forma *sphaerospora*, Grove, in Journ. Bot., vol. xxii, p. 132, pl. 245, f. 5 (1884) ; *Pilobolidae*, p. 335, pl. 4, f. 9.

Similar to *P. Kleinii*, but usually smaller. Spores yellow or orange, more or less globose, 10–20 μ in diam., varying greatly in size, often with granular contents, diffusing very easily in water, and having a thin wall composed of only one coat.

On dung of man, horse, cow, etc. Europe.

The spores vary considerably in size. I have always found this in company with *P. Kleinii* at the beginning of its growth in a culture, but changing and passing gradually into the normal form.

Palla (*l.c.*) says that this appearance is due to the growth of the two species in a mixed (impure) culture, but that *P. sphaerosporus* produces its fruit-bodies from one to several days before those of *P. Kleinii* show themselves. I do not entirely agree with this; it does not explain the transition stages, which may always be found on looking for them, like those represented by Coemans' *intermedia* and Klein's variety *c* of his *P. crystallinus*, where both ellipsoid and round spores may be seen in the same sporangium.

Illustration: Fig. 102. The figure here given, from Brefeld's Untersuchungen, is called by him *P. oedipus*; it is not that species, but exactly represents my *P. Kleinii* forma *sphaerospora*.

6. **Pilobolus heterosporus** Palla, *Zur Kenntniss der Pilobolus-Arten*, in Oesterr. Bot. Zeitschr. vol. 50, p. 349, pl. 10, f. 1–5 (1900); a *Résumé* of this article, with plates and descriptions, is given by R. Ferry in Revue Mycologique, 1904, pp. 19–33.

Sporangiophore 2–3 mm. high; trophocyst usually buried in the substratum, ellipsoid, rarely globose, 300–400 μ long. Subsporangial swelling ovoid or ellipsoid, 500–600 μ high, provided at the insertion of the columella with a thin narrow

FIG. 102.—*Pilobolus sphaerosporus* Palla. Fruit-body and spores. Copied by A. H. R. Buller from Brefeld's *Untersuchungen* (his *P. oedipus*) and enlarged by one-third. Magnification, 40 and 400.

annular zone. Sporangium shaped like a convex cap, deep-black, 400 μ broad; columella more or less deeply constricted in the middle, rounded at the apex, reaching nearly to the top of the sporangium; spores yellow or orange-red, varying in form and size in the same sporangium, narrow- or roundish-ellipsoid, with all intermediate forms, 8–20 (or even 25) μ long, and 6–12 μ broad.

On cow dung, Graz (Styria).

I have drawn up this description from Palla's rambling and diffuse account as given in his article. Palla says that he

cultivated his species, and found that it retained its characters up to fifteen generations. But it is in no way different from my forma *sphaerospora* of *Pilobolus Kleinii*. The irregularity of the spores is exactly what I found in my cultivations, and the retention of the characters may be due merely to the persistence of similar conditions throughout the whole series of his fifteen generations. If I am right in my opinion, the three species here numbered 4, 5, and 6 are all identical, and the variations in form are dependent upon the surrounding circumstances.

Illustration : Fig. 103.
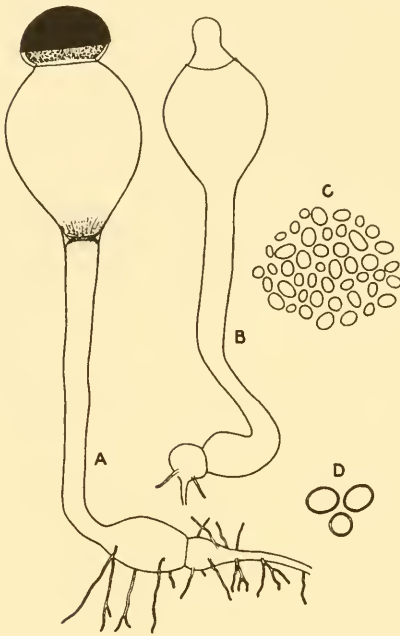
FIG. 103.—*Pilobolus heterosporus* Palla. A, fruit-body, after dehiscence of the sporangium. B, fruit-body, after removal of the sporangium, showing the columella. C and D, spores. Copied by A. H. R. Buller from Palla's *Zur Kenntniss der Pilobolus-Arten* and reduced to two-thirds. Magnification: A and B, 30 ; C, 214 ; D, 434.

7. **Pilobolus oedipus** Mont. *Mémoire sur le genre Pilobolus,* in Ann. Soc. Linn. de Lyon, pp. 1–7, f. *a–i* (1828). Coemans, *Monographie,* pp. 59–60, pl. 1, f. 1–20. Grove, *Pilobolidae,* p. 308, pl. 4, f. 14–15 ; and in Journ. Bot. vol. xxii, p. 131, pl. 245, f. 3. Sacc. Syll. vii. 186. Palla, *l.c.,* p. 400. Bainier, *Étude,* pp. 43–4, pl. 2, f. 1–10.

> *P. crystallinus* Cohn, *Entwicklungsgeschichte des Pilob. crystallinus,* with pl. 51, 52 (1851).
> *P. reticulatus* van Tiegh. in Ann. Sci. Nat. ser. 6, vol. iv, p. 336. Not the *P. oedipus* of Brefeld or of Klein, nor perhaps that of van Tieghem (*Nouv. Rech.* 1875, p. 43).

Sporangiophores yellow or reddish, usually short and thick, about 2–3 (or even 5) mm. high, rising singly from a roundish

trophocyst which projects somewhat above the substratum, but several of these are often so closely aggregated together that they form little tufts. Sporangium about 500 µ broad, not quite so broad as the ovoid subsporangial swelling, almost hemispherical, black ; columella conical or subcylindrical, slightly narrowed in the middle, sometimes so high that it reaches almost to the top of the sporangium ; spores globose, yellowish-red, 9–16 µ diam. ; with a wall composed of two distinct layers, of which the outer (epispore) is thick and often bluish.

On dung of horses, cows, pigs, goats, and mules ; it has also been found on human excrement, and it often occurs on decaying vege-table substances such as Algae (Spirogyra, Conferva, Oscillaria, and so on), and therefore also on the mud of river-banks (Thames, Oder, Red River of Winnipeg, etc.). It has been suggested that perhaps its spores do not require to pass through the alimentary canal of an animal, but this seems unlikely, since in a discharged sporangium they adhere together

Fig. 104.—*Pilobolus oedipus* Mon-tagne. A, young fruit-body. B, mature fruit-body. C, another mature fruit-body, with the spor-angium more flattened than usual. D, spores. Copied by A. H. R. Buller from Coemans' *Monographie* and reduced to two-thirds. Magnification : A, 53 ; B and C, 40 ; D, 200.

just as in other species of Pilobolus. They germinate, however, easily in water. The name of this species should be spelled *oedipus* (adjectival), not *OEdipus*.

Europe, E. Africa, N. America. Its round thick-walled spores are characteristic, also the deep colour which it displays owing to the crowded visible trophocysts. Chlamydospores (*Mycogone anceps* Coem.), lying in the mycelium, globose or ovoid, orange-yellow, oily-granular, 20–30 µ in diameter, are assigned to this species by Coemans, and by Ellis, North American Fungi (no. 3360) ; these may be azygospores (*cf.* Fig. 106, G.).

Illustration : Fig. 104.

8. **Pilobolus umbonatus** Buller, in his Researches on Fungi, vol. VI, pp. 177–178, figs. 81–84, 87–91 (1934).

Fruit-body 3–9 mm. high, arising from an oval to turnip-shaped
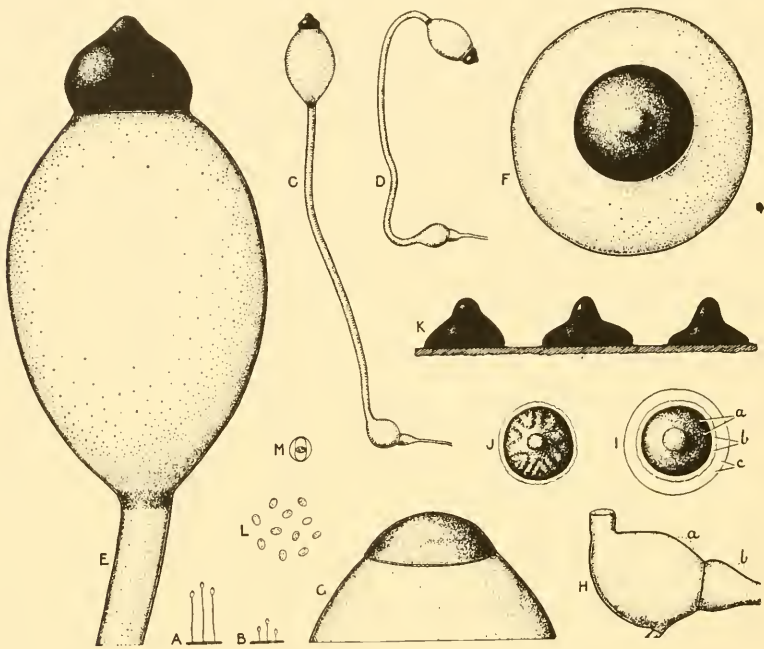
Fig. 105.—*Pilobolus umbonatus* Buller.   A, well-grown fruit-bodies 7–8 mm. high.   B, less well-grown fruit-bodies 1·5–3·0 mm. high.   C and D, whole fruit-bodies, showing sporangium, subsporangial swelling, stipe, and basal swelling attached to an apophysis ; C was 4 mm. high, and D 3·5 mm. high.   E, the upper part of a fruit-body, showing the umbonate sporangium, the barrel-shaped subsporangial swelling, and part of the stipe.   F, the same seen from above ; ratio of width of sporangium to width of subsporangial swelling = $\frac{1}{2}$.   G, the greyish columella, seen after removal of the sporangium from the top of the fruit-body.   H, a basal swelling a, attached to an apophysis b.   I, a discharged sporangium : a, the black sporangium-wall ; b, the colourless fringe of the sporangium-wall ; and c, adhesive jelly.   J, a similar sporangium, but jelly is not shown ; the surface of the sporangium-wall in I was smooth, in J wrinkled.   K, three discharged and dried-up sporangia seen in lateral view.   L, spores.   M, diagram showing a spore of *P. umbonatus* enclosed by a spore of *P. Kleinii*, in turn enclosed by a spore of *P. longipes*.   Drawn by A. H. R. Buller from material obtained at Winnipeg.   The drawings C and D reproduced from pencil drawings made by Hans Ritter.   Magnification : A and B, natural size ; C and D, 15 ; E–K, 75 ; L and M, 255.

basal swelling or trophocyst which may be terminal or intercalary, single or dispersed at intervals along a coarse stolon-like main hypha.

Stipe increasing slightly in diameter from below upwards, until just beneath the subsporangial swelling it is about 0·1 mm. in diameter. Subsporangial swelling ellipsoid, its maximum diameter being about midway between the base of the sporangium and the top of the stipe, 0·65 mm. long and 0·46 mm. broad ; a pale orange-red band of protoplasm at the junction of the stipe and the subsporangial swelling. Sporangium decidedly umbonate and more or less conical, 0·21–0·23 mm. in diameter or about one-half the diameter of the subsporangial swelling, shrinking on drying after discharge and becoming acutely pointed ; columella very bluntly conical or rounded (when removed from a discharged sporangium its edge is turned inwards towards the axis), greyish, distinctly darker than the subsporangial swelling. Spores ellipsoid, singly almost colourless, but yellow in mass, 5·0–6·0 × 3·0–3·8 μ.

On horse dung, Winnipeg, Canada, and, according to a communication from the late Dr. Roland Thaxter (who observed the species forty years ago but did not describe it), more frequently on sheep dung, at Boston, U.S.A.

Easily distinguished from all other species of Pilobolus by its decidedly umbonate sporangium and its minute ellipsoidal spores. With a hand-lens one can readily make out the acutely-pointed umbonate shape of the dried discharged sporangia when these are seen in lateral view.

Illustration : Fig. 105. Other illustrations in this volume : Figs. 81, 82, 83, 84, 87, 88, 89, 90, and 91.

B. *Sporangium yellow when projected ; provided with an apophysis.*[1]

9. **Pilobolus nanus** van Tiegh. *Trois. Mém.* in Ann. Sci. Nat. ser. 6, vol. iv, pp. 340–2, pl. 10, f. 16–22 (1876). Grove. *Pilobolidae,* p. 336, pl. 6, f. 2. Sacc. Syll. vii. 186. Palla, *l.c.,* p. 398.

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[1] In most Piloboli, the subsporangial swelling is not constricted below the level of its attachment to the columella and the wall of the sporangium. In *P. nanus,* according to van Tieghem, the subsporangial swelling is constricted a short way below that level so that it is divided into two unequal parts, a lower globular subsporangial swelling proper and an upper shallower swelling—the so-called *apophysis.*

Sporangiophores erect, not more than 1 mm. high, collected into groups of two or three (even four or five), all rising from contiguous buried intercalary trophocysts. Subsporangial swelling subglobose, colourless. Sporangium globular, about as wide as the subsporangial swelling, with the membrane of the upper part cutinised and
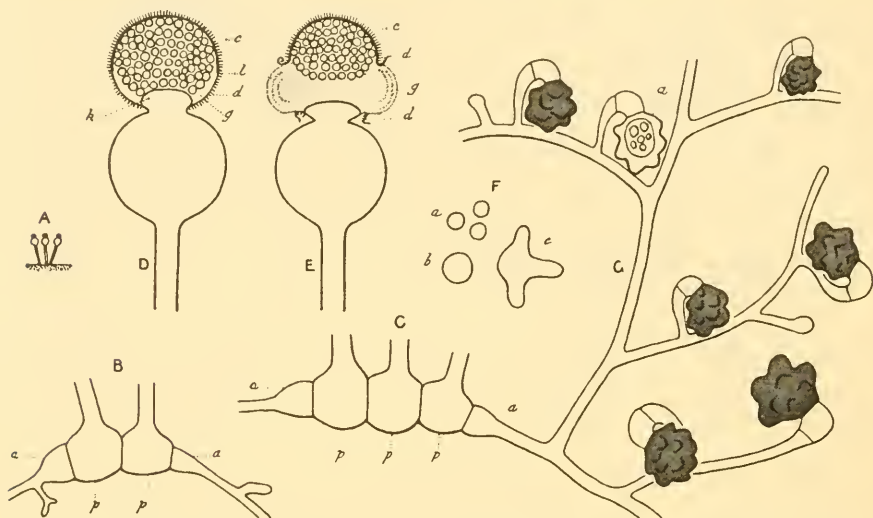
FIG. 106.—*Pilobolus nanus* v. Tiegh. A, three fruit-bodies. B, two basal swellings *p p*, and C, three basal swellings *p p p*, between two apophyses *a a*. D and E, optical sections through the upper part of a fruit-body, before and after dehiscence of the sporangium respectively ; *c*, yellow cuticularised wall of the sporangium ; *d*, diffluent region ; *l*, limiting ring of cuticularisation ; *g*, jelly ; *k*, columella. F, spores : *a*, resting ; *b*, swollen ; *c*, germinating. G, resting spores (azygospores), with tuberculate membranes, on a mycelium ; *a*, in section to show the thick cell-wall. Copied by A. H. R. Buller from van Tieghem's *Troisième Mémoire*. Magnification, not given.

yellow ; columella depressed-convex ; spores globose, colourless, 3·5–4 μ in diameter.

On excrement of rat. France.

Not observed by anyone but van Tieghem. The protoplasm of the sporangiophores is said by him to be colourless, as is also that of the mycelium. On the mycelium grew oblong-roundish, faintly yellow, coarsely verrucose chlamydospores, 15–20 μ in diameter, resting on short curved pedicels ; these are probably azygospores. The sporangia were shot off as in other Piloboli and stuck to the surrounding objects, while still yellow. When the explosion took

place, the rupture occurred at the constriction between the apophysis and the subsporangial swelling.

There is a possibility that this species is an abnormal form due to the unfavourable environment in which it was growing. Sporangia with defective black pigment in their walls have been observed by van Tieghem himself in his *P. oedipus* and by Buller in *P. longipes* (*vide* these *Researches*, vol. iv, p. 9, and this volume, p. 54).

Illustration : Fig. 106.

INSUFFICIENTLY KNOWN SPECIES

10. **Pilobolus Morinii** Sacc. Syll. xvii. 505 (1905).

> *Pilobolus* sp. Morini, in Mem. Accad. Sci. Ist. Bologna, ser. 5, vol. viii, p. 85, with plate (1899–1900).

Sporangiophores solitary, 600–800 μ high, each rising from an erect ovoid trophocyst. Subsporangial swelling subglobose or ovoid, somewhat narrowed above, and attenuated below into the sporangiophore, almost colourless when mature. Sporangium globular, somewhat flattened above, black, 130–200 μ in diameter ; columella obtusely conical, colourless, rounded above ; spores globose, orange-yellow (not colourless), 4·5–6 μ in diameter.

On dry human excrement. Montese, Bologna, Italy. Resembling *P. nanus* van Tiegh., except for its black sporangium and its sporangiophores not arising in groups.

11. **Pilobolus Borzianus** Morini, in Mem. Accad. Sci. Ist. Bologna, ser. 6, vol. iii, p. 126 (or 396), f. 3–10 (1909). Sacc. Syll. xxi. 827.

Sporangiophores 2·4–5 mm. high, growing two or three together from one trophocyst, which is ovoid and often imperfectly developed. Subsporangial swelling globose or shortly ovoid, 200–360 μ high, almost colourless or quite hyaline. Sporangium globose, very much flattened from above, 160–250 μ wide, intense bluish-black ; columella hemispherical or shortly conical, without apophysis ; spores spherical, deep yellow, 16–23 μ in diameter. Oval chlamydospores are developed in the mycelium.

Zygospores black, globose, about 180 μ across, with a thick and glabrous epispore (Morini, *l.c.* f. 3, 9).

On dung in the north of Italy.

Morini (in Mem. Accad. Sci. Ist. Bologna, ser. 6, vol. vi, pp. 123–4, f. 6–8, 1909) describes a var. *geminata* of this species. He says that the trophocyst may produce two hyphae at a time or, if one only, the one may branch into two above. He asserts that these forms remained constant in repeated artificial cultures. I suspect that the var. *geminata* is nothing more than an abnormal form ; for, since normally a Pilobolus sporangiophore collapses at the moment of discharging its sporangium, a forked sporangiophore can shoot away only one of its sporangia and not both. Abnormal branched sporangiophores are occasionally met with in other species, and are figured by Coemans in *P. crystallinus*, by Klein in *P. Kleinii* (his *P. crystallinus*), and have been seen by Zopf and Grove also in *P. Kleinii*.

12. **Pilobolus argentinus** Spegazzini, *Fung. Argent.* I, p. 176 (1880). Sacc. Syll. vii. 187.

Sporangiophores " immersed," here and there densely gregarious, 5–6 mm. high, at first cylindric-clavate, then filiform below, with a trophocyst and an ellipsoid subsporangial swelling, the whole plant entirely yellow. Sporangium globose, 100–125 μ diam., olive-black above, greenish-yellow below ; spores spherical, 12–15 μ diam., thick-walled, filled with a greenish-yellow granular protoplasm.

On horse dung, in grassy places alongside the Rio de la Plata. Judging by the description, one would consider this only a form of *P. oedipus*.

13. **Pilobolus roseus** Speg. *Fung. Argent.* I, p. 175 (1880). Sacc. Syll. vii. 187.

Sporangiophores densely gregarious, 2–4·5 mm. high, at first clavate, rosy-orange, truncate at the rounded apex, then filiform below, ellipsoid or ventricose-spheroid above, very beautifully rosy-hyaline. Sporangium black, hemispherical, 300–400 μ diam. ; spores ellipsoid, obtusely rounded at the ends, granular within, rosy-hyaline, 12–16 × 7–8 μ.

On cow dung, near the Rio de la Plata. This species might well be merely a form of *P. Kleinii* ; its chief distinction seems to reside in its colour, but a rosy hue is not unknown in some other species of Pilobolus.

14. **Pilobolus minutus** Speg. *Fung. Argent.* I, p. 176 (1880). Sacc. Syll. vii. 186.

Sporangiophores superficial, loosely gregarious, 2–5 mm. high, at first filiform-clavate, then ventricose and ellipsoid above, always hyaline, more or less elongated and filiform below. Sporangium black, lenticular, 125–145 μ diam. ; spores spherical or ellipsoid, granular, hyaline or faintly greenish-yellow, 7–8 μ diam.

On cow dung, in shady places near the Rio de la Plata. This species may well be only *P. Kleinii* var. *minor* Dewèvre.

15. **Pilobolus pullus** Massee, in Kew Bulletin, 1901, p. 160. Sacc. Syll. xvii. 506.

Sporangiophore about 1 mm. high, nearly colourless, inflated and ventricose above. Sporangium depressed-hemispherical, black, smooth, 250–300 μ broad ; columella convex, often constricted in the middle ; spores ellipsoid, 10–12 × 8–9 μ, with an orange epispore.

On cow dung. Tasmania (Rodway). Perhaps akin to a form of *P. Kleinii*.

Petch, in recording a species under this name in Annals Roy. Gard. Peradeniya, vol. vii, part 4, p. 297, says : " Scattered ; total height 0·6–0·8 mm., clavate, expanding almost from the base, 0·3 mm. diameter above. Sporangia oval, black, 0·25 × 0·15–0·2 mm. ; wall black-brown, smooth, not areolated. Spores oval, pale-yellow, 8–10 × 5–6 μ.

" On cow dung, Hakgala, Ceylon, December, 1917."

16. **Pilobolus Schmidtii** Sacc. Syll. xxiv. 11 (1926). *Pilobolus* sp. Alf. Schmidt, in Jahresber. d. Schles. Gesell. xc. 19 (1912).

Sporangiophores standing singly ; trophocyst ovoid, yellowish, 560–720 × 340–400 μ. Stipe 4 mm. high ; subsporangial swelling ovoid, up to 1 mm. high, 640–800 μ broad ; columella colourless, 250–370 μ high. Sporangium hemispherical, black, 430–510 μ broad ; spores ellipsoid, thin-walled, yellow or yellowish, 6·5–8·5 × 5–6 μ, not diffusing readily in water.

On dung of mules. Reared at Breslau, Germany, on mule dung

brought from Amani, German East Africa.   Allied to *P. Kleinii*, but differing in the dimensions of its parts.

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Pilaira van Tieghem, *Nouv. Rech.* in Ann. Sci. Nat. ser. 6, vol. i, p. 51 (1875).

Fruit-body consisting of a sporangiophore and a sporangium. Sporangiophore arising from the mycelium without a septum at its base.   It is evenly cylindrical and has no basal or subsporangial swelling.   Sporangium black, separated from the sporangiophore by a columella, and having its wall highly cutinised and persistent when immersed in water.   When mature the sporangiophore collapses and there is no projection of the sporangium.   The species are all coprophilous.

Pilaira differs from Pilobolus in that its sporangiophore has no septum at the base, no basal swelling, and no subsporangial swelling, and in that its sporangium is not violently projected.

In 1930 Fitzpatrick, in his book on the Phycomycetes, suggested that the genus Pilaira is " based on abnormal material of Pilobolus." There can be no doubt that this view is erroneous.   Not only van Tieghem and Brefeld but also Buller, I myself, and others have observed and cultivated the type of the genus, *Pilaira anomala*, and have found it to be quite distinct from any abnormal form of Pilobolus.

Five species of Pilaira are known.   The key to them, given below, is founded on that of Ling Yong.

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KEY TO THE SPECIES OF PILAIRA

{ Spores round	*nigrescens*
{ Spores oval or elongated	1
1 { Sporangiophores branched	*Saccardiana*
{ Sporangiophores simple	2
2 { Sporangiophores only about 1 mm. high . .	*dimidiata*
{ Sporangiophores up to 10 cm. high . . .	3
3 { Spores oval, up to 10 μ long	*anomala*
{ Spores elongated, up to 22 μ long . . .	*Moreaui*

1. **Pilaira anomala** (Ces.) Schröter, *Pilze*, in Cohn's Kryptogamen-Flora von Schlesien, vol. iii, p. 211 (1889). Sacc. Syll. vii. 188.

> *Pilobolus anomalus* Cesati, in Klotzsch, *Herb. viv. mycol.*, no. 1542 (1851). Brefeld, *Botan. Untersuch.* part 4, pp. 60–5, pl. 4, f. 18, 23–28.
> *Ascophora Cesatii* Coemans, *Monographie*, p. 63, pl. 2, f. E (1861).
> *Pilobolus Mucedo* Brefeld, *Botan. Untersuch.* part 1, p. 27, pl. 1, f. 25, 26 (1872).
> *Pilaira Cesatii* van Tiegh. in Ann. Sci. Nat. ser. 6, vol. i, p. 52, pl. 1, f. 14–24 (1875). Bainier, *Étude*, pp. 29–32, pl. 1, f. 16–18. Grove, in Journ. Bot., vol. xxii, p. 132, pl. 245, f. 6 ; *Pilobolidae*, p. 337, pl. 6, f. 7, 8.

Sporangiophore cylindrical, colourless, at first erect, 1–2 cm. high, then growing to a height of 9–12 (or even 20) cm., at length shrivelling and falling down on the substratum. Sporangium at first yellow, black when mature, more or less globular, 120–250 μ diam., then hemispherical with a small granular apophysis below ; columella colourless, hemispherical, but somewhat depressed ; spores ovoid, nearly colourless (but yellowish in mass), 8–12 × 6–7 μ.

Zygospores black, globose or ovoid, up to 115 μ diam., epispore covered with numerous minute papillae (Brefeld. *l.c.* part 4. f. 26–28).

FIG. 107.—*Pilaira anomala* (Ces.) Schröter. A, three fruit-bodies, collapsing. B, longitudinal optical section of a sporangium. C, upper and lower part of a fruit-body, after the dehiscence of the sporangium ; *m*, protruding mucilage. D, sporangium in air, before dehiscence. E, columella, seen from below, after the fall of the sporangium. F, a similar columella, in lateral view ; there are crystalloids in both the stipe and columella. G, spores. H, I, and J, three successive stages in the formation of a zygospore. Copied by A. H. R. Buller from van Tieghem's *Nouvelles Recherches*. Magnification : A, natural size ; B–F, 90 ; G, 380 ; H–J, 200.

On dung of sheep, goats, gazelles, hares. rabbits. goose. pig. ass, and horse. Europe, U.S.A. (Pennsylvania). Rather uncommon.

The zygospores were found by Brefeld on horse dung which had borne luxuriant crops of fruit bodies (*cf.* Fig. 107, H–J).

Illustration : Fig. 107.

2. **Pilaira nigrescens** van Tiegh. in Ann. Sci. Nat. ser. 6, vol. i, p. 60, pl. 1, f. 25–8 (1875). Grove, *Pilobolidae*, p. 337. Sacc. Syll. vii. 189.

Sporangiophore shorter than in the preceding species (1·5–2 cm.) and more slender. Sporangium also smaller, but having a similar granular apophysis ; columella blackish-violaceous or bluish, hemispherical, and ending in a conical papilla. Spores globose, colourless, 5–6 μ in diameter.
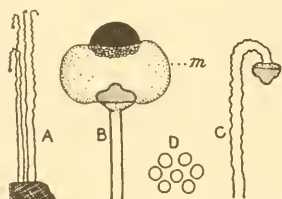
Fig. 108.—*Pilaira nigrescens* v. Tiegh. A, four fruit-bodies, collapsing. B, upper part of a fruit-body after dehiscence of the sporangium; *m*, protruding mucilage. C, upper part of a fruit-body after the fall of the sporangium. D, spores. Copied by A. H. R. Buller from van Tieghem's *Nouvelles Recherches.* Magnification : A, natural size; B and C, 90; D, 380.

On dung of rabbit. France ; rare. Distinguished by its size, its spores, and its conical and coloured columella.

Illustration : Fig. 108.

3. **Pilaira dimidiata** Grove, in Journ. Bot. vol. xxii, p. 132, pl. 245, f. 7 *a–d* (1884) ; *Pilobolidae*, p. 338, pl. 6, f. 10 *a–c.* Sacc. Syll. vii. 189.

Sporangiophore slender, cylindrical, and while erect not more than 0·5–1·0 mm. high, then bending down towards the substratum and becoming 3–4 mm. long. Sporangium at first yellow, then black, hemispherical, 100–120 μ diam. ; immediately beneath it the sporangiophore is widened somewhat
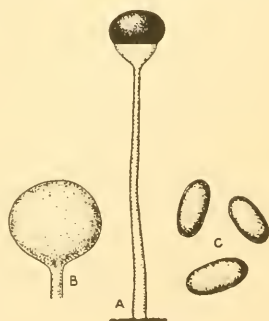
Fig. 109.—*Pilaira dimidiata* Grove. A, mature fruit-body. B, columella. C, spores. Drawn by A. H. R. Buller from sketches made by W. B. Grove in 1883 without the use of a *camera lucida.* Cf. Plate 245, Fig. 7, *a–d*, in Grove's *New or Noteworthy Fungi*, 1884. Magnification : A, 116 ; B, 240 ; C, 660.

like the apophysis of a moss-capsule (Funaria). Spores ellipsoid, almost colourless, 12–14 × 5–6 μ.

On dog's dung. England (Worcestershire); found only once, March and April. Distinguished from *Pilaira anomala* not only by its much smaller size, but also by its peculiar apophysis, which is almost as large as the sporangium, but slightly less in diameter, and not granular. It was growing luxuriantly on a rich substratum.

Illustration : Fig. 109.

4. **Pilaira Saccardiana** Morini, first mentioned in Rendic. Sess.
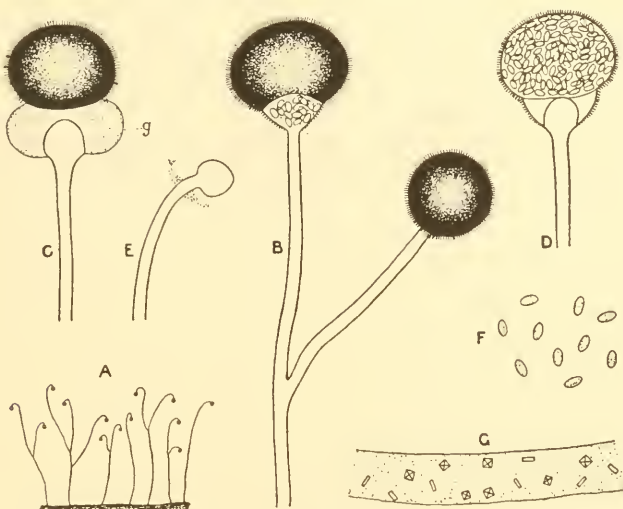
FIG. 110.—*Pilaira Saccardiana* Morini. A, fruit-bodies, sporangiophores branched ; sporangia mature and now hanging down. B, a branched sporangiophore bearing two sporangia, one in side view and the other seen from above. C, a sporangium after dehiscence ; *g*, swollen jelly. D, optical longitudinal section of a sporangium, showing the spores and the columella. E, the sporangium has gone ; the columella remains and, about its base, can be seen a gelatinous substance derived from the liquefaction of the inferior zone of the sporangial membrane. F, spores. G, part of a sporangiophore whose sporangium had not yet ripened ; in the protoplasm are numerous crystalloids of mucorine. Copied by A. H. R. Buller from Morini's *Ricerche intorno ad una nuova forma di Pilaira*. Magnification : A, natural size ; B–G, not stated.

R. Accad. Sci. Ist. Bologna, 1904, with plate, and then named *P. Saccardiana* in Mem. R. Accad. Sci. Ist. Bologna, ser. 6, vol. iii, p. 128 (1906). Sacc. Syll. xxi. 827.

Sporangiophore rarely emerging from a rudimentary trophocyst (which is usually wanting), slender, simple or branched, with at most two branches. Sporangium globose, faintly depressed from above, 90–130 μ in transverse diameter, brown above, then blackish, lower zone not cutinised and forming a broad annulus of the membrane by the gelatinisation of which the spores are afterwards set free ; columella shortly conical, deep-violet in colour ; spores oval, 7–10 μ long, hyaline, but with a smooth pallid violaceous membrane.

On dung, in the north of Italy.

Illustration : Fig. 110.

5. **Pilaira Moreaui** Ling, in " Étude morphologique, cytologique
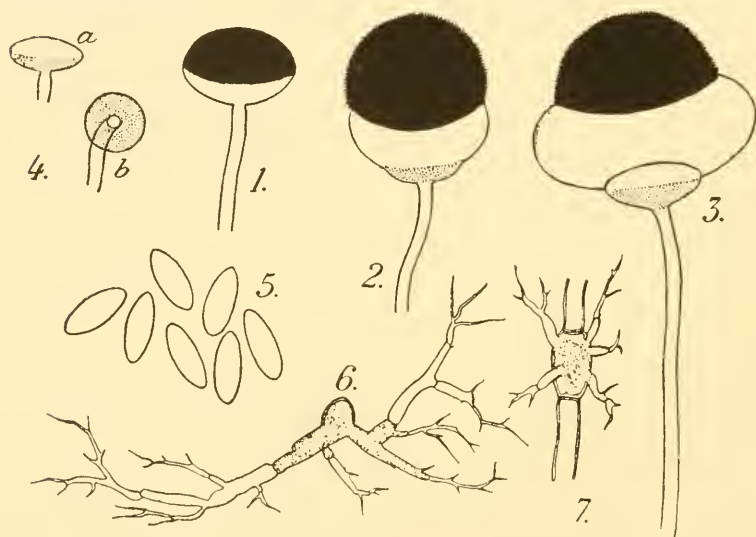
Fig. 111.—*Pilaira Moreaui* Ling. No. 1, young sporangium. No. 2, mature sporangium, seen in moist air. No. 3, the dehiscence of the sporangium under the influence of water which swells its lower part. No. 4, columellae after the fall of the sporangium : *a*, in profile ; *b*, from below. No. 5, spores. No. 6, a germinating spore. No. 7, a germinating chlamydospore. From Ling Yong's *Étude*. Magnification : nos. 1–4, 60 ; no. 5, 300 ; nos. 6 and 7, 70.

et microchimique d'une nouvelle Mucorinée, Pilaira Moreaui " (1926). See also Rev. générale de Botanique, xlii. 743.

Sporangiophores lax, hyaline, not branched, erect, soon decumbent, 10–12 cm. high, 30 μ broad. Sporangia at first yellow, depressed, when mature globose, intensely bluish-black, 300–400 μ

in diameter (in dwarfed specimens only 80 µ), membrane encrusted, the upper zone cutinised, persistent, the lower gelatinous, deliquescent ; columella flattened, broadly adnate, 150–180 × 90–100 µ. Spores cylindric-ellipsoid, smooth, hyaline, 18–20 × 8–10 µ (sometimes as much as 24 µ long), granular within, agglutinated ; chlamydospores few, intercalary in the submerged mycelium ; zygospores not seen.

On dung of horses and rabbits. France. Allied to *P. anomala*, from which it differs chiefly in its larger spores. It forms a gall with *Chaetocladium Jonesii*.

Illustration : Fig. 111.

Bibliography.—The following list includes all those papers and books which refer more particularly to the species of the Pilobolidae and their differentiation. Some additional works, which treat of the physiology, ecology, and other aspects of the group, are cited in the preceding Chapters written by Professor Buller.

BIBLIOGRAPHY OF THE PILOBOLID.E.

Anderson, R. S.—" The Validity of the genus Pilaira," in University of Iowa Studies, vol. xv, pp. 3–5, pl. 1 (1933).

Bainier, G.—" Étude sur les Mucorinées," Paris (1882) : pp. 33–48, *P. oedipus*, f. 1–10, *P. longipes*, f. 11–13, *P. Kleinii*, f. 14, 15, *P. roridus*, f. 16, *P. exiguus*, f. 17 ; pp. 29–32, *Pilaira Cesatii*, f. 16–18 (on another plate).

Baker, Henry—" Natural History of the Polype Insect," chap. xi, pl. 22, f. 9, 10 (1744).

Bary, A. de—" Vergleich. Morph. u. Biol. der Pilze," p. 77, f. 38, *P. oedipus* (1884).

Berkeley, Rev. M. J.—" Fungi," in Smith's English Flora, vol. v, part 2, p. 231 (1836).

Bolton, James—" An History of Fungusses growing about Halifax," vol. iii : pl. 132, f. 4, *Mucor roridus* ; pl. 133, f. 1, *Mucor urceolatus* (1789).

Bonorden, H. F.—" Handbuch der allgemeinen Mykologie," p. 128 (1851). His figure 203, called *Pilobolus crystallinus*, represents a form of *P. Kleinii*.

Brefeld, O.—Mittheilung in Sitzungsber. d. Gesellsch. naturf. Fr. zu Berlin : " Copulirende Pilze " in Bot. Zeitung, vol. xxxiii, pp. 850–3 (1875).

Brefeld, O.—"Botanische Untersuchungen über Schimmelpilze," Heft 1, p. 27, pl. 1, f. 25–26, *Pilobolus Mucedo* (1872); Heft 4, pp. 60–5, pl. 4, f. 18, 23–28, *P. anomalus*; pp. 69–70, pl. 3, f. 1–10, pl. 4, f. 11–14, "*P. oedipus*"; pl. 4, f. 15, "*P. crystallinus*"; f. 16, 19–22, "*P. microsporus*"; f. 17, "*P. roridus*" (1881).

Buller, A. H. R.—"*Pilobolus umbonatus*, a New Species" in his Researches on Fungi, London, vol. vi, pp. 169–178 (1934).

Bulliard, P.—"Herbier de la France," vol. i, p. 111 (1784), pl. 480, f. 1, *Mucor urceolatus* (1789).

Cesati, V. de—in Klotzsch, "Herbarium vivum mycologicum," *Pilobolus anomalus*, no. 1542 (1851).

Coemans, E.—"Monographie du genre Pilobolus, Tode, spécialement étudié au point de vue anatomique et physiologique," in Mém. cour. et des Sav. étrang. Acad. roy. de Belgique, vol. xxx, pp. 1–68; pl. 1 and 1 bis, *Pilobolus oedipus*; pl. 2, *P. crystallinus*, with fig. B. of *P. roridus* copied from Bolton (1861).

Coemans, E.—"Spicilegium Mycologicum," in Bull. Acad. Belg., ser. 2, vol. xvi, p. 71 (1863).

Cohn, F.—"Die Entwicklungsgeschichte des Pilobolus crystallinus," in Nova Acta Acad. Caes. L.-C. Nat. Cur., vol. xxiii, pp. 493–535, pl. 51–2 (1851).

Corda, A. C. J.—"Anleitung zum Studium der Mycologie," pp. 71–2, and p. lxix, pl. C, f. 25 (1842).

Corda, A. C. J.—"Icones Fungorum," vol. i, p. 22, *Pilobolus lentiger*, pl. vi, f. 286 (1837), afterwards placed in a new genus *Pycnopodium*, vol. v, p. 18 (1842). Also in vol. vi, p. 12, *Pilobolus crystallinus*, pl. 2, f. 32 (ed. Zobel, 1854).

Currey, F.—"On a species of Pilobolus," in Journ. Linn. Soc. Lond. Bot., vol. i, pp. 162–7, pl. 2, f. 1–10 (1857).

Dewèvre, A.—"Contribution à l'Étude des Mucorinées: Pilobolées," in Grevillea, vol. xxii, pp. 69–79 (1894).

Dickson, J.—"Fasciculi Plant. Crypt. Brit.," part 1, p. 25, pl. 3, f. 6 (1785).

Fitzpatrick, H. M.—"The Lower Fungi, Phycomycetes," ed. 1, pp. 251–253 (1930).

Fries, Elias M.—"Systema Mycologicum," vol. ii, pp. 308–9 (1823); vol. iii, p. 312 (1829). In both volumes *P. crystallinus* and *P. roridus* are marked "*v.v.*"

Greville, R. K.—"Flora Edinensis," p. 448, *P. crystallinus* (1824).

Grove, W. B.—"New or Noteworthy Fungi," in Journ. Bot. (1884), p. 131, pl. 245, f. 3, *P. oedipus*; p. 131, pl. 245, f. 4, *P. Kleinii*; p. 132, pl. 245, f. 5, *P. Kleinii*, forma *sphaerospora*; p. 132, pl. 245, f. 6, *Pilaira Cesatii*; p. 132, pl. 245, f. 7, *P. dimidiata*.

Grove, W. B.—"On the Pilobolidae," a Monograph (reprinted from the Midland Naturalist, Birmingham, pp. 1–40, with plates IV and VI (1884).

Klein, J.—"Mykologische Mittheilungen, I. Die Formen des Pilobolus," in Verhandl. Zool.-Bot. Ges. Wien, vol. xx, pp. 547–556 (1870).

Klein, J.—"Zur Kenntniss des Pilobolus," in Jahrb. f. wiss. Bot., vol. viii, pp. 305–381, pl. 23–30, f. 1–52, *P. Kleinii* (miscalled "*P. crystallinus*"); f. 53–67, *P. roridus* (miscalled "*P. microsporus*"), (1872).

Krafczyk, H.—"Die Zygosporenbildung bei Pilobolus cristallinus," in Ber. d. Deutsch. Bot. Gesell., vol. xlix, pp. 141–6, with two figs. (1931).

Kunze u. Schmidt—"Mykologische Hefte," Heft 2, pp. 70–6 (1823).

Ling, Yong.—"Étude morphologique, cytologique et microchimique d'une nouvelle Mucorinée, *Pilaira Moreaui* (Clermont-Ferrand, 1926).

Ling, Yong.—" Étude biologique des phenomènes de la sexualité chez les Mucorinées "
  in Revue générale de Botanique, vol. xlii, pp. 742-3 (1930).

Link, H. F.—" Observationes in Ordines plantarum naturales," Diss. I, p. 32, pl. 2,
  f. 50, *P. crystallinus* (1809).

Montagne, C.—" Mémoire sur le genre Pilobolus," in Ann. Soc. Linn. de Lyon,
  pp. 1–7, f. *a-i* (1828).

Morini, F.—" Ricerche sopra una nuova Pilobolea," in Mem. R. Accad. Sci. Ist.
  Bologna, ser. 5, vol. viii, p. 85, with plate (1900).

Morini, F.—" Ricerche intorno ad una nuova forma di Pilaira," in Rendic. Sess. R.
  Accad. Sci. Ist. Bologna, with plate (1904).

Morini, F.—" Materiali per una Monografia delle Pilobolee," in Mem. R. Accad. Sci.
  Ist. Bologna, ser. 6, vol. iii, pp. 111–129, with plate (1906).

Müller, Otto F.—" Von einem Kristallschwämmchen," in Kleine Schriften aus der
  Naturhistorie, p. 122, pl. 7 (1782).

Palla, E.—" Zur Kenntniss der Pilobolus-Arten," in Oesterr. Bot. Zeitschr., vol. 50,
  pp. 349–370, 397–401, pl. 10, *Pilobolus heterosporus* (1900).

Persoon, C. H.—" Observationes Mycologicae," part 1, p. 76, pl. 4, f. 9–11, *P.
  crystallinus* (1796).

Persoon, C. H.—" Synopsis methodica Fungorum," part 1, pp. 117–8, *P. crystallinus,
  P. roridus* (1801).

Petiver, James—" Gazophylacium," pl. 105, f. 14, *Fungus virginianus* (1711).

Plukenet, L.—" Phytographia," pl. 116, f. 7 (1691). " Almagestum Botanicum,"
  p. 164 (1696).

Purton, Thomas—" A Botanical Description of British Plants," Appendix to the
  " Midland Flora," vol. iii, part 1, pp. 323-5, pl. 31, *P. urceolatus* (1821).

Ray, John—" Historia Plantarum," vol. ii, p. 1928 (1688) ; vol. iii, p. 24 (1704).

Ray, John—" Synopsis methodica," ed. 2, Appendix, p. 322, " Fungus ex stercore
  . . ." (1696) ; ed. 3, pl. 13 (1724).

Relhan, R.—" Flora Cantabrigiensis," ed. 3, p. 579 (1820).

Roze, E. et Cornu, M.—" Une culture florissante du *Pilobolus crystallinus*," in Bull.
  Soc. Bot. France, vol. xviii, pp. 298–9 (1871).

Saccardo, P. A.—" Sylloge Fungorum," vol. vii, pp. 184–9 ; vol. xvii, pp. 505–6 ;
  vol. xxi, p. 827 ; vol. xxiv, p. 11.

Schmidt, Alf.—" Beitrag zur Kenntnis der deutsch-ostafrikanischen Mistpilze " in
  Jahresber. d. Schles. Gesell., vol. xc, pp. 17–25 (1912).

Scopoli, J. A.—" Flora Carniolica," ed. 2, vol. ii, p. 494, *Mucor obliquus* (1772).

Sowerby, J.—" English Fungi," pl. 300, *Mucor urceolatus* (1803).

Spegazzini, C.—" Fungi Argentini," pugillus primus, in Anal. de la Sociedad Cientif.
  Argent., vol. ix, pp. 158–192 (1880).

Tode, H. J.—" Beschreibung des Hutwerfers," in Schrift. der Berlin. Gesell. naturf.
  Fr., vol. v, p. 46, pl. 1 (1784).

Tode, H. J.—" Fungi Mecklenburgenses selecti," part 1, p. 41 (1790).

Vahl, Martin—" Flora Danica," vol. vi, f. 1080, *Pilobolus crystallinus* (1792).

Van Tieghem, Ph.—" Nouvelles Recherches sur les Mucorinées," in Ann. Sci. Nat.,
  ser. 6, vol. i, pp. 41–61, pl. 1, f. 7–13, *Pilobolus roridus* ; f. 14–24, *Pilaira Cesatii* ;
  f. 25–28, *P. nigrescens* (1875).

Van Tieghem, Ph.—" Troisième Mémoire sur les Mucorinées," in Ann. Sci. Nat.,
  ser. 6, vol. iv, pp. 335–349, pl. 10, f. 4, 5, *P. crystallinus* ; f. 6–10, *P. Kleinii* ;
  f. 11–15, *P. longipes* ; f. 16–22, *P. nanus* (1876).

Wiggers, F. H.—"Primitiae Florae Holsaticae," pp. 110–111 (1780). In the preface the binomial name (*Hydrogera crystallina*) is said to have been conferred upon the plant by his tutor, Weber.

Withering, W.—"Botanical Arrangement of all the Vegetables naturally growing in Great Britain," vol. ii, p. 784, *Mucor roridulus*, "Ray, Syn. p. 13" (1776).

Withering, W.—"A Systematic Arrangement of British Plants," ed. 4, vol. iv, pp. 394–5, *Mucor urceolatus*, *M. roridus* (1801); ed. 5, vol. iv, pp. 437–8 (1812).

Zopf, W.—"Zur Kenntniss der Infectionskrankheiten," in Nova Acta k. L.-C. Deutsch. Akad. Naturf., vol. lii, no. 7, pp. 352–8, pl. 6, f. 1–19, "*P. crystallinus*" (1888).

PART II

THE PRODUCTION AND LIBERATION OF SPORES IN THE DISCOMYCETES

CHAPTER I

THE PHENOMENON OF PUFFING IN SARCOSCYPHA
PROTRACTA AND OTHER DISCOMYCETES

Introduction.—In all the Basidiomycetes which violently dis-
charge their basidiospores, namely, the Hymenomycetes, the
Uredineae, Tilletia, and the Sporobolomycetes, every basidiospore
is shot away as soon as it has attained maturity ; and, since the
basidiospores ripen in succession, they are discharged in succession.
The result is that the liberation of basidiospores from the fruit-
bodies of the Hymenomycetes, from the teleuto-sori of the Rust
Fungi, from cultures of the mycelium of Tilletia, and from cultures
of Sporobolomycetes is a steady and continuous process, occupying
many hours, days, weeks, or even months, according to the species
or the cultural conditions. Spore-deposits made from any of these
fungi become denser and denser as the hours go by. Of all the
numerous species included in the groups of Basidiomycetes here
under discussion there is not one in which the basidiospores are given
off in sudden dense clouds at intervals determined by internal
organisation or external conditions.

In the Discomycetes, on the other hand, as is well known, there
are many species the fruit-bodies of which exhibit the phenomenon
of puffing, *i.e.* which, when subjected to certain changed conditions,

pass from a quiescent to an active state and suddenly liberate a cloud of spores. Among the larger Discomycetes which puff may be mentioned such common species as *Aleuria vesiculosa*, *Galactinia badia*, and *Peziza aurantia*.

Not only do most large Discomycetes puff but also many of the very small ones. The small ones often occur in large numbers gregariously on wood, etc., and all the fruit-bodies of a single group may puff simultaneously either when the log of wood or other substratum on which they grow is disturbed or when the tin box in which they have been collected is opened subsequently in the laboratory. Dr. Jessie S. Bayliss Elliott has kindly informed me that her record of very small Discomycetes which puff includes the following species :

Arachnopeziza aurata.	Lachnea setosa.
Ascobolus Crouani.[1]	Mollisia cinerea.
Chlorosplenium aeruginosum.	Orbilia xanthostigma.
Dasyscypha virginea.	Rhytisma acerinum.[2]
Helotium scutula.	

Historical Remarks.—The first reference to the phenomenon of puffing appears to be that of Micheli,[3] the discoverer of reproduction in fungi,[4] who in his celebrated *Nova Plantarum Genera* published in 1729 says of Fungoides (= Peziza, etc.) : " All the Fungoides are provided on the upper surface with very minute round or oval seeds, which are afterwards ejected upwards like smoke or

[1] The dung upon which this fungus was growing was kept in a large damp-chamber. The hymenial surface of the discs, at first pale, became black with mature asci containing the dark spores. When the door of the damp-chamber was suddenly opened, all the tiny apothecia puffed simultaneously and, in a flash, became pale again (J. S. B. Elliott *in litt.*).

[2] In the genus Rhytisma the apothecia open by a cleft. In including Rhytisma in the Discomycetes I have followed Boudier (*Histoire et Classification des Discomycètes d'Europe*, Paris, 1907, p. 177).

[3] P. A. Micheli, *Nova Plantarum Genera*, Florentiae, 1729, p. 204, Plate 86, Fig. 17.

[4] For a discussion of Micheli's discovery of spores not only in the Discomycetes but in fungi generally and for a translation into English of the record of the experiments by which he proved that spores are reproductive bodies *vide* A. H. R. Buller, " Micheli and the Discovery of Reproduction in Fungi," *Transactions of the Royal Society of Canada* (Presidential Address to Section IV), Series III, Vol. IX, 1915, pp. 1–25, Plates I–IV.

sparks, either by a contraction of the fibres while the plants are
expanding or by the lightest shake of even a gentle breeze."
Micheli's illustration of a puffing Discomycete is reproduced in
Fig. 112.

In 1791, Bulliard [1] described the phenomenon of puffing in
Helvella, Peziza, etc., and illustrated it as it occurs in a species of
Otidea.   He says : " If you shake these fungi or blow upon them
from above, their seeds ascend like steam ; you may blow strongly
afterwards, you may break the fungus into pieces, but you will not
see a second jet of seeds follow the first one immediately ; to obtain
a second jet there must pass an interval of some
two or three hours, depending on whether the
air is more or less free from moisture ; the parts
which compose the apparatus of the fructification
are as a rule too delicate to allow one to dis-
tinguish the mechanism of discharge, however
carefully one observes them with the best in-
struments."   Bulliard's diagram with which he
attempted to explain how puffing takes place
shows that he knew nothing of the true structure
of asci, and Tulasne's criticism of Bulliard's views
on puffing was quite justified.   Tulasne [2] says :
" Bulliard's ideas on this point are based on an
imaginary structure of the organs and the author's great want of
knowledge.   It is indeed doubtful whether he really knew from his
own experience that the smoke rising from Pezizas consists entirely
of their seeds."

In 1801, Persoon [3] recorded puffing in *Rhytisma salicinum* (his
Xyloma salicinum), for he says " In spring-time, the seed-dust was
blowing away from the cracks like smoke."   In 1805, during a rainy
May, Albertini and Schweinitz [4] saw *R. salicinum* " sending up clouds
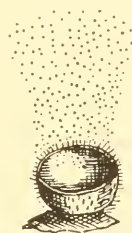
Fig. 112.—Micheli's
illustration of
puffing in the
Discomycetes,
published 1729.
Copied by A.
H. R. Buller.

[1] P. Bulliard, *Histoire des Champignons de la France*, Paris, 1791, pp. 51–52,
Plate II, Fig. 6.

[2] L.-R. and C. Tulasne, *Selecta Fungorum Carpologia*, Paris, Vol. I, 1861, p. 42 ;
also in the English Translation by W. B. Grove, Oxford, Vol. I, 1931, p. 44.

[3] C. H. Persoon, *Synopsis methodica fungorum*, Gottingae, Vol. I, 1801, p. 103.

[4] J. B. de Albertini et L. D. de Schweinitz, *Conspectus fungorum in Lusatiae
superioris agro Niskiensi crescentium*, Lipsiae, 1805, p. 62.

of smoke through the cracks of the cortex which was broken into irregular shield-like pieces." Tulasne,[1] in 1865, remarked that from the ascophorous hymenium of *Rhytisma acerinum*, when it has dehisced in spring, " the endospores escape like smoke."

In 1817, Kunze and Schmidt [2] remarked that they had observed puffing from the apothecia of two very small Discomycetes, by them included in the genus Phacidium but now regarded as species of Coccomyces. In their description of *Phacidium trigonum* (= *Coccomyces trigonus*) they say : " When in damp weather the disc is fully exposed, there follows after the least touch, just as in *P. coronatum*, the ejection of the spores in the form of a fine grey-green powder."

Desmazières,[3] in 1845, observed puffing in *Helvella ephippium* Lév. and stated that the vapour of the seeds was discharged into the air with a faint report. Tulasne [4] suggested that Desmazières had " been misled by some error " in supposing that he had heard his Helvella puff ; but, as we shall see in Chapter III, Desmazières's observation has been supported by subsequent investigations.

De Bary,[5] in the first edition of his well-known text-book of mycology published in 1866, treated of puffing in the Discomycetes in a modern manner. He showed : that the asci are turgid cells ; that, when puffing of a fruit-body takes place, a large number of the asci open apically at one and the same moment ; and that the spores of each ascus are shot up into the air owing to the contraction of the elastic ascus wall.

In 1909, I [6] showed that, if a section of a ripe hymenium of *Aleuria vesiculosa* is first submerged in water, the subsequent application to it of solutions of sodium chloride, potassium nitrate, grape sugar, or glycerine, all of which withdraw water from the cell-sap of the asci, does not cause the asci to explode, but that the asci

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[1] L.-R. and C. Tulasne, *loc. cit.*, Vol. III, 1865, p. 117 (Eng. Trans., p. 109).

[2] G. Kunze und J. C. Schmidt, *Mykologische Hefte*, Leipzig, 1871, p. 41.

[3] J. B. H. J. Desmazières, *Plant. crypt. France*, 2nd ed., fasc. XIX, 1845, No. 914. Cited from Tulasne.

[4] L.-R. and C. Tulasne, *loc. cit.*, p. 42 (Eng. Trans., p. 44).

[5] A. de Bary, *Morphologie und Physiologie der Pilze, Flechten, und Myxomyceten*, Leipzig, 1866, pp. 141–143.

[6] These *Researches*, Vol. I, 1909, pp. 238–241, 268.

explode readily when their tips are allowed to come into contact with iodine or certain other poisonous substances such as mercuric chloride, silver nitrate, copper sulphate, sulphuric acid, and alcohol ; and I further showed that asci which have contracted considerably owing to withdrawal of water from their vacuoles by potassium nitrate, etc., explode when brought into contact with iodine.   As a result of these experiments I suggested that puffing is caused by a stimulus given to the protoplasm in contact with the ascus lid.

In 1926, Ziegenspeck [1] reviewed the literature upon, and gave an account of his own investigations upon. the discharge mechanism of the asci of Ascomycetes (Discomycetes, Pyrenomycetes, Lichens). He came to the conclusion that, in fruit-bodies which puff, sudden slight changes in the environment of the asci, by setting up a sudden slight increase in the tension of the wall where the wall is weakest, cause the asci to explode.   Ziegenspeck regards the breaking open of an ascus during the phenomenon of puffing not as being due to the action of a stimulus but as a *purely mechanical phenomenon* comparable with the breaking of glass in which strains have been set up by unequal heating or cooling : there is a zone or line of weakness at the end of the wall of each ascus ; when the distension of an ascus wall has become great, it needs only a slight additional strain to cause the wall to break ; a very slight shaking, a very slight warming, a very slight external pressure, or a sudden withdrawal of water and therewith a reduction of elasticity, can lead to a sudden increased strain inside the membrane, with the result that the membrane relieves itself by splitting where its cohesion is least. Ziegenspeck, by employing a microscope with special optical arrangements, obtained evidence that strains are actually set up in the walls of the ends of the asci of Peziza, Ascobolus, etc., just before the asci explode.   On looking down on ripe moist asci, their ends appeared smooth and shining ; but, as soon as the asci were blown upon, the ascus tips became iridescent and exhibited Newton's rings.   Immediately after showing these signs of strain, the walls of the asci broke open and the spores were discharged.

Ziegenspeck's explanation of the cause of the bursting of

[1] H. Ziegenspeck, " Schleudermechanismen von Ascomyceten," *Botanisches Archiv* (Herausgeber, Carl Mez), Bd. XIII, 1926, pp. 341–381.

thousands of asci at the moment when a discomycetous fruit-body puffs either in the field or in the laboratory is simpler than the one suggested by myself in 1909 and is in good accord with his experimental observations. I am therefore inclined to accept it. However, that another factor, in addition to that of purely physical strains in the ascus wall, may have something to do with the bursting of an ascus seems to be shown by the already recorded results of my experiments on hymenia of *Aleuria vesiculosa* submerged in water. We can scarcely suppose that non-poisonous substances (sodium chloride, potassium nitrate, grape sugar, and glycerine) do not set up strains in the ascus wall whereas poisonous substances (iodine, mercuric chloride, silver nitrate, copper sulphate, sulphuric acid, and alcohol) do. The fact that poisonous substances cause submerged asci to burst, whereas non-poisonous substances do not, seems best explained by assuming that poisonous substances stimulate the protoplasm at the end of each ascus to act upon the ascus wall in such a way as to cause the operculum to open outwards and that non-poisonous substances do not so stimulate the protoplasm.

In recent years Richard Falck [1] has studied the conditions which affect the puffing of Discomycetes and permit of the spores escaping from the fruit-bodies ; and, as a result of his investigations, he has divided the fruit-bodies of Discomycetes into two groups : (1) the *radiosensitive, i.e.* those which emit spores when warmed by radiant heat given out by a lamp or the sun, etc., *e.g.* Morchellaceae, Gyromitra, and Verpa, and (2) the *tactiosensitive, i.e.* those which puff when touched or blown upon, *e.g.* Aleuria, Galactinia, Otidea, Peziza, and Pustularia.

Puffing illustrated by Photography.—The spore-cloud which, at the moment of puffing, is emitted from the fruit-body of one of the larger Discomycetes, is made up of tens of thousands or even millions of spores and can readily be seen with the naked eye. Dickson and Fisher,[2] working with *Sclerotinia sclerotiorum* (= *S.*

[1] R. Falck, " Ueber die Sporenverbreitung bei den Ascomyceten. I. Die radiosensiblen Discomyceten," *Mycologische Untersuchungen und Berichte* von R. Falck, Jena, Bd. I, Heft II, 1916, pp. 77–144 ; " Ueber die Sporenverbreitung bei den Ascomyceten. II. Die taktiosensiblen Discomyceten," *ibid.*, Heft III, 1923, pp. 370–403.

[2] L. F. Dickson and W. R. Fisher, " A Method of Photographing Spore Discharge from Apothecia," *Phytopathology*, Vol. XIII, 1923, pp. 30–32.

libertiana), have succeeded in photographing it in the following
manner.   Large numbers of sclerotia were germinated and, as soon
as the stipitate apothecia had attained full size, some scores of the
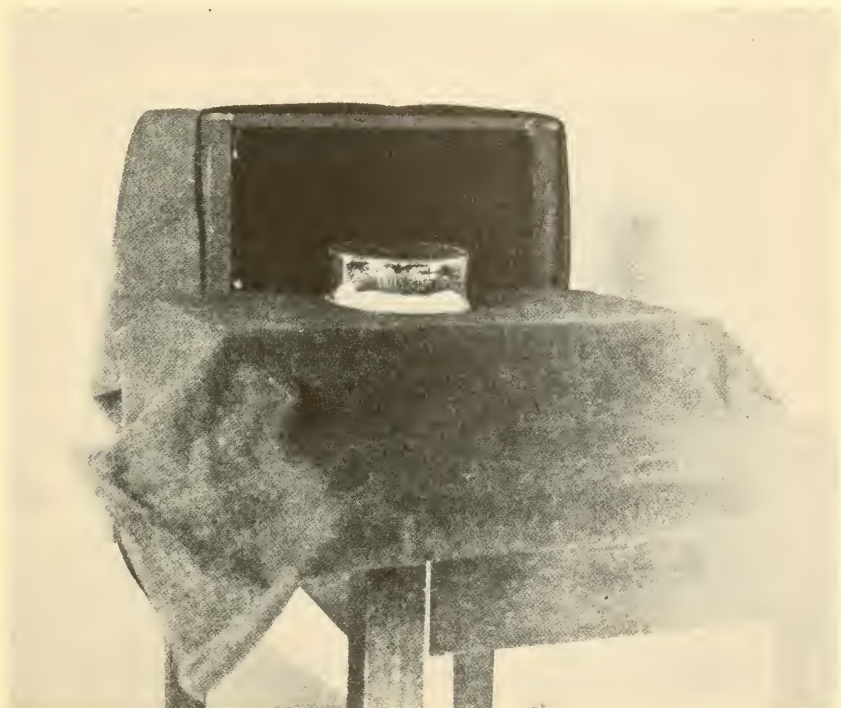
Fig. 113.—Arrangement of apparatus in preparation for photographing a cloud of
spores liberated during the puffing of the apothecia of *Sclerotinia sclerotiorum*
(= *S. libertiana*).   An inverted glass dish, 6 inches wide, on the table, covers a
number of stipitate apothecia developed from sclerotia.   Bright sunlight falls on
the fruit-bodies, and behind them is a black back-ground.   A photographic
camera (not here shown) is focussed on the centre of the dish.   By means of
suitable screens, the air around the dish is kept as still as possible.   When the
dish was raised a cloud of spores was emitted, as shown in the next illustration.
Photographed by L. F. Dickson and W. R. Fisher at Cornell University.   About
one-eighth the actual size.

sclerotia and their apothecia were arranged upon a layer of wet
cotton wool on a glass plate.   The plate and fungi were then
covered with an inverted crystallising dish six inches in diameter.
Thereupon the whole was placed out-of-doors in a shaded spot, and
it was left there for three days whilst the ascospores matured.
About two hours before the photograph was to be made, the dish

containing the apothecia was taken into a corridor of the laboratory and placed in the position shown in Fig. 113. A box lined with black paper was used to provide a black back-ground. The table was so arranged that direct sunlight illuminated the apothecia without shining into the box. A small object was then placed on the

Fig. 114.—A photograph showing spore-clouds which have been emitted by the puffing of numerous apothecia of *Sclerotinia sclerotiorum* (= *S. libertiana*) grown from sclerotia. It was taken in bright sunlight with an exposure of about one-hundredth of a second about one second after the dish (shown in Fig. 113) covering the fruit-bodies had been raised. The simultaneous discharge of the contents of millions of asci doubtless produced air currents which were a factor in raising the spore-clouds above the apothecia. Photographed by L. F. Dickson and W. R. Fisher at Cornell University. About two-fifths the natural size.

top of the middle of the dish and focussed with the camera. When everything was prepared, the photographer stood ready at the bulb of the camera and, at a given word, the dish was quickly removed from the apothecia. Nothing happened for about one second, and then the apothecia suddenly discharged clouds of spores into the air. At the right instant an exposure of a very rapid photographic plate was made for one-hundredth of a second, with the result shown in Fig. 114.

The Significance of Puffing.—The puffing of Discomycetes has excited the wonder of every field mycologist and is frequently mentioned in mycological literature. Yet, hitherto, no one, excepting Richard Falck, seems to have enquired whether or not the phenomenon is of any benefit to the fruit-bodies from the point of view of the dispersal of the spores. Conceivably, the asci of a Peziza might discharge their contents one by one in the order of ripening. Why then does a Peziza puff ? Can it be that simultaneous ascus-discharge in the Discomycetes is more advantageous to these fungi than successive discharge ; and, if so, wherein does this advantage lie ?

Falck has supposed that the dependence of Morchella, Gyromitra, Verpa, etc., on a sufficiently high temperature for the discharge of their spores causes spore-discharge to be delayed until the heat of the sun brings into existence air-currents which may assist in spore-dispersal [1] ; and he also holds that the dependence of many Pezizaceae on the wind for the discharge of their spores causes spore-discharge to be delayed in these fungi until the wind is sufficiently strong to be effective in carrying away the spores.[2] In these ecological theories there may well be some truth.

It was in the hope of throwing more light on the phenomenon of puffing that the investigation on *Sarcoscypha protracta* about to be recorded was undertaken.

The Genus Sarcoscypha.—The genus Sarcoscypha, according to Boudier,[3] is a very natural one. It includes species remarkable for their epixylous habit and the brilliant scarlet-red colour of their hymenium. The receptacles are more or less stipitate, bell-shaped, and tomentose on their outer side. The asci are very long, thin, and at their base attenuated and flexuous. The paraphyses are much branched, rather more pointed than clavate, and they contain red granules which turn green with iodine. The spores are large and oblong, and may or may not contain oil-drops ; in the latter case their contents are finely granular.[4] Boudier includes in

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[1] R. Falck, *loc. cit.*, I, pp. 124–126, 134.   [2] *Ibid.*, II, pp. 402–403.
[3] É. Boudier, *Histoire et Classification des Discomycètes d'Europe*, Paris, 1907, p. 55.
[4] *Ibid.*

Sarcoscypha nine species, among them being the well-known *S. coccinea*, found on sticks in damp woods in both Europe and North America, and the much more rare *S. protracta*.

Sarcoscypha protracta.—*Sarcoscypha protracta* Fr. (Fig. 115), according to Saccardo,[1] has various synonyms : *Microstoma hiemale* Milde ; *Peziza mirabilis* Borsz., under which name it is illustrated in M. C. Cooke's *Mycographia* ; *Sclerotinia baccata* Fuck. (the rooting base is not a sclerotium, hence the generic name Sclerotinia was a misnomer) ; and *Anthopeziza Winteri* Wettst., under which name it is illustrated in Kerner von Marilaun's *Natural History of Plants*. There is no description or illustration of the fungus in Boudier's *Icones Fungorum*.

Sarcoscypha protracta occurs in both Europe and North America, and appears to be a northern species. In Europe it has been found in Scandinavia, Finland, Germany, and Austria,[2] and it was observed by the late Professor J. H. W. Trail [3] in the month of May growing in clusters of two to six fruit-bodies among grass on the banks of the Dee near Ballater in Scotland. In Canada it has been found by myself in Manitoba and by Dr. E. H. Moss [4] in Alberta. Miss Hone [5] states that *S. protracta* is found in the State of Minnesota ; but, since her specimens were solitary and had no guttulae in their spores, the identification seems doubtful. My own specimens agree well with the descriptions given by Fries, Rehm, and other European mycologists.

Karsten,[6] in Finland, found fruit-bodies of *Sarcoscypha protracta* on Alder branches (*Alnus incana*) buried in the ground. According to Rehm,[7] the asci are 250–550 μ long and 18–24 μ wide ; and in

[1] P. A. Saccardo, *Sylloge Fungorum*, Vol. VIII, 1889, p. 155.

[2] For literature *vide* H. Rehm in Rabenhorst's *Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz*, Die Pilze, Vol. III, 1896, pp. 1072–1073.

[3] *Vide* W. Phillips, " British Discomycetes," *Grevillea*, Vol. XVIII, 1889–90, p. 83.

[4] I have seen Dr. Moss's specimens, collected 800 miles west of Winnipeg, and they exactly resemble those found by myself.

[5] Daisy S. Hone, "The Pezizales, Phacidiales and Tuberales of Minnesota," *Minnesota Botanical Studies*, Vol. IV, 1909, pp. 96–97.

[6] P. A. Karsten, *Mycologia Fennica*, Helsingfors, Vol. I, 1871, p. 44. I suspect that the buried branches (ramulos) were in reality roots (*cf.* my own Fig. 116).

[7] H. Rehm, " Hysteriaceen und Discomyceten," in Rabenhorst's *Kryptogamen-Flora*, 2 Aufl., Bd. I, Abt. III, 1896, p. 1073.

fruit-bodies found by me at Winnipeg some of the asci (Fig. 119, p. 245) just exceeded 600 μ in length. In these very large asci are contained very large spores, so large indeed that they were regarded by Fuckel [1] as the largest spores in all the Pezizae. The size of the spores has been recorded : by Fuckel [2] as $52 \times 20 \mu$; by Karsten [3] as 36–58 (mostly 42–48) $\times$ 15–17 μ ; and by Rehm [4] (Finnish and German specimens) as 36–40 $\times$ 15–17 μ. The size of the spores in Canadian fruit-bodies, as measured by myself, was found to agree with that given for European fruit-bodies by Karsten and Rehm. While the spores of *S. protracta* are very large for Pezizae, yet they are smaller than the spores of a few other Discomycetes, *e.g. Ascobolus immersus* in which the spores (Vol. I, Fig. 82, p. 254), exclusive of their broad gelatinous investment, measure 55–65 $\times$ 35–45 μ, and *Ptychoverpa bohemica* in which the spores measure 60–80 $\times$ 17–22 μ [5] The large size of the asci and spores in *Sarcoscypha protracta* was a factor in making this fungus favourable material for my investigation on the phenomenon of puffing.

Fruit-bodies of *Sarcoscypha protracta* (Fig. 115) come up singly or in clusters of two to eleven in young Poplar bush (*Populus tremuloides*) at River Heights, a suburb of Winnipeg, in the last weeks of April and the first weeks of May ; and they were first found there as follows. One day near the end of April, 1925, when the winter's snow had just melted but before there were any leaves on the trees, Charles aged eleven and Dennis aged eight, sons of my former colleague, Dr. C. H. O'Donoghue, were roaming the bush when their eyes were attracted by the beautiful scarlet apothecia standing up in little groups amid the leaf-mould. They very naturally took pleasure in gathering these firstlings of the spring and in taking them home to their parents. Dr. O'Donoghue kindly brought me some of the booty. Then, on April 30, Dr. O'Donoghue, his two sons as pioneers, and I visited the Poplar woods, and together we found some hundreds of fruit-bodies scattered here and there in the leaf-mould under the trees. On this occasion and

[1] L. Fuckel, *Symbolae Mycologicae. Beiträge zur Kenntniss der rheinischen Pilze*, Wiesbaden, 1869–70, p. 331.

[2] *Ibid.*   [3] P. A. Karsten, *loc. cit.*, p. 44.

[4] H. Rehm, *loc. cit.*, p. 1073.   [5] *Ibid.*, p. 1200.

subsequently, fresh material was gathered for investigation as it was required.

Dr. O'Donoghue's sons discovered for themselves that the
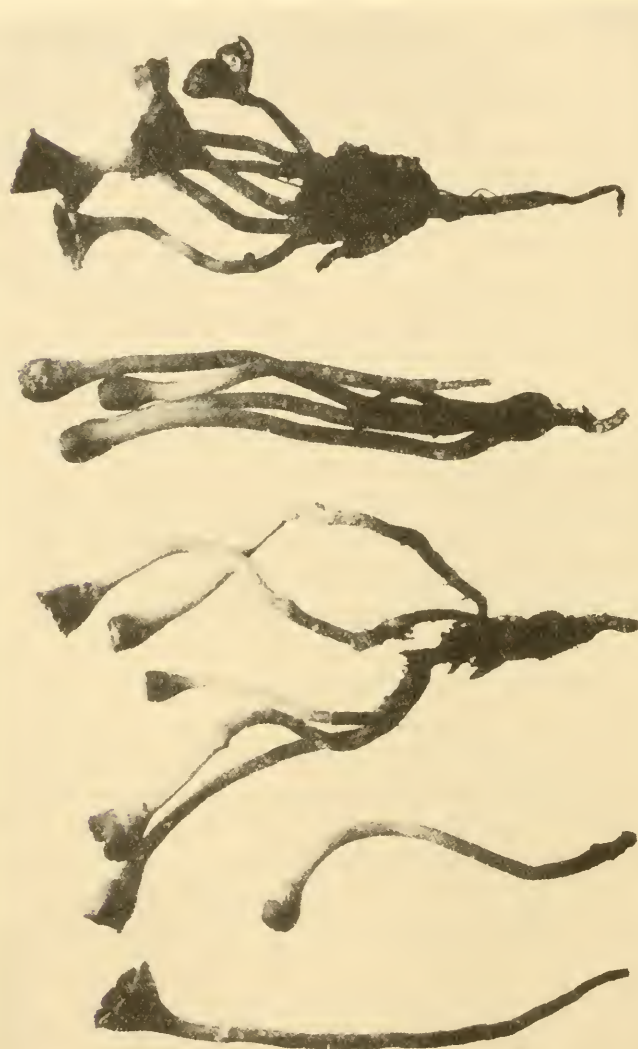
Fig. 115.—*Sarcoscypha protracta*. To the right, three groups of fruit-bodies each of which springs from a pseudorhiza which was attached to a buried root. To the left, two fruit-bodies, one unexpanded, the other fully expanded. The fully expanded fruit-bodies, which had a scarlet hymenium, possessed ripe asci and puffed strongly after being brought into a warm laboratory. Obtained in a Poplar wood at Winnipeg, Manitoba, about April 27, 1925. Natural size.

ungathered fruit-bodies of *Sarcoscypha protracta*, when tickled lightly with a little stick, send out a cloud like smoke and hiss as they do so (*cf.* Fig. 116); and Dr. O'Donoghue has informed me that he him-

self saw fruit-bodies puff on several occasions when he was picking them from the ground. We thus have good evidence that the fungus may puff under field conditions.[1]

The Perennial Pseudorhiza.—Investigations made in the bush convinced me that every fruit-body of *Sarcoscypha protracta* is epixylous. The mycelium grows in the wood of roots which are about 0·3–1·5 cm. thick and buried in the leaf-mould to a depth of 1–10 cm. The roots presumably are those of Poplar. When the mycelium in a root has progressed sufficiently, it usually gives rise to a single solitary fruit-body which grows upwards through the leaf-mould as a slender rod or stipe terminated by a rudimentary apothecium. The growth in length of the stipe is intercalary and takes place just below the cup. In this way the cup is pushed up through the leaf-mould and raised somewhat above its surface. After being brought by the intercalary growth of the stipe to the surface of the ground, the apothecium expands and thus the mature fruit-body comes to resemble the one shown in Fig. 116 or the one shown on the extreme left in Fig. 115. The stipe may be considered as having two parts : (1) a subterranean part which is blackened by the soil, slender, easily broken, which corresponds exactly to the " rooting base " of such Hymenomycetes as *Collybia radicata* and *C. fusipes*, and which is best called a *pseudorhiza*, and (2) an aerial part, whitish and highly tomentose, which may be referred to as the *aerial stipe*.

At the end of the first spring, the solitary fruit-body just described does not entirely disappear. While its apothecium and its aerial stipe die and rot away, the lower part of the pseudorhiza persists in the living condition until the second spring. Then it proliferates at its upper end and gives rise not to one but to several new fruit-bodies, each of which has a pseudorhiza and aerial stipe of its own. Thus in the second year there is developed a cluster of fruit-bodies varying in number up to eleven. Three such clustered fruit-bodies are shown on the right side of Fig. 115.

At the end of the second spring, the cluster may die away completely, but investigation shows that sometimes at least, while the

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[1] An account of the investigations recorded in this Chapter was given at the International Botanical Congress, held at Ithaca, U.S.A., in August 1926. *Vide* the *Proceedings* of the Congress, Vol. II, 1929, pp. 1627–1628.

secondary apothecia and aerial stipes decay and disappear, the bases of the secondary pseudorhizae persist until the third spring, when the ends of some of them proliferate and then give rise to tertiary fruit-bodies. We thus find that our original pseudorhiza produced in the first spring is either biennial or perennial. In *Collybia fusipes* we have an exactly similar arrangement. It is a remarkable fact that a perennial pseudorhiza should have been developed in two such diverse fungi as *Sarcoscypha protracta* and *Collybia fusipes* ; but both grow on buried roots, and we can only suppose that, in the course of evolution, in response to this common condition of their environment, they have reacted in a similar manner.

The Direction of Puffing and the Campanulate Form of the Apothecium. —When a fruit-body puffs, the spores are shot straight outwards from the cup in directions which are parallel to the cup's longitudinal axis. If the fruit-body is upright, as shown in Fig. 116, the spore-cloud is shot up vertically into the air, but if the fruit-body is oblique the spore-cloud is shot away obliquely.

A large number of fruit-bodies which were gathered at a temperature only just above the freezing-point of water, whilst occasional flakes of snow were
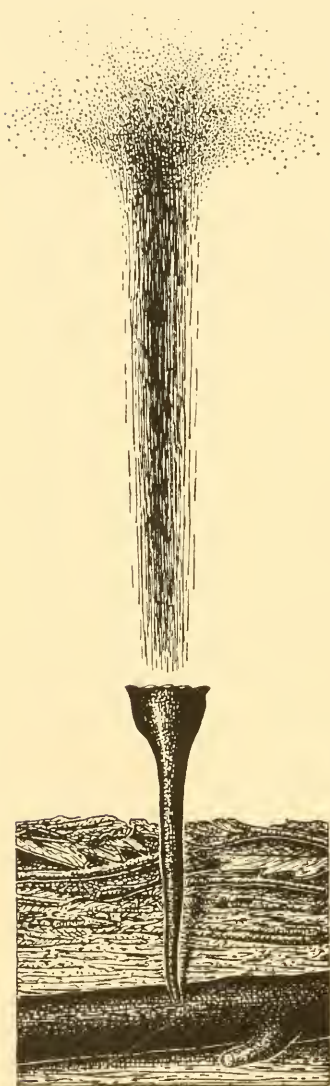
FIG. 116.—*Sarcoscypha protracta*. A vertical section through leaf-mould in a Poplar wood at Winnipeg, Manitoba, to show a single fruit-body attached by its rooting base or pseudorhiza to a buried root. The scarlet hymenium which covers the conical interior of the cup has just puffed and the spore-stream is represented diagrammatically as rising vertically to a height of about three inches above the top of the fruit-body. Natural size.

falling, appeared to be mature but could not be caused to puff in the open, presumably owing to their being too cold. The fruit-bodies were put into a vasculum and taken to the laboratory the air of which had a temperature of about 70° F. As soon as the laboratory had been entered, the fruit-bodies were removed from the vasculum, separated from one another, and placed in a series of Petri dishes. During these operations not a single fruit-body puffed. However, after an hour, when the fruit-bodies had become warmed up to the temperature of the room, puffing occurred freely whenever a mature fruit-body was removed from a Petri dish and exposed to the relatively dry room-air. Advantage was taken of this fact to observe the direction and distance of discharge of three particular fruit-bodies. In turn, each was quickly removed from its Petri dish with the fingers and held horizontally in the air of the laboratory. Within two seconds from the beginning of this operation puffing took place. The spore-cloud produced by each fruit-body travelled forward horizontally (*cf*. Fig. 116). One of the spore-clouds went 8 cm. before dispersing irregularly, another 12 cm., and another 17 cm. (= 7 inches).

The results yielded by the experiments just described prompted the following question : the apothecia being campanulate in form, how comes it that the spores are shot straight out of the cup in a direction more or less parallel to the cup's axis ? To solve this problem it was necessary to ascertain : (1) the exact shape of the apothecium at the moment of puffing, (2) the arrangement of the asci in the hymenium, and (3) the manner in which the asci open.

Variations in the shape of the apothecium, nearly three times the natural size, are shown in Fig. 117. At A is an end-view and at B a vertical section of a young apothecium which is about to expand. Its hymenium, as indicated in B, was lining the cavity of the cup, but the asci had not yet developed any spores. C is a well-expanded apothecium seen from above. Its dark central cavity was lined by a deep-scarlet hymenium containing ripe ascospores, whilst the lighter marginal crenations were orange-yellow and sterile. The six drawings D–I show vertical sections through six apothecia which had discharged or were about to discharge their spores. The average

angle of aperture of these and of other mature apothecia was about 50°. Within, the apothecia are rather pointed at the base and never well-rounded like those of *Galactinia badia, Aleuria vesiculosa,* etc.
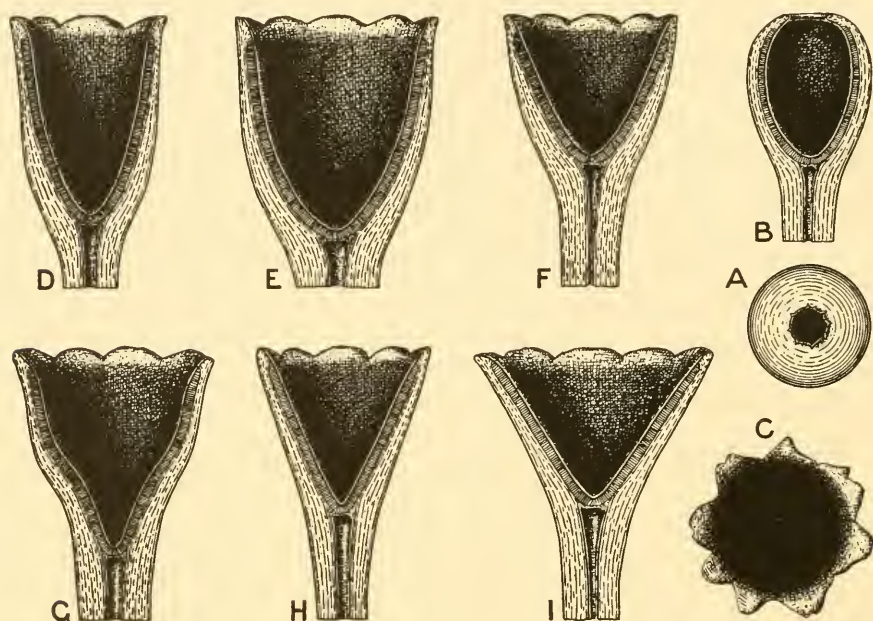
Fig. 117.—*Sarcoscypha protracta.* To show the exact forms of the cups in particular fruit-bodies. A, a young unexpanded fruit-body, seen from above. B, the same, seen in vertical section : the hymenium lining the inner surface of the cavity contains young asci which as yet have not developed any spores. C, a mature fruit-body seen from above : the cup is fully expanded and the hymenium contains asci enclosing ripe spores. D–I, vertical sections through six fully expanded fruit-bodies which had discharged, or were about to discharge, their spores. D and G had already shot away all their spores. The angle of aperture of the cup is smallest in D and largest in I. Several of the fruit-bodies were observed to puff in the laboratory. Fruit-bodies obtained in a Poplar wood at Winnipeg, Manitoba. Magnification, 2·6 natural size.

Moreover, they never open out in such a manner as to flatten the hymenium.

At A and B in Fig. 118, enlarged six times, is shown a diagrammatic drawing of a vertical section through an average cup. The cup is 8 mm. deep and has an angle of aperture of 50°. The asci in the hymenium have straight shafts and are arranged perpendicularly to the surface of the hymenium. The arrows indicate in A the

direction in which one might expect the asci to shoot their spores, and in B the direction in which the asci actually shoot their spores. How comes it that the asci do not discharge their spores in the direc-
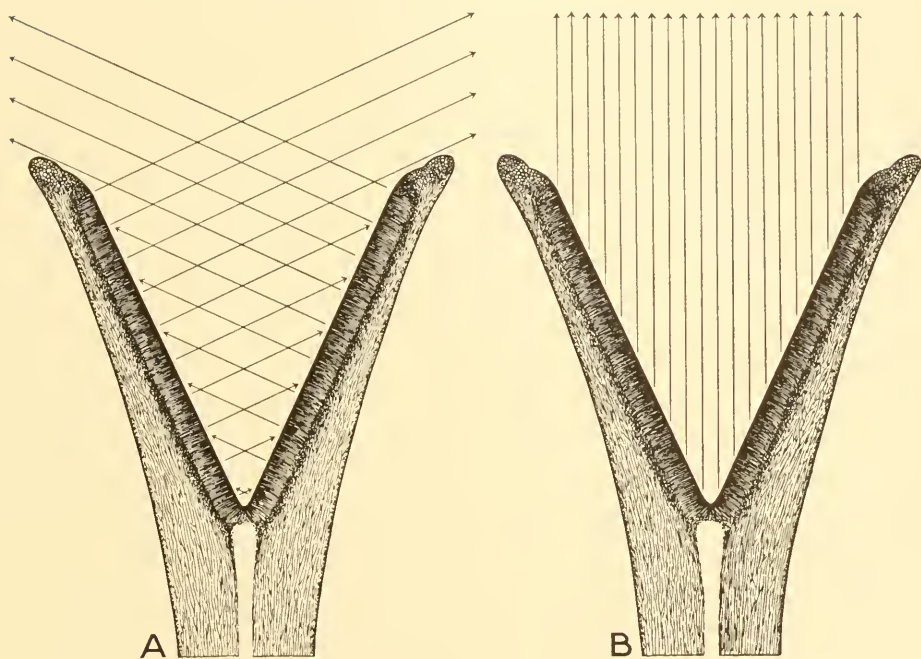
Fig. 118.—*Sarcoscypha protracta.*   Two diagrammatic drawings of a vertical section through a cup, 8 mm. deep, showing the average slope of the hymenium and the direction of the long axes of the asci.   The angle of aperture of the cup is 50°. The arrows indicate trajectories of the spores shot out by single asci.   In A the arrows show the direction in which twenty sets of spores would be shot, if they were discharged from the ends of the asci, in the direction in which the asci point, in still air : the asci in the lower two-thirds of the cup would shoot their spores against the opposite walls of the cup ; and the spores which escaped from the cup, as shown by the arrows, would travel obliquely and not vertically upwards.   In B the arrows show the general direction in which twenty sets of spores are actually shot.   Owing to each ascus having its operculum on the upward-looking side of its end, all the spores escape from the cup and are shot vertically upwards.   Magnification, 6 times the natural size.

tion in which their ends point ?   The answer must be sought for in a minute study of the individual ascus.

The Ascus as an Explosive Mechanism.—The hymenium of *Sarcoscypha protracta,* like that of other Discomycetes, consists of sterile paraphyses and of asci, each ascus containing eight ascospores.

A single paraphysis (Fig. 119, *d*) is a thin, much branched, septate structure, the cells of which contain deep-red particles. It is these particles which give to the hymenium as a whole its fine scarlet colour. The paraphyses constitute more than one-half of the hymenium and, as shown in Figs. 120, A, and 121, A, they lie between the asci and isolate them from one another. The branches of the same paraphysis or of adjacent paraphyses are closely packed together and become anastomosed with one another. Although the paraphyses tightly ensheathe each individual ascus, their walls and the ascus-wall, where they come into contact, are never fused together but are free to move over one another. This freedom is important, for it permits a young ascus to grow in length and an exploding ascus to contract in length and slide downwards in the hymenium between the paraphyses without any serious disturbance to the hymenium as a whole (*cf.* A and B in Fig. 120). With iodine the red particles in the paraphysal cells turn green.

The ascus is a long thin cylinder with a narrower, often undulating base and a straight shaft. In Fig. 119 there are shown : at *a* an almost ripe ascus which as yet does not protrude beyond the general level of the hymenium as determined by the tips of the paraphyses *d* ; at *b* a mature, fully protuberant ascus ; and at *c* an ascus just after it has exploded and shot away its spores. In both *a* and *b* the cell-walls are very thin and are lined internally by an equally thin layer of cytoplasm. This cytoplasmic layer encloses a single large central vacuole, filled with colourless cell-sap, and eight spores. The spores are situated in the upper end of each ascus. They adhere to one another and to the top of the ascus, so that they cannot fall down to the bottom of the vacuole, from which position they could not be discharged when the ascus exploded. It seems probable that they are held together and are attached to the ascus-apex by cytoplasm. In any case the adhesive substance is weak ; for, when an ascus explodes, the top spore is freed from the top of the ascus and all eight spores are separated from one another.

The eight spores in each ascus (Fig. 119, *a*, *b*) are long and oval or almost fusiform, and they all slope obliquely in the same direction. The upper end of the top spore *rests against the operculum*

that is to make its appearance when the ascus explodes.   Now since, as we shall see, each operculum is situated so that it looks toward the opening of the mouth of the apothecium, the

FIG. 119.—*Sarcoscypha protracta.*
The elements of the hyme-
nium. The transverse dotted
line indicates the general sur-
face of the hymenium as
defined by the outer ends of
the paraphyses. *a*, a young
and, as yet, non-protuberant
ascus showing : a thin cell-
wall lined by a thin layer of
cytoplasm, a large vacuole
filled with cell-sap, and eight
spores.   The wall is slightly
thickened at the end of the
ascus, and all the eight spores
are sloped in the same direc-
tion, the uppermost being
directed towards the future
operculum.   *b*, an older
ascus with maximum pro-
tuberancy above the general
level of the hymenium ; it
is ripe and ready to discharge
its spores. *c*, an exploded
ascus which shot out its eight
spores and much of its cell-
sap through an obliquely
situated operculate ostiole.
The direction of spore-dis-
charge, shown by the arrow,
was parallel to the vertical
axis of the cup.   A compari-
son of *b* and *c* shows that,
owing to the elasticity of
the cell-wall, the volume of
the ascus *c*, during the ex-
plosion, contracted to about
one-half. *e*, some isolated
spores shot out from an
ascus : their contents include
several oil-drops.   *d*, a
branched paraphysis, with
its upper divisions anasto-
mosing and densely com-
pacted.   The dots in the
cells represent the red parti-
cles which make the hy-
menium a deep scarlet. The
magnification of every part,
as shown by the scale, is 266.
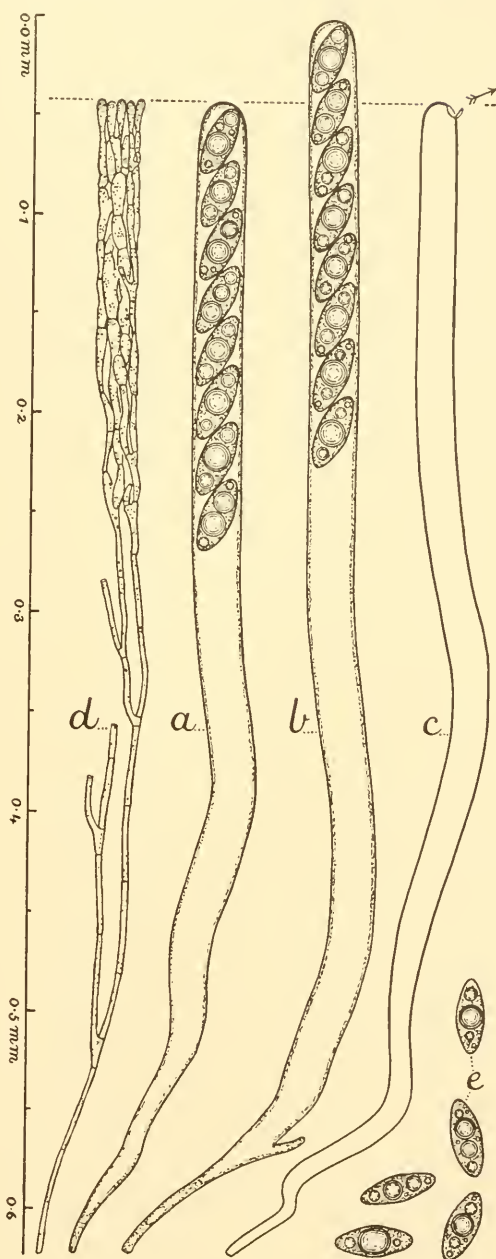
ascospores in a mature upright apothecium (Fig. 116, p. 240) are *all inclined slightly upwards.* This is well shown in Fig. 120, A.

A mature ascus, such as the one shown in Fig. 119 at *b*, is turgid owing to the high osmotic pressure of the cell-sap contained within its great central vacuole, and its thin elastic cell-wall is stretched like that of a toy balloon blown up with air. When the ascus explodes, it opens at its apex by means of a hinged operculum (Fig. 119, *c*), but *the aperture is formed not symmetrically about the longitudinal axis of the ascus* as in most other Ascomycetes, *but obliquely on one side of it.* Moreover, as shown in Fig. 120, B, *the aperture so formed always looks upwards toward the mouth of the apothecium.* In the light of these facts, we can readily perceive why it is that the ascus does not shoot its spores in the direction of its long axis and why, when puffing takes place, the spore-cloud is projected straight forward from the mouth of the apothecium in a direction parallel to the apothecium-axis. A comparison of Figs. 116, 118, B, and 120 will make all this clear.

When an ascus explodes, the elastic cell-wall which has been distended by the osmotic pressure of the cell-sap contracts with considerable force and great rapidity, in consequence of which the ascus shortens and becomes thinner. The ascus-apex is drawn down to the level of the paraphyses (Figs. 119, *c*, and 120, B) and no further, so that the total shortening of the ascus as a whole is only to the extent of about one-sixteenth of its original length or about 6·3 per cent.' An ascus, when shooting out its eight spores one after the other, is continuously contracting in length. If, before all its spores had been discharged, its apex were to be drawn down below the external surface of the hymenium as defined by the paraphyses, it is clear that the remaining spores could not be discharged properly ; they would strike against the paraphyses and so never get into the air. It is thus seen that the shortening of an exploding ascus by an amount just,equal to what at first protruded and no more is a factor in the organisation of the apothecium which makes for success in the dispersion of the spores. When an ascus explodes, its diameter becomes reduced to about three-quarters of what it was originally. Therefore the ratio of the circumference of a turgid ascus to that of the same ascus after explosion is about 100 : 75 ; or, in other words,

the ascus-wall, when an ascus explodes, contracts circumferentially to the extent of 25 per cent. of its original measure. As we have seen, an ascus on exploding contracts in length only about 6·3 per cent. It is clear therefore that, *per* linear unit, an ascus-wall contracts about four times as much in the circumferential direction as in the longitudinal.

From the fact that the ascus-wall is four times as elastic in the circumferential direction as in the longitudinal we may infer that the physical structure of the ascus-wall is not homogeneous, but is different in the one direction from what it is in the other. This difference in structure makes for efficiency in the working of the ascus when the ascus is considered as a mechanism for squirting away ascospores ; for it is important : (1) for the ascus to contract longitudinally as little as possible, so that before discharge it will not be obliged to project much beyond the paraphyses ; and (2) for the ascus to contract as much as possible circumferentially (and therefore radially), so that the eight spores and the sap-drops shall be shot out of the ascus-mouth with as great a velocity as possible.

Owing to the contraction of the cell-wall, an ascus, on exploding, reduces its volume to about one-half. Therefore an exploding ascus must discharge about one-half its contents through its apical aperture. The half of the contents which becomes discharged must be the apical half, and this includes all the eight spores and a mass of cell-sap having a volume perhaps equal to that of the spores.

The ascus, as a gun, is a squirt from which a jet of watery fluid containing eight spores is ejected by the pressure of a contracting elastic cell-wall. The hydrostatic pressure of the cell-sap within is transmitted equally in all directions through the fluid and thus acts upon the cell-wall equally at all points. So long therefore as the ascus-gun during discharge is held firmly fixed in one position by the paraphyses which surround it, it makes little or no difference, so far as the range of the gun is concerned, whether the ascus-jet is shot through a terminal opening in the ascus-wall, as in most Discomycetes, or through an oblique subterminal opening, such as that which is present in *Sarcoscypha protracta*. Thus we obtain a physical explanation for the success of a gun which has the strange peculiarity of not firing its projectiles in the direction of its long axis but obliquely

thereto. Action and reaction being equal and opposite, the asci of *S. protracta*, since they have an oblique direction of discharge, might well fail in effectiveness were it not for the well-developed paraphyses which prevent the end of each ascus from moving in a direction opposite to that of the projectiles (spores and drops of cell-sap) whilst the discharge is actually taking place.

That the discharge of the spores from each ascus takes place in a line which is very oblique to the ascus-axis can be well demonstrated by two experiments, one made in the air and the other in water, as follows :

(1) Using a sharp scalpel and working rapidly, one cuts through a fruit-body which has been lying in a Petri dish and is ready to puff, so as to isolate a piece of the apothecium extending from the rim to the base. This fragment is taken in the fingers and held horizontally with the hymenium looking upwards. Puffing then takes place, and one can observe that the spores are shot away not perpendicularly to the hymenial surface but obliquely upwards towards the rim-end of the fragment. For an illustration of what one sees, one may imagine that the surface of the hymenium shown on the right-hand side of Fig. 120, A, has been placed in a horizontal position and that the direction in which the spores are shot is shown by the arrows in their new positions.

(2) With a hand-razor one cuts a longitudinal-radial section through one side of an apothecium containing almost ripe asci and mounts it in water under a cover-glass. It has the appearance of the right or the left half of Fig. 120, A. One then places a drop of iodine at the edge of the cover-glass so that it gradually diffuses over the hymenium beginning at one end and passing slowly to the other end. As the iodine acts on the asci, they explode one by one in succession, and one can readily observe that the eight spores of each ascus are shot obliquely through the water toward the rim-end of the hymenium. One sees each ascus explode and contract (*cf.* A and B in Fig. 120), but the spores are shot away with such speed that one does not see them passing through the water until they have almost been brought to rest by the resistance of the water. As the iodine continues to diffuse over the section, hundreds of asci explode and the spores accumulate on the surface of the slide as a thick
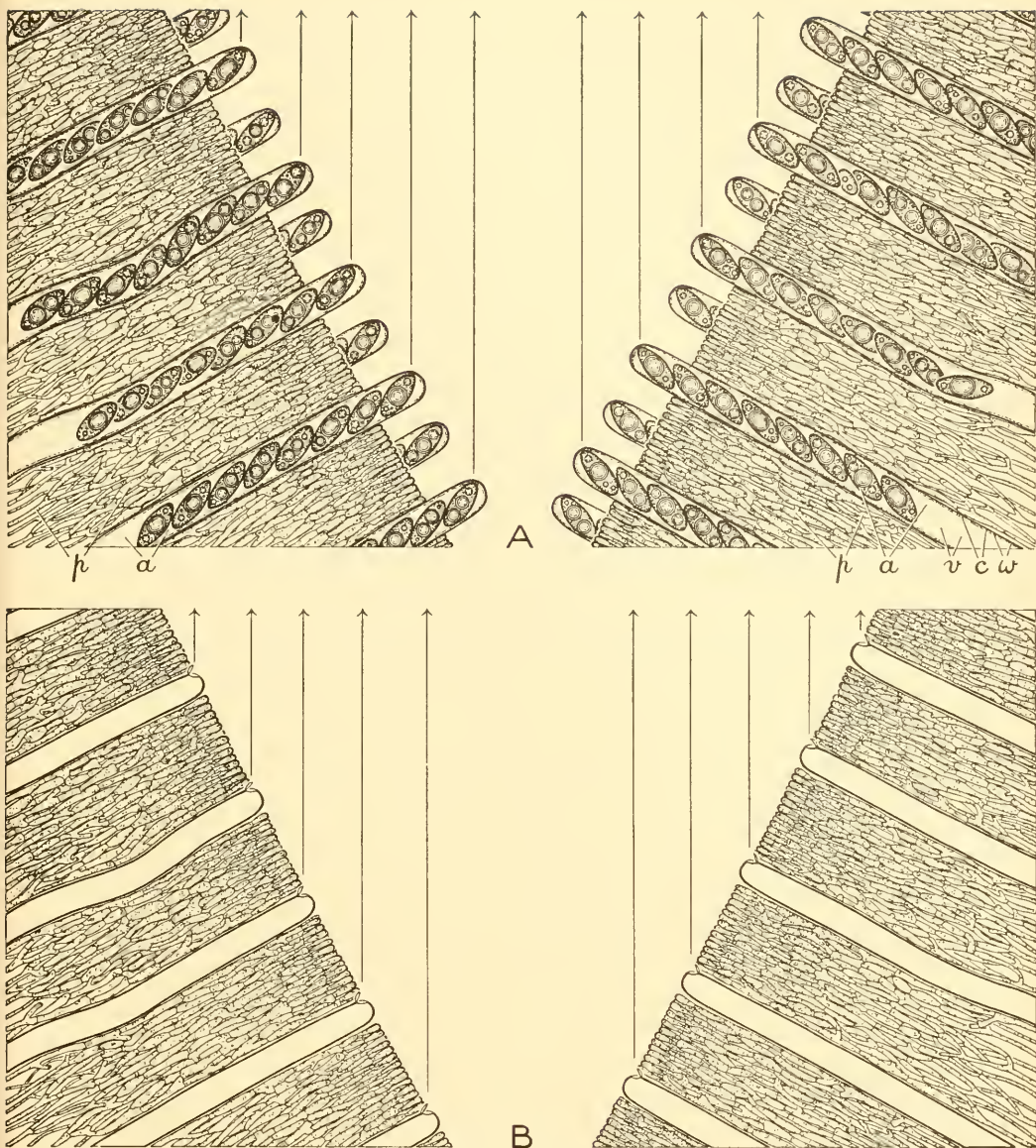
Fig. 120.—*Sarcoscypha protracta*. A semi-diagrammatic vertical section through the two sides of a cup to show the appearance of the hymenium before and after spore-discharge. The two sides of the cup have been approximated to bring them both within the same drawing. A, just before the fruit-body puffed. The hymenium consists of : *p p*, paraphyses containing the red particles which give the hymenium its scarlet colour ; *a a*, asci, each bounded by a thin elastic cell-wall *w*, and containing a lining layer of cytoplasm *c*, a large vacuole *v*, and eight spores enclosing oil-drops. The asci are fully protuberant and the spores are all ripe and ready for discharge. The uppermost spore in each ascus has its upper end resting against the part of the ascus-wall which is to become the operculum. All the spores in each ascus look obliquely upwards. The arrows show the direction in which the ascospores and part of the ascus-sap will be shot. B, the same section as A, one second after puffing has taken place. The spores and part of the ascus-sap have been shot upwards in the directions shown by the arrows through operculate ostioles which look upwards to the mouth of the cup. Each operculum has its hinge on the side of the ostiole farthest from the end of the ascus. In each ascus there has been a reduction of the diameter to about three-quarters, and of the length by an amount equal to the part of the ascus that formerly was protuberant. Magnification, 200.

spore-deposit in front of the hymenium.[1]   The eight spores of any one ascus come to rest at a distance of about 1 mm. from the hymenium, but the discharges of neighbouring asci set up momentary currents which gradually carry these eight spores farther from the hymenium, and thus the final spore-deposit comes to be situated at a distance of 1–2 mm. from the hymenium.   If one examines the exploded and empty asci, one observes : (1) that their openings are all obliquely situated, as shown in Fig. 120, B, and look in the direction in which the spores have been shot ; and (2) that their opercula are all hinged on that edge of the opening which is toward the ascus-base (Fig. 120, B).

Radial-longitudinal Sections and Surface Views of the Hymenium.—The key to understanding the fruit-body of *Sarcoscypha protracta* as an organ for the production and liberation of spores is a thorough study of the hymenium in radial-longitudinal sections and in surface views.   Only by such a study can one obtain satisfactory evidence of the spatial relations of the asci and paraphyses, and of the relation of the asci with the mouth of the apothecium.   Hitherto, so far as I know, no one has ever studied Discomycetes in this way, so that Figs. 120, 121, and 122 are the first of their kind in mycological literature.

A radial-longitudinal section of an apothecium is, of course, one which, if continued, would pass through the apothecium's longitudinal axis ; and, if complete, it would resemble the section shown in Fig. 118 (p. 243).   Pieces from each side of such a section are shown in Fig. 120, but, for convenience of illustration, the two pieces have been brought nearer together than they would usually be in an actual section.   At A is shown the appearance of the hymenium just before puffing and at B the appearance of the same hymenium one second after puffing.

In Fig. 120, A, the following points may be observed : (1) the only elements in the hymenium are asci and paraphyses ; (2) adjacent asci which lie nearly or exactly in the same vertical plane are separated by relatively thick masses of paraphysal branches ;

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[1] For a discussion of the mode of action of iodine and of other poisonous substances in causing the explosion of ripe and nearly ripe asci of *Aleuria vesiculosa* (there misnamed *Peziza repanda*) *vide* these *Researches*, Vol. I, 1909, pp. 238–240.

(3) the eight ascospores are all situated in the outer halves of the asci ; (4) the terminal spore of each ascus has its upper end against the operculum that will make its appearance when the ascus explodes; (5) the spores in each and every ascus are all inclined upwards so that the asci have a remarkably uniform appearance ; (6) the asci are fully protuberant ; (7) the cells of the paraphyses contain dark particles (these are red in living sections) ; and (8) the asci do not
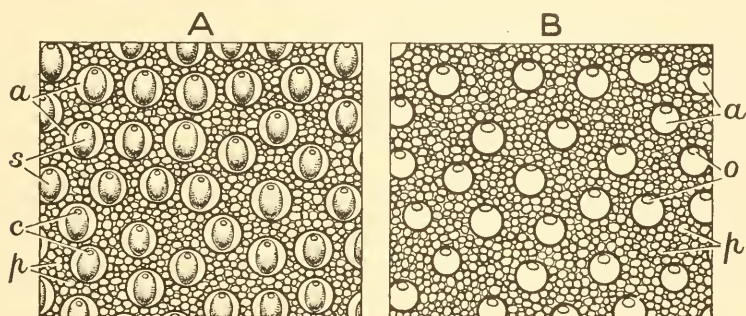
Fig. 121.—*Sarcoscypha protracta.* Surface views of the hymenium before and after spore-discharge. A and B correspond respectively with A and B in Fig. 120. A, just before the fruit-body puffed : *a*, asci ; *s*, ascospores, all obliquely situated with their upper ends pressing against the future operculum ; *c*, the area of contact of a spore with the future operculum ; *p*, the paraphyses. B, another surface view, similar to A, but one second after puffing has taken place : *p*, the unaltered paraphyses ; *a*, the empty asci shrunken to three-quarters of their original diameters ; *o*, the upward-looking openings of the asci (the opercula attached to the upper rims of the openings are not here shown on account of the small scale of the drawing). The positions of the asci in both A and B, and also the ostioles in the asci of B, were drawn with the *camera lucida*, while the spores and the areas *c* in A, as well as the paraphyses in A and B, have been represented semi-diagrammatically. Magnification, 267.

contain any dark particles, but their spores enclose characteristic oil-drops.

In Fig. 120, B, the following points may be observed : (1) the asci have contracted in length so that they are no longer protuberant ; (2) the asci have contracted to about three-quarters of their former diameter ; (3) the ends of the asci are at the level, and not below the level, of the ends of the paraphyses ; (4) the openings of the asci are all obliquely situated and look upwards in the directions shown by the arrows ; and (5) the opercula are all hinged in the same manner, *i.e.* each hinge is on that side of the opening which is toward the base of the ascus.

In Fig. 121, A, is shown a surface view of a hymenium just be ore puffing and therefore corresponding with the radial-longitudinal section shown in Fig. 120, A. In Fig. 121, A, the following points may be observed : (1) the only elements in the hymenium are asci
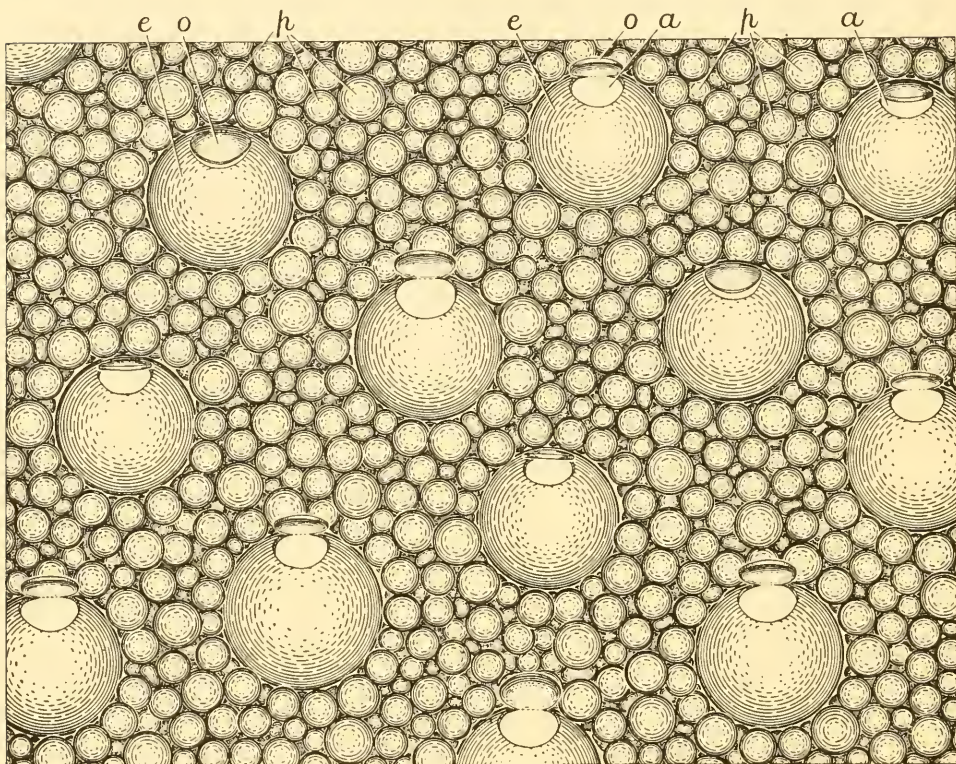
Fig. 122.—*Sarcoscypha protracta.* Semi-diagrammatic surface view of the hymenium, about one second after puffing has taken place and all the asci have exploded : *p p*, the paraphyses in which the asci are embedded ; *e e*, the empty asci ; *a a*, the apertures of the asci, through which the ascus-jets consisting of spores and drops of cell-sap were discharged ; *o o*, the opercula, each one hinged to the upper edge of the ascus-wall. Magnification, 1143.

and paraphyses ; (2) the paraphyses occupy at least one-half of the hymenium ; (3) the uppermost spore in each ascus is pointing obliquely upwards and is resting against the operculum that will be formed when the ascus explodes ; and (4) there is a large amount of cell-sap surrounding the spores.

In Fig. 121, B, is shown a surface view of a hymenium just after

puffing and therefore corresponding with the radial-longitudinal section shown in Fig. 120, B. In Fig. 121, B, the following points may be observed : (1) the asci have contracted so that their diameters are about three-quarters of what they were before spore-discharge took place ; and (2) the openings of the asci (the opercula are not shown) are all obliquely situated on the upper side of the end of each ascus.

In Fig. 122, which supplements Fig. 121, B, an exhausted hymenium in surface view is shown on a larger scale. In Fig. 122 one may again notice : (1) the wide separation of the asci by the paraphyses, and (2) the upper oblique positions of the ascus-apertures ; but, in addition, one may observe (3) that the opercula are all hinged in the same manner, *i.e.* on the side of the openings nearest the bases of the asci.

Figs. 120, 121, and 122 taken together provide data which allow us to form a clear conception of the spatial relations of the elements of the hymenium to one another and to the cup-shaped apothecium as a whole.

Correlations and Fruit-body Efficiency.—It is evident that the hymenium is wonderfully well fitted for producing and liberating spores from an apothecium with a more or less obconical interior. Correlated with this form is the upward-looking opening of each ascus, made when discharge of the spores takes place. If the ascus-openings did not look upwards toward the mouth of the apothecium, but looked sideways or were terminal, the spores could not be ejected efficiently from the apothecial cavity. The upward inclination of the top spore of each ascus and of the other seven spores in the spore-chain is correlated with the position of the opening that will be formed when the ascus explodes. If the ascospores were inclined downwards instead of upwards, when an ascus opened the exit of the spores through the opening would be rendered much more difficult if not impossible.

The area taken up by the paraphyses in the hymenium is somewhat greater than that taken up by the asci. Why should there not be fewer paraphyses and more asci ? Would not this make for greater efficiency ? Let us attempt to answer these questions. Firstly, it is to be remarked that paraphyses form an essential part

of the hymenium : they protect the young asci when they are developing and pushing upwards, and they support the mature asci mechanically, preventing them from having their apices forced downwards when they are firing their jet of spores and sap upwards. If the paraphyses were reduced to such a minimum that the asci were in lateral contact with one another, many of the asci would not be able to discharge their spores properly. To make this clear, let us imagine that on the right-hand side of Fig. 120, A, the five asci most in view were in contact one below the other. Then to secure the aerial dispersion of all the spores it would be necessary for the five asci to be discharged in succession from above downwards and in no other order ; for, if of any two adjacent asci in the row of five the lower one discharged before the upper one, since the opening of the exploding ascus looks upwards and since an exploding ascus contracts in length, most of the eight spores of the lower ascus would be shot against the lower side of the upper ascus and so would not escape from the mouth of the apothecium. From these considerations it is seen that it is best in *Sarcoscypha protracta* for the asci to be well separated from one another, especially in the vertical direction. If one regards the right-hand side of Fig. 120, A, one can see that, if one of the middle asci explodes, as it contracts in length and ceases to be protuberant all the spores can be shot away without any of them hitting the lower side of the ascus which is above it. Evidently, the number of the paraphyses is correlated with the spatial arrangement of the asci necessary to secure that all the spores from each ascus shall be efficiently discharged through the cavity of the apothecium into the outer air. A reduction in the number of paraphyses in the hymenium would therefore be disadvantageous.

Summing up, we may say that correlated with the conical form of the cavity of the apothecium in *Sarcoscypha protracta*, and making for efficiency in the production and liberation of spores by the hymenium, are the following arrangements : (1) the oblique upward-looking openings of the asci formed at the moment of spore-discharge ; (2) the obliquely upward inclination of the spores in each ascus ; and (3) the relatively distant separation of adjacent asci by paraphyses, particularly in respect to vertical planes.

What Factor determines the Oblique Position of the Opening of each Ascus ?—It is known that the asci of many Ascobolaceae and, as we shall see in the next Chapter, of many other Discomycetes belonging to the Humariaceae, the Pezizaceae, and the Morchellaceae are positively heliotropic so that, during their development, they bend their ends toward the light. The length of the part of the ascus which may bend, relatively to the length of the ascus as a whole, varies considerably in different species. Thus bending of an ascus through 45° is effected in *Aleuria vesiculosa* by the outer half of the ascus (Fig. 140, p. 293 ; *cf.* Fig. 123, A), but, in *Ciliaria scutellata*, by no more than the last sixth of the ascus (Fig. 135, p. 284 ; *cf.* Fig. 123, B). In all probability the ascus of *Sarcoscypha protracta*, like that of so many other Discomycetes, is positively heliotropic ; but, if so, its *bending is confined to its extreme apex.* We have only to suppose that the operculum originates in the first place in a radially symmetrical position at the end of the ascus and that, subsequently, the end of the ascus, after it has emerged at the surface of the hymenium and has received unilateral light from the mouth of the cup, makes a positively heliotropic curvature by growing in length on its darker side just sufficiently to swing the operculum round through an angle of about 45° until the operculum comes to face the strongest incident rays of light directly (Fig. 123, C). The assumption that such a heliotropic reaction takes place serves to explain not only why the opercula of all the asci in the hymenium look upwards toward the mouth of the cup (*cf.* Fig. 120, A and B, p. 249) but also why they are obliquely situated on the ends of the asci. Granted that the heliotropic reaction just suggested takes place, then, in a ripe ascus, while the apparent axis is straight, the axis of organisation is in reality curved at its extreme end (Fig. 123, C).

Seaver [1] has supposed that the oblique position of the opening

[1] F. J. Seaver, *The North American Cup-fungi*, New York, 1928, pp. 17–18. Seaver states that the apothecia of *Phillipsia Chardoniana* " are as flat as a pancake yet, so far as observed, the ascostomes are always eccentric, or, at least, are conspicuously and predominantly so." However, he does not inform us whether or not the apothecia under investigation were unilaterally lighted during their development and whether or not the opercula all faced the source of greatest light. As will be shown in the next Chapter, when the flat apothecium of *Ciliaria scutellata* is unilaterally lighted, the tips of all the asci bend toward the light, and the exploded asci have opercula which are sub-oblique (*cf.* Fig. 135, *l l*, p. 284).
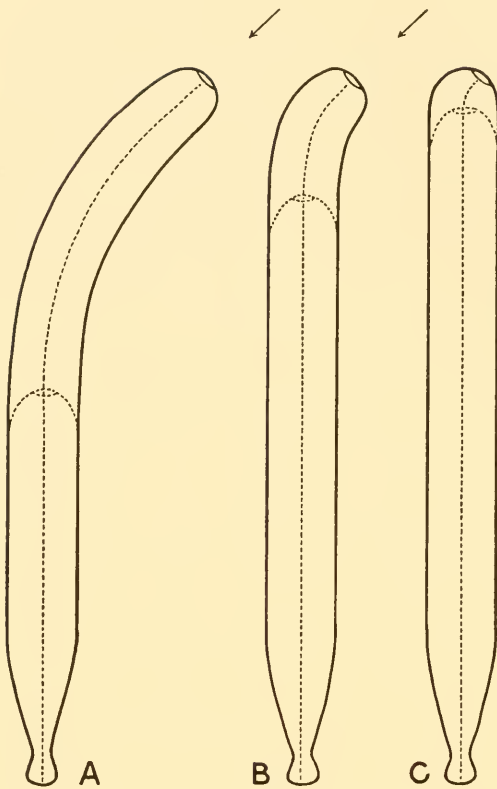
Fig. 123.—Diagram illustrating variations in heliotropic curvature in the asci of different species of Discomycetes. The arrows indicate the direction in which the asci were illuminated during their development. All the asci, at first, were straight, and their appearance (including an indication of the position of origin of the rudiment of each operculum), just before they began to bend, is indicated in part by broken lines. Heliotropic curvature toward the source of light began : in A (as in *Aleuria vesiculosa*) when the ascus was only half-grown in length ; in B (as in *Ciliaria scutellata*) when the ascus was about five-sixths grown in length ; and in C (as in *Sarcoscypha protracta*) when the ascus was almost full-grown in length. The curvature of the central broken line which represents the morphological axis of each ascus indicates that even in C, which externally has the appearance of a straight cylinder, the lateral position of the operculum has been attained, as in A and B, by a heliotropic curvature.

[1] *In litt.*, 1927 and 1928.

of the ascus, which he calls the *ascostome*, in a number of tropical Discomycetes, *e.g.* species of Phillipsia and Cookeina, is a purely "morphological character" not influenced by the external stimulus of light, but he admits [1] that he has not observed the fruit-bodies in the living condition and has no experimental basis for his assumption. In view of my own investigations on the obliquely situated ascostomata of *Sarcoscypha protracta* which always look toward the strongest incident rays of light, supported by other investigations on the position of the ascostomata of the asci of the discoid fruit-bodies of *Ciliaria scutellata*, etc., recorded in the next Chapter, I am unable to accept Seaver's assumption. It is my opinion that the internal tendency of every ascus is to produce its ascostoma in a radially symmetrical position at the ascus

apex and that the ascostoma subsequently takes up its oblique position
owing to a response of the ascus end to a heliotropic stimulus. Thus
the oblique position of an ascostoma at the end of any ascus is regarded
by me not as a purely " morphological character " depending for
its origin on internal growth stimuli only, but as a physiological
character depending on an external stimulus, namely, that of light.

**Experimental Proof that a Fruit-body, when it puffs, produces
a Blast of Air.**—When a fruit-body of *Sarcoscypha protracta* is held
horizontally in the still air of the laboratory and it puffs, the cloud
of spores set free travels horizontally to a distance varying from
about 8 to 17 cm. (7 inches), according to the intensity of the puff.
The greater the number of spores set free at one time, the louder is
the hiss made by them as they pass through the air and the farther
do they travel before becoming dispersed irregularly. As I watched
the puffing of a number of fruit-bodies tested one by one in the
laboratory, it seemed that the spore-cloud—at any rate in the latter
part of its journey—was being borne along as if by a blast of air.
I therefore decided to test this idea by means of experiment. On
May 9, 1925, I went to the Poplar wood where *Sarcoscypha protracta*
grew and gathered a considerable number of fruit-bodies. As the
temperature of the air in the wood was only a few degrees above the
freezing point of water, the fruit-bodies would not puff ; but, after
being brought into the laboratory and left in closed Petri dishes at
a temperature of about 70° F. for about an hour, many of them puffed
freely when they were removed from their Petri dishes and were
held in the fingers in the relatively dry air of the laboratory.

The apparatus employed for demonstrating that a fruit-body of
Sarcoscypha protracta, when it puffs, produces a blast of air is shown
in Fig. 124. What is represented was spread out in a horizontal
plane, and the observer is supposed to be looking down on it from
above. A test-tube *a* with an open side-pipe *b* (shown in section at *c*)
is held in a horizontal plane by the clamp *e* which is attached by
the clamp *d* to the vertical rod of an iron stand. The position of a
card-board screen is shown at *f* and *g*. The apparatus was set up
on a laboratory table near a window, so that strong daylight fell
upon it. In front of the mouth of the side-pipe *b*, as shown around *i*.
there was a black back-ground.
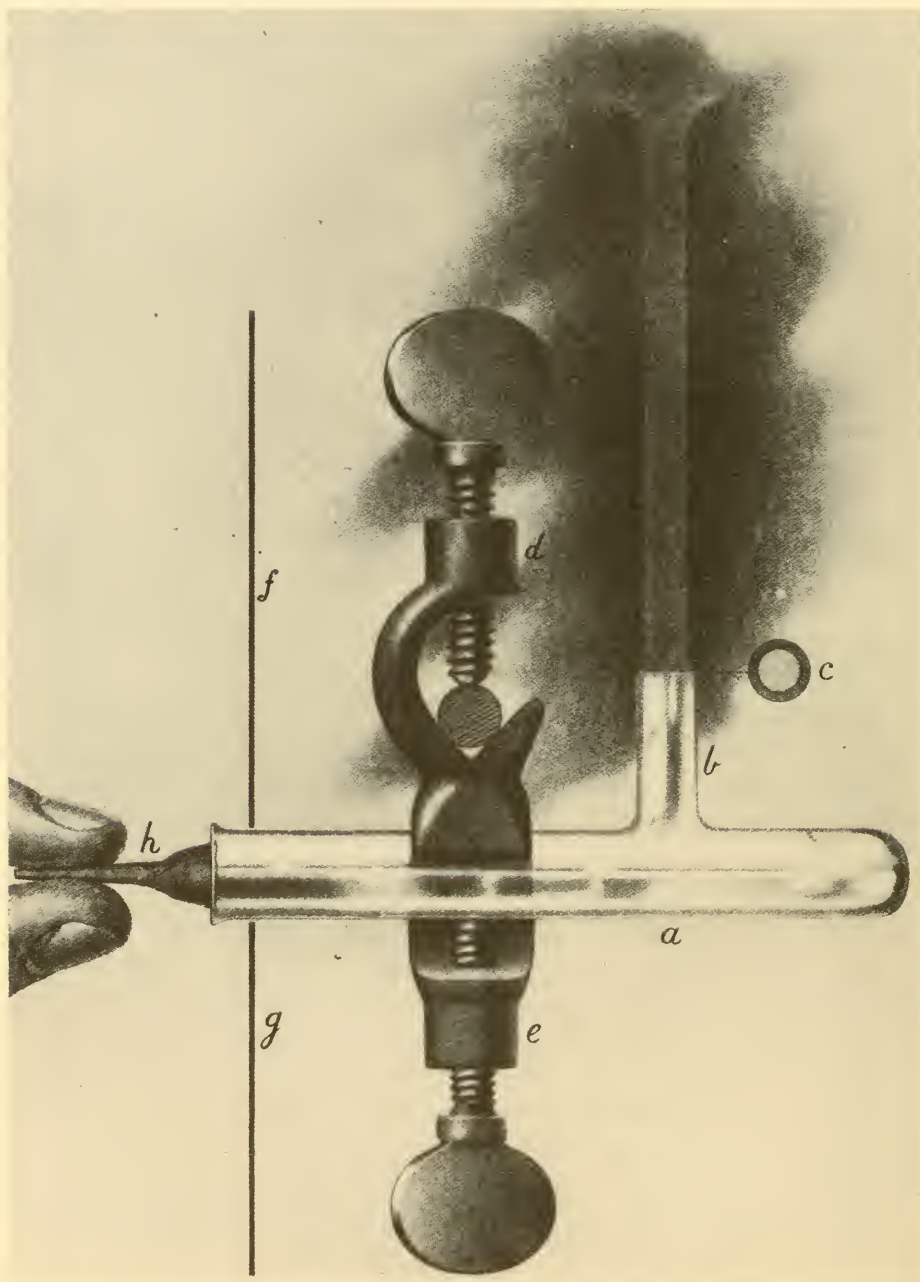
When the apparatus was set up, fruit-bodies were removed one by one from the Petri dishes with the fingers as required and were held at the mouth of the test-tube as shown in Fig. 124 at h. It was necessary to carry out each of these operations as rapidly as possible because, if a fruit-body was in a condition to puff, it usually puffed within three seconds of raising the Petri-dish lid. With the procedure just described some of the fruit-bodies were caused to puff into the mouth of the test-tube a.

Immediately after placing a fruit-body at the mouth of the test-tube a, I looked towards the black back-ground shown at i and then, within a second, if the fruit-body puffed strongly, I heard the fungus hiss for about two seconds and saw a cloud of spores shoot out from the side-pipe b. In the most successful experiments the spore-cloud was carried horizontally, as shown at i in Fig. 124, a distance of about three inches beyond the mouth of the side-pipe b before irregular dispersion took place ; but in several other experiments the distance was not more than about 1–1·5 inches. Since the spores cannot be shot by any ascus around a corner, it seems clear from these experiments that the fruit-bodies, on puffing, must create a blast of air which can carry the spores passively with it.

To vary the experiment just described, I suspended a strip of thin tissue-paper four inches long, one-tenth of an inch wide at the top, and six-tenths of an inch wide at the bottom, so that its lower end was just in front of and touching the mouth of the side-pipe b in Fig. 124. As before, several fruit-bodies in succession were caused to puff into the mouth of the test-tube a. As each puff took place, I heard the hiss and saw the lower end of the strip of tissue-

Fig. 124.—Apparatus, seen from above, as used for demonstrating that a fruit-body of *Sarcoscypha protracta*, when it puffs, produces a blast of air : a, a test-tube with an open side-pipe b (shown in section at c), held in a horizontal plane by the clamp e which is attached by the clamp d to the vertical rod of an iron stand ; f g, a cardboard screen ; h, a fruit-body removed from a Petri dish and placed at the mouth of the test-tube about three seconds ago. The fruit-body has just puffed. The million or more spores and sap-droplets which were shot into the tube have impinged upon the air and set it in motion, thus producing a blast of air which is now emerging horizontally through the mouth of the pipe b and bearing the spores with it, as shown at i. The cup of the fruit-body is somewhat smaller than the mouth of the test-tube ; so that, as the air is driven forward in the body of the tube a, more air enters at the mouth of the tube near h. The column of spore smoke, i, was observed in bright daylight against a black back-ground. Natural size.

paper blown away from the mouth of the side-pipe.   As the blast
of air subsided, the tissue-paper returned to its former position and
again covered the mouth of the side-pipe.   In some of the experi-
ments the tissue-paper was blown a distance of 1 cm. and, in the
most successful experiment, made with a fruit-body which puffed
with great vigour, the tissue-paper was blown a distance of 2 cm.
The blast of air in each experiment carried spores with it and the
spore-cloud could be seen being deflected laterally by the obstacle
of the tissue-paper.

When a fruit-body puffs, it not only shoots away many tens of
thousands of spores but also vast numbers of drops of ascus-sap.
When about twelve fruit-bodies had puffed into the test-tube, so
much ascus-sap in the form of extremely fine droplets had fallen
to the bottom of the test-tube in the region lettered a in Fig. 124
that, when the tube was tilted, the liquid could just be caused to
run in the form of a shallow drop.   A microscopic study of asci
before and after explosion goes to show that, in all probability, the
volume of ascus-sap shot out from any ascus is at least equal to the
volume of the eight spores and may possibly be greater, so that the
gathering of moisture on the base of the tube a was in accordance
with theoretical expectation.

The Cause of the Blast of Air.—When an apothecium puffs, it
does not contract its rim or alter its general shape, and there is no
reason to suppose that the blast of air emerges from the apothecial
cavity.   It seems most probable that the blast of air is brought into
existence by the forward movement of the spores and drops of ascus-
sap.   It was calculated that from one fruit-body, which was found
to have all its asci empty after it had puffed, a million spores had
been discharged.   Probably these million spores were accompanied
by at least a million drops of ascus-sap.   From the fruit-body,
therefore, there were shot forward in the same direction some two
million projectiles.   At the beginning of their flight from an apothe-
cium each of the projectiles, moving as it does at a high speed, must
drag a certain amount of air forwards.   Very quickly the velocity
of each projectile is greatly reduced by the resistance of the air ;
and, by this time, we may suppose, the air has been bombarded by
the projectiles to such an extent that it moves forward *en masse*

with a speed of several centimetres a second and carries many of the spores passively forward with it. The rate of fall of the spores is much less than the speed of the air. Hence it is possible for the blast of air to carry the spores through the side-pipe *b* and even some 1–3 inches beyond its mouth.

The forward movement of the air in the test-tube caused by the bombardment of the air by the projectiles does not create a partial vacuum in the tube, because the mouth of the cup of the fruit-body is somewhat smaller than the mouth of the tube to which it is applied ; so that, as the air is driven forward in the body of the tube (Fig. 124, *a*), more air enters at the mouth of the tube (Fig. 124, near *h*).

The ballistic phenomenon under discussion may be treated mathematically as follows :

Let M_1 = the mass of the projectiles (the spores and the cell-sap) shot out from all the ascus-guns,

v_1 = the velocity of projection,

M_2 = the mass of the air set in motion by the impact of the projectiles, and

v_2 = the common velocity of the air and projectiles after impact.

Then, by the principle of the conservation of energy,

$$M_1 \, v_1 = (M_1 + M_2) \, v_2$$

i.e. the momentum of the blast of air and of the projectiles which the air carries along with it is equal to the momentum of the projectiles at the moment when they left the asci. It therefore follows that the blast of air is caused by the air being bombarded by the projectiles.

After a blast of air has been started, its velocity is progressively diminished by friction with the still air through which it moves. Hence the blast of air produced by the apothecium, like a smoke-ring blown from the mouth, does not travel very far.

Since action and reaction are equal and opposite, when puffing takes place the stalked apothecium of *Sarcoscypha protracta* must tend to recoil with a momentum equal to that of the projectiles

which are shot away. I have not investigated the recoil in *S. pro-tracta*, but Dr. E. C. Stakman has informed me that he has observed the recoil in the thin-stalked apothecia of *Sclerotinia sclerotiorum* : as puffing took place, the cups containing the asci were visibly pressed backwards. Such a recoil gives an indication of the energy with which the projectiles discharged from the asci bombard the air and set it in motion.

The Blast of Air and the Dispersal of the Spores.—If the asci of a *Sarcoscypha protracta* fruit-body were to explode one after the other as they ripen, it is probable that the eight spores of each ascus would not be shot up into the air for more than 3–4 cm., and no appreciable blast of air could come into existence to raise them still further. On the other hand, when a hundred thousand asci explode together, *i.e.* when puffing takes place, the bombardment of the air by the spores and ascus-drops collectively is sufficient, as we have seen, to create a blast of air which can carry the spores with it for several inches after they have lost the velocity given to them by the ascus-guns. Consider now a fruit-body under natural conditions in a Poplar wood. Its stipe is negatively geotropic and its apothe-cium therefore looks upwards to the sky. If the asci were all to be discharged in succession as they ripen, none of the spores (we may suppose) would travel upwards more than an inch or two ; but, if the asci ripen one by one and then wait before exploding until some suitable stimulus sets them all off together, then the spores may be carried upwards by the blast of air, which they bring into existence, to a height of 5–7 inches. After being carried upwards into the air, the spores are borne away more or less horizontally by the wind and are thus dispersed. The nearer to the ground, the less wind there is ; and every extra inch the spores can be raised into the air by discharge from the apothecium, the stronger will be the wind which they will encounter and the greater the chance that they will be dispersed widely and find suitable conditions for propagating the species. Once more we are provided with an instance where union is strength and where collective action is better than individual action.

Concluding Remarks.—Our knowledge of the phenomenon of spore-discharge in the Discomycetes is still very incomplete. With

a view to its extension individual apothecia of a series of typical species should be observed in the field. Such apothecia should be left *in situ* and not be touched by man ; and, for each apothecium, during the whole spore-discharge period, a continuous record, not only of the times of spore-discharge, but also of the conditions of spore-discharge (temperature, moisture, wind-movements, etc.) should be made.

CHAPTER II

THE HELIOTROPISM OF THE ASCI AND THE DISCHARGE OF THE SPORES IN ASCOBOLI, CILIARIA SCUTELLATA, ALEURIA VESICULOSA, THE MORCHELLACEAE, AND OTHER DISCOMYCETES

Introduction—The Heliotropism of the Asci of *Ascobolus magnificus*—*Ascobolus stercorarius*—Our Present Knowledge of *Ciliaria scutellata*—The Heliotropism of the Asci of *Ciliaria scutellata*—The Heliotropism of the Asci of *Melastiza miniata* and *Cheilymenia vinacea*—*Aleuria vesiculosa* and its Identification—Results of a Previous Investigation on *Aleuria vesiculosa*—The Heliotropism of the Asci and the Discharge of the Spores in *Aleuria vesiculosa*—Puffing of *Aleuria vesiculosa* under Natural Conditions—The Heliotropism of the Asci and the Discharge of the Spores in *Galactinia badia*—*Urnula Craterium*—*Otidea onotica* and *O. leporina*—The Heliotropism of the Asci and the Discharge of the Spores in the Morchellaceae—The Helvellaceae—Concluding Remarks.

Introduction.—*Sarcoscypha protracta*, which was treated of in the last Chapter, is a rather specialised Discomycete, for : (1) its hymenium, which lines the inside of the cup, has more or less the shape of an inverted cone ; (2) its asci are straight ; and (3) the opercula of its asci are not situated symmetrically at the ends of the asci but obliquely, so that they look upwards toward the mouth of the cup.

Doubtless, there are a number of other Discomycetes which resemble *Sarcoscypha protracta* in their general form and their mode of producing and liberating spores. However, a very large number of Discomycetes differ from *S. protracta* in that : (1) they have a flat hymenium, *e.g. Ciliaria scutellata*, or a more or less hemispherical hymenium, *e.g. Aleuria vesiculosa* and *Galactinia badia*, or a cavernous hymenium, *e.g. Morchella crassipes* and *M. conica* ; (2) their asci are not straight, but are more or less curved at their free ends where the spores are situated ; and (3) the mouth of each of their asci is symmetrically situated at the end of the ascus.

The ripe asci of the Ascoboleae protrude considerably above the

outer surface of the hymenium, so that the disc is made papillate by their prominent summits, and it has long been known that these protuberant asci bend toward the light and are therefore positively heliotropic.   Zopf [1] illustrated the heliotropism of the asci of *Ascobolus denudatus* and of a species of Saccobolus in his well-known text-book in 1890 (Figs. 125 and 126).

As a result of my own observations, I have no doubt that the asci of *Ciliaria scutellata*, *Aleuria vesiculosa*, *Galactinia badia*, *Morchella crassipes*, and other similar Discomycetes, like those of the Ascoboleae, are heliotropic, and that the response of the asci to the directive stimulus of light serves to explain how it is that the ascospores discharged from the interior of hollow cups, such as those of *Aleuria vesiculosa*, *Galactinia badia*, etc., and from the interior of cavernous depressions at the surface of the caps of the Morchellae are shot out into the external air without striking opposing chamber walls.

In the summer of 1926, at the International Congress of Plant Sciences, I gave a paper [2] on the discharge of spores in the Higher
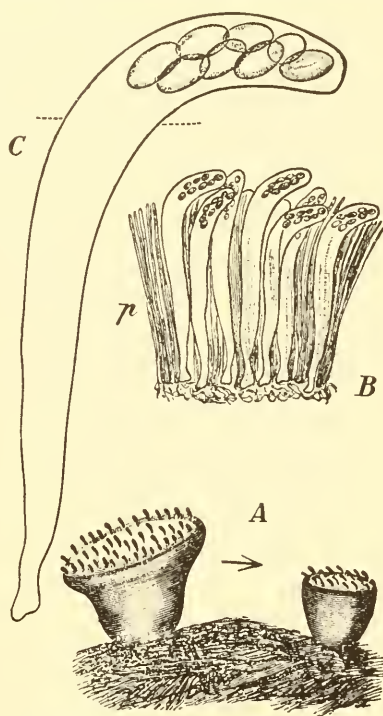
FIG. 125.—*Ascobolus denudatus*. Heliotropism of the asci.   A, a smaller and a larger cup-like fruit-body on a fragment of dung.   The asci protruding above the hymenium have all become heliotropically bent toward the light, the direction of which is indicated by the arrow.   B, a vertical section through one of the fruit-bodies showing the hymenium in which are the heliotropically bent asci, each containing eight spores.   C, a single ascus from B ; it is strongly bent toward the source of the light.   The dotted line indicates the top of the hymenium.   Drawn by W. Zopf. From his *Die Pilze* (1890, p. 205). Magnification :   A,   25 ;   B,   80 ; C, 300.

[1] W. Zopf, *Die Pilze*, Breslau, 1890, pp. 205–206, Fig. 64.

[2] A. H. R. Buller, " Some Observations on the Discharge of Spores in the Higher Fungi," *Proceedings of the International Congress of Plant Sciences*, Vol. II, 1929, pp. 1627–1628.

Fungi in which. after treating of *Sarcoscypha protracta*, I said : " In most Discomycetes, *e.g. Morchella conica, Aleuria vesiculosa, Galactinia badia, Lachnea scutellata*, and *Ascobolus immersus*, the asci are heliotropic, *i.e.* their outer ends bend so that they point toward the direction from which the strongest light comes, and the operculum is situated symmetrically at the apex of each ascus. The result of these arrangements is that, when the asci explode, the spores are successfully shot away from the fruit-bodies into the outer air."

In 1927, J. S. B. Elliott,[1] in a brief paper on *Aleuria repanda*, described the response of the apothecia to unilateral light. Apothecia came up on an old jute hearth-rug in a garden tool-shed into which light penetrated through a hole in the door. She found that, in preparation for spore-discharge, each apothecium undergoes two successive adjustments : (1) a *coarse* adjustment concerned with the apothecium as a whole ; and (2) a *fine* adjustment concerned with the individual asci. In the coarse adjustment, the stalk of each young apothecium bends toward the source of light and thus causes the mouth of the cup to become directed toward the source of light (Fig. 127) ; while, in the fine adjustment which follows

FIG. 126.—Saccobolus. Heliotropism of the asci. A, an ascus of a Saccobolus from sheep dung ; it is straight, so the direction of the incident light may have been parallel to its long axis. The spores are stuck together to form a single projectile. B, the spores shown in detail. C, a single ascus of a Saccobolus heliotropically bent toward the source of light. A mass of jelly surrounds the eight spores and attaches the spore-mass to the apex of the ascus. Drawn by W. Zopf. From his *Die Pilze* (1890, p. 205). Magnification : A, 450 ; B, 900 ; C, 540.

[1] Jessie S. Bayliss Elliott, "*Aleuria repanda* Pers.," *Trans. Brit. Myc. Soc.*, Vol. XII, June, 1927, pp. 166–169.

the coarse one, each ascus bends toward the source of light and thus causes its free end to become directed toward the source of light.

The coarse and fine *heliotropic* adjustments which have just been described take place not only in *Aleuria repanda* but, as I have observed, in other stipitate Discomycetes when grown in unilateral light ; and they are analogous to the coarse and fine *geotropic*

Fig. 127.—*Aleuria repanda*.   Heliotropism of fruit-bodies.   The fruit-bodies were growing on an old jute hearth-rug in a garden tool-shed and, during their development, were illuminated solely by light coming through a hole in the door.   The stipes, which were positively heliotropic, have turned toward the source of light and have thus caused the upper hymenial surfaces of the cups to face the light. Photographed at Tanworth-in-Arden, England, by Jessie S. Bayliss Elliott.   About one-half the natural size.

adjustments which take place respectively in the stipe and gills of many agarics, *e.g. Psalliota campestris*.[1]   In both the Discomycetes and the Agaricaceae, by means of these adjustments, the fungus guns (asci and basidia respectively) come to rest in positions in which they point toward open spaces, from which spaces the pro-jectiles (ascospores and basidiospores), immediately or shortly after their discharge, can be readily carried off by the wind.

[1] These *Researches*, Vol. I, 1909, p. 56.

In 1916, Richard Falck [1] published a paper on the discharge of spores in the Discomycetes in which he pointed out that *Morchella esculenta, Gyromitra esculenta*, and other Morchellaceae emit clouds of spores when they are warmed by heat radiated from a lamp (Fig. 128), etc. ; and he called the Discomycetes which emit spores when warmed the *radiosensitive* Discomycetes.[2] He did not investigate the shape of the asci in the hymenial chambers of Morchella, etc., and thus failed to observe that they point toward the openings of the chambers. In the belief that the asci are straight and directed perpendicularly to the surface of the hymenium, he [3] said : " In the chambers of the Morchellae the opposing walls are at most 1 cm. and, as a rule, only a few mm. from one another ; so that, if no secondary forces came into play, these walls would bespatter one another with spores. Still more unfavourable are the conditions in the narrow and winding passages of Gyromitra." He [4] placed cover-glasses against the sides of some of the fruit-bodies and noticed that, when clouds of spores were emitted, the spores were deflected from their courses by the cover-glasses as if they were being carried by air-currents. He [5] therefore argued that, when a Morchella or a Gyromitra fruit-body is irradiated by heat from a lamp, etc., air and water-vapour currents (*Luft- und Wasserdampf-strömungen*) are produced in the hymenial chambers and passages and these currents bear the spores along and thus enable the spores to escape from the fruit-body. Thus, according to Falck, not only the discharge of the spores but also the escape of the spores from Morchella, Gyromitra, etc., is dependent upon the fruit-bodies being warmed by radiation.

I am unable to accept Falck's theory of the escape of spores from the fruit-bodies of the Morchellaceae because it is based on

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[1] Richard Falck, " Ueber die Sporenverbreitung bei den Ascomyceten. I. Die radiosensiblen Discomyceten," *Mycologische Untersuchungen und Berichte von R.* Falck, Bd. I, Heft II, 1916, pp. 77–144.

[2] In a second paper, " Ueber die Sporenverbreitung bei den Ascomyceten. II. Die taktiosensiblen Discomyceten " (*ibid.*, Heft III, 1923, pp. 370–403), Falck treats of spore-discharge in those Discomycetes which puff when touched by a solid object or blown upon ; and he calls such Discomycetes the *tactiosensitive* Discomycetes.

[3] R. Falck, " I. Die radiosensiblen Discomyceten," *loc. cit.*, p. 133.

[4] *Ibid.*, p. 133.   [5] *Ibid.*, p. 134.

two false assumptions : (1) that the asci in the hymenial chambers and folds are not heliotropically curved but are straight ; and (2) that the air-currents produced during puffing are caused by a difference in temperature between the fruit-body and the outer air.

In the preceding Chapter, I have shown by means of a simple
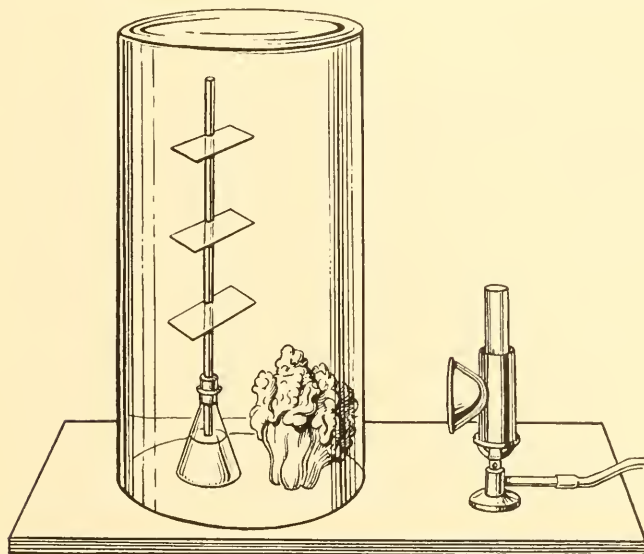
Fig. 128.—An experiment on spore-discharge in *Gyromitra esculenta*. A fruit-body with ripening asci has been kept in a moist chamber. A pencil of rays from an incandescent lamp is now suddenly directed on to the fruit-body. The fruit-body reacts to being heated by emitting spores vigorously. The clouds of spores rise in the chamber and some of the spores settle on the glass slides suspended therein. Copied for the author by G. Atkinson in black-and-white from Olga Falck's half-tone drawing in R. Falck's *Mycologische Untersuchungen und Berichte*, Heft II, 1916, Fig. 1.

experiment that, when a fruit-body of *Sarcoscypha protracta*, which has not been warmed above the temperature of its surroundings, puffs, the discharged spores which are shot out from the mouth of the cup impinge upon the air, set the air in motion and, in the end, are borne along by the air for a much greater distance than they would go were the air to remain still.[1] The air-currents which Falck perceived in his experiments with the Morchellaceae are doubtless

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[1] *Vide supra*, pp. 257–260.

caused by the same means as those of *Sarcoscypha protracta*, *i.e.* they are not warm-air and water-vapour currents, but are produced mechanically by thousands of spores violently striking the air in the same general direction and at the same time, and thus setting the air in motion.

To explain the phenomenon of puffing in Morchella and Gyromitra, when the fruit-bodies are warmed, we have only to suppose : (1) that the asci are sensitive to heat and, when ripe, always explode when their temperature is raised above a certain degree, so that puffing may be initiated by warming a fruit-body ; (2) that the asci are curved heliotropically toward the mouths of the hymenial cavities instead of being straight ; and (3) that the air-currents which arise at the moment of puffing are due to the spores and ascus sap-drops bombarding the air and setting it in motion mechanically and are not due to the fruit-body sending out blasts of warm air.

In what follows an attempt will be made to show how spore-discharge takes place in a number of the commoner Discomycetes which have heliotropic asci.

The Heliotropism of the Asci of Ascobolus magnificus.—For the purpose of verifying and extending Zopf's statement that the asci of certain Ascoboleae are positively heliotropic, I have investigated the effect of light on the direction of growth of the asci of *Ascobolus magnificus*.

Ascobolus magnificus, originally described by B. O. Dodge [1] from North America, occurs on horse dung and, as its specific name indicates, is remarkable for the great size of its fruit-bodies. Dr. Dodge kindly supplied me with cultures of the mycelium.

When spores of *Ascobolus magnificus* have been sown on sterilised horse dung in a warm laboratory, the fruit-bodies begin to appear on the surface of the dung at the end of seven days, are about half-grown at the end of ten days (Fig. 129), and are fully grown and expanded at the end of about fourteen days (Fig. 103, Vol. IV, p. 179). Mature fruit-bodies have a flat pale-greenish-yellow hymenium which becomes darkened with protuberant asci containing

[1] B. O. Dodge, " Artificial Cultures of Ascobolus and Aleuria," *Mycologia*, Vol. IV, 1912, pp. 218–221.

violet spores. Dodge gives the diameter of the fruit-bodies as 0·5–2·7 cm.

Observations made on artificial cultures in the laboratory showed that the asci of *Ascobolus magnificus*, like those of other Ascoboleae, are positively heliotropic. When the hymenium is *illuminated from above*, as indicated in Fig. 130, A, the asci grow perpendicularly upwards and the axis of the apical end of each

FIG. 129.—*Ascobolus magnificus.* Young fruit-bodies growing on sterilised horse dung in a glass jar. Spore-discharge has not yet begun. Photographed by B. O. Dodge. Natural size.

ascus remains parallel to the incident rays of light ; and, when the hymenium is *obliquely illuminated*, as indicated in Fig. 130, B, the asci make a growth curvature until the axis of the apical end of each ascus becomes parallel to the incident rays of light.

The asci of *Ascobolus magnificus*, like those of *A. immersus* and other Ascoboleae, are, as compared with typical Pezizaceae such as *Aleuria vesiculosa* and *Galactinia badia*, remarkably protuberant (*cf.* Fig. 130 with Figs. 140, p. 293, and 147, p. 306). Almost one-third of a ripe ascus of *Ascobolus magnificus* projects freely into the air, and it is this terminal portion of the ascus which bears the
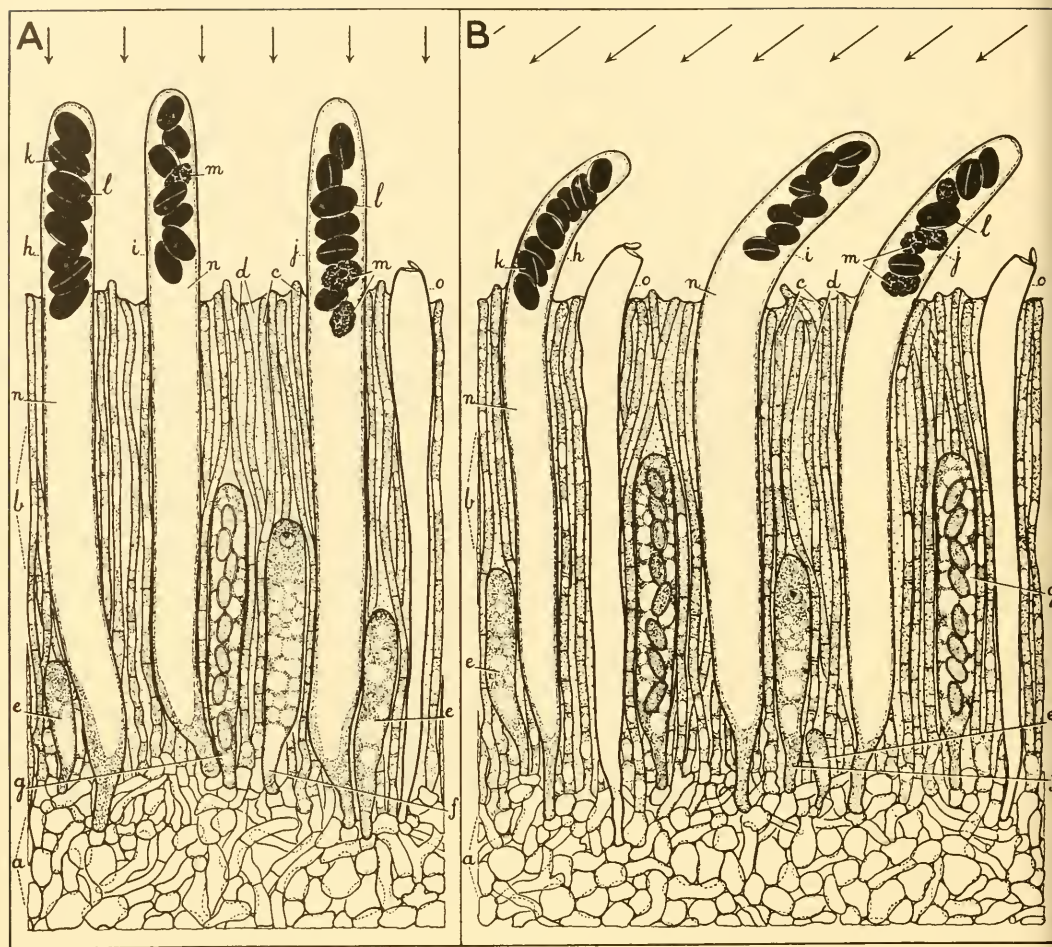
FIG. 130.—*Ascobolus magnificus.* Two vertical sections through the living hymenium and subhymenium, to illustrate the heliotropism of the asci. In A the ripe asci *h*, *i*, and *j* point vertically upwards toward the source of light which illuminated them directly from above, as indicated by the arrows. In B the free ends of the ripe asci *h*, *i*, and *j* are curved toward the source of light which illuminated them obliquely, as indicated by the arrows. A : *a*, the subhymenium, the contents of the cells are not shown ; *b*, the hymenium, the contents of the cells are shown by shading. The hymenium consists of slender unbranched paraphyses *c*, embedded in a yellowish gelatinous matrix *d*, and of asci : *e e*, very young asci containing vacuolated protoplasm ; *f*, a slightly longer ascus with a nucleus near its apex ; *g*, an older ascus, containing 8 young ascospores, pushing up between the paraphyses ; *h*, *i*, and *j* three ripe asci all pointing in the direction of the source of light and now ready to discharge their spores ; *k*, a purple spore with a white longitudinal line in view ; *l l*, spores which happen to show the external gelatinous meniscus which each spore bears on one side ; *m*, exceptional spores with roughened walls ; *n*, a large central vacuole filled with cell-sap ; *o*, an ascus which has discharged its spores and has collapsed, its open end still projects slightly beyond the paraphyses and its operculum is visible at its apex. B, letters with the same significance as in A. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 280.

eight violet spores and also executes the heliotropic curvature toward the source of light.

The paraphyses of *Ascobolus magnificus* are slender and loosely arranged, and they are embedded along with the asci in a gelatinous matrix ; moreover, they are not responsive to heliotropic stimuli. In being anheliotropic they differ from the paraphyses of some Pezizaceae, such as *Aleuria vesiculosa*. Whereas in *Ascobolus magnificus* the heliotropic curvature of the asci is accomplished above the level of the apical ends of the paraphyses, in *Aleuria vesiculosa* it is accomplished chiefly below that level (*cf.* Figs. 130 and 140, p. 293).

Ascobolus stercorarius. — *Ascobolus stercorarius* (= *A. fur-furaceus*), an apandrous Discomycete, the archicarp (scolecite) of which was first seen by Tulasne [1] in 1866, has yellowish discoid fruit-bodies, $0 \cdot 5$–$5 \cdot 0$ mm. in diameter, which in Europe and North America commonly appear in pastures on the dung of horses, cows, and other herbivora.

The apothecium of *Ascobolus stercorarius*, as found by Janczewski [2] in 1871, is at first angiocarpous. The stages in its development have been represented diagrammatically by Corner [3] (Fig. 131) and have thus been described by him. An archicarp arises on the mycelium and one of its cells becomes the ascogonium which collapses about the time when the asci appear. " In the first stage (Fig. 131, *a*) sterile hyphae arise from the stalk of the archicarp or from adjacent mycelial hyphae and envelop the ascogonium in a loose weft (*b*). The hyphae branch profusely, especially to the inside, which suggests a sympodium, and the branches apparently

[1] L.-R. and C. Tulasne, " Note sur les phénomènes de copulation que présentent quelques champignons," *Ann. de Sci. Nat.*, sér. V, T. 6, 1866, p. 215. A median-longitudinal section of a young fruit-body of *A. furfuraceus*, schematically represented by Sachs on the basis of a drawing by Janczewski (*Bot. Zeit.*, 1871, Taf. IV), is reproduced in de Bary's *Vergleichende Morphologie und Physiologie der Pilze* (1884, pp. 201 and 223). R. A. Harper (*Jahrb. f. wiss. Bot.*, XXIX, 1896) and E. J. Welsford (*New Phytologist*, VI, 1912) found that *A. furfuraceus* lacks antheridia and is therefore apandrous.

[2] E. v. G. Janczewski, " Morphologische Untersuchungen über *Ascobolus furfuraceus*," *Botanische Zeitung*, Jahrg. XXIX, 1871, pp. 271–276, Taf. IV, Fig. 21.

[3] E. J. H. Corner, " Studies in the Morphology of the Discomycetes. II. The Structure and Development of the Ascocarp," *Trans. Brit. Myc. Soc.*, Vol. XIV, 1929, pp. 277–278.
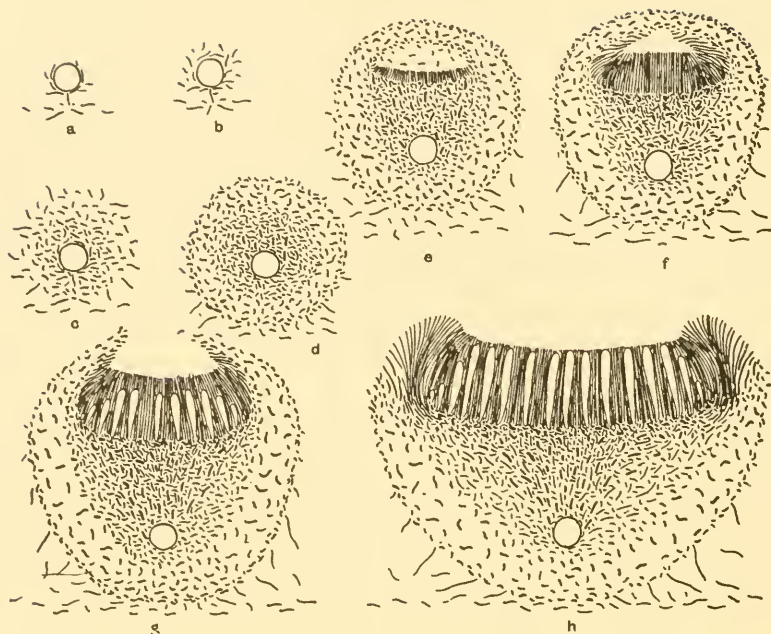
FIG. 131.—Diagram showing stages in the development of an angiocarpic apothecium, *e.g.* that of *Ascobolus stercorarius*. The hyphae are represented by short lines the direction of which indicates the course of the main hyphae in the different tissues. The ascogenous hyphae are represented by short thick lines diverging from the ascogonium which is drawn as a circle near the base of the apothecium solely for reference ; actually it is obliterated by the growth of the surrounding tissue at an early stage in development. Asci are drawn in successive stages at the margin of the hymenium in *g* and *h* to indicate the radial growth of the ascogenous hyphae, but no attempt has been made to show similarly their intercalary growth. The cortical parenchyma is shown by means of thick well-spaced lines without special direction except at the margin, for the palisade arrangement is commonly lost in the mature tissue. Hyphae which grow from the underside of the apothecium as excrescent cells into the substratum and which constitute a secondary mycelium are shown by means of a few longer lines. *a* and *b*, hyphae growing upwards and enveloping the ascogonium ; *c*, a ball of interwoven hyphae surrounding the ascogonium ; *d*, tissue differentiation has begun, the cortex is distinguishable ; *e*, a mucilage cavity has formed in the upper part of the spherical mass internal to the cortex, and a palisade layer of hyphae is growing upwards into it, also ascogenous hyphae are growing from the ascogonium toward the palisade layer ; *f*, the palisade layer can now be seen not only on the floor but on the sides of the mucilage cavity, and the hyphae which compose it are being converted into paraphyses ; *g*, the internal tissues, by expanding, have now ruptured the cortical sheath which formed the roof of the mucilage cavity, the hymenium has thus become exposed, and the ascogenous hyphae have given rise to asci which are pushing upwards among the paraphyses ; the primary parts of the apothecium have now been completely formed ; *h*, the secondary period of development has begun for marginal growth is now taking place, the marginal hyphae are developing sympodially giving rise to paraphyses above and cortical cells below. Drawn by E. J. H. Corner and published by him in *Trans. Brit. Myc. Soc.*, Vol. XIV, 1929.

grow in all directions so that a ball of interwoven hyphae with the ascogonium near the centre results (c). Then tissue differentiation begins (d). The cells of the outer layers enlarge and often become thick-walled, forming a pseudoparenchymatous cortex, while the internal hyphae remain thin-walled, and continue to branch, so that the spaces which would be formed by the expansion of the cortex are filled. A mucilage cavity then forms in the upper part of the spherical mass internal to the cortex (e). It originates partly by the stretching of the internal tissue but mainly by the dissolution of the internal hyphae. Numerous hyphae grow into the cavity as a palisade layer which spreads from the floor over the sides to the roof. Some hyphae are outgrowths from cells lining the cavity, but others are said to originate from the neighbourhood of the ascogonium and to grow up with the ascogenous hyphae. The ascogenous hyphae are produced from the ascogonium after its envelopment and on reaching the floor of the cavity begin to form asci. The upgrowth of the palisade hyphae is soon arrested and they are transformed into paraphyses, which act marks the beginning of the hymenium (f).

" Subsequently the cortical layer overlying the mucilage cavity is ruptured by the expanding internal tissue, and about the same time the first asci are formed (g). The intercalary growth of the hymenium, due to the production of asci and paraphyses, presses the sides of the apothecium apart, exposing the disc (g), while the apothecium passes through a characteristic turbinate shape before becoming lenticular.

" Finally, in stage (h), a marginal growing-point is formed, and apparently from the hyphae which grew from the sides of the mucilage cavity and which, on exposure of the disc, form a sympodium : the outer laterals are converted into cortical hyphae and the inner ones into paraphyses.

" Evidently primary and secondary parts can be distinguished in the angiocarpic apothecium. The primary part consists of the archicarp, the investing hyphae, and the initial part of the hymenium, and the primary period of development comprises all stages up to (g) (Fig. 131). The secondary part consists of all tissues derived from the marginal growing-point, and the secondary
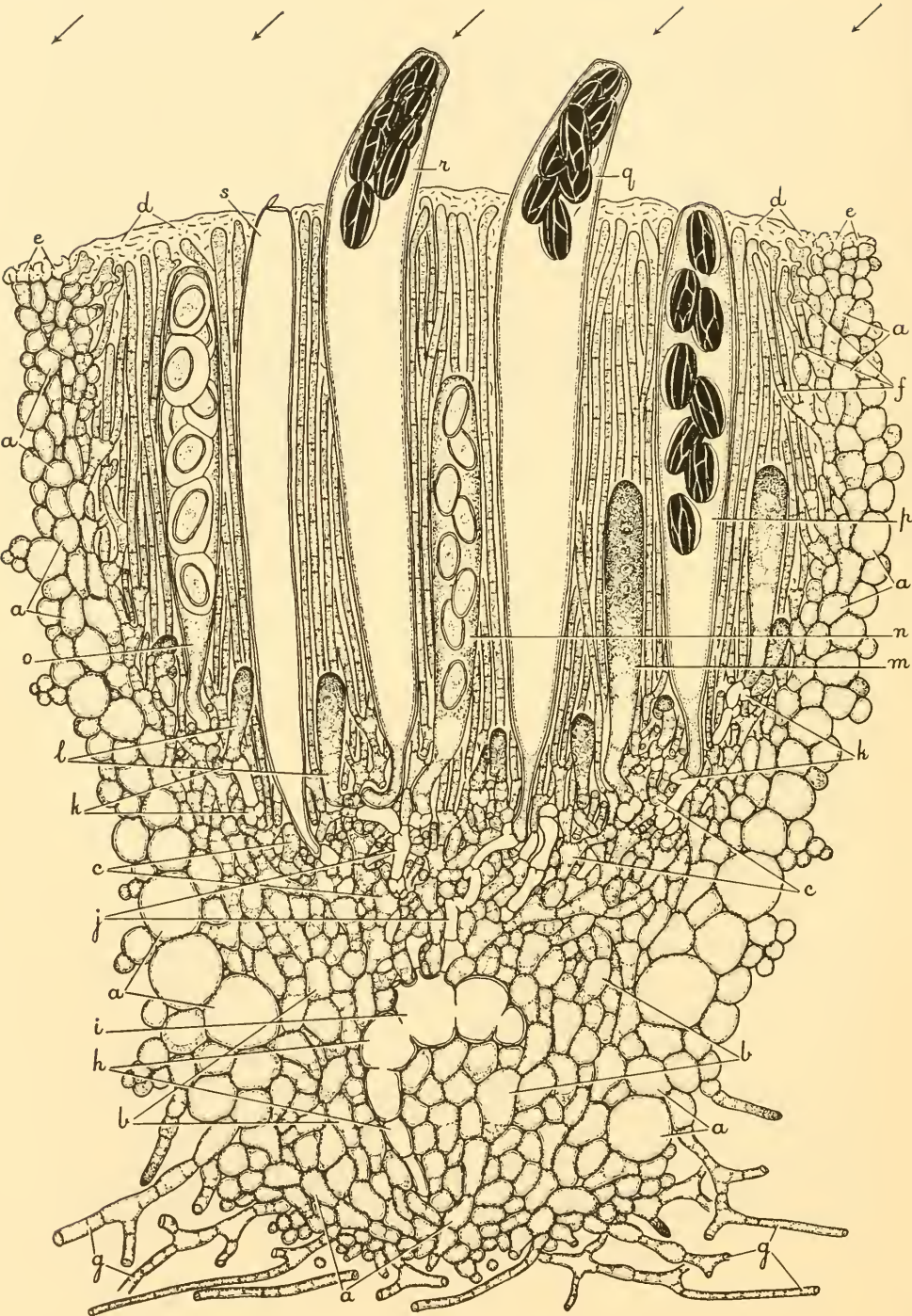
period of development extends from the initiation of marginal growth to maturity."

The asci of *Ascobolus stercorarius* push upwards between the paraphyses and at first are straight. However, shortly before they discharge their spores, they protrude considerably beyond the general level of the hymenium, and their aerial parts bend heliotropically toward the source of greatest light. Two such heliotropically curved asci are shown in Fig. 132 in which is reproduced Corner's illustration (arrows added by myself) of a median-vertical section through the whole of a small fruit-body.[1]   As in other

Fig. 132.—*Ascobolus stercorarius*, a common coprophilous Discomycete which has heliotropic asci and which puffs audibly. Median-vertical section of a very small apothecium. The apothecium is turbinulate in form ; it was angiocarpic in origin, and the primary sheath of cortical cells which overlay a mucilage cavity and the disc became ruptured at *e e* owing to the expansion of the internal tissues, and thus the disc was exposed. The apothecium was built up originally around the archicarp from sympodial clusters of cortical hyphae and paraphyses, the intercalary parts of which now form the medulla. Details of structure are shown as follows : the *excipulum*, consisting of the *cortex a a* and the *medulla b b* ; *c c*, the *hypothecium* (in which the ascogenous hyphae are situated) ; the *hymenium*, consisting of *asci* and *paraphyses*, above the hypothecium ; *d, mucilage* ; *e e*, the broken edges of the primary cortical sheath that at first covered the mucilage cavity and disc ; *f*, rudimentary paraphyses, evidently forming part of the sympodial clusters of hyphae from which the apothecium was constructed ; *g, secondary mycelium* composed of hyphae which have grown from the underside of the apothecium as excrescent cortical cells into the substratum ; *h*, the *archicarp (scolecite)* consisting of a bent chain of cells with a wide pore in the centre of each septum ; *i*, the *ascogonium*, one of the cells of the archicarp, which gave rise to the ascogenous hyphae ; *j*, ascogenous hyphae passing upwards through the hypothecium ; *k*, hooks and hook-cells which were formed at the ends of ascogenous hyphae ; *l*, young asci pushing up between the paraphyses, each containing a fusion nucleus ; *m*, an older ascus containing four nuclei ; *n*, an ascus containing eight young spores ; *o*, an ascus containing eight older spores, each surrounded by jelly ; *p*, an ascus containing mature or nearly mature spores each of which has a dark-violet cell-wall marked with interlacing white lines and bears on one side a lenticular mass of jelly ; *q* and *r*, two highly turgid and fully expanded asci, each containing a thin layer of protoplasm lining the cell-wall, a large central vacuole filled with cell-sap, and massed at the apex eight ripe spores which are about to be violently discharged : the asci *q* and *r* protrude beyond the layer of mucilage and their aerial parts are bent heliotropically toward the source of strongest light the direction of which is indicated by the arrows ; when the operculum of the ascus *q* or the ascus *r* opens, the elastic ascus-wall will contract and drive out through the operculum not only the eight spores but also a large amount of cell-sap which will be scattered in the air in the form of a fine spray of spherical droplets ; *s*, an ascus which has discharged its eight spores and has shrunk to about one-half of its original volume ; at its apex the hinged operculum can be seen. Drawn by E. J. H. Corner, originally published by him in *Trans. Brit. Myc. Soc.*, Vol. XIV, 1929, but now, with his consent, altered slightly, lettered, described, and reproduced on a larger scale than formerly by A. H. R. Buller. Magnification, about 340.

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[1] E. J. H. Corner, *loc. cit.*, p. 286.

Ascoboli, the paraphyses of *A. stercorarius* are anheliotropic (*cf.* Fig. 132).

Our Present Knowledge of Ciliaria scutellata.—This fungus (Fig. 133), so well-known under its old name *Lachnea scutellata*, was transferred to the genus Ciliaria by Boudier,[1] in 1885, in his masterly revision of the classification of the Discomycetes. *Ciliaria scutellata* commonly occurs [2] on damp rotten wood in shady places in woods in the British Islands, Europe, Canada, the United States of America, Java, Tasmania, and probably many other parts of the world. I myself find it every autumn in the woods of Manitoba and of western Ontario.[3] Owing to its brilliant red colour and its dark marginal hairs, it attracted the attention of the early botanical systematists and its existence is recorded in the works of Ray [4] (1696), Dillenius [5] (1718), Vaillant [6] (1727), and Micheli [7] (1729). Linnaeus [8] described the fungus in the second edition of his *Flora Suecica* (1755) and called it *Peziza scutellata*, under which name it also appears in his *Species Plantarum* [9] (1753). Illustrations of the fruit-bodies are given in various works on systematic mycology among which may be mentioned those of Cooke,[10] Rehm,[11] Lindau,[12] and Hard.[13]

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[1] É. Boudier, " Nouvelle Classification Naturelle des Discomycètes Charnus," *Bull. Soc. Myc. France*, T. I, 1885, p. 105. *Vide* also his *Histoire et Classification des Discomycètes d'Europe*, Paris, 1907, p. 61.

[2] M. C. Cooke, *Mycographia seu Icones Fungorum*, London, Vol. I, 1879, p. 73.

[3] G. R. Bisby and A. H. R. Buller, " Preliminary List of Manitoba Fungi," *Trans. Brit. Myc. Soc.*, Vol. VIII, 1922, p. 95.

[4] John Ray, *Synopsis Methodica Stirpium Britannicarum*, ed. II, London, 1696, p. 29, No. 41.

[5] J. J. Dillenius, *Catalogus Plantarum sponte circa Gissam nascentium*, Franco-furti, 1718, p. 194.

[6] S. Vaillant, *Botanicon Parisiense*, Leide et Amsterdam, 1727, p. 57, No. 8.

[7] P. A. Micheli, *Nova Plantarum Genera*, Florentiae, 1729, p. 206, No. 12.

[8] C. Linnaeus, *Flora Suecica*, Stockholmiae, 1755, p. 458.

[9] C. Linnaeus, *Species Plantarum*, 1753, p. 1181.

[10] M. C. Cooke, *loc. cit.*, Plate XXXIV, Fig. 131 (coloured).

[11] H. Rehm in Rabenhorst's *Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz*, Ed. II, Bd. I, Die Pilze, Abt. III, Ascomyceten, p. 1034. Figs. 1–4. Only 1–2 oil drops are shown in each ascospore, instead of a large number as observed by Boudier and myself.

[12] G. Lindau in Engler und Prantl's *Die nat. Pflanzenfamilien*, Teil I, Abt. I, Pezizineae, p. 181, Fig. 147, A and B.

[13] M. E. Hard, *The Mushroom, Edible and Otherwise*, Columbus, Ohio, U.S.A., 1908, p. 509, Fig. 433 (a good photograph).

The fruit-bodies of *Ciliaria scutellata* are usually gregarious, sessile, almost closed and subglobose at first, then expanded and plane, fixed by a central point, 3–10 mm. varying up to 15 mm. in diameter, and 1–2 mm. thick.   The upper surface of the disc is deep carmine or almost vermilion but sometimes pallid, while the lower surface is yellowish-red or brownish.   The outer covering of the fruit-body is furnished with large brown thick-walled septate hairs (Fig. 134), 300–600 μ long [1] and 15–20 μ wide, the longest and last developed [2] ones being at the periphery.   At first, when the fruit-body is small and almost spherical, the marginal hairs are turned centripetally over the top of the hymenium ; but, later, as
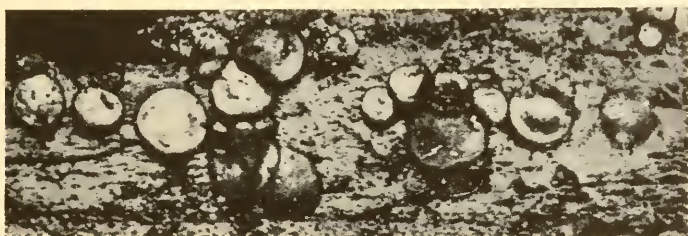
Fig. 133.—*Ciliaria scutellata* (= *Lachnea scutellata*).   Fruit-bodies on rotting wood.   Part of Fig. 433 in M. E. Hard's *The Mushroom, Edible and Otherwise,* published at Columbus, Ohio, U.S.A., 1908.

the fruit-body expands and flattens, they turn outwards centrifugally.   Thus the mature fruit-body, when seen from above, has the appearance of a carmine disc fringed with projecting brown-black hairs.   It was this fringe of dark hairs, reminding one of eye-lashes, that suggested to Quélet the name of the genus, *Ciliaria*, to which Boudier has transferred the species under discussion.   The flesh or trama of the fruit-body consists of an upper layer of fine interwoven hyphae and of a lower layer of pseudoparenchyma. The hymenium, made up of asci and numerous paraphyses, is about 0·3 mm. thick.   The asci (Fig. 135) are fairly large,

[1] J. Lagarde in his " Contribution à l'étude des Discomycètes charnus " (*Ann. Myc.*, Jahrg. IV, 1906, pp. 207–208), in giving details of the anatomical structure of *Ciliaria scutellata*, states that the length of the hairs varies from 150 to 300 μ and that those at the margin may attain 700 μ.

[2] *Vide* W. H. Brown, " The Development of the Ascocarp of *Lachnea scutellata*," *The Botanical Gazette*, Vol. LII, 1911, p. 276.

cylindrical except at the very base, not turning blue with iodine, 271–305 μ long and 20–22 μ wide (in the fruit-bodies investigated), and after explosion shortened to about 265 μ. The paraphyses (Fig. 135, *d*) are simple or branched at the very base, septate, orange-red, narrowly cylindrical but club-shaped terminally, con-
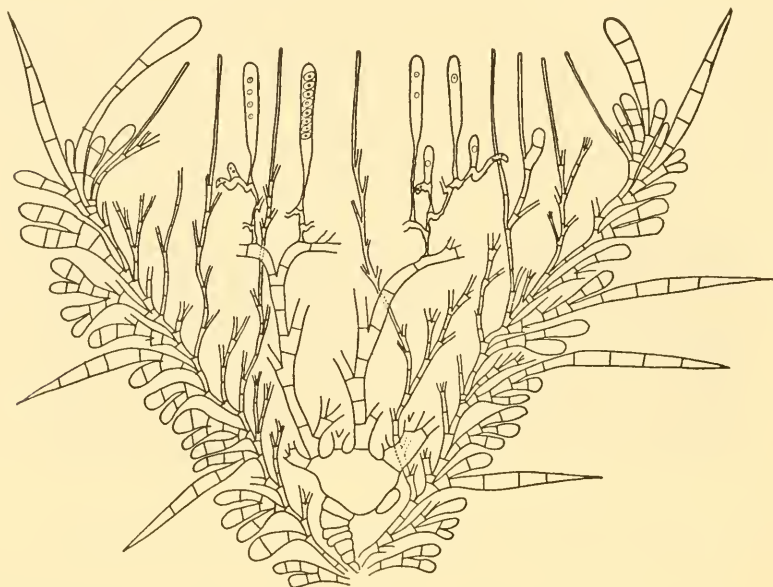
FIG. 134.—W. H. Brown's diagram illustrating the mode of development of a fruit-body of *Ciliaria scutellata*. The penultimate cell of the archicarp has become swollen to form the ascogonium (ascogenous cell) and has given rise to hyphae which are forming the asci. The vegetative hyphae at the base of the archicarp have grown in length, have branched, and have produced the paraphyses, the trama, and the outer covering of the fruit-body including the setae.

taining a few granules, and turning green with iodine.[1] The spores (Fig. 135) are colourless, smooth, ovoid-elliptic, furnished within with numerous oleaginous granules which fill them completely, 20–22 μ long and 11–13 μ wide.

No one has yet grown *Ciliaria scutellata* in artificial cultures. Brefeld [2] tried but did not succeed in germinating the spores.

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[1] É. Boudier, in his *Icones Mycologicae* (Paris, 1905–1910, T. IV, p. 207), by inadvertence, states that the paraphyses turn *blue* with iodine, but he correctly illustrates them in his Plate CCCVIII as turning green.

[2] O. Brefeld, *Untersuchungen über Pilze*, Heft X, 1891, p. 331.

W. H. Brown [1] has given us an interesting account of the development of the fruit-body.   He discovered that, if fruit-bodies which are developing on a log of wood are picked off, a new crop of fruit-bodies soon makes its appearance.   Taking advantage of this fact, he obtained a series of very young fruit-bodies which he was able to investigate with the help of the microtome.   He found that each fruit-body develops (*cf.* Fig. 134) from an archicarp,[2] the penultimate cell of which swells up greatly and becomes the ascogonium or ascogenous cell.   This ascogenous cell, which is multinucleate, sends out ascogenous hyphae which grow upwards, branch and rebranch and, finally, after hook-formation and conjugate nuclear divisions, produce the asci.   Each ascus contains two nuclei which fuse together.   The fusion nucleus then undergoes the usual subdivisions, so that eight nuclei result.   The eight nuclei eventually become enclosed in the eight spores.   No antheridium could be observed, so that there is no evidence that the ascogenous cell receives any male nuclei.   The vegetative hyphae at the base of the archicarp grow in length, branch freely, and produce the paraphyses, the trama, and the outer covering of the fruit-body including the setae.   Brown's diagram illustrating the mode of development of the fruit-body is reproduced in Fig. 134.

The red colouring matter of *Ciliaria scutellata*, like that of Pilobolus, the Uredineae, *Polystigma rubrum*, *Nectria cinnabarina*, *Peziza aurantia*, etc., is held in tiny oil drops and is therefore a *lipochrome*.[3]   The lipochromes are regarded as carotins.[4]   They are insoluble in water, but soluble in alcohol, ether, chloroform, etc. When dry, with concentrated sulphuric acid, they give the typical

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[1] W. H. Brown, *loc. cit.*, pp. 275–305, with 51 Figs. in the text and one Plate.

[2] M. Woronin was the first to see and illustrate the archicarp of *Ciliaria scutellata*. He said that it consisted of a chain of 2–5 cells which soon became surrounded by vegetative hyphae which obscured its further development.   *Vide* his " Die Entwicklungsgeschichte des *Ascobolus pulcherrimus* und einiger Pezizen " in de Bary and Woronin's *Beiträge zur Morphologie und Physiologie der Pilze*, No. 11, 1866, pp. 5–6, Plate II, Figs. 1–3.

[3] W. Zopf, " Über das mikrochemische Verhalten von Fettfarbstoffen usw.," *Zeitschr. f. wiss. Mikroscopie*, Bd. VI, 1889, p. 172 ; also *Die Pilze*, Breslau, 1890, pp. 146–147.

[4] *Cf.* J. Zellner, *Chemie der Höheren Pilze*, Leipzig, 1907, pp. 12, 139–142 ; and Hans Molisch, *Mikrochemie der Pflanzen*, 1923, p. 221.

carotin reaction, *i.e.* they turn blue ; and, with iodine dissolved in potassium iodide, they turn blue-green.[1]   The pigmented oil-drops of *C. scutellata* are contained in large numbers in the protoplasm of the cells making up the paraphyses and subhymenium.   The pigment was obtained by Zopf [2] by extracting with alcohol and forming an ester with caustic soda.   The ester, when treated with petrol-ether, yielded a yellow substance that, after being dried, turned blue when moistened with nitric acid.   When examined spectroscopically by Bachmann,[3] the pigment gave two absorption bands like those of the lipochrome of the Uredineae.

The Heliotropism of the Asci of Ciliaria scutellata.—Since the disc of *Ciliaria scutellata* is flat or almost so, it is not difficult to make heliotropic experiments with its asci.   All that is required is to let the fruit-body develop on the top of a board lying on the ground in unilateral light and then, by microscopical investigation, to find out whether or not the tops of the ripe asci have become curved toward the source of illumination.

The conditions for such a heliotropic experiment as that just suggested were provided by one of the ice-houses of the Arctic Ice Company of Winnipeg.   This Company, not far from Winnipeg on the banks of the Red River, has a series of large wooden ice-houses each of which is 100 feet long, 30 feet wide, and about 50 feet high, windowless, and provided at one end with a large double door. The floors are covered with sawdust including bits of planks, etc., derived from a saw-mill.   The ice-houses are filled during the winter with great blocks of ice taken from the Red River, and are gradually emptied in the spring and summer.   After the ice has been removed from an ice-house, the sawdust and pieces of wood on the floor are left undisturbed for some months, during which time lignicolous fungi develop upon them in abundance.   As after the removal of the ice from an ice-house the double door is left more or less open, the fungi which develop on the floor are subjected to unilateral daylight.   Among the flat Discomycetes found growing in the ice-houses were : *Ciliaria scutellata, Melastiza miniata,* and *Cheilymenia vinacea.*

[1] J. Zellner, *loc. cit.*, p. 139.   [2] W. Zopf, *Die Pilze*, Breslau, 1890, p. 146.

[3] E. Bachmann, cited from Zopf, *loc. cit.*

Several fruit-bodies of *Ciliaria scutellata* which were expanded horizontally on the top of boards in one of the ice-houses were chosen for investigation.   First, the direction in which the light from the door of the ice-house had illuminated them was clearly marked upon their upper surfaces, and then they were removed to the laboratory and studied by means of transverse hand-sections taken vertically downwards in a direction parallel to the direction of the rays of light which had illuminated them (*cf.* Fig. 135).   The sections, after being mounted in water, were still alive.

On observing the sections it was found that, in every fruit-body, the ripe asci all across the discs were bent at their free ends in the direction of what had been the source of light.   It was thus proved that the asci of *Ciliaria scutellata* are heliotropic.   Some of the heliotropically curved asci are shown in Fig. 135.   Each of them is bent terminally through an angle of 45°.

A mature fruit-body of *Ciliaria scutellata*, which was about 1·25 cm. in diameter, was spread out as a flat disc on a board on the floor of one of the ice-houses over 50 feet away from the doorway through which came the light which had illuminated it unilaterally. I touched the fruit-body and had the satisfaction of seeing that, as it puffed, it shot away its spores in the direction of the source of illumination.   The direction of puffing could only have been due to the fact that the asci were positively heliotropic and, during their development, had bent their free ends toward the light.

The paraphyses of *Ciliaria scutellata*, as in the Discomycetes generally, come to maturity before the asci, and the asci push up between them (*cf.* Fig. 135).   At first, each ascus is quite straight and the young spores are relatively small with the uppermost one at some distance from the end of the ascus.   As a young ascus continues its development, the spores grow in size and move upwards so that the top one of the chain of eight becomes appressed to the operculum, while the ascus as a whole, through increase in length, comes to push its apex about 30 μ above the general level of the paraphyses.   It is during the emergence of the end of the ascus into the free air that the ascus responds positively to the heliotropic stimulus of light, and the actual curvature is confined to the last sixth (40–50 μ) of the ascus length (Fig. 135, *h–k*).

When an ascus of *Ciliaria scutellata* explodes, it shortens by
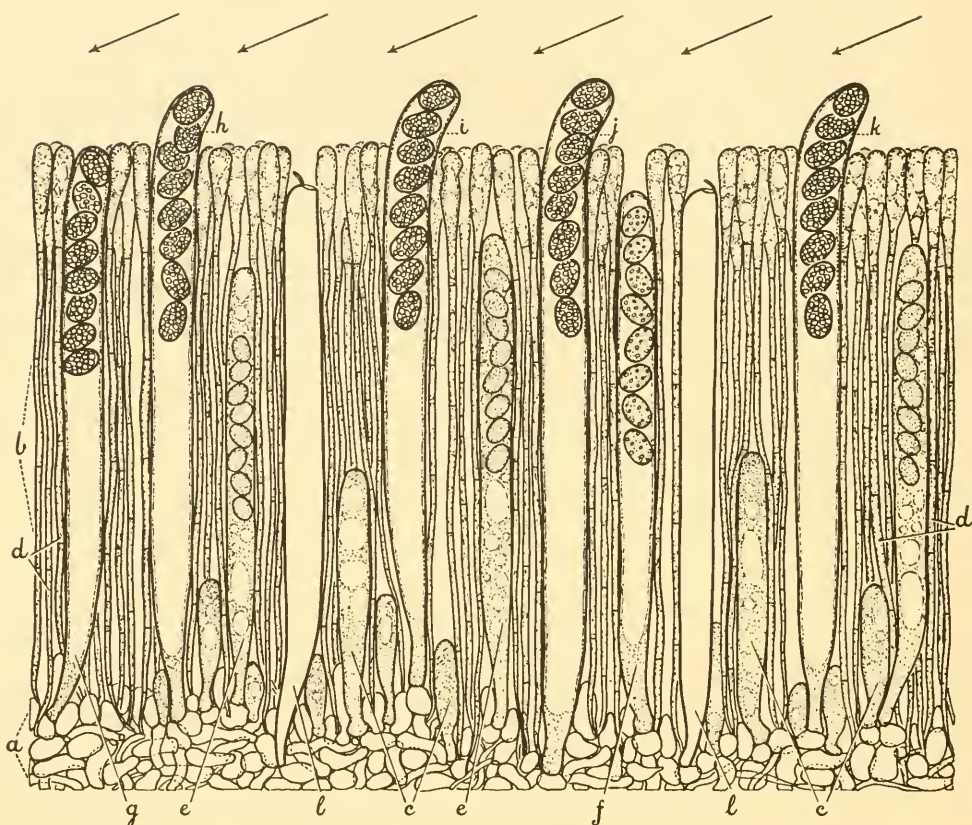
FIG. 135.— *Ciliaria scutellata* (= *Lachnea scutellata*). Vertical section through the subhymenium *a* and the hymenium *b* of a fruit-body which grew in unilateral light, to illustrate the heliotropism of the asci. The arrows indicate the direction of the incident rays of light, and the asci at their extreme free ends have bent toward the source of the light. In the hymenium the following structures may be distinguished : *c c*, young asci pushing upwards among the paraphyses *d d* ; *e e*, older asci containing spores ; *f*, a still older ascus with more mature spores ; the asci *e*, *e*, and *f* all have a straight axis ; *g*, an ascus with mature spores just pushing up above the paraphyses, its end is becoming heliotropically curved ; *h*, *i*, *j*, and *k*, four ripe asci with their free ends heliotropically curved toward the source of light, their spores containing oil-drops ; *l l*, two asci which have just discharged their spores and have contracted in length, so that their ends are below the ends of the paraphyses; in contracting, these two asci, *l l*, have straightened somewhat so that their openings (each with an operculum laterally attached) appear to be asymmetrically situated on the side of the end of the ascus nearest to the source of light. Magnification, 294.

about 30-40 μ, and thus its apex becomes drawn down to just below the level of the paraphyses. During the contraction in length, the

end of the ascus becomes straighter, and then the operculum can be seen occupying a more or less oblique position at the end of the ascus (Fig. 135, *l l*). Investigation of fruit-bodies of *C. scutellata* grown under various conditions of light seems to indicate that the operculum really originates symmetrically at the end of the ascus, but that, owing to heliotropic curvature of the end of the ascus. the operculum becomes pushed to one side—the side which is nearest to the source of light. Thus the more or less oblique position of the mouth of the ascus of *Ciliaria scutellata* and of other similar Discomycetes is not a purely "morphological character" as Seaver [1] thought, but is a physiological character caused by the response of the ascus to the heliotropic stimulus of light.

The Heliotropism of the Asci of Melastiza miniata and Cheilymenia vinacea.—Fruit-bodies of these species came up in the ice-house on pieces of planks and on sawdust along with those of *Ciliaria scutellata* which they resemble in their flattened discoid form.[2]

The fruit-body of *Melastiza miniata* (Fuck.) Boud.[3] is an orangered disc which varies in large specimens from 1 to 2 cm. in diameter. It is covered on its outer side by short blunt yellowish-brown hairs. Its paraphyses are club-shaped at their ends. With iodine the paraphyses turn green [4] but the asci do not turn blue. The spores are oval-elliptic, colourless, verrucose, and often blunt at the ends.

The fruit-body of *Cheilymenia vinacea* (Rabenh.) Boud. is a yellow disc, $0 \cdot 5–1 \cdot 0$ cm. in diameter (Fig. 136, A and C). It bears on its outer side numerous long pointed septate hairs or setae (B). Its paraphyses are cylindrical and not swollen at their free ends (F). With iodine the asci do not turn blue. The spores are ellipticoblong, colourless, smooth. devoid of oil-drops, and about 20 μ long.[5]

[1] F. J. Seaver, *vide supra*, pp. 255–257.

[2] The identification of *Melastiza miniata* and *Cheilymenia vinacea* was made with the help of the excellent descriptions and illustrations given by Boudier in his *Icones Mycologicae*.

[3] According to Seaver (*The North American Cup-fungi*, New York, 1928, p. 103), this is a synonym for *Melastiza Chateri* (W. G. Smith) Boud.

[4] Boudier (*Icones Mycologicae*, T. IV, p. 218), by inadvertence. states that the paraphyses do not turn green with iodine, but he correctly illustrates them as turning green in his Plate CCCLXXXVI.

[5] The asci of *Cheilymenia vinacea* are described by Boudier as 270 μ long. In my specimens they were only 230 μ long.

Just as with the fruit-bodies of *Ciliaria scutellata*, the fruit-bodies of *Melastiza miniata* and *Cheilymenia vinacea* were marked before being gathered in the ice-house and then were removed to the laboratory and sectioned by hand. The investigation of the hymenia revealed that in both species the asci, all across each disc, had become slightly curved at their free ends toward the door of the ice-house, *i.e.* toward the source of light, and were therefore positively heliotropic (Fig. 136, C, D, and E ; the arrows in C indicate the directions in which the asci pointed).

The heliotropic curvature of the asci of *Melastiza miniata* and *Cheilymenia vinacea* in unilateral light is confined, as in *Ciliaria scutellata*, to the free ends of the asci and, in the ice-house, it resulted in the opercula being pushed over toward the light, so that the spores were probably shot out from each ascus obliquely upwards at an angle of 45° to the vertical, the inclination being toward the source of light. Here, as in *Ciliaria scutellata*, it was observed that, as the asci discharge, they straighten themselves out somewhat so that the curvature of their ends almost disappears and the operculum in an exploded ascus comes to be obliquely set at the end of the ascus but always on the side from which the light has come (Fig. 136, *cf.* D and E). The oblique position of the operculum in an exploded ascus in such a fungus as *Cheilymenia vinacea* is, as in *Ciliaria scutellata*, not a purely "morphological character" but a physiological one.

Fig. 136.—*Cheilymenia vinacea.* A and C, a fruit-body. B, a marginal hair. D and E, heliotropically curved asci. F, a paraphysis. A and C, nat. size ; B × 150; D–F × 300.

Aleuria vesiculosa and its Identification.—*Aleuria vesiculosa* is one of the largest and best known of the Pezizaceae, and from its vesicular appearance, it has been called the *Bladdery Peziza*[1] and

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[1] M. E. Hard, *The Mushroom, Edible and Otherwise*, Columbus, Ohio, U.S.A., 1908, p. 508.

also the *Bladder Elf-cup*.[1]   It commonly occurs on heaps of horse dung and on manured soil in Europe and North America.

The fruit-bodies of *Aleuria vesiculosa* are often clustered so that they more or less distort one another by mutual pressure (Fig. 137) but, not infrequently, they occur as isolated individuals. A single fruit-body varies in diameter from 3 to 8 cm.   At first, it is globose and almost closed ; but, later, it expands to form a more or less hemispherical cup which is brittle, somewhat translucent and, at the base, about 3 mm. thick. The margin of the cup usually becomes notched and may either turn outwards to a greater or less degree or remain incurved.   Below, where it enters the substratum, the flesh of the cup is contracted centrally to form a short stem-like base or stipe which breaks up in the substratum into strands of mycelium (Fig. 138). The inner hymenial surface of the cup is pale brown or fawn-coloured, while the outer lower surface is brownish and coarsely granular or furfuraceous from the presence of minute irregular warts. The flesh or trama is parenchymatous, the central cells being large and watery. Sometimes the trama splits so that a cavernous air-space is formed between the hymenium and the base of the cup. The hymenium is about $0 \cdot 36$ mm. thick. The paraphyses are unbranched and septate, the terminal cell being slightly club-shaped and coloured yellow with oil-drops and the shaft-cells at first cylindrical but becoming swollen at their sides as the fruit-body becomes older. The asci are fairly large, about 360 μ long and 20 μ wide, slightly attenuated downwards, turning blue with iodine, those at the base of the cup straight and those at the sides heliotropically curved toward the mouth of the cup.   The spores are elliptic, colourless, smooth, without internal oil-globules, bearing on one side a little lenticular mass of colourless jelly which swells up after discharge in water, about 20–24 μ long and 11–13 μ wide.

The fruit-bodies of *Aleuria vesiculosa* used in my investigations were obtained in part from manured ground at the Manitoba Agricultural College and in part from laboratory cultures.   To procure living fruit-bodies during the winter months at Winnipeg

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[1] E. W. Swanton, *Fungi and How to Know Them*, London, 1909, p. 182.

my procedure was as follows. Frozen horse dung from a street or fresh horse dung from a stable (the dung-balls unbroken) was spread in a layer about 3 inches thick on the zinc-lined floor of a large

FIG. 137.—*Aleuria vesiculosa* (= *Peziza vesiculosa*). A group of fruit-bodies not yet fully expanded, growing among grass, presumably on dunged ground. Photographed by A. E. Peck in Yorkshire, England. Natural size.

damp-chamber ($3 \cdot 5 \times 2 \times 2 \cdot 25$ feet) kept on a table in the laboratory a few feet away from a window.   The dung was watered from time to time to keep it moist.   After about a month, on some of the dung-balls, fruit-bodies of *Aleuria vesiculosa* began to appear. Sometimes they were clustered, at other times solitary.   They were never very large and, owing to the conditions under which they were grown (excessive moisture and relatively feeble light),

Fig. 138.—*Aleuria vesiculosa*.   Vertical section through a very large fruit-body.   Its cupulate form was developed by marginal growth.   The hymenium *a* lines the inside of the fruit-body.   The asci in the upper regions of the fruit-body at *b* and *c* would be at a disadvantage in discharging their spores were they not heliotropic and therefore directed toward the cup's mouth.   Outline of the fruit-body copied by the author from Boudier's *Icones Mycologicae*, Pl. 257, *c*.   Natural size.

they tended to be abnormal : firstly, in sometimes having a well-pronounced stipe and, secondly, in becoming more expanded than usual (Fig. 167, p. 333).   The expansion was due to the paraphyses swelling laterally prior to the discharge of the spores from the asci.

In Volume I of these *Researches* I gave an account of the production and liberation of the spores of the fruit-bodies just described but, unfortunately, misnamed the species *Peziza repanda*.   From a comparison of my fruit-bodies grown in cultures with the fruit-bodies of *Aleuria vesiculosa* growing in the open, I am now

confident that my culture fruit-bodies are only a form of *Aleuria vesiculosa*.[1]

Results of a Previous Investigation on Aleuria vesiculosa.—The following is a summary of the results of my investigation on the production and liberation of spores in *Aleuria vesiculosa* (erroneously identified as *Peziza repanda*) recorded in 1909 in Volume I of these *Researches* [2] :

" The spores of *Aleuria vesiculosa* are shot up into the air to a height of 2–3 cm. The eight spores from an ascus separate from one another almost immediately after leaving the ascus mouth, and are then carried off by the wind. The fact that the ascus jet breaks up on leaving the ascus was observed by means of the beam-of-light method.

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[1] In 1910 I sent some of my culture fruit-bodies to Dr. E. J. Durand, who wrote to me as follows regarding them. " The plant does not seem to me to be *P. repanda* but to belong more properly in the vicinity of *P. vesiculosa* as you suggest. It has seemed to me for some time that the latter species is very variable or that several species are confused under this name, which can ultimately be separated. Typical *vesiculosa*, as I understand it, is usually pale in colour, sessile, semitransparent, and the opening contracted and small. This evidently is not your plant. The so-called variety *cerea* comes nearer to it ; but, still nearer, it seems to me is the plant described by Vuillemin in the *Proc. of the French Ass. for the Adv. of Science* for 1886 under the name *Aleuria Asterigma*, p. 491, pl. 10. This you will notice is stipitate and repand, and has an Aspergillus-like conidial stage. Rehm (*Discomycetes*, p. 1018) mentions *Oedocephalum fimetarium* as the conidial stage of *P. vesiculosa*, and this is very similar to and doubtfully distinct from the conidial stage mentioned by Vuillemin. To be sure, the conidia of the latter are slightly smaller than in *Oe. fimetarium* and the germination of the spores is slightly different. The last feature may well be due to difference in culture medium. It seems to me we have here three very closely related forms which may be regarded as forms of one variable species, or which cultures may finally show to be distinct in the conidial condition. Your plant I think belongs to the form now known as *Aleuria Asterigma* (or *Peziza Asterigma*), but cultures will be necessary to determine the nature of the conidial stage."

In making the remarks just quoted Durand took into account not only the fruit-bodies sent to him but also my drawings, including the conidial stage, shown in Vol. I of these *Researches*, Figs. 77, 78, and 79 (pp. 235, 236, and 241). Boudier in his *Icones Mycologicae* illustrates *Aleuria Asterigma* and shows every fruit-body with a well-developed stipe. My culture fruit-bodies differ from those shown in Boudier's illustrations in that while some have well-developed stipes others are quite sessile like typical *A. vesiculosa*.

[2] These *Researches*, Vol. I, 1909, pp. 233–250, Figs. 77–79 ; summary on p. 268. In quoting from the summary, as above, I have substituted the correct name *Aleuria vesiculosa* for the erroneous one *Peziza repanda*.

" Puffing is probably not due (as de Bary supposed) to the mere withdrawal of water from the asci. Solutions of grape sugar, glycerine, sodium chloride, and potassium nitrate, which merely withdraw water from the ripe asci of *Aleuria vesiculosa*, do not cause their explosion. On the other hand, solutions of many poisonous substances, *e.g.* iodine, mercuric chloride, silver nitrate, acetic acid, and alcohol, give rise to marked puffing. Two alkalies—sodium hydroxide and sodium carbonate—kill the asci without causing them to discharge their contents. It seems probable that puffing is caused by a stimulus given to the protoplasm in contact with the ascus lid.[1]

" The physics of the ascus jet in *Aleuria vesiculosa* has been discussed. It seems probable that the separation of the eight spores of an ascus during their upward flight into the air is due to considerable differences in the initial velocities given to the individual spores upon their discharge. Surface tension probably plays but a minor part in breaking up the ascus jet. When an ascus is regarded as an apparatus for squirting out a jet in such a manner that the jet immediately breaks up into eight parts so that each part contains a spore, its structure becomes more intelligible.

" The eight spores in an ascus of *Aleuria vesiculosa* are loosely attached together, and the row of spores is anchored to the ascus lid by a special protoplasmic bridle. De Bary's hypothesis of currents is unnecessary in accounting for the means by which the spores are caused to take up their characteristic positions in the ascus."

Whilst making the investigation just summarised, I observed that some of the asci of the fruit-bodies of *Aleuria vesiculosa* were curved whilst some were straight ; but I did not then know the cause of the difference and, in my illustrations (Vol. I, Fig. 79, p. 241), represented only straight asci, such as are actually found at the centre of the base of a well-formed fruit-body. The curvature of the curved asci, as we shall see in the next Section, is not a mere accident attributable to the kinetics of slicing the living fruit-bodies but is due to the developing asci bending heliotropically.

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[1] For a discussion of the cause of the bursting of asci when a fruit-body puffs under normal conditions *vide supra*, pp. 230–232.

The Heliotropism of the Asci and the Discharge of the Spores in Aleuria vesiculosa.—When a fruit-body of *Aleuria vesiculosa* is more or less hemispherical in shape, like those shown in Fig. 137, the hymenium is illuminated by daylight which comes to it from above ; so that, in a fruit-body of the shape postulated, if the asci
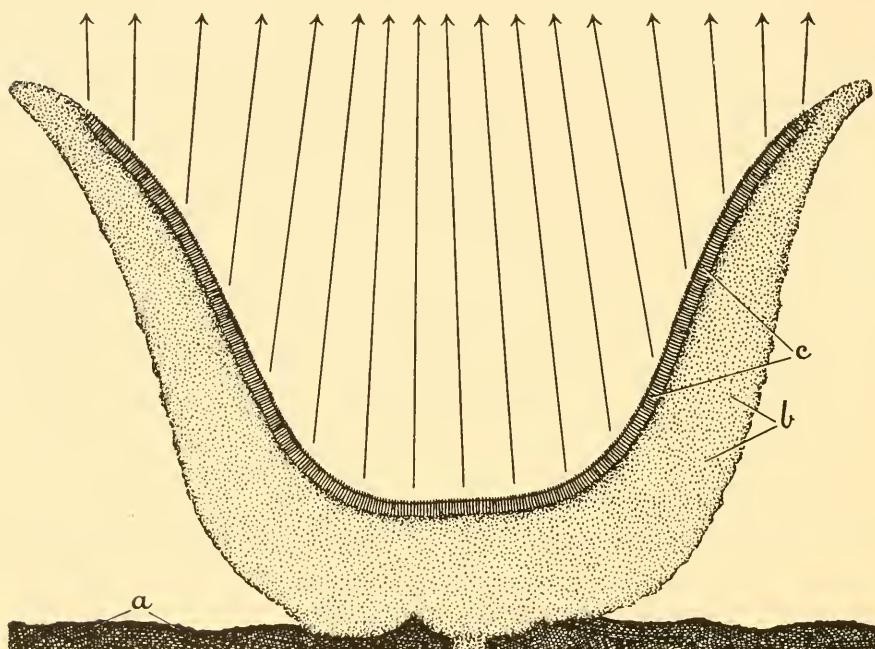
FIG. 139.—*Aleuria vesiculosa* (= *Peziza vesiculosa*). Semi-diagrammatic median vertical section through a small, fully expanded fruit-body which grew in the open on dunged soil at Winnipeg, Canada. To show the heliotropic curvatures of the ends of the asci and the general directions in which the asci would discharge their spores : *a*, dunged soil ; *b*, the receptacle or fruit-body flesh ; *c*, the hymenium. The asci are everywhere bent upwards toward the source of the strongest daylight, and the arrows indicate the direction in which the spores would be discharged. Magnification, 6.

are heliotropic, they should all point upwards toward the fruit-body's mouth. Whether or not the asci of a more or less hemispherical fruit-body do actually point toward the fruit-body's mouth can be determined : (1) by examining the hymenium in radial-longitudinal sections ; (2) by examining the hymenium in surface view ; and (3) by observing the direction of the discharge of the spores in the air with the naked eye or in water with the help of the microscope.
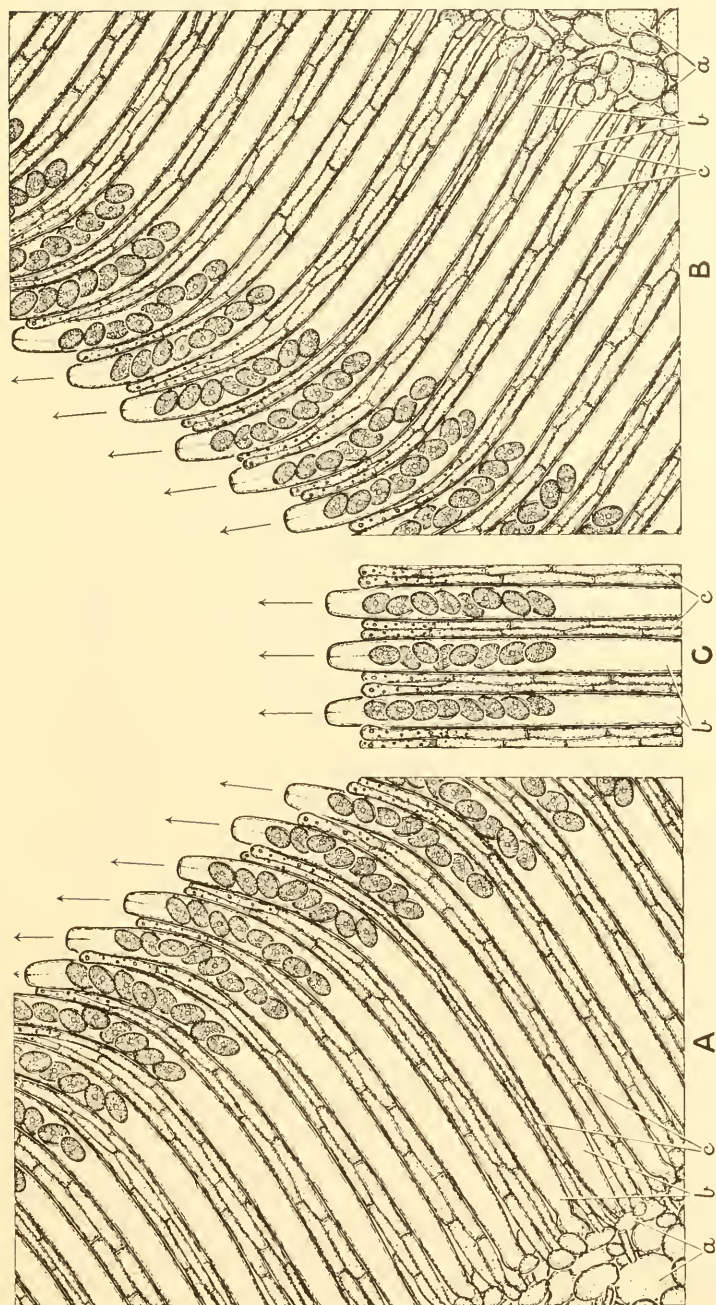
Fig. 140.—*Aleuria vesiculosa*. Semi-diagrammatic representation of three sections taken in a median-vertical plane through a cupulate fruit-body (*cf.* Fig. 139), to show how the asci and paraphyses, in response to heliotropic stimuli, are directed toward the fruit-body's mouth. The fruit-body developed under constant very moist conditions and all its asci have ripened their spores and are ready to discharge them. A and B, sections from two opposite sides of the fruit-body; C, a section from the base of the fruit-body: *a*, the hypothecium; *b*, asci, each having a thin layer of protoplasm lining the cell-wall, a large central vacuole filled with cell-sap and, near the apex, eight uniseriate spores. The arrows indicate the directions in which the asci point; everywhere the asci are directed toward the source of greatest light and therefore toward the open mouth of the fruit-body. Magnification, 220.

Some more or less hemispherical fruit-bodies of *Aleuria vesiculosa* were obtained from an open garden at the Manitoba Agricultural College in October, 1925, and they were examined in the manner just indicated. In each fruit-body, as shown in Figs. 139 and 140, it was found that the asci at the base were straight and looked directly upwards and that the asci on the sides were curved in such
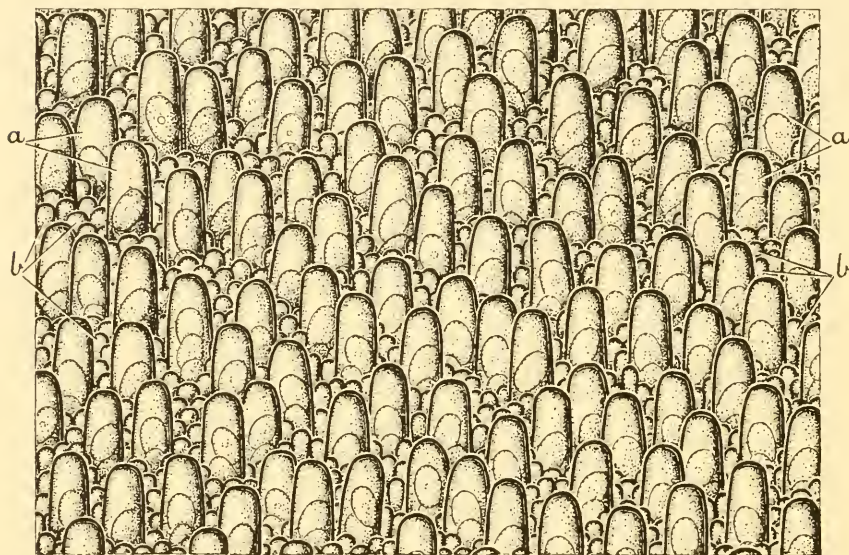
Fig. 141.—*Aleuria vesiculosa.* Semi-diagrammatic surface view of the hymenium half-way up the side of a cupulate fruit-body (*cf.* Figs. 138 and 139). Asci *a* and paraphyses *b* are turned upwards heliotropically so as to be directed toward the source of the strongest light and, therefore, toward the fruit-body's mouth. Magnification, 293.

a way that their apices looked toward the fruit-body's mouth. Every ascus which, owing to its position of origin, had developed in unilateral light had, during its development, bent its free end toward the fruit-body's mouth, *i.e.* toward the source of light.

Surface views of the hymenium on the sides of more or less hemispherical fruit-bodies have the appearance represented semi-diagrammatically in Fig. 141, from which one may conclude once more that all the asci point in the same general direction, namely, toward the fruit-body's mouth.

Radial-longitudinal sections from the sides of a more or less

hemispherical fruit-body (*cf.* Fig. 140, A) were mounted in water and then a drop of iodine was run under the cover-glass so that it came into contact with the outer surface of the hymenium.   The wall of the free end of each mature and projecting ascus turned blue as it absorbed the iodine, and then the ascus suddenly shot out its eight spores into the surrounding fluid.   All the asci in sections of one fruit-body discharged their spores within a few seconds, so that the iodine caused the phenomenon of puffing to take place in a watery medium.   Whilst the discharge of the spores was taking place, it was easy to see that each ascus shot away its spores in the direction in which it was pointing, *i.e.* at an angle of about 45° to the outer surface of the hymenium.   This direction of discharge, had the asci been left undisturbed in their fruit-body under aerial conditions, would have enabled the asci to shoot their spores upwards and through the fruit-body's mouth.

If a piece of the wall is quickly taken from the side of a mature fruit-body of *Aleuria vesiculosa* which has been enclosed for some time in a glass case, and is held horizontally in the air until it puffs, since the asci are inclined in the direction of the rim, the spores should be discharged into the air in the general direction of the rim and the cloud of spores should be seen thus travelling with the naked eye.   Unfortunately, when I wished to make this experiment with *Aleuria vesiculosa*, no more suitable fruit-bodies of that species were available ; but the proposed experiment came off quite success-fully with the very similar fruit-bodies of *Galactinia badia* (Fig. 149, p. 308) : the spores were shot toward the rim of the piece of fruit-body in a cloud at an angle of about 45° with the outer horizontally-placed surface of the hymenium.

A fruit-body which developed in the large damp-chamber in the laboratory was abnormally wrinkled and flattened out in the manner shown in the transverse section in Fig. 142, A ; and it happened to be so arranged that it faced the incident light.   I very carefully examined the central convex ridge both in radial-longitudinal and in surface sections, and I found that the asci at the top of the ridge were straight and faced the light while those on the sides were curved upwards so that they also faced the light, *i.e.* that all the asci on the convex central ridge were pointing toward the light

(Fig. 142, B). Had the hymenium of the ridge been bent concavely downwards—as it is in normal fruit-bodies—instead of convexly upwards, the asci would have looked toward the light in the manner shown in Figs. 139 and 140 ; but there is this difference between the arrangement of the asci in the concave and the convex hymenium : in the concave hymenium (Fig. 140) the asci are turned away from the centre of the hymenium, whereas in the convex hymenium (Fig. 142) they are turned toward the centre of the hymenium. The arrangement of the asci on the central convex ridge of our abnormally shaped fruit-body allows us to draw the conclusion that the asci in a normal, more or less hemispherical fruit-body do not turn away from the centre of the hymenium because they are influenced by the organisation of the fruit-body as a whole but because they are stimulated by light which causes them to curve heliotropically.

FIG. 142.—*Aleuria vesiculosa*. A, vertical section of irregular fruit-body ; arrows indicate general direction of light. B, diagrammatic representation of the central ridge of A : the asci are heliotropically directed toward the light. A, 1·3 natural size ; B, much enlarged.

Sufficient evidence has now been brought forward to justify the conclusion that the asci of *Aleuria vesiculosa* are positively heliotropic.

The relations of the heliotropic asci with the paraphyses will now be discussed. The asci of *Aleuria vesiculosa*, when ripe, protrude beyond the paraphyses for a distance of not more than 30 μ (Fig. 140). Now the heliotropic curvature of an ascus on the side of a hemispherical fruit-body of *A. vesiculosa* is not confined as in *Ciliaria scutellata*, *Melastiza miniata*, and *Cheilymenia vinacea* to the very end of the ascus—the part that protrudes beyond the paraphyses—but begins about the middle of the ascus, *i.e.* deep down in the hymenium, and includes the whole of the spore-bearing portion (Fig. 140). It may therefore be asked : how comes it that the asci, notwithstanding that they are hemmed in by paraphyses, are able to bend at so great a depth in the hyme-

nium ? The answer to this question is provided by a study of the paraphyses.

As in other Discomycetes, the paraphyses of *Aleuria vesiculosa*
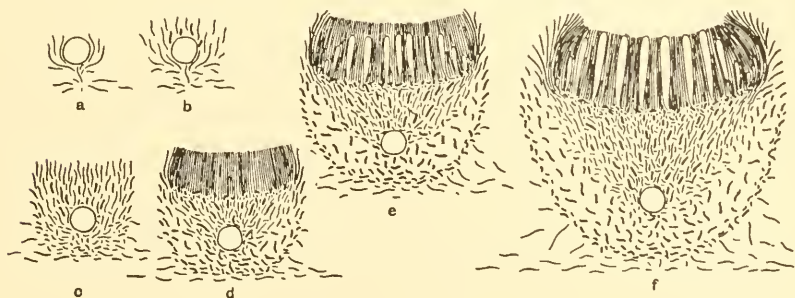
FIG. 143.—Diagram of the development of a gymnocarpic apothecium, *e.g.* that of *Ascobolus magnificus* or *Pyronema confluens*. The hyphae are represented by short lines the direction of which indicates the course of the main hyphae in the different tissues. The ascogenous hyphae are represented by short thick lines diverging from the ascogonium which is drawn as a circle near the base of the apothecium solely for reference ; actually it is obliterated by the growth of the surrounding tissue at an early stage in development. Asci are drawn in successive stages at the margin of the hymenium in *e* and *f* to indicate the growth of the ascogenous hyphae ; but no attempt has been made to show similarly their intercalary growth. The cortical parenchyma is shown by means of thick well-spaced lines without special direction except at the margin, for the palisade arrangement is commonly lost in the mature tissue. Hyphae which grow from the underside of the apothecium as excrescent cells into the substratum and which constitute a secondary mycelium are shown by means of a few longer lines. *a* and *b*, hyphae growing upwards and enveloping the ascogonium ; these investing hyphae do not form a closed sheath as they do in an angiocarpic apothecium (*cf.* Fig. 131) ; they continue to grow upwards as a palisade layer which becomes better defined as new elements are added from below, as in stages *c* and *d* ; the thickness of the apothecium depends primarily upon the extent of this upgrowth and, as the upgrowth ceases, first in the centre the distal parts of the hyphae mature into paraphyses. Thus the formation of the hymenium is started and continues centrifugally until maturity. About the same time the cells in the lower part of the primordium begin to enlarge ; the process extends outwards and upwards to the margin, forming a pseudo-parenchymatous cortex. The ascogenous hyphae arise at about stage *b* and they grow upwards with the sterile hyphae ; the formation of asci begins shortly after the appearance of the paraphyses and is shown in *e* and *f* ; in *f* marginal growth is beginning. Drawn by E. J. H. Corner (*Trans. Brit. Myc. Soc.*, Vol. XIV, 1929).

attain maturity before the asci (*cf.* Figs. 143 and 144). The paraphyses soon grow to their maximum length and then the asci push up between them. Observations on fruit-bodies grown in the laboratory have taught me that *the paraphyses are positively heliotropic.* As shown in Fig. 145, which represents a section through

a young hymenium taken from the side of a fruit-body, the outer ends of the paraphyses are all turned toward the light. This heliotropic bending of the paraphyses facilitates the heliotropic bending of the asci ; and it may therefore be said that in *A. vesiculosa* the paraphyses prepare a way for the asci which come after them and eventually push out beyond them. At first the young asci are straight, as shown in Fig. 145 ; but, as soon as they become about half-grown in length, they respond to the stimulus of light and, in
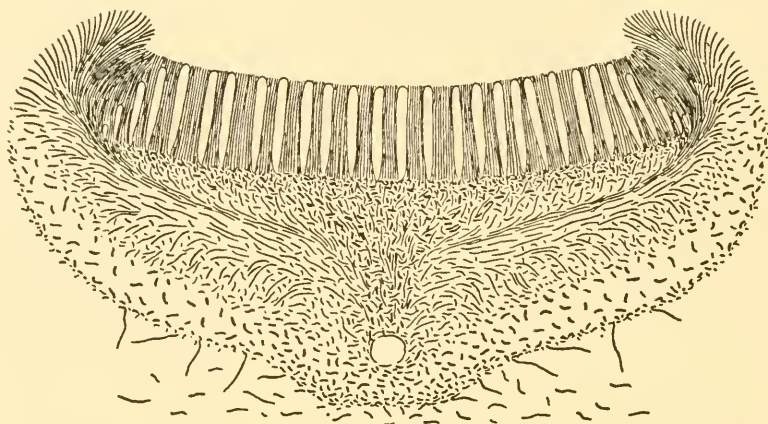
FIG. 144.—Diagram of the construction of a sessile apothecium of one of the Discomycetes. Marginal growth is taking place. The marginal hyphae all around the periphery of the disc are developing sympodially, giving rise to paraphyses above and cortical cells below. It will be seen that, at the margin of the hymenium, the paraphyses are formed first and that afterwards asci grow up between them. Drawn by E. J. H. Corner (*Trans. Brit. Myc. Soc.*, Vol. XIV, 1929).

so doing, make their way between the curved paraphyses without disarranging them (*cf.* Fig. 140, p. 293).

The paraphyses, at first, are only about 3 μ in diameter and are quite cylindrical (Fig. 145) ; but, as the fruit-body ripens, they undergo a considerable change in form. By the time the first asci are ripening or are ready for spore-discharge, their terminal cell has become somewhat clavate and their shaft-cells have increased considerably in diameter (*cf.* Fig. 140, p. 293). Finally, by the time the fruit-body has become fully expanded and all the asci have ripened and some or many have burst, their terminal cell may have become markedly clavate and their shaft-cells have become

bulged out laterally so as to attain diameters often of 12–16 μ
(Vol. I, Fig. 79, D, p. 241).   The lateral expansion of the shaft-cells
of the paraphyses under moist conditions of development is one of
the factors which cause a fruit-body to open out hemispherically
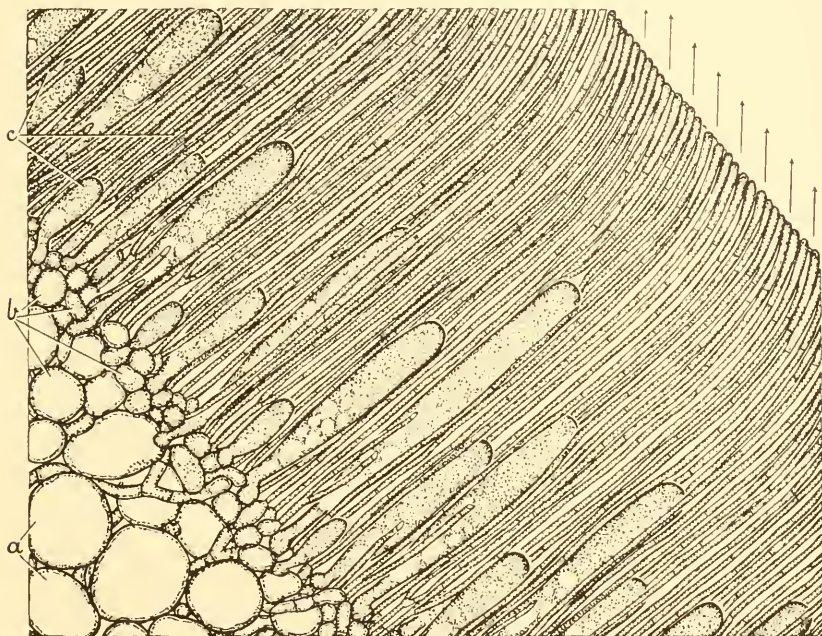
Fig. 145.—*Aleuria vesiculosa.*   Heliotropism of paraphyses.   Median-vertical sec-
tion of the side of a young fruit-body : *a*, cortex ; *b*, hypothecium ; *c*, hymenium
made up of long slender paraphyses and of young asci pushing outwards and
upwards between the paraphyses.   The paraphyses, which developed before
the asci, have become heliotropically turned toward the source of light and
therefore upwards as indicated by the arrows.   The asci, which are not yet
half-grown, are straight and have not yet begun to bend toward the source of
light.   The heliotropic curvatures which the asci will make will coincide in
direction with those already made by the paraphyses.   Magnification, 293.

or, in the laboratory, to become flattened and even recurved over
the substratum.   The asci do not all begin their development at
the same time nor do they all attain maturity at the same time,
but some have the start of others.   In the laboratory, and doubtless
also under natural conditions, the fruit-body may discharge spores
several times in succession by puffing.   When the first set of ripe
asci has discharged its spores, the paraphyses swell up laterally and

fill up the gaps in the hymenium which the contraction and collapse of the asci have made and thus they assist in maintaining the mechanical stability of the hymenium as a whole.

The paraphyses of *Aleuria vesiculosa* and of the Discomycetes in general function : (1) by forming the framework of the hymenium in the young apothecium and thus preparing a place for the asci ; (2) by protecting the developing asci against mechanical injuries and loss of moisture ; and (3) by supporting the mature asci in fixed positions whilst the spores are being discharged.

In the Discomycetes in general, the paraphyses, during the early stages of their development, grow straight outwards from the hypothecium (subhymenium) and, when fully grown, they are either straight, *e.g.* in *Ascobolus stercorarius* (Fig. 132, p. 276) and *Ciliaria scutellata* (Fig. 135, p. 284), or are straight below and heliotropically curved at their free ends, *e.g. Aleuria vesiculosa* (Fig. 140, p. 293). Moreover, when fully grown, they are, as a rule, simple and unbranched or, if branched, the branches are bent forwards so that their long axes are parallel with those of the parent hyphae (Fig. 119, *d*, p. 245). Furthermore, as a rule, the paraphyses in a hymenium do not anastomose with one another.[1] The general straightness, the simplicity of structure, and the separateness of individual paraphyses facilitate mechanically the outward growth of the asci and the taking up by the asci of positions that are advantageous for spore-discharge. The slenderness of paraphyses permits of the paraphyses fitting into and filling up the spaces between the relatively very thick asci, and the club-shaped swellings so characteristic of the apical cells of paraphyses serve to close up the hymenium on its exterior and thus to make it more compact and better adapted for the protection and support of the asci. In some Discomycetes, *e.g. Ascobolus magnificus* (Fig. 130, p. 272), *A. stercorarius* (Fig. 132, p. 276), and *Rhizina inflata*, the outer wall of the end of each paraphysis becomes mucilaginous and the mucilage so produced assists in closing up the exterior of the hymenium.

[1] To this rule I know of only one exception, *Sarcoscypha protracta*. In this Discomycete the upper part of each paraphysis is much branched and the branches are packed closely together. I have observed anastomoses between some of the branches (*vide supra*, p. 244).

In the Discomycetes the asci and usually also the ascospores [1] are colourless, and the coloration exhibited by the hymenium, *e.g.* scarlet in *Sarcoscypha coccinea, S. protracta*, and *Ciliaria scutellata*, orange in *Peziza aurantia*, brown in *Aleuria vesiculosa* and *Galactinia badia*, and yellow in *Cheilymenia vinacea*, is due to pigments contained within the paraphyses. These pigments may be nothing but by-products of metabolism. On the other hand, there is the possibility that they may have some significance for the production and liberation of the spores. It is difficult to avoid the supposition that, in species where the fruit-bodies are exposed to the sun, a pigment, by absorbing the sun's rays, must be a factor in raising the temperature of the hymenium and, therefore, indirectly, a factor in hastening ascus-development and spore-discharge. Falck has actually observed that, when quiescent fruit-bodies of *Morchella esculenta* which contain a brown pigment are exposed to direct sunlight, not only is the temperature of the hymenium raised but spore-discharge is initiated.[2]

An ascus, like most hyphae with a free end, increases in length by apical growth. It therefore seems probable that the part of the ascus which is sensitive to the heliotropic stimulus of light is its apex. It may well be that in every ascus the operculum originates in a radially symmetrical position at the end of the ascus and that the ascus attains heliotropic equilibrium only when the protoplasm underlying the operculum is symmetrically lighted. This theory of heliotropism can be extended to all the Discomycetes with heliotropic asci. It has the advantage of simplicity and is in accordance with the modern view of the nature of heliotropic reactions held by Blaauw and others. It accounts for the fact that in all Discomycetes with heliotropic asci the opercula (or the equivalent in the inoperculate species) at the end of the heliotropic reaction do actually face the strongest rays of light.

The advantage to the fruit-body as a whole in the opercula facing

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[1] The Ascoboli are exceptional in having ascospores with pigmented walls. The colour of the disc of *Ascobolus stercorarius* is somewhat greenish owing to the yellow colour of the mucilage (derived from the paraphyses) combining with the violet colour of the spores. The Bulgariae, *e.g.* the well-known *Bulgaria inquinans*, have brown spores.

[2] *Vide infra*, p. 321.

the light lies of course in this : that it enables the asci to discharge
their spores in the direction of the strongest incident rays of light,
i.e. in the direction most free from obstacles. In the more or less
hemispherical fruit-bodies of Discomycetes like *Aleuria vesiculosa*,
the direction is toward the mouth of the fruit-body.

The chain of eight spores at the end of an ascus of *Aleuria vesi-
culosa*, as described in Volume I, is held in position by a protoplasmic
bridle. The terminal spore in the chain in a heliotropically curved
ascus usually has a slope counter to that of the curvature of the
ascus, as shown in Fig. 140 (p. 293), while the other spores of the
chain are not arranged so regularly. All the eight spores are held
together by a string of cytoplasm. The gelatinous meniscus on one
side of each spore (Fig. 140) swells up when the spore is shot into
water (Vol. I, Fig. 79, H, p. 241) and doubtless, like the similar
meniscus on the side of the spores of *Ascobolus stercorarius* and of
Coprinus sterquilinus (Vol. III, Fig. 95, p. 227), serves to fasten the
spore to the herbage on which it may settle and thus to keep it there
until it is swallowed with the herbage by a herbivorous animal such
as a horse. There can be but little doubt that the spores of *Aleuria
vesiculosa*, like those of many other coprophilous fungi, *e.g.* Piloboli
and many Ascoboli and Coprini, are able to pass through the
alimentary canal of a horse unharmed, so that, when excreted
within the substance of the solid faeces, they find a suitable medium
in which to germinate. The horse is evidently a very important
factor in the dispersion and the maintenance of *Aleuria vesiculosa*
as a species.

In *Aleuria vesiculosa*, as in the Discomycetes already treated of in
this Chapter, an ascus, on discharging its spores, not only contracts
but becomes somewhat straighter. In a contracted ascus the
operculum is usually symmetrically placed at the end of the ascus,
but in a much curved ascus it may be very slightly inclined toward
the shorter and concave side of the ascus.

In my damp-chamber cultures of *Aleuria vesiculosa* the cup-shaped
fruit-bodies with incurved edges did not contain any mature asci and
the spores became ready for discharge only as the fruit-bodies opened
out and more or less flattened themselves against the substratum.
I gathered an expanded and mature fruit-body from the horse dung

on which it was growing and transferred it to a small crystallising dish covered with a glass plate.   On three successive days thereafter I removed the plate and quickly took out the fruit-body from the dish.   Each time, on coming into contact with the dryer outer air of the laboratory, the fruit-body puffed vigorously.   The blast of spores was emitted for 1–2 seconds and could be readily heard. Falck [1] states that, in *Aleuria vesiculosa*, puffing cannot be caused by a rise of temperature but can be caused, at first, by touching the hymenium with a solid object or by stroking it with a hair-pin and also, later, by blowing upon it.   Whether or not my fruit-body puffed because it was touched, or because air passed rapidly over its surface, or because the tips of its asci were exposed suddenly to dry air was not determined.

Puffing of Aleuria vesiculosa under Natural Conditions.—Dr. H. T. Güssow has seen fruit-bodies of *Aleuria vesiculosa* puff intermittently when growing under purely natural conditions and untouched by man.   His observations, which he kindly communicated to me *in litt.*, were as follows.   " Toward the end of August, 1928, I sat on the shore of Penlake, Muskoka, in Ontario, underneath hemlock, spruce and maple.   The ground was very moist and the fungus flora plentiful.   Occasionally, with the swaying of the branches, a sunbeam or two flitted across the ground to disappear again and throw things into a dense shade.   In the light of a sunbeam something like a sparkling cloud was thrown straight up. I looked more closely.   Perchance it was a falling drop of water splashing ; but no, there was nothing to see !   Another cloud was shot up not more than forty inches from my eyes, and then I perceived that it had come from the Waxy Peziza, *Aleuria vesiculosa*, wonderfully camouflaged against the débris of the ground. I watched the fungi for some minutes.   Deep shade,   Nothing happened. There !   A sunbeam momentarily fell upon the cups, and at once there was a puff of glistening sparks !   Soon the sun began to shine directly upon the cups and then, twice in succession, more clouds of spores appeared.   Would puffing take place in the shade ?   I held

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[1] R. Falck, " Ueber die Sporenverbreitung bei den Ascomyceten. II. Die taktiosensiblen Discomyceten," in his *Mycologische Untersuchungen und Berichte*, Cassel, Bd. I, Heft III, 1923, p. 374.

my hat so as to cast a shadow upon the cups, and I waited and watched for several minutes, but all in vain. Perhaps they had exhausted their ammunition. Away came my hat. The sun again struck the cups. At once two puffs, like a pyrotechnic display! I tried to measure the height to which the spores had been shot but could only estimate it at 4–6 cm."

Dr. Güssow's observations show that, under natural conditions, the fruit-bodies of *Aleuria vesiculosa* not only puff but also shoot up their spores to some distance above the mouths of the apothecia. Doubtless the direction of discharge of the clouds of spores which he saw was in part determined by the heliotropic curvatures of the asci.

Dr. Güssow's observations also suggest that his *Aleuria vesiculosa* fruit-bodies puffed as a result of their becoming suddenly illuminated or heated by a beam of sunlight. However, it is certain that sunlight is not necessary for the puffing of *A. vesiculosa* ; for. in the laboratory, ripe fruit-bodies of this species puff when removed from damp air and brought into relatively dry air without any appreciable change in the conditions of light or heat.

With a view to throwing further light on spore-discharge in the Discomycetes it is desirable that Dr. Güssow's observations should be extended, *i.e.* that particular fruit-bodies of various species growing under perfectly natural conditions and untouched by man should be studied throughout their spore-discharge period and that the number of times they puff and the exact conditions under which they puff should be recorded.[1]

The Heliotropism of the Asci and the Discharge of the Spores in Galactinia badia.—*Galactinia badia* is another large, commonly occurring, more or less hemispherical Discomycete (Fig. 146). It differs from *Aleuria vesiculosa* in habitat, for it is not coprophilous and grows on the ground in open or shady places in woods. I have gathered specimens at the Royal Gardens, Kew, and also at Minaki on the Lake of the Woods in central Canada.

[1] Among the Discomycetes that I have seen puff in the open as they were being touched or gathered are : a Ciboria that grows on male Birch catkins, *Ciliaria scutellata*, *Galactinia badia*, *Rhizina inflata*, and *Urnula Craterium*. Probably, had these fruit-bodies been watched instead of being touched, sooner or later they would have been seen to puff.

The fruit-bodies of *Galactinia badia* are often caespitose but sometimes solitary, 3–8 cm. in diameter, brown, and furfuraceous externally. A single fruit-body soon becomes cup-shaped and then, with further expansion, more widely opened until it becomes more or less bowl-shaped or saucer-shaped, with the margin entire or nearly so and the sides of the cup wavy. Below, the fruit-body is contracted into a very short stem-like base. The upper hymenial surface is fawn-coloured varied with tints of olive and red,[1] while the under side is paler brown and minutely granular. The paraphyses are cylindrical but with the apical cell somewhat thickened at the top, septate, and almost colourless. The asci are cylindrical, slightly attenuated downwards, eight-spored and the spores uniseriate, turning blue with iodine, 320–340 μ long and 16–18 μ wide. The spores are elliptic-ovoid, colourless, minutely warted externally, containing one or usually two oil-drops, 17–19 μ long and 9–10 μ wide.

Fig. 146.—*Galactinia badia*. One-half of a fruit-body. The arrows indicate the directions in which the asci point and, therefore, the directions in which the ascospores are discharged. Natural size.

Falek [2] found fruit-bodies of *Galactinia badia* on damp sandy open land and, on testing them, observed that they puffed vigorously when blown upon, but not when their temperature was raised. In younger specimens puffing could be caused by stroking the hymenium with a hair-pin, but not by blowing upon it.

I gathered some living fruit-bodies of *Galactinia badia* from a wood at Minaki, took them to the laboratory at Winnipeg, and there studied them by means of radial-longitudinal sections in the manner already described for *Aleuria vesiculosa*.

One of the fruit-bodies investigated is illustrated in Fig. 146. It was found that the asci at the base of the cup were straight and

[1] This description of the colour is taken from Boudier's *Icones Mycologicae*, Vol. IV, p. 155. Rehm (*Discomyceten*, p. 1011) states that the discs are umber-brown or olive-green, and remarks on the variability of the colour as noted by Fries and others. My own specimens were decidedly brown.

[2] R. Falek, *loc cit.*, p. 273.

thus pointed directly upwards (Fig. 147, A), and that the asci on
the sides of the cup were all curved upwards toward the central
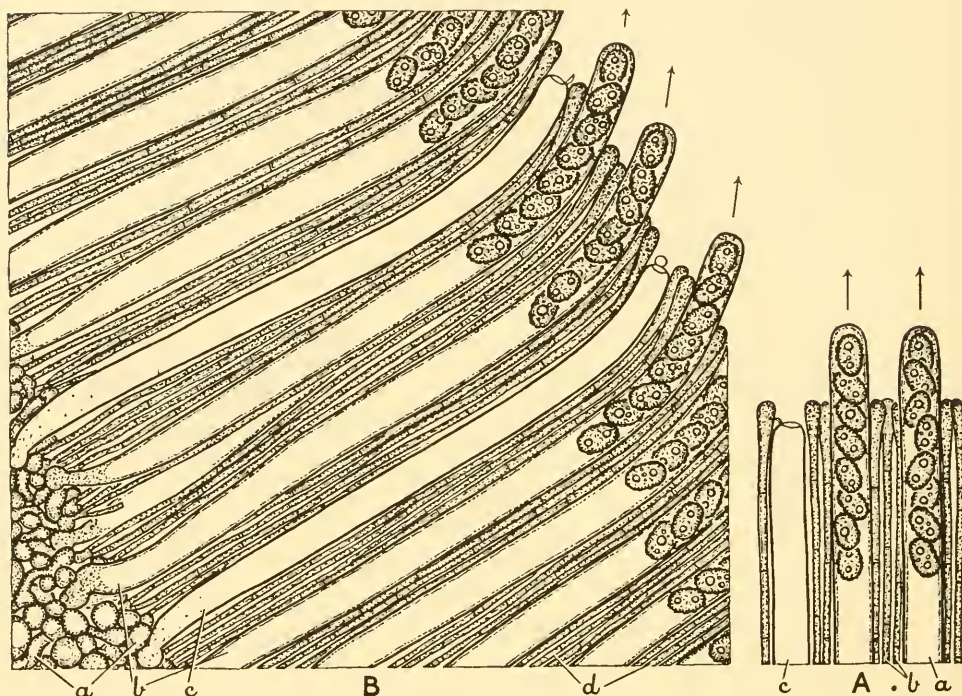
FIG. 147.—*Galactinia badia.* Heliotropism of the asci and paraphyses. Semi-
diagrammatic representation of two sections taken in a median-vertical plane
through a cupulate fruit-body. The arrows indicate the directions in which
the asci point. A, part of the hymenium at the base of a fruit-body (*cf.* Fig.
146): *a*, an ascus ready to discharge its spores; *b*, paraphyses; *c*, an ascus
which has discharged its spores; the asci and paraphyses point toward the
source of light and, therefore, straight upwards toward the middle of the fruit-
body's mouth. B, part of the hypothecium (subhymenium) and hymenium on
the left side of a fruit-body (*cf.* Fig. 146): *a*, the hypothecium; *b*, two asci
ready to discharge their spores; *c*, an ascus which has discharged its spores and
which clearly shows its operculum attached to the side of its ascostoma;
d, paraphyses; the asci and paraphyses are heliotropically bent toward the
source of strongest light and are therefore directed toward the fruit-body's
mouth. The ripe spores have rough outer walls and, as a rule, they each
contain two oil-drops. Magnification, 293.

point of the cup's mouth (Fig. 147, B). The maximum bending of
the asci about half-way down the sides of the cup was through an
angle of about 45°. It thus became clear that all the asci, wherever
situated in the hymenium, were, as in *Aleuria vesiculosa*, turned

toward the mouth of the cup, *i.e.* toward the source of light.   There can be but little doubt that this arrangement of the asci was due to these organs reacting heliotropically during their development.

A piece of a fruit-body taken from the side of a cup, including a portion of the rim, was laid flat on a slide and its hymenium was examined in face view with the microscope.   The overlapping of the ends of the asci as they pointed in the general direction of the rim of the cup could be readily seen (Fig. 148).

A fruit-body had been kept in a small closed vessel all night.   In the morning this vessel was suddenly opened and a piece of the side of the fruit-body was quickly cut away, removed to the outer air of the laboratory, and held horizontally with the hymenium looking upwards.   Immediately after this had been accomplished, the fruit-body puffed ; and it was then observed by both Mr. T. C. Vanterpool and myself that the cloud of spores emitted from the hymenium

Fig. 148.—*Galactinia badia*.   Semi-diagrammatic surface view of hymenium on the side of, and near the top of, a cup-shaped fruit-body (*cf.* Fig. 146). Asci *a* and paraphyses *b* turned upwards so as to point to the source of strongest light and, therefore, to the fruit-body's mouth.   Magnification, 345.

was not shot vertically upwards but obliquely upwards at an angle of about 45° toward the rim of the piece of fungus (Fig. 149). This direction of puffing was, of course, due to the fact that the asci had curved toward the mouth of the fruit-body and were therefore inclined toward the nearest point of the fruit-body's rim.   It may be added that the cloud of spores was seen to travel obliquely forward for about an inch and that its emission was audible.

Puffing of the fruit-bodies of *Galactinia badia* takes place not only after confinement in a laboratory but also in the open.   In an excursion in Kew Gardens Miss E. M. Wakefield and I found some large brown fruit-bodies of *G. badia* amid grass under some young trees.   I picked one of the fruit-bodies from the ground and

immediately thereafter it puffed vigorously and, in so doing, gave out an audible sound.   Had the fruit-body been left undisturbed where it had developed, probably it would have puffed sooner or later owing to its being touched by a passing animal, blown upon by rising wind, or partially dried by the loss of water vapour to the surrounding atmosphere as this became less humid.

Urnula Craterium.—*Urnula Craterium*, the *Burnt-out Crater Fungus*, is somewhat rare in Europe but is common in North America, and I have met with it several times in Canada on the shores of the Lake of the Woods and of Lake Winnipeg. It grows on the ground in woods and is attached to buried or partly buried sticks (Fig. 150) or to saw-dust, etc. (Figs. 169 and 170, pp. 335 and 336). The fruit-body is stipitate and the stipe is continued upwards into a deep black cup.   A mature fruit-body in the open, when touched, puffs vigorously, and the spore-smoke is shot out of the mouth of the cup. Here, as in *Aleuria vesi-*

FIG. 149.—*Galactinia badia.*   Diagram to illustrate an experiment on the direction of discharge of the asci.   A fruit-body matured its spores in a damp-chamber.   A piece of its side was then very quickly cut away, removed from the damp-chamber and, with the hymenium looking upwards, was laid flat on a sheet of glass in the laboratory. About two seconds thereafter, the piece of fruit-body puffed and the cloud of spores was shot away from the hymenium in the manner shown : *a* the basal end and *b* the apical end of the piece of fruit-body (shown in section) ; *c*, the sheet of glass ; the arrows indicate the direction in which the asci discharged their spores.   Natural size.

culosa and *Galactinia badia*, the safe exit of the spores from the cup is due to the ends of the asci being heliotropically turned to the source of brightest light and therefore to the opening in the top of the fruit-body.

The paraphyses of *Urnula Craterium* are thin, septate, much branched and brown above.   Their end-branches are straight and show no signs of heliotropic curvature, thus contrasting with the asci.   In *U. Craterium*, therefore, while the asci are heliotropic, the paraphyses are anheliotropic.

Otidea onotica and O. leporina.—In *Otidea onotica* (Fig. 151) and
O. leporina (Fig. 164, p. 329), the fruit-bodies are not regularly cup-

Fig. 150.—*Urnula Craterium*, the *Burnt-out Crater Fungus*, a lignicolous Discomycete common in North America but rare in Europe. It has deep and black cup-like fruit-bodies. These, at maturity, when touched in the field, puff vigorously, the spores being shot two inches or more above the mouth of each cup. The asci, here as in other cup-like fruit-bodies of Discomycetes, are positively heliotropic and have their ends pointed toward the cup's mouth. Collected near Ottawa by W. S. Odell. Photographed by the Photographic Division of the Geological Survey of Canada. Natural size.

shaped but are relatively long and narrow and are characteristically
split down one side. Owing to the split, the light strikes upon the

hymenium more from the side of the fruit-body than from the free end. Probably, therefore, in *Otidea onotica* and *O. leporina* the asci are bent heliotropically toward the open side of the fruit-body rather than to the free end. The Otideae puff freely when their mature fruit-bodies are touched, and then the clouds of spores which they emit through their asymmetrical openings can be readily observed.
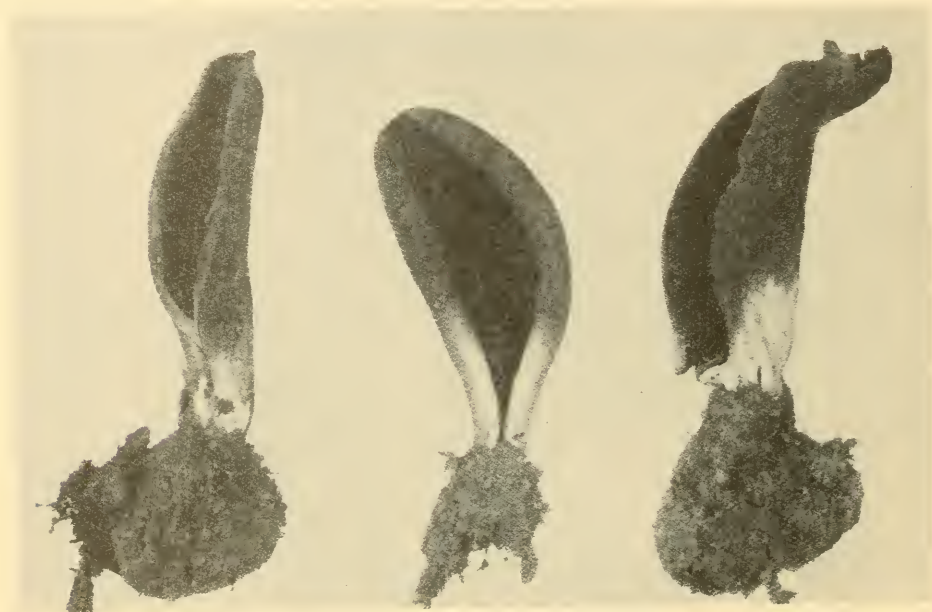
Fig. 151.—*Otidea onotica*, a Discomycete with auriculate fruit-bodies. The long lateral opening down the side of each fruit-body enabled the light to strike the hymenium more or less perpendicularly and thus, doubtless, to stimulate the asci heliotropically, so that the ends of the asci turned themselves toward the opening. When spore-discharge took place, therefore, the ascospores could readily be shot away from the fruit-body into the open air. Fruit-bodies shown were collected at the Experimental Farm, Ottawa, by W. S. Odell. Photographed by the Photographic Division of the Geological Survey of Canada. Natural size.

The Heliotropism of the Asci and the Discharge of the Spores in the Morchellaceae.—In the Morchellaceae, which includes the genera Morchella and Mitrophora : the fruit-body or *receptacle* is differentiated into a stipe and a pileus ; the pileus is hollowed out externally into pits or *alveoli* ; the hymenium is broken up into separate portions, each of which lines the interior surface of one of the alveoli ; and the alveoli are separated from one another by *ribs*

FIG. 152.—*Morchella esculenta.* A fruit-body (receptacle) with its stipe and pileus. The pileus is hollowed out externally into pits (alveoli) each of which is lined by a hymenium. The various hymenia are separated from one another by ribs which are sterile along their outer edges. The asci of each hymenial pit are heliotropically curved toward the pit's mouth and, therefore, they shoot their spores away from the fruit-body. Photographed in Yorkshire, England, by A. E. Peck. Natural size.

FIG. 153.—*Morchella esculenta.*   Upper left and right-hand fruit-bodies, showing the external appearance of the hymenial pits or alveoli.   Lower left fruit-body in median vertical section, showing the hollow structure of the stipe and pileus-flesh and the depth of the pits.   Lower middle fruit-body, a median transverse section through the pileus.   The asci are heliotropically curved toward the mouths of the pits and, therefore, shoot their spores away from the fruit-body. Photographed by the Geological Survey of Canada.   Natural size.

which are sterile exter-
nally (Figs. 152 and 153).
The fruit-body of the Mor-
chellaceae, owing to its
possessing a subdivided
hymenium, is said to be
compound.[1]

The alveoli on the
exterior of the pilei of the
Morchellaceae, as defined by
their exterior sterile ribs,
may be irregularly rounded,
irregularly polygonal, or
more or less longitudin-
ally elongated. When the
alveoli are rounded or
polygonal or but slightly
elongated, they remain
simple, although their in-
ternal lateral walls are
usually plicate (Fig. 152).
On the other hand, when
the alveoli are much
elongated longitudinally
(Figs. 154 and 155), they
are usually broken up by
more or less well developed
t r a n s v e r s e hymenium-
covered ridges. Thus a
much elongated *primary
alveolus* may be subdivided
into *secondary a l v e o l i*.
Each species has alveoli
of characteristic shape.
Thus the alveoli are : in

FIG. 154.—A fruit-body of *Morchella deliciosa*.
The pits (alveoli) are much elongated and
are divided transversely into secondary
alveoli. Collected at Ottawa by W. S.
Odell. Photographed by the Photographic
Division of the Geological Survey of Canada.
Natural size.

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[1] É. Boudier, *Histoire et Classification des Discomycètes d'Europe*, Paris, 1907, p. 30.

Morchella crassipes and *M. esculenta* (Fig. 152), irregularly rounded and plicate within ; in *M. rotunda*, irregularly quadrangular ; in *M. conica*, *M. deliciosa* (Fig. 154), *M. angusticeps* (Fig. 155), *M. costata*, *M. elata*, and *Mitrophora hybrida*, much elongated and divided transversely into secondary alveoli.[1]

Each hymenium-lined alveolus of a Morchella or of a Mitrophora, whether it be rounded, polygonal, or elongated, is comparable with the single hymenium-lined cavity that is characteristic of cupulate Pezizeae, *e.g. Galactinia badia* and *Lachnea hemispherica* ; and we can therefore think of such a fruit-body as that of *Morchella conica* or *M. crassipes* as being constructed, as it were, of a series of Peziza-cups which have coalesced to form a conical-ovate compound structure. To discover the secret of the escape of the spores from a Morchella fruit-body it is necessary to investigate the alveoli individually and to find out how the asci in their walls are arranged and in what direction they point. Such an investigation has been made on the fruit-bodies of both *Morchella conica* and *M. crassipes*.

In June, 1925, at Winnipeg, at my request, my colleague Mr. C. W. Lowe obtained some fruit-bodies of *Morchella conica* and investigated them whilst they were still fresh

Fig. 155.—A fruit-body of *Morchella angusticeps*. The primary pits (alveoli) of the pileus are divided transversely into secondary pits. Collected at Ottawa by W. S. Odell. Photographed by the Photographic Division of the Geological Survey of Canada. Natural size.

[1] For descriptions and excellent coloured illustrations of these species, except *M. deliciosa* which is not illustrated and *M. esculenta* which he has split up into *M. rotunda* and *M. vulgaris*, *vide* É. Boudier's *Icones Mycologicae*, Tomes II and IV, Paris, 1905–1910.

and in good condition.   With a hand-razor he cut transverse sections through the alveoli, mounted them in water, and studied them under the microscope ; and he convinced himself that, in each alveolus, the asci were so arranged that their apices were turned toward the mouth of the alveolus, *i.e.* in the direction from which the asci had been illuminated during their development (Fig. 156).   The asci on the sides of an alveolus were curved outwards often through an angle of about 45°, whilst those at the base of a chamber were straight.   Subsequently, using dried and pickled material (the former cut with a hand-razor and the latter with the help of a microtome), I confirmed Mr. Lowe's observations (Figs. 157 and 158).

The curvatures of the asci in an alveolus of *Morchella conica* are strictly comparable to the curvatures of the asci in the cup of *Aleuria vesiculosa* or *Galactinia badia*, and there can be little or no doubt that they are due to reactions to the heliotropic stimulus of light.

A thin median-vertical section of a fruit-body of *Morchella conica*, such as that shown in Fig. 157, A, not only permits one to perceive the disposition of the hymenium within the alveoli, but also reveals the very light physical framework which serves to support the hymenium and to hold it in a position favourable for the discharge of the spores : the flesh of the stipe and of the pileus roughly constitutes a cylinder, and the interior of the cylinder is occupied by a relatively very large air-space.   Here, as in the stipes of many Agaricaceae, *e.g. Coprinus sterquilinus* (Vol. IV, Fig. 66, p. 116), and as in the stems of many Phanerogams, *e.g.* Gramineae and Umbelliferae, the disposition of the supporting substance in the form not of a solid cylinder but of a hollow one increases the power of the organ to withstand mechanical stresses and strains.

On June 13, 1928, at Victoria Beach on Lake Winnipeg, Dr. G. R. Bisby and I hunted the woods for Morchellaceae and found several large fruit-bodies of *Morchella crassipes*.   With the help of a hand-razor and a travelling microscope set up in the field-station of the Manitoba Natural History Society, I examined the alveoli in cross-section and found that, just as in those of *M. conica*, the asci were curved towards the mouths of the alveoli.   Apparently they had all responded to the heliotropic stimulus of light.   Dr. Bisby, who was good enough to examine my preparations, confirmed my observations.

Boudier,[1] in his illustrations of *M. crassipes*, represents four asci all of which are curved at their ends. The upper parts of two
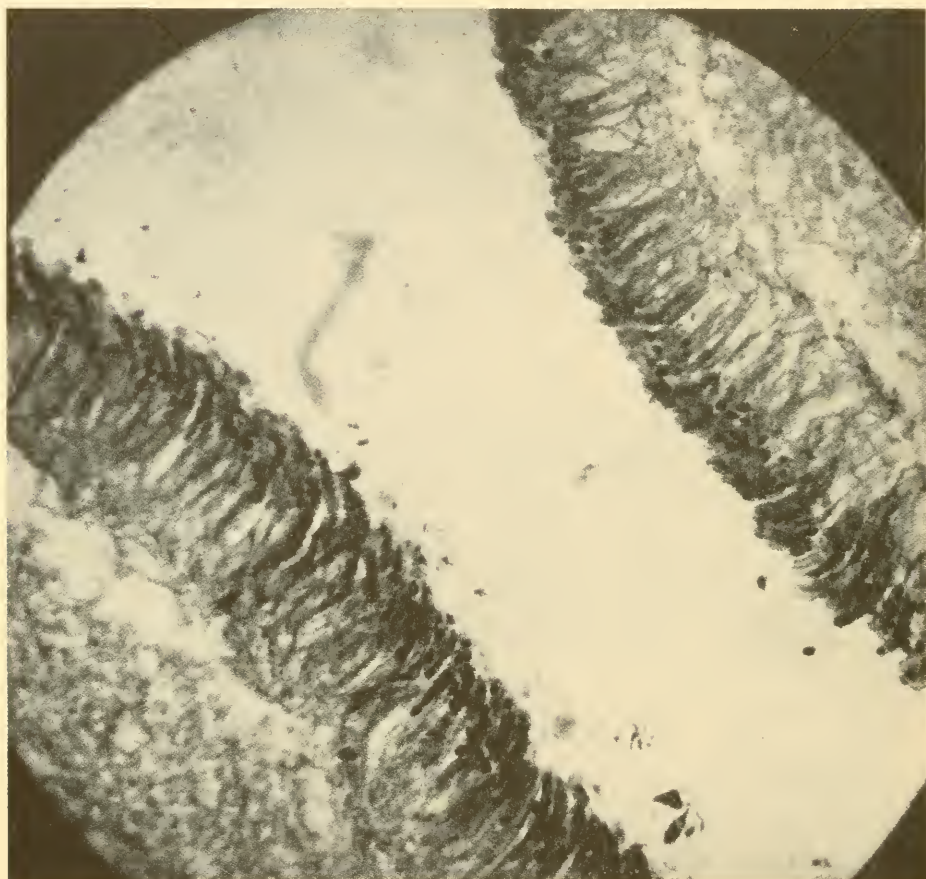
Fig. 156.—Photomicrograph of part of a transverse section through a pit (alveolus) of *Morchella conica*, to show the asci heliotropically curved toward the pit's mouth. The material was fixed, stained, and cut with a microtome. The opposing walls of the pit are nearer together than they were before the material was fixed. The ends of the asci are curved through an angle of about 45°, so that the spores, if the fruit-body had not been disturbed, would have been shot away from the fruit-body without hitting any opposing hymenial wall. Photographed by C. W. Lowe at Winnipeg. Highly magnified.

of the asci (Fig. 159), one containing spores and the other after discharge, enlarged 820 times, are both drawn with their ends curved

[1] É. Boudier, *loc. cit.*, Tome II, Plate CXCIV, *b, d, e.*

through an angle of about 45°.   Doubtless the curvature of these
asci was heliotropic in origin.

Since, in a fruit-body of a Morchella, the asci in each alveolus
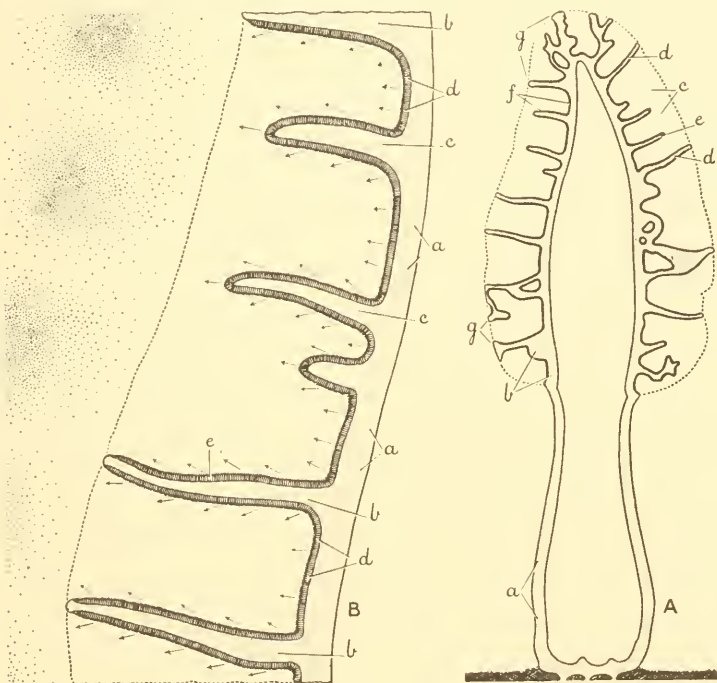
FIG. 157.—*Morchella conica.*   A, a thin median-vertical section through
a receptacle (fruit-body) : *a*, the stipe ; *b*, the pileus ; *c*, a longi-
tudinally elongated alveolus (pit) separated from neighbouring alveoli
by dissepiments *d d* and having transverse ridges *e* within.   The
hymenium *f* covers the interior surface of each alveolus and is
absent only on the free edges of the dissepiments, which form sterile
ribs *g g*.   The broken line indicates the outermost limits of the alveoli.
B, two alveoli of A, shown on a larger scale : *a*, the pileus-flesh ; *b b*,
dissepiments ; *c c*, transverse ridges ; *d*, the hymenium covering the
interior of the alveoli, but absent from the exterior edges (ribs) of
the dissepiments ; *e*, a portion of the hymenium illustrated on a
larger scale in Fig. 158.   The asci are all directed toward the source
of strongest light and therefore toward the mouth of each alveolus.
The arrows indicate the general directions in which the spores are
shot, and the clouds of spores outside the mouths of the alveoli have
just been formed in consequence of the receptacle having suddenly
puffed in response to the application of heat from a lamp (*cf.* Fig. 128).
A, natural size.   B, four times the natural size.

point toward the mouth of the alveolus and since the operculum of
each ascus is situated symmetrically at the end of the ascus, it is

clear that, when spore-discharge takes place, the spores must all be shot through the mouths of the alveoli into the open air (Fig. 157, B). Thus, so far as the escape of the spores from a fruit-body of Morchella is concerned, we have a direct and very simple explanation, one that is in conformity with what is known concerning the escape of the spores from the fruit-bodies of simpler Discomycetes, such as species of Ciliaria, Aleuria, and Galactinia, and one which, clearly, must take the place of Falck's *Temperaturströmungen* theory already discussed and criticised in the Introduction to this Chapter.[1]

The asci on the walls of an alveolus of a Morchella fruit-body all look toward the mouth of the alveolus and toward that part of the mouth from which come the strongest incident rays of light. Therefore, when puffing takes place, the spores of any single alveolus are all shot outwards in great numbers in the same general direction. This being so, the same results accrue as those already experimentally investigated in connexion with puffing from the cup of *Sarcoscypha protracta* : the spores discharged from the alveolus strike the air almost simultaneously, in the same general direction, and very violently ; they set the air in motion ; and they cause an air-current to come into existence sufficiently strong to carry them farther from the fruit-body than they could travel by their own momentum.[2] It was mechanically produced air-currents of the kind just described which Falck perceived in his investigations on the discharge of spores in Morchella and its allies and which he mistook for his "Temperaturströmungen."

Fig. 158.—*Morchella conica.* Semi-diagrammatic vertical section through the hymenium on the upper side of a horizontal dissepiment between two alveoli (*cf. e* in Fig. 157, B). The asci, owing to their having responded to the stimulus of light, are all curved at their ends toward the mouth of the alveolus in which they grew. The arrows indicate the directions in which the spores would be shot. Magnification, 293.

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[1] *Vide supra*, pp. 268–270.   [2] *Vide supra*, pp. 257–260.

The formation of radially-projecting dissepiments and ridges on the exterior of a pileus in the Morchellaceae (Fig. 157) is comparable with the formation of gills on the under side of the pileus in the Agaricaceae ; for in both groups of fungi the same mechanical advantage is obtained, namely, a considerable increase in the area of the spore-bearing layer or hymenium without the hymenophore losing its compactness as a whole.   The gills of the Agaricaceae are often

closely packed : indeed, the upper part of the interlamellar space between two adjacent gills in many agarics, *e.g.* *Psalliota campestris* (*vide* Fig. 139 in Vol. II, p. 390), is often reduced to 1 mm. or less.   On the other hand, in most of the Morchellaceae, the alveolar spaces are usually several mm. in diameter.   Correlated with this difference in the spatial arrangement of opposing hymenial walls is the fundamental difference in the structure and power of the guns in the two groups of fungi. The basidia of the Agaricaceae are relatively small and feeble guns with a range of only 0·1–0·2 mm.,

FIG. 159.—*Morchella crassipes.*   Asci sharply curved at their ends.   Magnification, 820.   Copied by A. H. R. Buller from Boudier's *Icones Mycologicae*, T. II, Pl. 194.

and the basidia on one gill cannot therefore bespatter with spores the opposing hymenium of another gill, even when the interlamellar space is only 0·5 mm. in width (*cf.* Fig. 139 in Vol. II, p. 390).   In contrast with such basidia, the asci of the Morchellaceae are relatively large and powerful guns with a range of 1–3 cm., and they therefore require much more room for discharge than basidia. The actual space provided for them is what is necessary to permit of all the asci turning toward, and shooting their spores through, the mouths of the alveoli.[1]

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[1] For other remarks on the manner in which the basidium has influenced the structure of hymenomycetous fruit-bodies and the ascus has influenced the structure of discomycetous fruit-bodies *vide* these *Researches,* Vol. I, 1909, pp. 22–24 ; also *cf.* Vol. II, 1924, pp. 67–68.

As shown by data embodied in a comparative Table [1] in Volume I, the spores of the Discomycetes and of the Agaricaceae and Polyporaceae are of the same order of size, and they therefore have about an equal chance of being dispersed by the wind when they come within its sweep. In the Agaricaceae and Polyporaceae, the spores, after discharge, fall down between the gills or down the hymenial tubes and thus leave the fruit-body ; but, as if to facilitate the spores being carried off by the wind, the pilei which produce them are usually raised up on stipes or project laterally from tree-trunks, etc., a free subpilear space for the passage of the wind and the removal of the escaping spores thus being provided. In the Discomycetes, where the asci look upwards, as in the Pezizaceae, or more or less upwards or laterally, as in the Morchellaceae, the chances that the wind will carry off the spores are enhanced : (1) by the considerable range of the individual ascus guns ; (2) in Pezizaceae, etc., by the simultaneous discharge of many of the guns—the phenomenon known as puffing [2] ; and (3), in many species of Pezizaceae and in all the Morchellaceae and Helvellaceae, by the raising of the pilei above the surface of the ground by means of stipes comparable with those of the Agaricaceae and Polyporaceae.

It is characteristic of the Morchellaceae and many Helvellaceae that the fruit-bodies appear in the spring, from March to May, *i.e.* at a period of the year when the night temperatures are relatively low. Falck's observations in the laboratory have led him to suppose that, in a Morchella or Gyromitra growing under natural conditions : (1) at night and during the day when the sky is over-cast and the temperature is low, ripe asci accumulate in the fruit-bodies but no spores are discharged ; (2) the discharge of spores takes place only in the daytime when the fruit-bodies are warmed by the sun ; and (3), in bright sunshine lasting for some hours, the fruit-bodies discharge their spores continuously, *i.e.* in the order in which the asci ripen and not in intermittent clouds.[3] Thus, according to Falck, normal spore-discharge in a Morchella or Gyromitra does

[1] These *Researches*, Vol. I, 1909, p. 248.

[2] *Vide supra*, p. 262.

[3] R. Falck, " Ueber die Sporenverbreitung bei den Ascomyceten. I. Die radio-sensiblen Discomyceten," *Mycologische Untersuchungen und Berichte* von R. Falck, Bd. I, Heft II, 1916, pp. 126–127.

not take place below a certain temperature (about 10° C.) [1] and, when it does take place, it is accomplished not by a series of vigorous and intermittent puffs but by the successive bursting of asci which results in the liberation of a stream of spores like that which escapes from the pileus of one of the Hymenomycetes.

Falck found that two fruit-bodies of *Morchella esculenta* lying in the shade in a cool room had a temperature of 15·3° C. and were not discharging spores. He thereupon exposed the fruit-bodies to direct sunlight. After one minute a thermometer behind the irradiated hymenium registered 18° C. and spore-discharge had become active. After five minutes the thermometer registered 19·8° C. and spore-discharge continued. He then placed the fruit-bodies in the shade. After four minutes the thermometer registered 16·9° C. and the discharge of the spores had ceased.[2] These experiments strongly support Falck's conclusion that the sun, under natural conditions, must often be a prime factor in causing fruit-bodies, not only of Morchellae but of Gyromitrae, Verpae, and other vernal radiosensitive Discomycetes, to discharge their spores.

Falck [3] regards the brown colour of the caps of Morchella, Gyromitra, etc., as an aid to the absorption of the sun's rays and therefore as advantageous for the working of the spore-discharge mechanism.

The Helvellaceae.—The Helvellaceae, according to Boudier,[4] include the following genera : Ptychoverpa, Verpa, Gyromitra (Figs. 160 and 161), Physomitra, Helvella (Fig. 163, p. 328), and Leptopodia. In all the species of this family the fruit-bodies are stipitate, while the pileus is attached by its centre to the stipe, is reflexed in the form of a hood, and is more or less lobed.

Several illustrations of asci of various species of Helvellaceae drawn by Boudier and reproduced in his *Icones Mycologicae* suggest that the asci of the Helvellaceae are heliotropic. Among these illustrations are : (1) an ascus of *Gyromitra gigas* [5] with

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[1] R. Falck, " Ueber die Sporenverbreitung bei den Ascomyceten. I. Die radio-sensiblen Discomyceten," *Mycologische Untersuchungen und Berichte* von R. Falck, Bd. I, Heft II, 1916, pp. 91–92.

[2] *Ibid.*, p. 102.   [3] *Ibid.*, pp. 84, 114–115, 125.

[4] É. Boudier, *Histoire et Classification des Discomycètes d'Europe*, Paris, 1907, pp. 33–38.

[5] É. Boudier, *Icones Mycologicae*, Tome II, Pl. 221, *f*.

an operculum tilted at an angle of 45° to the slightly curved ascus axis; (2) two asci out of the three of *Ptychoverpa*
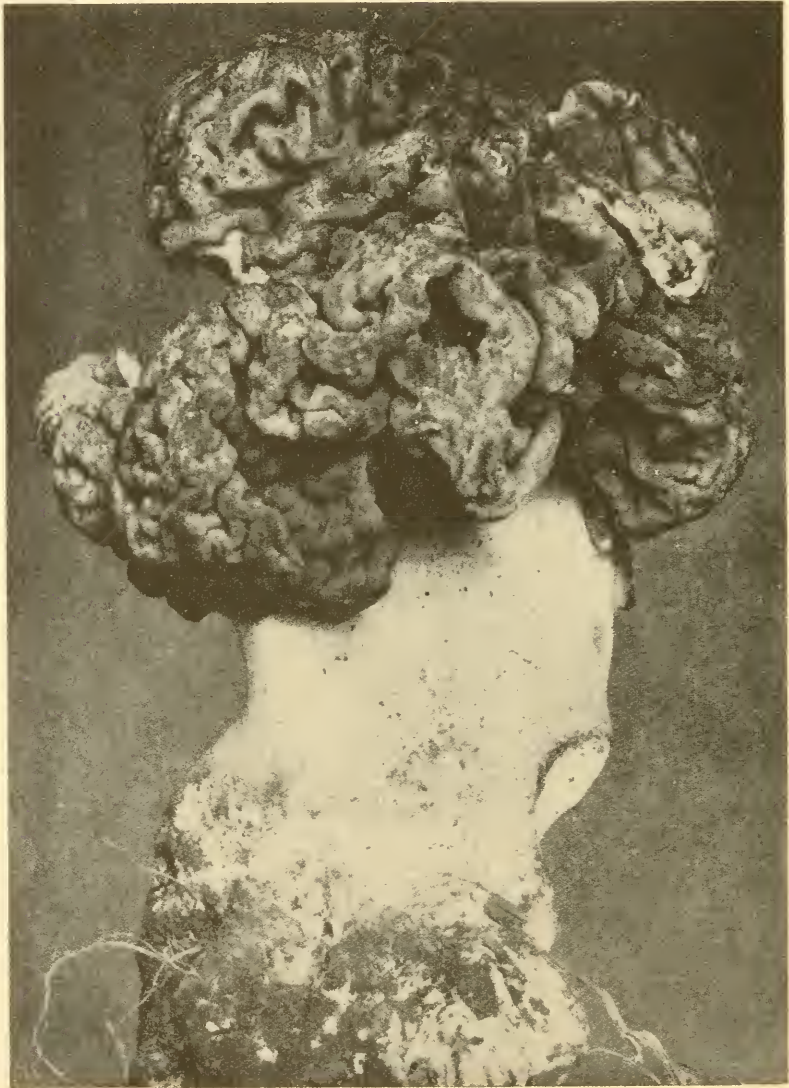
FIG. 160.—A fruit-body of *Gyromitra esculenta*. The hymenium covering the pileus is much folded. The escape of the spores from the sides and crevices of the folds is doubtless aided, here as in the Morchellaceae, by the heliotropic curvature of the ends of the asci. Photographed in Yorkshire, England, by A. E. Peck. Natural size.

bohemica[1] sharply turned at their ends through an angle of about 45°; (3) an enlarged outer part of an ascus of *Helvella sulcata*[2] turned at its end through an angle of 40°; and

FIG. 161.—Another fruit-body of *Gyromitra esculenta*. The folds in the hymenium are well developed. If the asci are heliotropic, as in the Morchellaceae, those on the sides of the folds could readily shoot their spores away from the fruit-body. Photographed at Ottawa by W. S. Odell in the spring, 1927. Natural size.

(4) a discharged ascus of *Leptopodia elastica*[3] with its operculum in face view very obliquely situated on the ascus end.

The only member of the Helvellaceae which so far I have investigated is *Ptychoverpa bohemica* (Corda) Boud., often referred to as *Verpa bohemica*. The hymenium differs from that of the species

[1] É. Boudier, *Icones Mycologicae*, Tome II, Pl. 218, *d*.
[2] *Ibid.*, Pl. 229, *g*.   [3] *Ibid.*, Pl. 232, *h*.

of Verpa in that it is folded into numerous longitudinal and rather freely anastomosing ribs, and the asci are remarkable in that they each contain two very large spores (15–18 × 60–80 μ, Seaver [1]) instead of the usual eight relatively small spores.

On May 21, 1932, my colleague, Dr. G. R. Bisby, kindly brought to me from the grounds of the Manitoba Agricultural College several rather old fruit-bodies of *Ptychoverpa bohemica*. I cut transverse sections through their pilei, examined these sections under the microscope, and at once perceived that in all the hymenial grooves and depressions the asci were curved outwards so that their opercula must have faced the strongest rays of light to which the ends of the asci had been subjected in the places where the fruit-bodies developed. Thus clear evidence was obtained that in the Helvellaceae, just as in the Morchellaceae, the asci are heliotropic.

Concluding Remarks.—From the investigations which have been recorded in this Chapter, we may conclude that, in a large number of Discomycetes belonging to various families : (1) the asci are positively heliotropic ; and (2) this response to the stimulus of light is of biological significance in that it permits of a fruit-body pointing and discharging its asci toward open spaces, thus increasing the chances that the spores will be carried off and be dispersed by the wind.

It is not without interest to note that, while in the Discomycetes heliotropism of the asci is a common phenomenon, in the Hymeno-mycetes heliotropism of the basidia is unknown. The ends of the basidia on the sides of the gills of the Agaricaceae and on the sides of the hymenial tubes of the Polyporaceae are of necessity illuminated from below, and yet they never turn downwards. Owing to the peculiar organisation of the fruit-bodies in these Hymenomycetes and to the very short range of the basidial guns, it is easy to see that a heliotropic response on the part of the basidia would not aid the escape of the spores from the hymenium into the interlamellar or tubular spaces but would actually hinder it. Thus, where a heliotropic response by a fungus gun is advantageous for spore-dispersal, as it is in the Discomycetes, it takes place ; and, where it would be disadvantageous for spore-dispersal, as in the Hymenomycetes, it does not take place.

[1] F. J. Seaver, *The North American Cup-fungi (Operculates)*, New York, 1928, p. 244.

CHAPTER III

THE SOUND MADE BY FUNGUS GUNS AND A SIMPLE METHOD
FOR RENDERING AUDIBLE THE PUFFING OF DISCOMYCETES

Fungus Projectiles—Type I : Sphaerobolus—Type II : Pilobolus—Type III :
    Ascobolus—Type IV : Peziza—A Simple Method for Rendering Audible the
    Puffing of Discomycetes—Type V : Empusa and Entomophthora, Uredineae
    and Hymenomycetes— Sounds Made by Fungus Guns are of No Biological
    Significance.

Fungus Projectiles.—The various projectiles of fungi, in order of
size, may be classified as follows :

   I. A gleba, containing a great number of spores, as in *Sphaero-
        bolus stellatus,*

  II. A sporangium, containing about 50,000 spores, with
        sporangiophore-sap attached, as in Pilobolus,

 III. An aggregation of eight spores forming a single mass,
        together with ascus-sap, as in *Ascobolus immersus,*

  IV. A chain of eight spores which breaks up into its eight com-
        ponents on leaving the ascus, as in Peziza and Pustularia,
        and

   V. Single spores, as in the conidia of Empusa and Entomoph-
        thora, and as in the basidiospores of the Uredineae and
        Hymenomycetes.

Type I : Sphaerobolus.—The gleba of *Sphaerobolus stellatus*—
the largest known fungus projectile—is a viscid, smooth, dark,
spherical body, 1–1·25 mm. in diameter (Vol. V, Fig. 145, p. 294),
and it is cast out of the Sphaerobolus gun (Vol. V, Fig. 172, *c*, p. 361)
by a catapult mechanism which has already been described.[1]   The
gun, when about to discharge its projectile, is 2–2·5 mm. in diameter.
Its maximum *horizontal* range, as measured by myself, was found

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[1] These *Researches*, Vol. V, 1933, pp. 310–325.

to be 18 feet 7 inches ; and its maximum *vertical* range, as measured by Leva B. Walker, was found to be 14 feet 5 inches.   For *Sphaero-bolus stellatus* var. *giganteus* and for *S. iowensis* Miss Walker found the maximum vertical range of the projectiles to be about 11 feet and about 12 feet respectively.[1]

The sound of the discharge of the Sphaerobolus gun is quite distinct and has long been known.   It was heard by E. Fischer [2] in 1884 ; and it was mentioned by de Bary [3] in 1884 and by Zopf [4] in 1890.   I heard it as a little snap when the gun was a foot in front of my eyes.[5]   Of all fungus guns the Sphaerobolus gun is not only the largest and most powerful, but also the loudest.   Its sound is much louder than that of the ascus of Peziza and appreciably louder than that of the sporangiophore of Pilobolus.

The projectiles of Sphaerobolus are shot away with considerable speed and, as they are of the size of very small shot, it is not sur-prising that, when they strike a hard object near their place of discharge, the impacts are audible.   These impact-sounds were remarked by Micheli [6] in 1729 and have been noticed by a number of observers since.   Some thirty years ago I had some Sphaerobolus fruit-bodies in a covered glass dish, and I remember very well hearing the impact of the glebae as they struck the glass cover and flattened out upon it.   The sound of a glebal projectile striking against glass in this way is distinctly louder than the sound of the discharge of the gun which fires the projectile.

Type II : Pilobolus.—The sporangium of the larger species of Pilobolus (Fig. 27, p. 69) and the sap of the subsporangial swelling which travels with it make up a projectile (Fig. 74, p. 151) which is nearly spherical and about 0·6 mm. in diameter.   The projectile can be fired upwards from the sporangiophore a distance of 3–6 feet and horizontally 4–8 feet.[7]   As with Sphaerobolus, two sounds can

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[1] These *Researches*, Vol. V, 1933, pp. 325–335.

[2] E. Fischer, " Entwicklungsgeschichte der Gastromyceten," *Bot. Zeit.*, 1884, Nos. 28–31.

[3] A. de Bary, *Vergleichende Morphologie und Biologie der Pilze*, Leipzig, 1884, p. 353.

[4] W. Zopf, *Die Pilze*, Breslau, 1890, p. 375.

[5] These *Researches*, Vol. V, 1933, pp. 325 and 326.

[6] P. A. Micheli, *Nova Plantarum Genera*, Florentiae, 1729, p. 221.

[7] *Vide supra*, pp. 66–68.

be heard when a Pilobolus gun goes off : (1) the sound of the exploding gun and (2) the sound of the projectile striking some object. W. B. Grove,[1] in 1884, was the first to hear the sound emitted by the gun, and in 1909 I confirmed his observations.[2]   More recently, in my laboratory, I listened to the shooting away of the sporangia of *Pilobolus longipes* and, each time a gun went off, I distinctly heard a minute snap.   I asked my colleague Mr. C. W. Lowe and my laboratory attendant to listen also, and they both declared that

FIG. 162.—*Acetabula vulgaris.*   Anton de Bary records (1884) that he heard a very perceptible hissing sound when large specimens of this fungus puffed vigorously.   Photographed by A. E. Peck at Scarborough, England.   Natural size.

the sound of each explosion was unmistakable.   In Volume I of this work, I described a simple experiment which enabled a listener to hear the striking of a Pilobolus projectile on a drum when he was 21 feet away from the place of impact.[3]

Type III : Ascobolus.—In a fruit-body of *Ascobolus immersus* (Vol. I, Fig. 81, p. 252) about half a dozen asci come to maturity and explode every day about noon.   The asci and ascospores are

[1] W. B. Grove, " Monograph of the Pilobolidae," *The Midland Naturalist*, Birmingham, England, 1884, p. 15.

[2] These *Researches*, Vol. 1, 1909, p. 259.   [3] *Ibid.*, pp. 259–260.

very large. The eight ascospores of each ascus have thick gelatinous outer walls, and adhere firmly together both before discharge and when being shot through the air. The projectile — an aggregate of eight spores surrounded by a film of ascus-sap—is oval in form, about 0·3 mm. long, and 0·15 to 0·2 mm. wide ; and it can be shot directly upwards 10 inches or occasionally 14 inches.[1] In respect to *A. immersus* no one, as yet, has heard either the sound of an ascus exploding or the sound of the projectile striking another object ; but it is probable that both these sounds might be detected by an attentive listener.

Type IV : Peziza.— In Peziza, Aleuria (Fig. 140, p. 293), Galactinia (Fig. 147, p. 306), Pustularia, etc., the ascus contains eight spores, as in *Ascobolus immersus* ; but the eight spores separate

FIG. 163.—*Helvella crispa.* Anton de Bary records (1884) that he heard a very perceptible hissing sound when large specimens of this fungus puffed vigorously. Photographed by A. E. Peck at Scarborough, England. Natural size.

from one another as soon as they are shot out of the ascus (Vol. I, Fig. 78, p. 236). There are many tens of thousands of asci in each

[1] These *Researches*, Vol. I, 1909, p. 252.

large fruit-body, and the fruit-body is usually more or less cup-shaped or wine-glass-shaped. An ascus, when exploding, makes a faint sound ; but this sound is so faint that, if each ascus were fired off in a solitary manner, it might have escaped detection. However, the fruit-bodies of Peziza, Pustularia, Urnula, Otidea, Rhizina, Helvella, and many other Discomycetes exhibit the curious

phenomenon known as *puffing* : when certain conditions surrounding a mature fruit-body are suddenly changed, thousands of asci may explode almost simultaneously and thus produce a cloud of white spores which can be seen floating away in the air (Fig. 114, p. 234).[1] The puffing of certain Discomycetes has been heard. Thus Desmazières,[2] in 1845, recorded that the spore-vapour of *Helvella ephippium* is discharged into the air with a faint report ; de Bary,[3] in 1884, stated that he

Fig. 164.—Some of the ear-like fruit-bodies of *Otidea leporina*. This fungus puffs audibly, when touched. Photographed in England by Somerville Hastings. Natural size.

had been able to hear " a very perceptible hissing sound produced by large specimens of *Peziza acetabulum* (Fig. 162) and *Helvella crispa* (Fig. 163) " ; and Stone,[4] in 1920, announced that in a

[1] *Vide supra*, pp. 227–234.

[2] J. B. H. J. Desmazières, *Plant. crypt. France*, 2nd ed., fasc. XIX, 1845, No. 914. Cited from Tulasne.

[3] A. de Bary, *Vergleichende Morphologie und Biologie der Pilze*, Leipzig, 1884, p. 98. (English Translation, 1887, p. 89.)

[4] R. E. Stone, " Upon the Audibility of Spore Discharge in *Helvella elastica*," *Trans. Brit. Mycol. Soc.*, Vol. VI, 1920, p. 294.

very quiet room at the Ontario Agricultural College at Guelph he distinctly and repeatedly heard the puffing of *Helvella elastica* even when the fruit-bodies were several feet distant.

Observations on the audibility of spore-discharge in Discomycetes have also been made by R. B. Johnstone.[1] This observer collected fruit-bodies of *Otidea leporina* (Fig. 164) at Perth, Scotland, in September, 1920, and took to Glasgow half a dozen mature

FIG. 165.—*Sarcoscypha coccinea*, a large Discomycete which comes up in early spring on sticks and which, owing to the very pure and beautiful scarlet colour of its hymenium, is attractive even to children. The puffing of its fruit-bodies when handled was heard by the late E. J. Durand. The fruit-bodies shown were collected near Ottawa by W. S. Odell. Photographed by the Photographic Division of the Geological Survey of Canada. Natural size.

specimens packed in a box, each one wrapped in paper. The box remained closed for 24 hours. " On opening the box," says Johnstone, " the specimens were placed on the table, and I attended to something else. While thus engaged I heard every now and then a slight hissing sound, but, being busy, paid no attention to it, till looking by chance at the table I saw one of the Otideae puff, and

[1] R. B. Johnstone, " Audibility of the Spore Discharge in *Otidea leporina*," *Trans. Brit. Mycol. Soc.*, Vol. VII, 1921, p. 86.

immediately heard a hiss.   The hiss was quite distinct, and required no effort to hear it, although I was fully six feet from the plants. Mr. Stone's experience occurred to me and, placing my six specimens in a row, I sat for some time watching my miniature field-battery at work.   First one fired (puffed), followed by the report (hiss), then another carried on, the others following in their turn at intervals of not more than two or three minutes.   I noticed as the time passed that the intervals between the puffs increased in length.   What surprised me was the frequency with which the fruit-bodies puffed.''

The late Professor E. J. Durand of the University of Minnesota, who studied the Discomycetes for many years, informed me that incidentally, in the course of his work, he had heard the puffing of the fruit-bodies of several species, among which were :

| Aleuria repanda, | Sarcoscypha coccinea (Fig. 165), |
| Aleuria vesiculosa, | Sclerotinia tuberosa. |

A Simple Method for Rendering Audible the Puffing of Discomycetes.—I have discovered that the puffing of any Discomycete can be readily heard, if one listens to it in a particular manner which is now to be described ; and this discovery was communicated to the British Mycological Society during the Minehead Foray in October, 1920.[1]

On August 7, 1920, in the company of Mr. W. B. Grove, I visited Queen's Cottage Grounds, Kew Gardens, and there found several cup-like fruit-bodies of *Pustularia catinus* (Holms.) Fuck. (Fig. 166). These were put in a small cardboard box where they remained during the night.   The next morning the box was taken to the Kew Herbarium and there opened for the purpose of identifying the fruit-bodies which it contained.   At once one of the fruit-bodies puffed, and a cloud of white spores could be seen escaping from the hymenium.   I heard nothing, but I thought of the sound one hears when one places a sea-shell against one's ear, and I wondered whether or not I should be able to hear the puffing of the Pustularia if I put one of its little cups to my ear.   No sooner thought of than done.

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[1] The Minehead Foray, *Trans. Brit. Mycol. Soc.*, Vol. VII, 1921, p. 4.

I put the fruit-body which had already puffed to my ear ; it puffed again ; and I distinctly heard the puffing which was like a rush of steam from a minute jet. I tried some of the other fruit-bodies with a similar result, and Mr. W. B. Grove who was examining the fungi with me confirmed my observations. In the course of the next two days, the puffing of the Pustularia fruit-bodies was distinctly heard by Miss E. M. Wakefield and by Dr. T. Petch. The little cups puffed each morning when the box was opened, for six consecutive days. If one puts a Pustularia cup to one's ear, when it puffs one can not only hear the sound of the puffing but also feel the spray from the asci as though the ear were being sprayed by a fine atomiser. When the fungus is close to an ear, the puffing can be heard even when the room is not quiet and one is talking to friends.
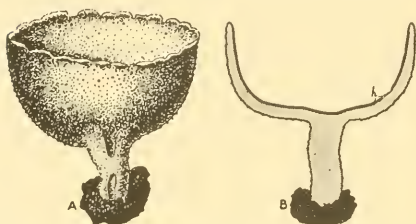
Fig. 166.—Fruit-bodies of *Pustularia catinus*. They puff audibly. A, a whole fruit-body. B, a median-vertical section ; *h*, the hymenium. Copied by A. H. R. Buller from Boudier's *Icones Mycologicae*, Tome II, Pl. 336. Natural size.

Using the method just described I have succeeded in hearing the puffing of the following species :

Aleuria repanda,
Aleuria vesiculosa,
Ascobolus stercorarius,
Caloscypha fulgens,
Ciliaria scutellata,
Galactinia badia,
Peziza aurantia,
Pseudoplectania nigrella,
Pustularia catinus,
Pyronema confluens,
Rhizina inflata,
Sarcoscypha protracta,
Sarcosphaera coronaria,
Urnula Craterium,
Urnula geaster.

Aleuria repanda puffs audibly in the same manner as *Pustularia catinus*. I gathered one of its fruit-bodies from an old log in a wood at Kenora on the Lake of the Woods, took it to Winnipeg, placed it in a closed glass dish, and kept it there undisturbed for 24 hours. I then took the cover from the dish, picked up the fruit-body, and at once placed it close to my ear. Immediately thereafter the fruit-

body puffed vigorously, the puffing continuing for about 2 seconds. During this time I felt my ear being sprayed with the contents of many thousands of asci, and I distinctly heard a loud hissing sound. Then the fruit-body became quite silent.

Aleuria vesiculosa (Fig. 167), which in the first volume of these *Researches* I incorrectly called *Peziza repanda*, puffs audibly like *A. repanda* and *Pustularia catinus*. Some horse-dung balls obtained from the streets of Winnipeg in a frozen condition were kept moist
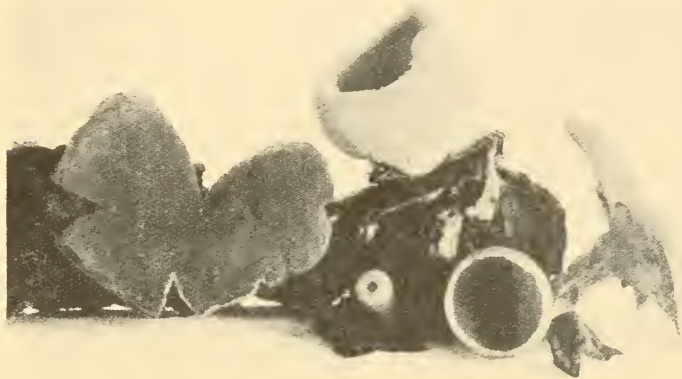
FIG. 167.—*Aleuria vesiculosa* (= *Peziza repanda* of Vol. I), a Discomycete that puffs audibly. The fruit-bodies came up spontaneously on horse dung kept in a large glass case in the laboratory at Winnipeg. They puffed only when fully expanded. Natural size.

in a large glass damp-chamber in the laboratory. The mycelium of *Aleuria vesiculosa* grew spontaneously in the dung-balls and, after about a month, gave rise to several beautiful clusters of fruit-bodies.[1] These were at first globose and closed, but they soon expanded, the extreme margins of the cups remaining more or less incurved. The discs were pallid-brown and the external surface furfuraceous, *i.e.* coarsely granular or warted. The discharge of the spores did not begin until the fruit-bodies had become more or less flattened out. I placed one of the flattened fruit-bodies in a small glass box. Next day I removed the lid of the box and at once put the fungus

[1] The clusters resembled the one in the photograph reproduced as Fig. 432, p. 508, in M. E. Hard's *The Mushroom, Edible and Otherwise*, Ohio, U.S.A., 1908.

to my ear. Puffing took place almost instantly, and I distinctly heard the little blast which lasted for 1–2 seconds. The fruit-body was then put back in the box. On listening as before, I heard the fruit-body puff on each of the next two succeeding days.

I found some large fruit-bodies of *Peziza aurantia* (Fig. 168) in
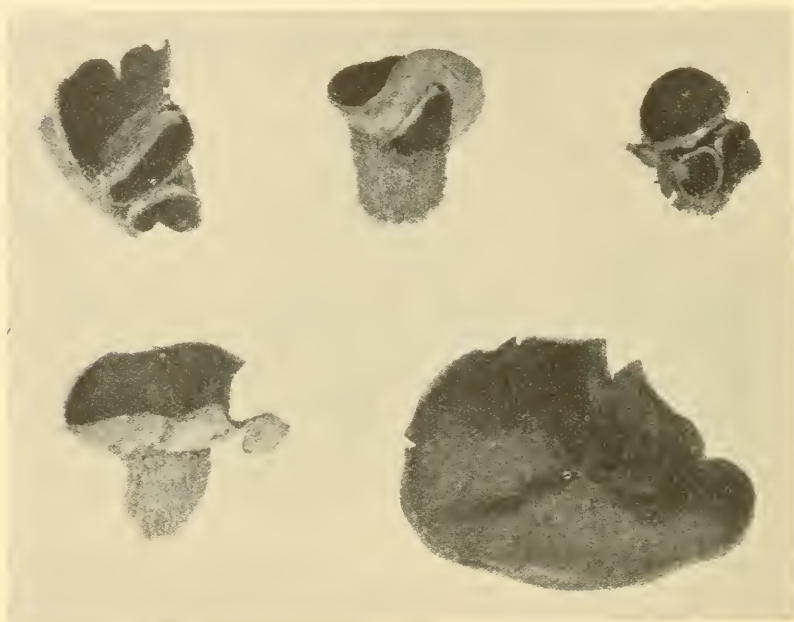
Fig. 168.—*Peziza aurantia*, a well-known Discomycete with deep orange or orange-red fruit-bodies. The puffing of individual fruit-bodies when placed against an ear was heard by the author. The fruit-bodies shown were obtained from above sand below a stump at Ottawa by W. S. Odell. Photographed by the Photographic Division of the Geological Survey of Canada. Natural size.

September, 1921, when at the Worcester Foray of the British Mycological Society, and kept them in a vasculum for about 24 hours. During this time they were slowly drying. I then opened the vasculum and at once put one of the fruit-bodies to my ear. It puffed loudly for about 1–2 seconds in the same manner as the species of Pustularia and Aleuria already described. I succeeded in demonstrating the audibility of puffing for this species to several of my companions.

During the Worcester Foray just mentioned Miss M. A. Brett

found some of the subferruginous, ear-shaped fruit-bodies of *Otidea leporina* (Fig. 164, p. 329) growing on the ground in Ockeridge wood. When she touched them with her hand, they emitted clouds of spores. She stooped down and, on placing her ear against one of them, distinctly heard it puff. Puffing, therefore, can be heard when fruit-bodies are growing under natural conditions in the open.

On September 5, 1924, in Kew Gardens, I found a cluster of fruit-bodies of *Galactinia badia*. On picking a large one and at once placing it against my ear, I heard it puff vigorously.

Another large Discomycete which I have seen puff vigorously in the open, and have also heard, is *Urnula Craterium* (Figs. 169 and 170). The fruit-bodies of this fungus are black or blackish-brown and they consist of a stem 3–4 cm. long and of a cup 3–4 cm. in diameter and 4–6 cm. deep. They come up in the spring on buried or partially buried sticks in woods, and I have met with them under these conditions both in western Ontario and in Manitoba. Some twenty years ago at Kenora on the Lake of the

FIG. 169.—Two fruit-bodies of *Urnula Craterium* growing on sawdust and chips of wood. The mature fruit-bodies of this fungus puff vigorously when touched, and the puffing can readily be heard if it takes place close to the ear. Collected near Ottawa by W. S. Odell. Photographed by the Photographic Division of the Geological Survey of Canada. Natural size.

Woods I found a group of mature *U. Craterium* fruit-bodies and was much impressed by seeing them, as I gathered them, shoot dense clouds of spores out from the mouths of their deep cups. Had I put one of them to my ear, doubtless I should have heard it puff loudly, but at the time I did not think of doing this. On May 8, 1933, I gathered a full-grown fruit-body of *U. Craterium* at Victoria Beach, Lake Winnipeg. On examining it in the laboratory I found that its asci were immature : their ends were already turned up heliotropically toward the mouth of the cup, but the spores had not

yet been formed.   The fruit-body was kept moist in a glass dish.
Five days after it had been gathered, I took it out of the dish, put
it to my ear, and distinctly heard it puff.   On the next day, by
which time more asci had ripened, it puffed more vigorously and,
in so doing, gave out a loud fizzing sound.

The smallest Discomycetes that I have been able to hear puff
are *Ciliaria scutellata*, *Pyronema confluens*, and *Ascobolus stercorarius*.

A fruit-body of *Ciliaria scutellata*, about 1 cm. in diameter (*cf.* Fig. 133, p. 279), was removed from a board and set in a covered dish. Four days later I opened the dish, put the fruit-body to my ear, and distinctly heard the fruit-body puff.

Some fruit-bodies of *Pyronema confluens*, 1–2 mm. in diameter and more or less confluent (*cf.* Vol. V, Fig. 61, p. 112), were growing on sterilised soil in a pot covered with a sheet of glass. On removing the glass and putting my ear down to the fruit-bodies, I at once heard them puff vigorously.

Fig. 170.—Another fruit-body of *Urnula Craterium.* Collected near Ottawa by W. S. Odell along with the two shown in Fig. 169. Photographed by the Photographic Division of the Geological Survey of Canada. Natural size.

A considerable number of fruit-bodies of *Ascobolus stercorarius* each about 2–4 mm. in diameter, had developed on a sterilised horse-dung ball in the laboratory.   One afternoon between 4 and 5 P.M., on removing the dung-ball from the culture dish and bringing it close to my ear, I heard the fruit-bodies puff very clearly.

Urnula geaster is a remarkable Discomycete which grows on roots of dead Elms in woods in the State of Texas, *i.e.* in a southern region of the United States of America.   There, on account of the

very noticeable cloud of spore-smoke which its mature fruit-bodies emit when they are gathered in the open or jarred in the laboratory, it is popularly known as the *Devil's Cigar*.[1]   A well-grown fruit-

Fig. 171.—*Urnula geaster*. To the left, a young fruit-body with the hymenial cavity still closed. To the right, a mature fruit-body beginning to expand and forming valves from above downwards. Found on roots of a dead elm, *Ulmus crassifolia*, at Denton, Texas, U.S.A., and photographed by W. H. Long.   Natural size.

body, just before opening and expanding, is club-shaped and resembles in form a stipitate Puff-ball (Fig. 171).   It is about 4 inches high, velvety brown without, and leathery in consistence.   The stipe is stout and about 2 inches long and continuous with it above

[1] *Fide* personal communication by F. McAllister of the University of Texas, December, 1926.

is a hollow thin-walled chamber which is oval, 1·5–2 inches in diameter, and lined within by the hymenium. At first the hymenial chamber is quite closed but, at maturity, it becomes slit from the

Fig. 172.—*Urnula geaster*. A fruit-body with six valves, almost fully expanded, the hymenium exposed to view. Found on a root of a dead elm, *Ulmus crassifolia*, at Denton, Texas, U.S.A., and photographed by W. H. Long. Natural size.

apex downwards into about six triangular segments (Fig. 172). These segments then bend outwards and downwards, reminding one of the segments of the outer peridium of a Geaster. Hence the specific name. The puffing stage, according to information sent

me by Dr. W. H. Long, is only attained when the segments have
become well reflexed (Fig. 173).   Professor I. M. Lewis, formerly

FIG. 173.—*Urnula geaster*.   Two fruit-bodies fully expanded, seen from above.
This is the puffing stage.   Found on roots of a dead elm, *Ulmus crassifolia*, at
Denton, Texas, U.S.A., and photographed by W. H. Long.   Natural size.

of the University of Texas, told me that, when puffing takes place, vast numbers of spores are liberated and the spores rise above the hymenium to a height of several inches ; and he kindly sent me some living fruit-bodies for study. Although these fruit-bodies, packed in moss, were six days in coming from Austin, Texas, they were still alive and able to discharge spores on arrival in Winnipeg. I not only saw the puffing of some of these fruit-bodies but heard it as well. The spores appeared to me to be shot straight outwards from the hymenium to a distance of 2–3 cm., and I came to the conclusion that their further ascent above a fruit-body was simply due to their being carried upwards by air-currents. The puffing took place, just as in *Aleuria vesiculosa*, immediately after a fruit-body had been removed from a closed chamber where it had lain undisturbed for some hours. On removing a fruit-body from its chamber and putting it to my ear, I distinctly heard the blast as numerous asci discharged themselves during 1–3 seconds.

Rhizina inflata, as is well known, has large, convex, inflated, chestnut-brown fruit-bodies which appear above the ground about the roots of certain Coniferae (Fig. 174). On September 27, 1920, at Rabbit Lake, Kenora, central Canada, I found some of these fruit-bodies growing on the surface of a sandy deposit above the roots of some Pines (*Pinus Banksiana*) which had been burnt and thus killed a year or two previously. I took several of the fruit-bodies to Winnipeg and, on September 28, put them in crystallising dishes covered with glass plates. On September 29, after the fruit-bodies had been kept in the dishes for about 24 hours, I took one of them out and held it in a beam of sunlight about 12 inches in front of my eyes. When thus brought into drier air, the fruit-body puffed vigorously for a few seconds and it continued to give off a few spores for more than a minute. So much I observed with my eyes, but I heard nothing. I then took another fruit-body out of one of the dishes and held it close to my ear. I distinctly heard it puff. The sound was not so loud as that made by *Pustularia catinus* and *Aleuria vesiculosa* but it continued longer. It was loudest during the first two or three seconds and then gradually died away in the course of about a minute. It reminded me of the effervescence of freshly poured champagne or mineral waters.

During the next eight days, with the exception of the seventh, I tested the fruit-bodies daily and on some days three times a day. They puffed audibly every time I tested them. Altogether, therefore, the fruit-bodies puffed audibly for a period of nine days. The puffing was heard not only by myself but also by Professor Frank Allen of the Physics Department of the University of Manitoba, by Professor Charles H. O'Donoghue of the Zoological Department,

FIG. 174.—*Rhizina inflata*. Large convex inflated chestnut-brown fruit-bodies on the ground above the roots of Coniferae. Photographed at Oxshott, England, by Somerville Hastings. Two-thirds the natural size.

and by the following members of the Botanical Department: Professor H. F. Roberts, Mr. C. W. Lowe, Miss K. Scott, and Miss I. Mounce. I also succeeded in demonstrating the audibility of the fruit-bodies to several students and to the members of the Scientific Club of Winnipeg.

To hear a fruit-body of *Rhizina inflata* puff, it is necessary to place it close against one's ear. I could hear the puffing of a fruit-body very distinctly when the fruit-body was close against my ear, only faintly when the fruit-body was removed to a distance of one inch from my ear, and not at all when the fruit-body was removed to a distance of three inches from my ear. This is easily

accounted for by the smallness of the amount of energy transmitted by the sound waves coming from the exploding asci and by the well-known law of inverse squares, namely, that the intensity of the sound travelling outwards in all directions from a given source varies, when heard by the ear, inversely as the square of the distance of the source of sound from the ear.

Taking advantage of the audibility of puffing in *Rhizina inflata*, one can tell in the dark, merely by listening and without using one's eyes, whether or not spore-discharge is taking place. Thus I kept some fruit-bodies for three days in a dark room. When, at intervals, I took them out of their dishes in the dark and put them successively to my ear, they puffed audibly just as they had done in the light in the laboratory. I could not see the fruit-bodies, but my ears assured me that their spore-discharging function was being vigorously carried out. These experiments in the dark room show incidentally that puffing is not dependent upon light.

The sound of puffing is a collective sound made up of the individual sounds produced by the explosion of the individual asci, and is comparable to the fizzing sound made by the bursting of the individual bubbles arising in freshly poured effervescent beverages. I believe that it is possible to hear even the individual asci explode. I put a fruit-body against my ear in a very quiet closed dark-room, and listened attentively to the later phases of the puffing phenomenon. The sound, after a short time, became discontinuous and comparable to the sound made by the first few large rain-drops falling at the beginning of a thunderstorm upon a tin roof. Just as one can hear each individual drop of rain under the conditions just described, so I thought I could hear each single ascus explode as puffing was ceasing.

The hissing sound produced by the puffing of *Pustularia catinus*, *Aleuria vesiculosa*, etc., is comparatively loud, but has a duration of only about two or three seconds. On the other hand, the effervescent sound produced by the puffing of *Rhizina inflata* is comparatively feeble, but has a duration of from about one to several minutes. Fruit-bodies of the Rhizina, kept in a closed crystallising dish for a day and then taken out and placed close to the ear, usually puffed audibly for upwards of a minute. One such .

fruit-body puffed audibly for 4·5 minutes and another for 9 minutes. The puffing is always strongest during the first few seconds and then gradually becomes feebler and feebler until it ceases to be heard. The fruit-body which puffed for 9 minutes was listened to by myself and Miss I. Mounce. We passed the fruit-body from one to the other at intervals, and we agreed in our observations as to the gradual dying away of the sound of the puffing and as to the approximate length of time during which it was audible.

On October 9, I revisited Rabbit Lake and found some more *Rhizina inflata* growing about the roots of the dead Pine trees. In one place several fruit-bodies had united laterally and had formed an irregular mass covering about 25 square inches of ground. The day was remarkably warm for the time of the year (70°–80° F.) and the sun had been shining directly upon the fruit-bodies for at least two hours. I broke off a small piece of the outer part of the fungus-mass. Thereupon, the untouched but slightly shaken remaining part of the fungus-mass puffed vigorously, and the cloud of spores floated away in the sunlight. A short time thereafter I stretched myself at full length upon the ground and placed an ear against the upper surface of the fungus-mass, thereby touching it ; and I thought I heard the fungus-mass puff for at least two seconds ; but, unfortunately, the wind was sighing in some trees not very far off so that conditions were not so still as were desirable, and there was no opportunity of repeating the observation. However, I have no doubt, from the observations made in the open with my eyes and ears, as just recorded, that not merely visible but also audible puffing sometimes takes place in the fruit-bodies of *Rhizina inflata* under natural conditions.

I have but little doubt that, by putting the fruit-bodies close to one's ear, one could hear the sound of spore-discharge of most, or perhaps all, of the Discomycetes which puff strongly. My method for making explosions of asci audible is as simple as that of Columbus for making an egg stand on end ; and, now that it has been described, it is probable that the fact that ascus-guns, when firing together as huge batteries, produce an audible sound will soon be generally confirmed.

Type V : Empusa and Entomophthora, Uredineae and Hymenomycetes.—Single conidia, together with conidiophore-sap,

are shot away from their conidiophores by Empusa [1] and Ento-
mophthora, often to a distance of 1–2 cm. ; and single basidiospores
are shot away from their sterigmata by Uredineae to a distance of
$0\cdot4$–$1\cdot0$ mm., and by Hymenomycetes to a distance usually of
$0\cdot1$–$0\cdot2$ mm. Up to the present, no sound has ever been heard
when spore-discharge has been taking place from the fruit-bodies
of these fungi ; and the projectiles are so minute and their pro-
pulsion so feeble that, probably, for the unaided human ear, Empusa
and Entomophthora and all Uredineae and Hymenomycetes will
for ever be silent plants. However, physicists have invented
microphones which magnify sounds very greatly ; and it is possible
that, with the aid of these instruments, the collective sound of the
simultaneous discharge of thousands of spores from a large fruit-
body of one of the Agaricaceae might be made audible. So far as
I know, no one has tested this possibility as yet. The best fruit-
bodies to use with a microphone in a trial experiment would probably
be a large Coprinus, such as *Coprinus comatus* or *C. sterquilinus*,
which has large spores and discharges its spores only in the zones
of spore-discharge bordering upon the autodigesting edges of the
gills. A large fruit-body of *Coprinus comatus* shoots away from
its gill-edges about 1,000,000 spores a minute or more than 10,000 a
second, and it is just possible that a very sensitive microphone might
make the collective sound of these numerous discharges faintly
audible.

**Sounds Made by Fungus Guns are of No Biological Signifi-
cance.**—Finally, it may be remarked that the sound given out by
fungus guns as they explode and the sound made when a fungus
projectile strikes some object are merely bye-products of the process
which has to do with the dispersion of the spores ; and, obviously,
they are neither of advantage nor of disadvantage to the fungi
concerned. While sounds are given out by, and are heard by, the
Higher Animals and some Lower Animals, so far as we know plants
never signal to one another by means of sound waves or respond
to sounds of any kind whatsoever.

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[1] For a photograph showing how far the spores of *Empusa muscae* are shot,
vide Vol. I, 1909, Fig. 83, p. 255. The magnification there given should have been
$1\cdot3$ and not $\frac{3}{4}$.

PART III

PSEUDORHIZAE AND GEMMIFERS AS ORGANS OF CERTAIN
HYMENOMYCETES

CHAPTER I

THE PSEUDORHIZA

Introductory Remarks—Collybia radicata—Mycena galericulata—Coprinus macrorhizus.

Introductory Remarks.—The stipes of the fruit-bodies of certain Agaricaceae are prolonged downwards through the soil for several inches by so-called *rooting bases*. For *rooting base* Fayod [1] has substituted the excellent term *pseudorhiza*. Among the species having fruit-bodies with a characteristic pseudorhiza may be mentioned : *Collybia radicata, C. longipes, C. pulla, Tricholoma macrorhizum, Pleurotus Ruthae, P. citrinatus, Pholiota radicosa, Flammula inopus, Coprinus macrorhizus,* and *Collybia fusipes.* In all these species, with the exception of *Collybia fusipes*, the pseudorhiza is annual and unbranched. In *Collybia fusipes*, as we shall see, the pseudorhiza is perennial and branched.

A pseudorhiza forms a link between a mycelium vegetating in a buried root or other buried nutrient substratum and the aerial part of the fruit-body which produces and liberates spores. In forming such a link, it is analogous to certain pseudo-sclerotia, sclerotia, and mycelial cords, but it differs from these structures in being a specialised part of a stipe of a fruit-body and not part of a mycelium.

The observations on the pseudorhizae of *Collybia radicata* and *Coprinus macrorhizus* about to be recorded were made for the most part in England during the years 1910–1915, and a brief account of them was presented at the Ontario meeting of the American Phytopathological Society in 1919.[2]

[1] V. Fayod, " Prodrome d'une Histoire Naturelle des Agaricinés," *Ann. Sci. Nat.*, T. IX, 1889, p. 214.
[2] A. H. R. Buller, "The Pseudorhiza of certain Saprophytic and Parasitic Agaricineae," *Phytopathology*, Vol. X, 1920, p. 316.

Collybia radicata.—This species appears in the late summer and autumn in woods and grassy places under certain trees, especially Beeches. In England I have found it frequently under Beeches (*Fagus sylvatica*) and once under a Horse Chestnut (*Aesculus Hippocastanum*). In central Canada, where no species of Beech grows, it is absent,[1] but it is common in the Beech woods of eastern Canada [2] and the United States.[3] It occurs in Japan.[4] Howitt [5] observed fruit-bodies coming up annually under an Ironwood tree (*Ostrya virginiana*) at Guelph, Ontario, and Grove [6] repeatedly has seen fruit-bodies coming up under an Oak (*Quercus Robur*), which was far distant from any Beech at Studley, England. *Collybia radicata*, therefore, is associated not merely with Beeches, but with Oaks, Ironwoods, Horse Chestnuts and, doubtless, a number of other trees.

The stipe of *Collybia radicata* is peculiar in that its aerial part, which is from four to seven inches in height and somewhat thickened below, is continued downwards beneath the soil. As it passes beneath the soil, it becomes much swollen and fusiform, and it then terminates in a long tapering root-like structure. The subterranean " rooting base " of the stipe is often four or more inches long (Fig. 175).

Fayod [7] investigated the so-called rooting base of the stipe of *Collybia radicata* and found that it originated solely by intercalary growth. He discovered that the carpophores come into existence in the first instance upon roots buried to a depth of 10 cm. beneath the soil ; and he suggested that the fungus is a root-parasite. The youngest fruit-bodies which he observed were bulbous and only 2 to 3 mm. long. A longitudinal section showed him that the bulbous base of a young fruit-body is composed of large barrel-shaped cells mixed with fine filamentous hyphae, and that the two

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[1] G. R. Bisby, A. H. R. Buller, and J. Dearness, *The Fungi of Manitoba*, London, 1929, p. 32.

[2] H. T. Güssow and W. S. Odell, *Mushrooms and Toadstools*, Ottawa, 1927, p. 116.

[3] C. H. Kauffmann, *The Agaricaceae of Michigan*, Lansing, U.S.A., 1918, Vol. I, p. 766, and Vol. II, Plate CLXVII.

[4] M. Shirai and K. Hara, *A List of Japanese Fungi*, ed. 3, 1927, p. 96.

[5] J. E. Howitt, personal communication.

[6] W. B. Grove, personal communication.

[7] V. Fayod, *loc. cit.*, pp. 214–215.

kinds of elements are disposed parallel to the longitudinal axis of the primordium. A study of successive stages revealed that the so-called root arises from the multiplication and elongation of the two sets of elements just mentioned. Thus, by intercalary growth, that part of the primordium which is to expand into the aerial stipe and pileus is gradually carried upwards to the surface of the soil where alone it attains its full development. Fayod's description of the development of the fruit-bodies of *Collybia radicata*, unfortunately, was not accompanied by any illustrations.

Fayod pointed out that the term *root* is unsatisfactory in describing the subterranean part of the stipe of such a fungus as *Collybia radicata*, because the so-called root has neither root-cap nor vascular bundles and arises exclusively by intercalary growth and 'not from an apical growing point. The so-called root also differs from a true root in that it grows vertically upwards instead of more or less downwards. In order to indicate that the basal subterranean part of the stipe of *Collybia radicata* and other similar Agaricaceae is something different from a true root, Fayod gave to it the name *pseudorhiza*.[1]

My own observations on *Collybia radicata*, so far as they have gone, confirm those made by Fayod. At Heyshott in England, in September, 1913, there were several fruit-bodies growing around the base of an old Beech tree. On carefully excavating the soil surrounding the pseudorhizae, I ascertained that each of the fruit-bodies was growing just above a Beech root. I succeeded in definitely tracing one of the pseudorhizae downwards through the soil and in making out its attachment to a buried root (Fig. 175, B). The pseudorhizae of the other fruit-bodies were traced downwards through the soil for several inches, but they broke off just before their points of connexion with a root were reached. Owing to the brittleness of the most slender and deepest part of a pseudorhiza and to the compactness of the soil, it is often not easy to free a pseudorhiza from the soil without breaking it or detaching it from the root upon which it has grown. However, in my father's garden at Birmingham, by taking more time and care than was possible at Heyshott, I succeeded in exposing two pseudorhizae 16 cm. long

[1] V. Fayod, *loc. cit.*, pp. 214–215.
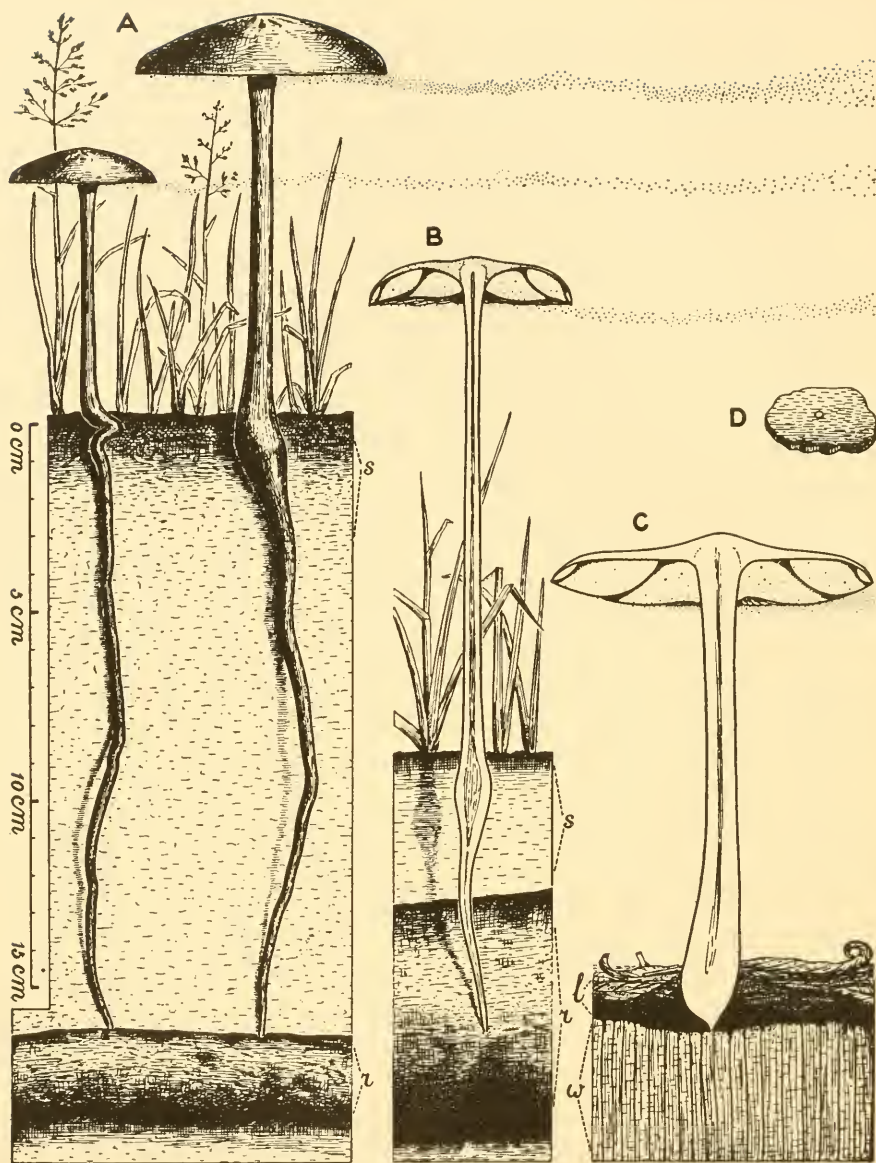
FIG. 175.—Fruit-bodies of *Collybia radicata* provided with pseudorhizae attached to roots of trees, etc., beneath the surface of the soil. A, two fruit-bodies on a Horse-Chestnut root ; *r*, the root ; *s*, the soil, excavated ; each fruit-body consists of an aerial stipe-shaft and pileus and of a long subterranean pseudorhiza ; the spores falling from the pilei are being carried off by the wind. B, a vertical section through a fruit-body attached to a thick Beech root near the surface of the soil ; *r*, the root ; *s*, the soil ; the pseudorhiza is solid to the centre. C, a vertical section through a fruit-body attached to the top of a Beech stump covered with a thin layer of leaf-mould ; *w*, wood ; *l*, the leaf-mould ; no pseudorhiza has been developed. D, a piece of the outer surface of a root ; the ring in its centre shows the very small point of attachment of the pseudorhiza of a large fruit-body. A, observed at Birmingham ; B, at Heyshott ; and C, at Kew. A, B, and C, reduced to one-half the natural size ; D, natural size.

and also the root of a Horse Chestnut to which they were attached without breaking the connexions (Fig. 175, A).   In Queen's Cottage Grounds, Kew Gardens, I found a fruit-body attached to the top of a Beech stump (Fig. 175, C).

The length of a pseudorhiza varies with the depth of the soil overlying the infected root, through which the rudiments of the pileus and aerial part of the stipe must be pushed up.   The deeper the overlying soil, the longer is the pseudorhiza, and *vice versa*. If a fruit-body begins to develop 16 cm. below the surface of the soil, the pseudorhiza becomes 16 cm. long (Fig. 175, A) ; if a fruit-body begins to develop 7·5 cm. below the surface of the soil, the pseudorhiza becomes 7·5 cm. long (B) ; while, if a fruit-body develops on the top of a stump where the soil is very thin, the pseudorhiza scarcely comes into existence at all (C).   It is probable that here, just as in *Coprinus sterquilinus*,[1] light regulates the length of the pseudorhiza by inhibiting the growth in length of the pseudo-rhiza as soon as this organ has pushed up the rudimentary pileus and aerial part of the stipe to the surface of the ground and has thus come into a position to receive a light stimulus.

The pseudorhiza thickens and becomes fusoid just beneath the surface of the ground.   It is this fusoid portion only which functions mechanically in supporting the weight of the whole of the subaerial part of the fruit-body.   The long tapering extremity of the pseudorhiza, which is very thin and weak, merely serves for the conduction of food materials.   The principle of economy in structure is here illustrated once more, for the thickening and mechanical strengthening of the pseudorhiza is limited to that part alone which has a mechanical function to perform.

The pseudorhiza and the aerial stipe-shaft are both solid, their central parts being filled with white fibrils ; and both of these organs are smooth on the exterior (Fig. 175, B).   The pileus has a somewhat slimy pellicle ; but, as experiment showed, drops of rain-water are not absorbed through it.   The fruit-bodies persist for some time after their expansion, and the spore-discharge period probably lasts for a week or ten days.   The discharge of the spores from beneath the pilei is represented in Fig. 175.

[1] These *Researches*, Vol. IV, 1931, pp. 112–117.

I have found fruit-bodies of *Collybia radicata* coming up on two dead stumps. One of these stumps was at Kew Gardens and the other at Birmingham. The former was *in situ* where it grew, but the latter had been pulled up some two or three years previously and had been laid upon the soil to support ivy. There can be no doubt, therefore, that *Collybia radicata* can live as a pure saprophyte ; and it may be that, even when it grows upon the roots of living Beech trees, it is merely destroying roots which have been killed by some other agency. However, it is not unlikely that the fungus is a wound-parasite, *i.e.* a parasite which first invades the dead tissues exposed at the surface of a wounded root and then slowly invades and kills the living tissues adjacent to the dead tissues first entered. The facts which lead one to suspect that *Collybia radicata* is a parasite are : (1) the fungus grows on the roots of living Beeches, Oaks, and Horse Chestnuts, (2) the fungus comes up year after year beneath trees which have once become infected, and (3) the fungus has developed a special organ—the pseudorhiza —by means of which it successfully meets the requirements of its subterranean mode of vegetation. Whether or not the fungus is really a parasite, however, can only be decided by exact experiment.

Collybia longipes [1] was found by Fayod above the roots of an Oak.[2] Its pseudorhiza is doubtless developed in the same manner as that of *Collybia radicata* ; and the fungus probably has relations with the Oak of the same nature as those of *C. radicata*.

Mycena galericulata.—This species lives on dead wood. Its fruit-bodies often develop at the surface of unburied stumps and sticks, etc. Under these conditions each fruit-body consists of a pileus and normal stipe only. However, it sometimes happens that the woody medium in which the mycelium vegetates lies several inches below the surface of the soil or vegetable mould. When this is so, the stipe of each fruit-body possesses a pseudorhizal prolongation similar to that of a stipe of *Collybia radicata*. The accompanying illustration, Fig. 176, shows the relation of a group of fruit-bodies to a block of wood which was buried about 4 inches below the top of a layer of leaf-mould. The three fruit-bodies

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[1] *Vide* Cooke's *Illustrations of British Fungi*, Pl. 201.

[2] V. Fayod, *loc. cit.*, p. 215.

came into existence, in the first place, at the surface of the wood,

and they then grew upwards through the overlying leaf-mould. The youngest fruit-body (*a*), at the time the fruit-bodies were found, had not yet emerged but, by means of a pseudorhiza, was gradually pushing its pileus upwards into the light. Its pseudorhiza appeared to be increasing in length solely by intercalary growth just beneath the pileus and the enclosed rudiment of the aerial stipe-shaft ; and its pileus was very small, hard, and conical. The free end of the fruit-body, in form and mode of elongation, thus closely resembled the root-tip of a Phanerogam and was admirably adapted for pushing aside obstacles in its upward progress. It differed from the root-tip of radicles, however, in that it was growing upwards instead of downwards. We may conclude from the
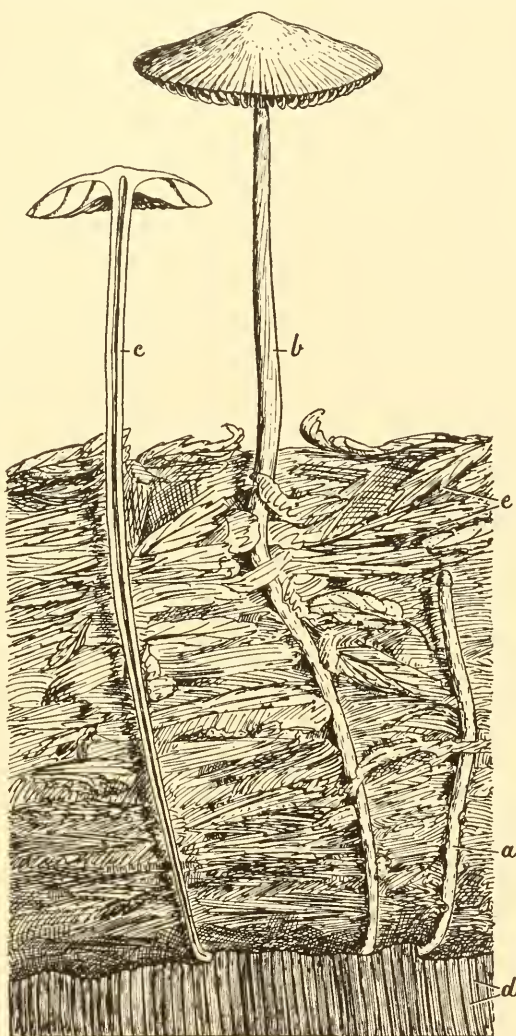
FIG. 176.—*Mycena galericulata.* Section through leaf-mould, *e*, and a buried stump, *d*, to show origin of fruit-bodies from the wood and mode of development of the pseudorhiza; *a*, a pseudorhiza which is elongating just beneath the rudimentary pileus; *b* and *c*, fully developed fruit-bodies shedding spores; in *c*, the pseudorhiza and stipe, which are continuous with one another, are both hollow in the centre. Reduced to two-thirds natural size.

study of such a set of fruit-bodies as that shown in Fig. 176 that the pileus of *Mycena galericulata* remains in a very rudimentary condition whilst travelling through the soil and only begins to expand when it has come to the soil's surface.

At its point of attachment to the woody substratum, a pseudorhiza of *Mycena galericulata* consists of a small, firm, yellowish swelling, and the shaft of the pseudorhiza, as it passes upwards through the soil, gradually increases in diameter, and thus attains its maximum diameter just beneath the surface of the ground. The outer surface of a pseudorhiza is white and covered with fine hyphae which attach themselves to leaf-débris, etc.; and the interior of a pseudorhiza is hollow like that of the aerial stipe-shaft with which it is continuous (Fig. 176, *c*).

A pseudorhiza, like an aerial stipe-shaft, grows upwards because it is negatively geotropic. Sometimes a pseudorhiza, instead of being straight, is crooked. When this is so, the crookedness has been brought about by the pseudorhiza, when pushing upwards, having encountered mechanical obstacles which could not be penetrated and which therefore were avoided by oblique growth like that which occurs with true roots under similar conditions.

Mycena galericulata, so far as I am aware, lives only as a saprophyte on dead wood, and I have never seen it in any situation which would suggest that it is a parasite. The fruit-bodies illustrated in Fig. 176 were rather large and more expanded than usual; but, after they had been gathered a day or two, the gills turned pinkish in the usual way.

The fruit-bodies of *Mycena galericulata* shown in Fig. 176 had bisporous basidia, which is in accord with the observations of Patouillard,[1] Ricken,[2] and Lange.[3] Ricken[4] states that the bisporous basidia of *M. galericulata* serve to distinguish this species from the very similar but four-spored *M. tintinnabulum*. However, that there is a four-spored form of *M. galericulata* as well as a two-

[1] N. Patouillard, *Tabulae analyticae fungorum*, Fasc. III, 1884, No. 214, p. 96.

[2] A. Ricken, *Die Blätterpilze*, Leipzig, Bd. I, 1915, p. 439.

[3] J. E. Lange, "Studies in the Agarics of Denmark," *Dansk Botanisk Arkiv*, Copenhagen, Bd. I, No. 5, 1914, p. 33.

[4] A. Ricken, *loc. cit.*

spored is indicated by Rea [1] who has described the basidia of *M. galericulata* as " generally with 2-sterigmata only " and by Bauch [2] who, in his list of the Hymenomycetes having two-spored forms, remarks that the fruit-bodies of *M. galericulata* found at Rostock were " meist zweisporig." Moreover, Rea has informed me that he has observed fruit-bodies of *M. galericulata* with quadrisporous basidia. It may be that, as Bauch [3] found in *Camarophyllus virgineus* (one of the Hygrophoraceae), the two-spored fruit-bodies of *M. galericulata* are haploid, while the four-spored are diploid. Another possibility is that both forms are diploid, but that two nuclei migrate into each spore of the two-spored form, while only one nucleus migrates into each spore of the four-spored form. The problem here suggested can be solved only by precise investigation.

Coprinus macrorhizus.—This species has been confounded with *Coprinus lagopus* (= *C. cinereus* = *C. fimetarius* [4]), and in Massee's *British Fungus-Flora* it is called *Coprinus fimetarius*, var. *macrorhizus*. A comparative study of the Coprini has taught me that *Coprinus lagopus* and *C. macrorhizus* are independent species. This conclusion is based not merely on studies in the field but also on a comparison of pure cultures. The following are some of the points by which the two species may be distinguished.

(1) *Exterior of the pileus.* The pileus of *C. macrorhizus* (Fig. 177) is rounded at the top, pale, and brownish-grey, the brown being particularly marked at the apex. The pileus of *C. lagopus* (Fig. 133, Vol. III, p. 305) is more conical, and ashy-grey except at the very apex which is brown. The floccose scale-hairs on the surface of the pileus of *C. macrorhizus* are more matted than those of *C. lagopus.*

(2) *Breadth of gills.* The gills of *C. macrorhizus* are distinctly broader than those of *C. lagopus.* The more conical form of the

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[1] Carleton Rea, *British Basidiomycetae*, Cambridge, 1922, p. 384.

[2] R. Bauch, " Untersuchungen über zweisporige Hymenomyceten. I. Haploide Parthenogenesis bei Camarophyllus virgineus," *Zeitschrift f. Botanik*, Bd. XVIII, 1925–1926, p. 344.

[3] *Ibid.*, pp. 354–382.

[4] *Cf.* these *Researches* : Vol. III, 1924, pp. 301–303 ; and Vol. IV, 1931, pp. 191–193.

pileus of the latter is associated with this fact (*cf.* Fig. 185, p. 367, and Fig. 138, Vol. III, p. 316).

(3) *Opening of the pileus.* Before spore-discharge and auto-digestion begin, the pileus of *C. lagopus* opens widely and thus becomes almost flattened. The gills are therefore pulled some distance apart from one another, with the result that, during spore-discharge, the cystidia do not connect adjacent gills but simply appear as free projections or *pegs* on the gill-sides (Fig. 146, Vol. III, p. 325). On the other hand, the pileus of *C. macrorhizus* begins to

Fig. 177.—*Coprinus macrorhizus.* Young fruit-bodies grow-ing on stable manure. Photographed at Birmingham, England. Natural size.

shed spores while it is still campanulate, and in this species—as may be readily observed in the field with a pocket lens or even with the naked eye—the cystidia *bridge the interlamellar spaces* and *connect adjacent gills* during the whole period of spore-discharge (*cf.* Fig. 122, Vol. III, p. 287). *C. lagopus* belongs to the Lagopus Sub-type of fruit-body organisation, whereas *C. macrorhizus* belongs to the Atramentarius Sub-type.[1]

(4) *Shape of the spores.* The spores of *C. macrorhizus* are dis-tinctly shorter in proportion to their width than those of *C. lagopus.* The spores of *C. macrorhizus* are oval in form and those of *C. lagopus* elongated-oval.

[1] *Cf.* these *Researches*, Vol. III, 1924, pp. 296, 301–302.

(5) *Stipe.* The aerial part of the stipe of *C. macrorhizus* is usually stouter and firmer than that of *C. lagopus* (*cf.* Figs. 180 and 185, pp. 360 and 367, with Fig. 133, Vol. III, p. 305). The stipe of *C. macrorhizus* frequently ends below in a long conspicuous sub-terranean "rooting base" or pseudo-rhiza. In *C. lagopus* such an organ is usually absent.

(6) *Substratum.* *C. lagopus* is extremely common on horse-dung balls, as is shown by the fact that fresh balls collected at Vancouver (British Columbia), Edmonton (Alberta), Shellbrook (Saskatchewan), Winnipeg (Manitoba), and in three different places near Birmingham, England, all yielded fruit-bodies of this species.[1] *C. macrorhizus*, on the other hand, very rarely comes up on isolated dung-balls but is extremely frequent on

Fig. 178.—*Coprinus macrorhizus.* Mature fruit-bodies shedding spores, growing on stable manure. The one on the extreme right has its pseudorhiza in view. Photographed at Birmingham, England. Natural size.

large masses of horse dung, such as are represented by manure piles, hot-beds, and the like. *C. macrorhizus* seems to be specially adapted to flourish in dung well mixed with straw and heated by the fermentation process.

[1] W. F. Hanna, "The Problem of Sex in Coprinus lagopus," *Annals of Botany*, Vol. XXXIX, 1925, pp. 433–434.

(7) *Cultures.* *C. lagopus* grows well on sterilised horse-dung balls, and on this medium new fruit-bodies can be obtained with certainty in about fifteen days after the spores have been sown. On the other hand, *C. macrorhizus* grows relatively poorly on sterilised horse-dung balls and takes a longer time to produce fruit-bodies. Both species retain their structural peculiarities when grown in parallel cultures side by side in the same laboratory.

(8) *Size of fruit-bodies.* The fruit-bodies of *C. macrorhizus* are as a rule larger and less fragile than those of *C. lagopus.* Both in
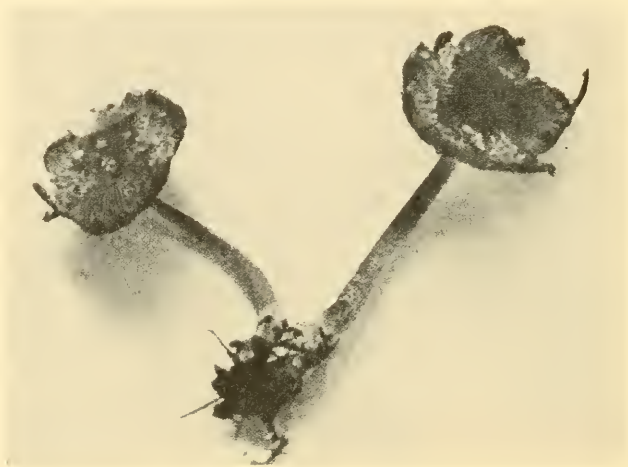
Fig. 179.—*Coprinus macrorhizus.* Two fully expanded, revolute, and almost exhausted fruit-bodies, removed from stable manure. Some of the spores have settled on the stipes. Photographed at Birmingham, England. Natural size.

the open and in culture the fruit-bodies of *C. macrorhizus*, if produced at all, attain a certain minimum size which is considerable. On the other hand, the fruit-bodies of *C. lagopus*, while able to attain a large size under favourable conditions, yet on exhausted horse dung or under conditions of competition may be extraordinarily small. The stipes of *C. lagopus* vary in length from about 10 cm. down to 1–10 mm. and their expanded pilei from about 3 cm. in diameter down to $0 \cdot 75$–$3 \cdot 0$ mm. Thus dwarf fruit-bodies occur in *C. lagopus* (Fig. 138, A, B, C, Vol. III, p. 316) but not in *C. macrorhizus.*

Hitherto, the pseudorhiza or rooting base of the stipe of *Coprinus macrorhizus* has nowhere been adequately described. In 1911, Weir gave an erroneous account of the way in which it arises. He referred to pseudorhizae definitely as roots (*Würzeln*). Translated from the German, one of his statements is as follows : " The direction of growth of the root in a homogeneous substratum is vertically downwards. Hard bodies are avoided by lateral twisting." [1] Again, in describing the origin of the " roots," he says that at first a knot of hyphae comes into existence something like that of the sclerotium of *Coprinus stercorarius*. " The knot . . . increases in size, begins to elongate, and behaves itself in its upper and lower halves in a different manner, in that the hyphae of the under half become positively geotropic. Soon after the formation of this rudiment (*Anlage*), the hyphae on the under side become matted at one point and grow downwards like a root. It is remarkable that the hyphae, even in the substratum, go on growing so as to form a firm unified structure by means of which they remove themselves more and more from the light and apparently seek the optimum conditions of moisture and food-materials." [2] His further remarks on this matter it is unnecessary to repeat as, like those just quoted, they are based on the erroneous supposition that the root-like organ of the fungus grows downwards through the substratum like the root of a Phanerogam. I shall now endeavour to show : that the root-like organ is really a pseudorhiza, like that of *Collybia radicata* ; that, unlike a root, it comes into existence not by apical growth, as Weir supposed, but by intercalary growth ; that it is negatively geotropic and not positively geotropic ; and that, in consequence, it grows upwards and not downwards through the substratum. It is the thin lower end of the pseudorhiza which is first formed and not the thick upper end.

For the purpose of my investigation, I examined a large number of fruit-bodies of *Coprinus macrorhizus* which were coming up on a long flat-topped pile of horse manure which had been removed from some stables and was well mixed with straw. The manure

[1] J. R. Weir, " Untersuchungen über die Gattung Coprinus," *Flora*, Bd. CIII. 1911, p. 313.

[2] *Ibid.*, pp. 312–313.

pile was in a state of fermentation, so that only a few inches from the surface it was decidedly hot to the hand. On digging down
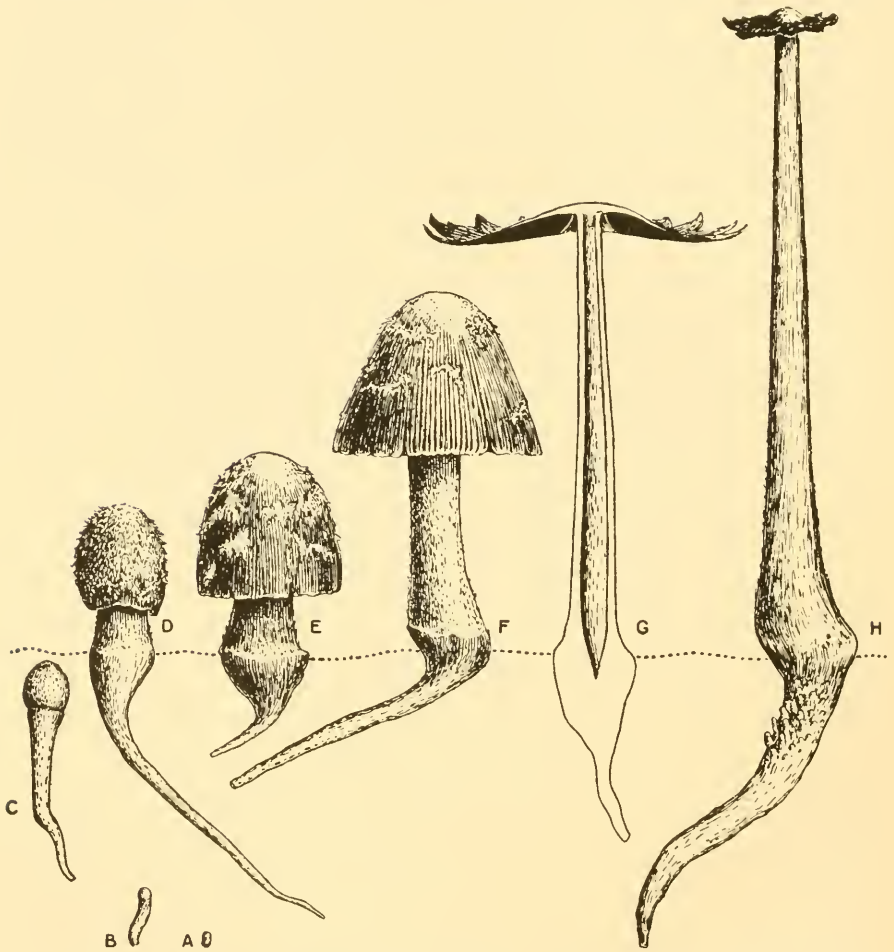
Fig. 180.—*Coprinus macrorhizus.* Fruit-bodies in various stages of development with short pseudorhizae. A and B, each with a pseudorhiza capped by a pileus growing upwards through the substratum. C, a fruit-body nearing the surface of the ground with an enlarging pileus. D, E, F, stages in the elongation of the stipe and the expansion of the pileus. G and H, the stipes have attained full length; in G, the gills are disappearing through autodigestion and spores are being shed; in H, the pileus has been almost completely destroyed through autodigestion, and spore-discharge has ceased. Natural size.

into the manure, I found that many of the fruit-bodies had very long rooting bases. Using Fayod's terminology, we shall now

refer to these rooting bases as *pseudorhizae*. Illustrations of some of the fruit-bodies are shown in Figs. 177–187.

I made excavations in the manure pile and discovered that
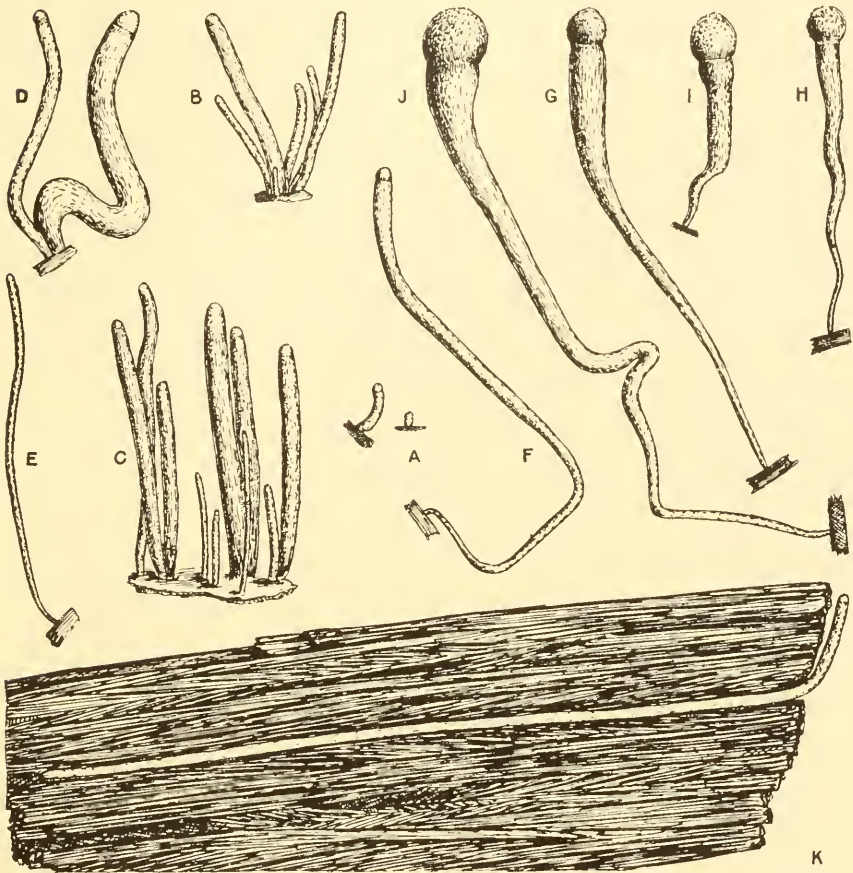
FIG. 181.— *Coprinus macrorhizus*. Fruit-bodies excavated, during the development of their pseudorhizae, from below the surface of a horse-dung manure heap. The position of the particular piece of the substratum from which each pseudorhiza was growing is shown in every case. A–F, elongation of the pseudorhiza by intercalary growth just below the rudimentary terminal pileus. G–J, pilei enlarging on being brought up to near the surface of the substratum by their respective pseudorhizae. K, a pseudorhiza growing through densely matted straw some inches below the surface of the substratum. Natural size.

young fruit-bodies, like those shown in Fig. 180 at A, B, and C and in Fig. 181 at A, B, and C, were pushing up to the light a few inches below the general surface of the manure. From a study of

a long series of intermediate fruit-bodies, some of which are shown in Figs. 181 and 186 (p. 369), it became clear that fruit-bodies which develop pseudorhizae arise as tiny primordia *not at the surface of the manure* as Weir would have us suppose, *but at some distance below it.* These primordia are at first minute balls about 1 mm. across (Fig. 181, A). They are attached to more solid parts of the substratum and frequently arise at the surface of a single straw making part of a bundle of straws. Their subsequent growth, no doubt, is accomplished at the expense of an extensive mycelium to which they are attached by their bases.

In Fig. 181, A–J, are shown pseudorhizae in various stages of development, after they had been excavated from the manure pile. In Figs. 180 and 185 (p. 367) are shown a number of fruit-bodies with shorter or longer fully developed pseudorhizae *in situ* with the surface of the manure pile indicated by means of a broken line.

Fig. 182.—*Coprinus macrorhizus.* Fruit-bodies which originated at the surface of the substratum and have therefore not developed pseudorhizae. A, B, C, E, successive stages of development. D, a section of E showing absence of pseudorhiza ; the spores on the gills are ripening from below upwards. Natural size.

It was found that, when fruit-bodies arise at the surface of the manure, as they sometimes do, no pseudorhizae whatever are formed. In Fig. 182, A to D, are shown stages in the development of fruit-bodies whose origin in the manure pile was superficial. It is clear that the production of a pseudorhiza depends simply on the position of origin of the fruit-body primordium. If the primordium is developed at the top of the substratum, no pseudorhiza is produced (Fig. 182) ; if it is developed one or two inches below the surface of the substratum, the pseudorhiza becomes about one or two inches long (Fig. 180) ; and, if it is developed about four inches below the

surface of the substratum, the pseudorhiza becomes about four inches long (Fig. 186, p. 369).

The development of a fruit-body primordium will now be described in detail. The pseudorhiza of a fruit-body which has originated a few inches below the surface of the manure and which has shed its spores and withered away aerially sometimes develops a large number of primordia ; and, occasionally, some of these primordia develop into fruit-bodies which produce pseudorhizae of their own, come above ground, and open out in the usual manner (Fig. 187, p. 371). The primordia arising on old pseudorhizae develop in just the same manner as those produced directly on the surface of a straw and are easier to procure. Old pseudorhizae bearing primordia were therefore used for studying the earliest stages of fruit-body development.

The material was fixed [1] in Fleming's fluid (weaker solution) for 24 hours, washed for 24 hours in running water, and then placed in 30 per cent. alcohol. It was then brought up gradually to absolute alcohol and, after being passed through xylol in the usual manner, was embedded in paraffin. Sections were cut with the microtome and double-stained in iron-alum-haematoxylin [2] and Bordeaux red.[3]

The earliest stages in the development of the fruit-bodies of *Coprinus macrorhizus* are very similar to those of *Psallio'a campestris* described by Atkinson [4] in 1906. The primordium of the carpophore as a whole in its youngest state is a homogeneous body composed of slender uniform dense hyphae which are woven in a complex manner into an even meshwork, and surrounded by a thin layer of hyphae which are looser and less dense in their arrangement. This outer looser layer can be considered as a universal veil. It is quite distinct in all the youngest stages (Figs. 183 and 184) and grows for some time. Finally, it ceases to grow and, as the pileus expands, splits up and thus gives rise to the hairy scales which are such a

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[1] *Cf.* Chamberlain, *Methods in Plant Histology*, Chicago, 1905, p. 248.

[2] This was the same as Haidenhain's solution (*vide* Chamberlain, *loc. cit.*, p. 250) except for the fact that 1·5 per cent. aqueous solution of iron alum was used instead of ferrous ammonium sulphate.

[3] A 1 per cent. aqueous solution for 3 minutes.

[4] G. F. Atkinson, " The Development of Agaricus campestris," *Botanical Gazette*, Vol. XLII, 1906, pp. 241–264.

marked characteristic of the mature pileus. In a very young primordium there is no evidence of a differentiation into stipe and pileus : the inner mass of hyphae surrounded by the universal veil appears to be homogeneous, just as in *Psalliota campestris*. As a primordium becomes somewhat larger and older, and before there is any evidence of an external annular furrow differentiating pileus and stipe from one another from without, stained longitudinal
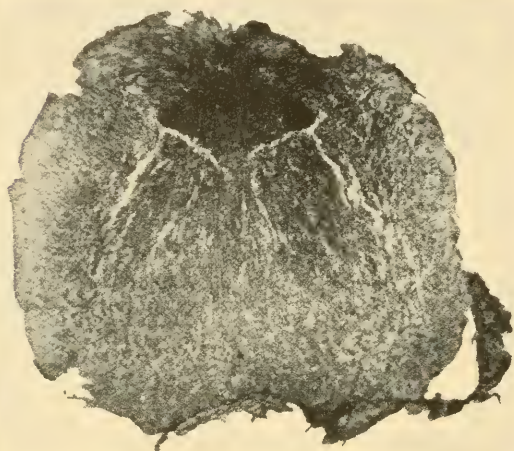
Fig. 183.—*Coprinus macrorhizus*. A median-vertical section of a very young fruit-body with its hymenophore just becoming differentiated. As yet no pseudorhiza has been developed. Magnification, 46.

sections exhibit, near the upper end of the young fruit-body and some distance in from the surface, two small deeply stained areas. These are really part of an annular area within the fruit-body, which is composed of hyphae which are densely compacted and rich in protoplasm. These hyphae make up the primordium of the gills (Fig. 183). It is to be noted, therefore, that the primordium of the

gills arises as in *Psalliota campestris*,[1] *i.e.* that it comes into existence internally at a time when the fruit-body is homogeneous and compact except for the looser envelope. After the primordium of the gills has come into existence, interlamellar spaces arise within it, and the plates of tissue thus separated from one another develop into the gills (Fig. 184 and in Vol. III, Fig. 140, p. 318). A gill-chamber apart from these interlamellar spaces was not observed. The gills in their youngest stage have their long axes directed perpendicularly to the long axis of the carpophore (Fig. 183) ; but, with further growth, they become obliquely situated, their inner

[1] *Cf.* G. F. Atkinson, *loc. cit.*, p. 249.

edges remaining in contact with, and in continuity with, the primordium of the stipe (Fig. 184).

With the differentiation of the primordium of the gills, the other parts of the young fruit-body become delimited. We can now distinguish the primordium of the pileus-flesh and the primordium of the stipe. The universal veil at this stage is seen to form a continuous layer which encloses both pileus and stipe (Fig. 184). Soon after the young gills have begun to grow downwards, a very slight annular furrow makes its appearance on the outside of the whole fruit-body (Fig. 181, B–D, p. 361). This furrow, which becomes more and more pronounced as the gills grow in size (Fig. 181, G–J), marks off the pileus which lies above it.

The annular furrow, just referred to, may be considered as dividing the stipe into two parts, an upper interpilear part which is enclosed between the gills, and a lower sub-pilear part which is entirely below the gills. These two parts are continuous with each other ; but, as we shall see, it is convenient to distinguish between them.

If a fruit-body develops directly on the surface of the substratum, as sometimes happens in nature (Fig. 182, p. 362) and in artificial cultures, the primordium of the

Fig. 184.—*Coprinus macrorhizus.* A median-vertical section through the upper part of a slender cylindrical fruit-body which was pushing its way upwards through manure (*cf.* Fig. 181, B and C ; also Fig. 186, *b* and *c*). The pseudorhiza is pushing up the rudimentary apical pileus by elongating in an intercalary zone of growth which is just below the level of the base of the gills. At this stage of development, the universal veil covering the apex of the fruit-body is compact and functions like a root-cap. Magnification, 19.

stipe as a whole becomes differentiated into two parts. The interpilear part becomes the primordium of the shaft of the

stipe, and the subpilear part the primordium of the base of the stipe.

The various primordia are then related as follows :

The primordium of the fruit-body as a whole producing :

- the primordium of the gills
- the primordium of the pileus-flesh

together forming the primordium of the pileus

- the primordium of the stipe producing :
  - the primordium of the stipe-shaft
  - the primordium of the stipe-base.

Now, when a fruit-body is produced on the surface of the sub-stratum, the primordium of the base of the stipe gradually thickens and elongates a little, the elongation taking place chiefly in its upper part ; and that is all. The primordium of the shaft of the stipe, however, elongates after a time enormously, and so carries the pileus high into the air (Fig. 182, p. 362).

When a fruit-body starts its development not at the surface of the substratum, but at some distance below it, the primordia originate in the same manner as that just described. But here the primordium of the base of the stipe becomes especially important. In its upper part, just below the annular furrow, the parallel hyphae of which it is composed grow enormously in length by intercalary growth. The result of this is that the primordium of the pileus (flesh, gills, and veil), together with the primordium of the shaft cf the stipe, is pushed upwards through the manure, and an elongating root-like structure, the *pseudorhiza*, comes into existence. The intercalary growth at the top of the pseudorhiza continues until the primordia of the pileus and stipe-shaft have been carried up to, or nearly up to, the surface of the substratum. It then ceases. As the pileus is carried upwards, the pseudorhiza not only elongates but, as a rule, gradually thickens, so that the diameter of the youngest and highest part is often very much greater than that of the oldest and lowest part (*cf.* Figs. 180 and 185, pp. 360 and 367).

As the pileus approaches the loosest and uppermost layer of

manure, it enlarges, and its gills become better differentiated. After the pseudorhiza has ceased its intercalary growth, the stipe-shaft
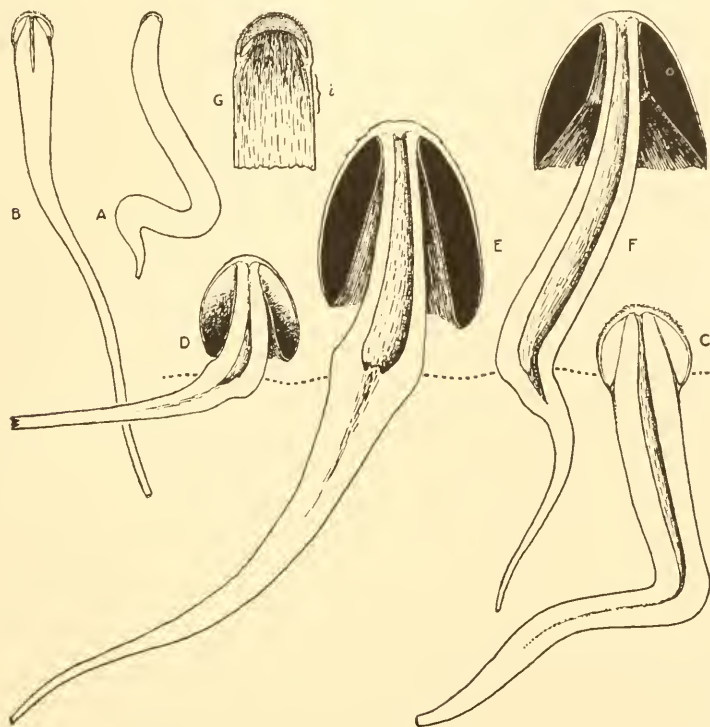
Fig. 185.—*Coprinus macrorhizus*. Sections of fruit-bodies in various stages of development, all with a pseudorhiza. A, the solid pseudorhiza is capped by a very rudimentary pileus which is shown enlarged at G ; the fruit-body had not yet reached the surface of the ground. B, a fruit-body approaching the surface of the ground : its pileus is enlarging. C, D, E, F, successive stages in the development of the pileus and subaerial stipe. The dotted line shows the general level of the substratum. In C, which has a hollow pseudorhiza, the spores have not yet been developed : the gills are white. In D, the spores are ripening from below upwards : the gills are therefore turning black from below upwards. In E, black spores are present all the way up the gills, the aerial stipe-shaft is elongating, and the pileus expanding. In F, the stipe-shaft has elongated, the gills are undergoing autodigestion, and spores are being shed. In G, which is the apex of A magnified, the gills and pileus-flesh can be clearly perceived ; *i*, the zone of intercalary growth of the pseudorhiza. A–F, natural size ; G, magnification, 3.

primordium, which has been enclosed between the gills, begins to elongate. This results in the pileus being torn away from its base. The universal veil is broken and a ring-like scar is usually left behind

at the base of the stipe (*cf.* Fig. 180, p. 360). As the stipe elongates, the pileus continues its development. The dimorphic basidia, the paraphyses, and the cystidia become differentiated, the cystidia grow in length, and soon the spores appear on each gill from below upwards. As the pileus, in opening out, becomes campanulate, the spores begin to fall down in the interlamellar spaces. With the production of spore-freed gill-surfaces arising from below upwards, autodigestion sets in and the gills are then gradually destroyed from below upwards in the manner which has been described for other Coprini (Figs. 180, p. 360, and 185). The pileus opens out relatively slowly, much more slowly than in *C. lagopus*.

The whole of a fruit-body (pseudorhiza, stipe, and pileus) is produced at the expense of a mycelium which vegetates deep down in the manure. This mycelium is present in the hard masses of manure, on the surface of which the primordia arise in the first instance, as shown in Fig. 186. We really have an arrangement similar to that already described for *Collybia radicata* and for *Mycena galericulata*, which grow on wood beneath the soil.

The lower end of a pseudorhiza is remarkable for the smallness of its diameter, but it must be remembered that the only function which it has to perform, after it has pushed up the primordium of the pileus through the manure, is that of conduction. There is no need for it to be mechanically strong, as it has no weight to support. Only on nearing the surface of the ground does the pseudorhiza become thickened. There can be little doubt that this thickening is correlated with the demands for mechanical support which must subsequently be made upon the pseudorhiza by the weight of the pileus and stipe.

From the surface of the pseudorhiza fine hyphae arise which pass out into the substratum and become attached to the manure. It will be remembered that similar hyphae occur on the pseudorhiza of *Mycena galericulata*. In all probability, these hyphae are used chiefly for fixing the pseudorhiza and thus enabling it to push the primordium at its end upwards with more ease. They may also conduct to the fruit-body a certain amount of water, but they are probably not nutritive in the sense that they collect food materials other than water. There can be but little doubt that the materials
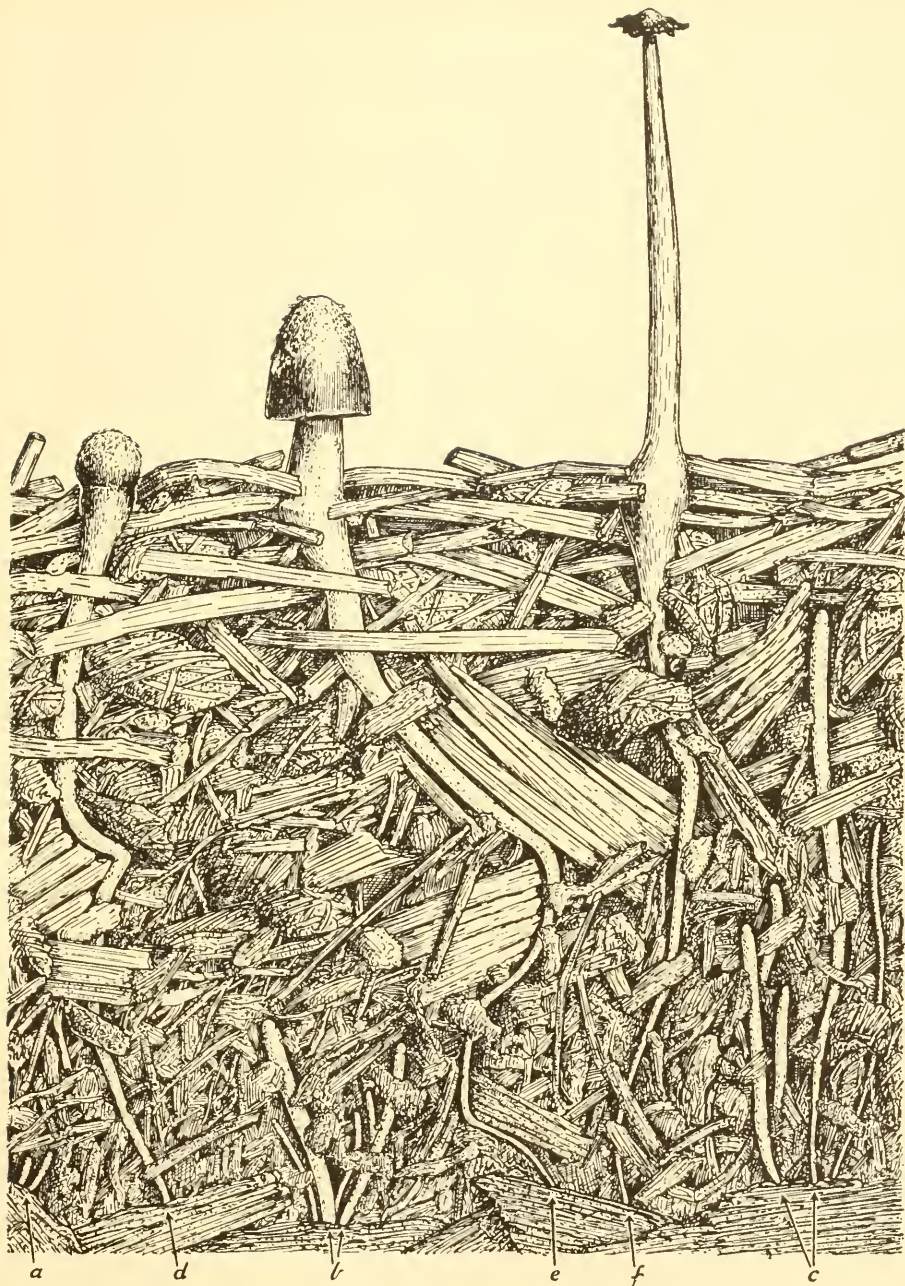
Fig. 186.—*Coprinus macrorhizus.* Development of a set of fruit-bodies with very long pseudorhizae, shown in a vertical section through a pile of stable manure. At the base of the Figure is more solid manure from which the pseudorhizae are springing ; toward the top, the manure is looser and the straw more evident. Stages in development, drawn from actual specimens, are indicated by the letters a to f. In a, b, and c, the blunt upper end of each pseudorhiza consists of a rudimentary pileus, already with tiny gills within ; in d, e, and f, the pseudorhiza, through intercalary growth, has brought each pileus to the surface of the substratum. At d, the pileus is swelling ; at e, the pileus is expanding and the aerial stipe-shaft elongating ; at f, the stipe-shaft is fully elongated and the pileus, which has shed its spores, is now greatly reduced owing to autodigestion. Natural size.

for the construction of the stipe and pileus are conducted upwards from a hard mass of manure through the base of the pseudorhiza. In support of this view may be cited the analogous case of *Collybia radicata* in which the whole fruit-body is produced at the expense of a mycelium vegetating in a tree-root buried several inches beneath the soil (Fig. 175, A and B, p. 350).

In passing upwards through the substratum, the pseudorhiza is doubtless directed, like the stipe, by the stimulus of gravity. Harder and softer parts of the substratum must be traversed. The softer parts are directly penetrated, but the harder ones, such as dense masses of straw, can only be avoided by oblique or lateral growth. Sooner or later, often by a very tortuous course of from three to six inches, the surface of the manure is reached. The twisted pseudorhizae shown in Figs. 181 (p. 361) and 186 tell a tale of obstacles which have been encountered during upward growth and have been grown around. In general, the length of a pseudorhiza varies with the depth in the manure at which the primordium of the fruit-body has originated.

The older and thinner parts of a pseudorhiza are quite solid, but the younger swollen parts, beneath the base of the stipe-shaft, although often solid (Fig. 185, D and E, p. 367), are sometimes hollow in the centre (Fig. 185, C). The hollow space, when present, is continuous with that of the stipe.

A pseudorhiza, before the pileus has shed its spores, is usually unbranched. Occasionally, however, I have observed indications of the putting out of a branch laterally (Fig. 187, E), but this is very rare. Also, before a fruit-body at the end of a pseudorhiza has shed all its spores, primordia—but primordia only—of a number of new fruit-bodies may be developed on the surface of the pseudorhiza (Fig. 187, B). Nevertheless, I have never seen anything corresponding to the illustration published by Weir [1] in which he shows one chief fruit-body as yet unexpanded and four long branches coming from the pseudorhiza and all bearing secondary fruit-bodies of considerable size. However, branching not infrequently takes place from an old stout pseudorhiza when the fruit-body at its end has shed its spores and withered to the base

[1] J. R. Weir, *loc. cit.*, Fig. 21, p. 317.

of the stipe (Fig. 187, C).   I should regard Weir's illustration as correct, so far as the branching is concerned, had the chief and oldest
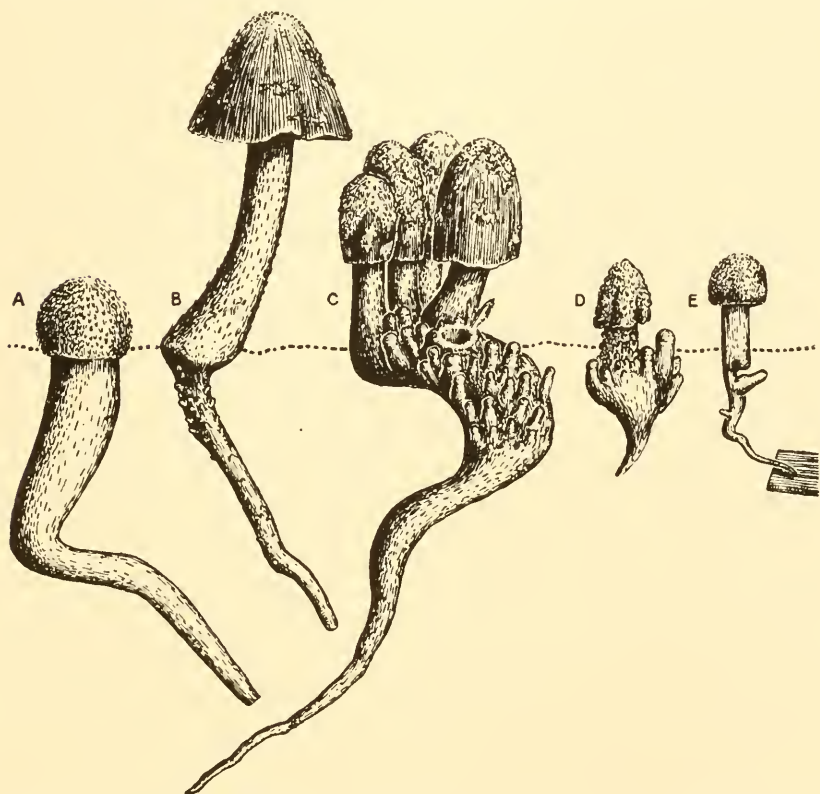
Fig. 187.—*Coprinus macrorhizus*.   Branching of the pseudorhiza.   The dotted line indicates the general level of the substratum.   A, a young fruit-body with a very stout pseudorhiza likely to produce new fruit-bodies when the first has been exhausted.   B, fruit-body beginning to shed spores, having a pseudorhiza which has already given rise to the rudiments of a number of new fruit-bodies. C, a very stout pseudorhiza which gave rise to a large subaerial fruit-body which shed its spores, died down, and is now represented merely by a stump.   The pseudorhiza subsequently gave rise to numerous rudiments of new fruit-bodies, four of which are now developing normally.   D, the terminal fruit-body was injured and has ceased to develop, and new fruit-bodies are arising on the pseudorhiza.   E, the pseudorhiza which is springing from a straw, has a terminal fruit-body and two lateral rudimentary ones.   Natural size.

fruit-body been represented as having rotted down to the top of the pseudorhiza : but this was not done.   Weir seems to have combined two stages of development in a single drawing.   From Weir's illustration, as it stands, one would judge that the main axis

and the four chief branches had all been produced simultaneously or nearly so ; but I do not think that this happened. Rather, a single stout pseudorhiza was first produced ; then the large fruit-body at its end opened, shed its spores, underwent autodigestion, and withered down to its base ; and, finally, from primordia which were produced on the pseudorhiza either whilst the first fruit-body was expanding or after it had shed its spores, new pseudorhizae and secondary pilei were subsequently developed.

The normal production of a number of secondary fruit-bodies from an old and large pseudorhiza which bears the stump of a large primary fruit-body was observed several times (Fig. 187, C). Injury to a primary fruit-body, so that this withers before it has expanded, may also lead to the production of secondary fruit-bodies (Fig. 187, D). The development of secondary fruit-bodies upon an old pseudorhiza is no doubt correlated with the fact that the food materials contained within the pseudorhiza have not been exhausted by the formation of the primary fruit-body. New fruit-bodies, as Weir also observed, are produced until exhaustion is complete. So far as my observations have gone, it is only the larger and more vigorous pseudorhizae which produce secondary fruit-bodies. Weak slender ones often show no trace of them. Doubtless, by injuring the pilei terminating pseudorhizae, one could cause secondary fruit-bodies to come into existence at will.

What is the function of the pseudorhiza of *Coprinus macrorhizus* ? The answer is as follows. The primordia of the fruit-bodies of *C. macrorhizus* are often produced on a deep-seated mycelium at a little distance below the surface of the nutrient substratum and, when this occurs, the pseudorhiza serves as an organ for pushing upwards to the surface of the substratum those parts of the fruit-body—the rudimentary pileus and the rudimentary stipe-shaft— which are destined to complete their development aerially. The frequent production of hundreds of fruit-bodies of *C. macrorhizus* on a manure pile where perhaps no other agaric is to be seen testifies to the success of this species in the struggle for existence.

When visiting a large mushroom cave near Paris, I observed that *Coprinus macrorhizus* was growing on some of the beds. The fruit-bodies had the normal size and were provided with the

characteristic pseudorhiza. Since the beds had been left in utter darkness from the time when they had been made up, it is clear that light could have had nothing to do with the development of the fruit-bodies. In particular, the direction of the growth of the pseudorhiza could not have been influenced by a heliotropic stimulus.

Among the conclusions to which Weir [1] came in his already mentioned investigations upon *Coprinus macrorhizus* was that this fungus possesses " a positively geotropic, root-like sclerotium (*ein positiv-geotropisches, wurze'ähnliches Sklerotium*)." This description of the pseudorhiza is entirely erroneous. The pseudorhiza, as we have seen, grows upwards instead of downwards, its mode of growth is intercalary and therefore not like that of a root, and there is no ground to justify the view that it is to be regarded as a sclerotium. A sclerotium is an independent more or less compact mass of mycelium in which food materials are stored and which, after resting for a longer or shorter time, gives rise to one or more sporophores or to a new mycelium. But a pseudorhiza does not consist of mycelium, for it is part of a sporophore ; nor is it a resting body ; and the food materials which it contains are not reserve food materials but materials in transit to the developing pileus and stipe-shaft.

[1] Weir, *loc. cit.*, p. 319.

CHAPTER II

THE PERENNIAL PSEUDORHIZA OF COLLYBIA FUSIPES

Introduction—Historical Remarks—Fruit-body Clusters and their Pseudorhizae—
The Supposed Sclerotium—Evidence that the Pseudorhiza Persists—Mode of
Development of a Compound Pseudorhiza—Lateral Grafting—Healing of
Pseudorhizal Wounds—The Mycelium and the Problem of Parasitism—The
Functions of the Pseudorhiza and the Significance of its Persistency—*Sarco-
scypha protracta*.

Introduction.—*Collybia fusipes*, one of the best-known of the fleshy
fungi, commonly occurs on the ground near the trunks of trees,
particularly those of Beeches (Fagus) and Oaks (Quercus). The
fruit-bodies are sometimes solitary but they usually come up in
smaller or larger clusters, and two such clusters are shown in a
Beech grove at Kew Gardens in Fig. 188. The pileus, when fully
expanded, is about 1·5–2·5 inches wide, but larger ones are not
rare and I have seen one six inches wide (the left fruit-body in
Fig. 189). The top of the pileus, when young, is reddish-bay ; but,
in age, it becomes dingy tan-colour and is often speckled with
numerous small dark spots and blotches as if it had been injured
in some way. The stipe varies from about 2 to 6 inches in length
and from about 0·25 to 1 inch in thickness ; and it is usually more
or less fusiform (hence the specific name), being thickest in the
centre, tapering somewhat upwards toward the pileus, and tapering
greatly toward its base where it is attached in the soil. Its exterior
wall is reddish-brown and cartilaginous, and within it is at first
fibrously stuffed, afterwards becoming somewhat hollow. The
spores are colourless, small, and thin-walled ; and each one passes
through all its developmental stages from its first origin to its dis-
charge from the sterigma in about one hour and five minutes.[1]

[1] These *Researches*, Vol. II, 1924, pp. 44, 49, and 54.

Collybia fusipes, whose clustered fruit-bodies are so often seen over the roots of Beeches and Oaks in Europe and England, seems to be somewhat rare in North America. McIlvaine and Macadam [1] state that in the West Virginia mountains it is frequent, but it is not recorded as occurring in North America by such experienced mycologists as Atkinson,[2] Kauffman,[3] and Murrill.[4] Dr. R. E. Stone has informed me that he saw fruit-bodies of *C. fusipes* under a Beech (*Fagus grandifolia*) at Guelph, Ontario, in 1920 ; but Güssow and Odell [5] do not mention *C. fusipes* in their account of the mushrooms and toadstools of eastern Canada. My colleague Dr. G. R. Bisby [6] and I have never found *C. fusipes* in central Canada.[7] All of the author's observations on *C. fusipes*, about to be recorded, were made in England.

Historical Remarks.—Bulliard,[8] as shown by the illustration in his Plate 106, appears to have found the fruit-bodies in a cluster attached to something looking like a small sclerotium. In 1843, Léveillé,[9] in his *Mémoire sur le genre Sclérotium*, called attention to Bulliard's illustration and gave the following account of the supposed sclerotium and of the origin of the fruit-bodies. " *Agaricus fusipes* Bull. grows in summer and autumn at the foot of trees, sometimes in groups and sometimes solitarily. Its structure is most curious. It is remarkable for its fusiform stipe deeply buried in the earth. Bulliard (Pl. 106) appears to have found it on a sclerotium : this is a supposition ; but, as we have met with it

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[1] C. McIlvaine and R. K. Macadam, *One Thousand American Fungi*, Indianapolis, 1902, p. 116, Plate XXIX, A, No. 4.

[2] G. F. Atkinson, *Studies of American Fungi. Mushrooms, edible, poisonous, etc.*, Ithaca, U.S.A., 1900.

[3] C. H. Kauffman, *The Agaricaceae of Michigan*, Lansing, U.S.A., 1918.

[4] W. A. Murrill, in " The Agaricaceae," *North American Flora*, Vol. IX, 1916, p. 375 ; also *Edible and Poisonous Mushrooms*, New York, 1916.

[5] H. T. Güssow and W. S. Odell, *Mushrooms and Toadstools, an Account of the more Common Edible and Poisonous Fungi of Canada*, Ottawa, 1927.

[6] G. R. Bisby, A. H. R. Buller, and J. Dearness, *The Fungi of Manitoba*, London, 1929, p. 32.

[7] It is possible that *Collybia fusipes* does not occur in Japan, as it is not mentioned in Shirai and Hara's *A List of Japanese Fungi*, 1927.

[8] P. Bulliard, *Herbier de la France*, Paris, 1782, Plate CVI.

[9] J. H. Léveillé, " Mémoire sur le genre Sclérotium," *Ann. Sci. Nat.*, sér. 2, T. XX, 1843, pp. 228–229.

Fig. 188.—*Collybia fusipes.* Two clumps of fruit-bodies coming up on grass, each developing from a subterranean persistent pseudorhiza attached to one of the roots of the Beech tree. Photographed in Kew Gardens. Much reduced from natural size.

several times in this condition, we can believe that the tubercle
from which Bulliard's fungi are springing is a true sclerotium. The
existence of this sclerotium, however, is not constant ; usually the
stipe is attached directly to dead roots or to old pieces of wood
embedded in the earth. When one has observed the fungus in a
certain place one year and one returns there at about the same time
the following year, one almost always again finds individuals of the
same species : if one then digs up the fungus carefully, so as to
obtain everything that is attached to it, one sees an elongated,
irregular, spongy, black body from which spring the new fruit-
bodies. This body is not a sclerotium, but rather the stipe of the
fruit-body of the previous year, which has served as the root-stock
(*souche*) for new out-growths and which has fulfilled the functions
of a sclerotium. In the following year, the stipes of the fruit-
bodies of the second year serve in their turn as root-stocks, and so
on, successively giving rise to new fruit-bodies ; in such a manner
that an *Agaricus fusipes* which three or four years before had its
stipe deeply buried in the earth ends up by finding itself at the
surface. This is the only example of growth of this kind that we
know in the numerous families of the Mushrooms and Toadstools
(*Champignons*)."

More than three centuries ago, in 1578, Clusius, who was living
in Hungary, was preparing to write his *Brevis Historia*—the first
monograph on fungi ever published ; [1] and he conceived the happy
idea of having a series of paintings made of those species which he
wished to describe. He therefore engaged an excellent artist who
produced a series of eighty-seven water-colour drawings which are
now known as the *Codex of Clusius*. This Codex, after having
been lost for two centuries, was discovered in the library of the
University of Leyden in 1874, and was published by Istvanffi at
Budapest in 1900 as a tercentenary celebration of the publication
of the first monograph on fungi.[2] Plate 78 of the Codex represented

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[1] Clusius, *Fungorum in Pannoniis observatorum brevis Historia*, 1601.

[2] G. Istvanffi, *A Clusius-Codex mykologiai méltatása adatokkal Clusius életrajzához.
Études et Commentaires sur le Code de l'Escluse, augmentés de quelques notices bio-
graphiques*. Enrichis de 22 figures et de 91 planches chromolithographiées, repro-
ductions du Code de l'Escluse, Budapest, 1900, pp. 1-287, published by the Author
in Magyar and French.

to Clusius, who had no scientific names for his fungi, the sixth species of the twenty-second genus of his *Fungi perniciales*. As exactly reproduced by Istvanffi, it gives us an excellent life-size coloured illustration of a group of seven *Collybia fusipes* fruit-bodies, all arising from the top of a dark, rod-like, somewhat obconical body about 1·5 inches long and 0·4 inch wide at the top. There can be no doubt whatever that this body was dug up from the ground with the fruit-bodies to which it is shown attached, and that it was simply a stipe-base which had persisted from the previous year in the manner described by Léveillé. It thus appears that the persistent stipe-base of *Collybia fusipes*, although not recognised as such, was observed and illustrated in its natural size in an admirable coloured drawing long before the time of Bulliard and Léveillé, some 350 years ago.

Fruit-body Clusters and their Pseudorhizae.—Without knowing of Léveillé's observations, I rediscovered the attachment of the fruit-bodies of *Collybia fusipes* to the roots of trees in 1912, and only subsequently was my attention called to Léveillé's paper written seventy years previously.[1] I shall now give an account of my own observations on *Collybia fusipes* which, so far as the persistence of the stipe is concerned, confirm and extend those of the French writer. It will be shown, however, that the supposed sclerotium is only a much modified stipe or combination of stipes, and is not a structure formed by the mycelium prior to the development of fruit-bodies.

In the month of July, 1912, fruit-bodies of *Collybia fusipes* were found in Queen's Cottage Grounds, Kew Gardens, coming up in clusters upon the vegetable mould near the trunks of certain Oaks and Beeches. Under one large Beech were found nine clusters of fruit-bodies which were distant from the tree-trunk as follows : one 2·5 feet, four about 3 feet, one 5 feet, one 9 feet, and two about 12 feet. In general appearance the tree seemed healthy, for it bore an abundance of green leaves and no big dead branches. One or two branches, however, had been cut away, and the largest of these, which had projected from the trunk ten feet from the ground,

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[1] I am indebted to Mr. J. Ramsbottom for kindly calling my attention to Léveillé's paper.

was six inches in diameter. There was nothing to be seen at the time above-ground to indicate that the tree was in a moribund condition ; yet moribund it actually was. In August, 1914, I revisited the tree and found that the clusters of *Collybia fusipes* fruit-bodies on the ground round about the trunk were even more numerous than in 1912, for there were fifteen clusters instead of nine. Ten of these clusters were within 3 feet of the trunk, two about 5 feet distant from the trunk, and one 9 feet distant. The distances of the other two groups from the trunk, owing to an over-sight, were not recorded in my notes. The tree was now evidently suffering from disease, for the bark on one side of the trunk appeared to be going rotten and adventitious roots which had grown out here and there in its cracks were already dead. There were leaves on all the chief branches, but they seemed to be fewer than on neighbouring trees of the same size. About two years later (1916 ?), during my absence from England, the tree, on account of its dying condition, was cut down and removed. As we shall see, the gradual death of this tree was accompanied by an extensive destruction of its roots by the mycelium of *Collybia fusipes*, and the clusters of fruit-bodies which appeared above the roots year after year were nothing but the visible 'subaerial signs of the destruction of the tree's subterranean root-wood.

When, in 1912, I beheld nine clusters of *Collybia fusipes* fruit-bodies scattered in the leaf-mould under the Beech tree as just described, it at once occurred to me that the fungus might be a root-parasite. I therefore carefully excavated the leaf-mould and soil covering the bases of several of the clusters. As a result I found that every fruit-body cluster was attached to a root. The mycelium of *Collybia fusipes* growing in the forest floor, therefore, is to be sought for not in the leaf-mould but in the wood of buried roots.

One of the *Collybia fusipes* clusters which, at a distance of 12 feet from the Beech trunk, was attached to a stout Beech root, is shown one-third its natural size in the photograph reproduced in Fig. 189. The cluster was made up of five large fruit-bodies, the smallest of which had a pileus two inches in diameter and the largest a pileus six inches in diameter. The five fruit-bodies were all connected

at their strongly tapering bases with a curious dark swollen stroma-like fungal strand ; and this strand, in its turn and at a depth of about six inches below the general surface of the ground, was

Fig. 189.—*Collybia fusipes*. A group of fruit-bodies resembling those shown in Fig. 188, after excavation. The fruit-bodies spring from a black, swollen, subterranean, persistent pseudorhiza which is attached to a rotten root of a Beech tree and is directed vertically upwards. The top of the root was about five and a half inches below the surface of the leaf-mould and soil. Photographed at Kew by Miss E. M. Wakefield and the author. Reduced to one-third the natural size.

attached to a horizontal Beech root. The root was 1·5 inches in diameter, but was so rotten that, with but little difficulty, I was able to sever it with my penknife. On examining the root in transverse section, I could see that it was infected with the mycelium

of the Collybia. The rotten wood was white. Upon the exterior
of the root, the mycelium had formed a thin black crust which was
continuous with the black outer covering of the stroma-like fungal
strand to which the fruit-bodies were attached. The root was
traced three feet toward the tree-trunk and found to be dead and
rotten throughout this distance ; but it could not be followed any
further owing to difficulties of excavation. Neighbouring roots
interlacing above and below the rotten one were living and quite
sound.

The stroma-like strand connecting the Beech root with the fruit-
bodies in the manner just described (Fig. 189) was, as comparative
observations made upon many similar strands has taught me,
nothing more or less than a swollen sympodium of persistent stipe-
bases or pseudorhizae, representing the remains of two or three
crops of fruit-bodies produced in as many years. In shape it was
irregularly cylindrical-obconic. Its height was about 3 inches, its
diameter at its base where it was attached to the root 0·3 inch, and
its maximum diameter, 2·5 inches above its base, about 2 inches.
From the swollen top of the persistent pseudorhizal strand arose
the newly formed fruit-bodies, all attached to it by black and
remarkably attenuated stipe-bases (pseudorhizae). The extreme
basal parts of the new stipes were only 0·1–0·2 inch in diameter ;
but, as the stipes passed upwards through some two or three inches
of leaf-mould, they thickened out and finally attained a maximum
diameter of 0·5–0·75 inch. Within each stipe were wavy parallel
strands of fibres. In addition to the five large fruit-bodies, the
cluster contained a number of rudimentary ones, which were attached
to the top of the persistent stipe-strand but which had never pushed
themselves up freely into the air. The upper parts of several of
these rudimentary fruit-bodies appeared to be in a state of decay.

A second fruit-body cluster, younger than that just described,
was situated only 1·5 feet from the trunk of the Beech tree (Fig. 190,
to the right). After the soil about its base had been removed, it
was found that the fruit-bodies were all attached by means of a
persistent pseudorhizal strand to a stout root which was oval in
cross-section and which measured 5·5 inches from above downwards
and 1·75 inches in width. In this instance the root, which was traced

right up to the tree-trunk, proved to be a buttress-root. Nevertheless, it was quite dead and rotted to a considerable extent. Neighbouring buttress-roots were quite sound. The top of the

Fig. 190.—*Collybia fusipes*. To the right, a cluster of young fruit-bodies springing from a massive compound pseudorhiza which was attached to the top of a large buttress-root of a Beech in Queen's Cottage Grounds, Kew. To the left (somewhat dried) two fruit-bodies attached to a long thin root-like pseudorhiza. Natural size.

root to which the persistent pseudorhizal strand was attached was buried 4 inches below the surface of the leaf-mould. The persistent pseudorhizal strand much resembled the one already described, for it was obconic in form, 3 inches high, 2 inches wide above, and attached to the root by a slender base. Upon its broad top there

were situated about forty young fruit-bodies, the largest of which had a stipe that as yet was only 1·5 inches long and a pileus as yet only 0·7 inch wide. Doubtless, if the cluster had been left undisturbed, only a few of the forty fruit-bodies would have completed their development and have shed spores, for I have observed that in all large mature *Collybia fusipes* clusters there are a few fully

Fig. 191.—*Collybia fusipes*. A large fruit-body with a thin pseudorhiza of its own attached to an irregularly cylindrical persistent pseudorhiza which resembled a piece of rotten wood. Obtained above a Beech root in Queen's Cottage Grounds, Kew. Natural size.

expanded fruit-bodies and a considerable number of much smaller, imperfectly developed, fruit-body rudiments. It is the rule, here as elsewhere in the Agaricaceae, that the fruit-body rudiments largely outnumber the fruit-bodies which are destined eventually to produce spores. In some clusters the bases of those fruit-body rudiments which are inhibited in their development appear to persist through the winter and, by undergoing a certain amount of renewed thickening, to add to the mass of the persistent pseudorhizal strand from which in the next summer the new fruit-bodies arise.

The persistent pseudorhizal strands of fruit-body clusters attached to the roots of a number of trees other than the Beech above described were also examined. Some of them were several inches long, narrowly cylindrical in shape, and scarcely or not at all swollen at their tops (Fig. 190, to the left ; also Fig. 191) ; and, when thus constructed, they usually bore only one, two, or at most
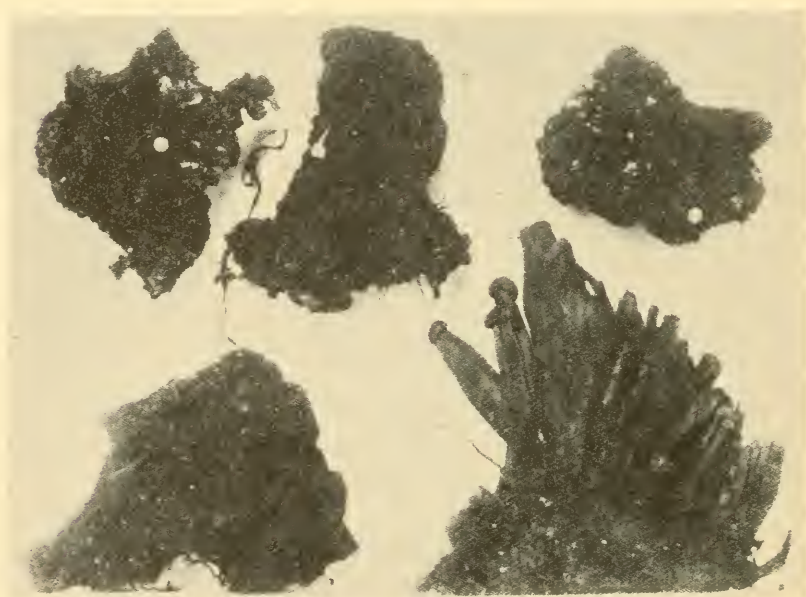
Fig. 192.—*Collybia fusipes.* A black, massive, spongy, compound pseudorhiza broken into five pieces. It doubtless took several years to form. A new crop of fruit-bodies is springing from its top. Found almost at the surface of the ground above a buttress-root of a Beech in Queen's Cottage Grounds, Kew. Natural size.

very few fruit-bodies. These particular strands resembled thin rotten roots in their form, blackness, and consistence to such a degree that they might easily have been taken for them. Indeed, I myself was deceived by the first one I found, but soon discovered my error.

The Supposed Sclerotium.—Certain other pseudorhizal strands, instead of being obconic or cylindrical, were massive and irregularly rounded ; and they were usually seated upon buttress-roots just below the surface of the ground. One of them, which was seated

upon the buttress-root of a Beech, was as large as a child's fist, blackish both without and within, and spongy in texture. After I had excavated it, I broke it up into five pieces all of which are shown in Fig. 192. This dark body appeared to be made up of a great number of irregularly fused, rather brittle, persistent stipe-bases or pseudorhizae ; and its formation was doubtless due to several years' growth. As it was black and spongy in texture, it resembled a sclerotium not a little ; and it was probably such a large black irregular structure as this that Léveillé had in mind when he stated that the fruit-bodies of *Collybia fusipes* sometimes spring from a sclerotium. However, we ought not to regard such a structure as a sclerotium : firstly, because it is composed of basal parts of fruit-bodies and not of mycelium ; and, secondly, because there is no evidence that, if isolated from the root to which it is attached, it could independently produce any fruit-bodies. Doubt-less it contains a certain amount of reserve food materials ; but, in the main, it is not a food reservoir but only a conductor of food materials from the mycelium in the wood of the root to the new fruit-bodies which develop at its apex.

Evidence that the Pseudorhiza Persists.—The evidence that goes to prove that the stroma-like fungal strand connecting a fruit-body cluster of *Collybia fusipes* with the root of a tree is really a persistent pseudorhiza or sympodium of pseudorhizae is of two kinds : (1) field observations, which show that a cluster of fruit-bodies often comes up above a root at exactly the same spot where a cluster appeared the previous year, and (2) comparative observa-tions upon the structure of pseudorhizae.

(1) Field observations. In 1916, Miss E. M. Wakefield and I made careful notes upon the positions of twenty *Collybia fusipes* fruit-body clusters which had come up above the roots of Beeches and Oaks in Queen's Cottage Grounds, Kew Gardens ; and, in order to find out whether or not new clusters would appear in 1917 at exactly the same places as clusters had appeared in 1916, we drove into the ground alongside of each cluster a long metal skewer. In 1917, on account of the war, I was unable to return to Kew, and Miss Wakefield was able to visit the skewers only once. This visit, which was made in early autumn, yielded positive results,

for Miss Wakefield reported to me that she had found six new fruit-body clusters coming up close by as many skewers in exactly the same places as those in which their predecessors had appeared the previous year. The remaining fourteen skewers, with the exception of two or three which had vanished, were also visited, but no fruit-body clusters had as yet appeared by any of them. The six positive observations, however, strongly support the view that the pseudorhizae are persistent from year to year and give rise to successive crops of fruit-bodies.

(2) Comparative observations on the structure of pseudorhizae. A considerable number of stroma-like strands connecting fruit-body clusters with buried roots were unearthed and carefully examined. In every instance their structure was in harmony with the view that a stroma-like strand is either a simple or compound persistent pseudorhiza.

Fig. 193.—*Collybia fusipes.* A, a vertical section through leaf-mould, *l*, and soil, *s*, showing a small Beech root, *r*, to which a persistent subterranean pseudorhiza, *p*, is attached. The pseudorhiza and root are represented as cut through in a vertical plane. The substance of the pseudorhiza is continuous with the sheet of mycelium *m* in the rotten root; *n*, another very thin sheet of mycelium in the region of the cambium. B, another drawing showing the root, *r*, and pseudorhiza, *p*, in longitudinal section (the pseudorhiza for convenience in representation has been bent through a right angle from its natural position shown in A); *m* and *n*, sheets of mycelium in the rotten root. Found in Queen's Cottage Grounds, Kew, by Miss E. M. Wakefield. Natural size.

Mode of Development of a Compound Pseudorhiza.—In order to follow the steps by which a compound persistent pseudorhiza is gradually built up, let us assume : (1) that a stout root of a Beech or an Oak buried some inches beneath the surface of the ground has become infected with the mycelium of *Collybia fusipes*, and (2) that the fungus plant is fruiting for the first time. The forms of many old compound pseudorhizae seem to indicate that often, in the first

fruiting year, only one large fruit-body is sent upwards from the root to the surface of the ground. Let us assume that this has happened in the theoretical case under discussion. The single fruit-body will consist of a pseudorhiza resembling the pseudorhizae of *Collybia radicata*, *Coprinus macrorhizus*, etc., already described in previous pages, an aerial stipe-shaft, and a pileus (Fig. 194, A). A first single fruit-body of this kind I have not as yet been fortunate enough to find. However, Miss Wakefield, when excavating a large fruit-body cluster attached to a buttress-root of a tree in Queen's Cottage Grounds in August, came across the solitary, un-branched, more or less obconic structure shown in Fig. 193. There can be no doubt that this structure, which was unearthed only by accident, was nothing more than the persistent pseudorhiza of a single fruit-body, which had developed the previous year, which had persisted in the soil for about twelve months, and the apex of which had not as yet given rise to any new fruit-bodies. The pileus and aerial stipe-shaft, which the pseudorhiza had supported, had rotted away, and the top of the pseudorhiza itself had become healed over by a protecting surface layer of tissue.

From the foregoing we may assume that, in the *first year* of fruiting, usually or often, one large fruit-body is sent upwards from a root (Fig. 194, A), and that this fruit-body, after sporulation, dies and rots away except for its subterranean pseudorhiza which persists through the ensuing winter (B). In the *second year* of fruiting, it often happens that the solitary persistent pseudorhiza of the first year produces several large fruit-bodies from rudiments which arise at or near its apex (C). Each of these new fruit-bodies has a pseudorhiza. These second-year fruit-bodies, after shedding their spores, die and rot away except for their pseudorhizae which persist. Thus, in the second winter, the persistent subterranean structure consists of a central first-year pseudorhiza bearing a number of second-year pseudorhizae (D). In the *third year* of fruiting, some or all of the second-year pseudorhizae, in their turn, may give rise to new fruit-bodies (E). These, after sporulation, would die down leaving their living pseudorhizae behind (F). Thus, in the suc-ceeding winter, the subterranean structure would consist of a

sympodium of the stipes produced during the three preceding summers. In the *fourth year* of fruiting, one or more of the persistent third-year pseudorhizae would give rise to new fruit-bodies (G) ; and then these fruit-bodies and the subterranean sympodium
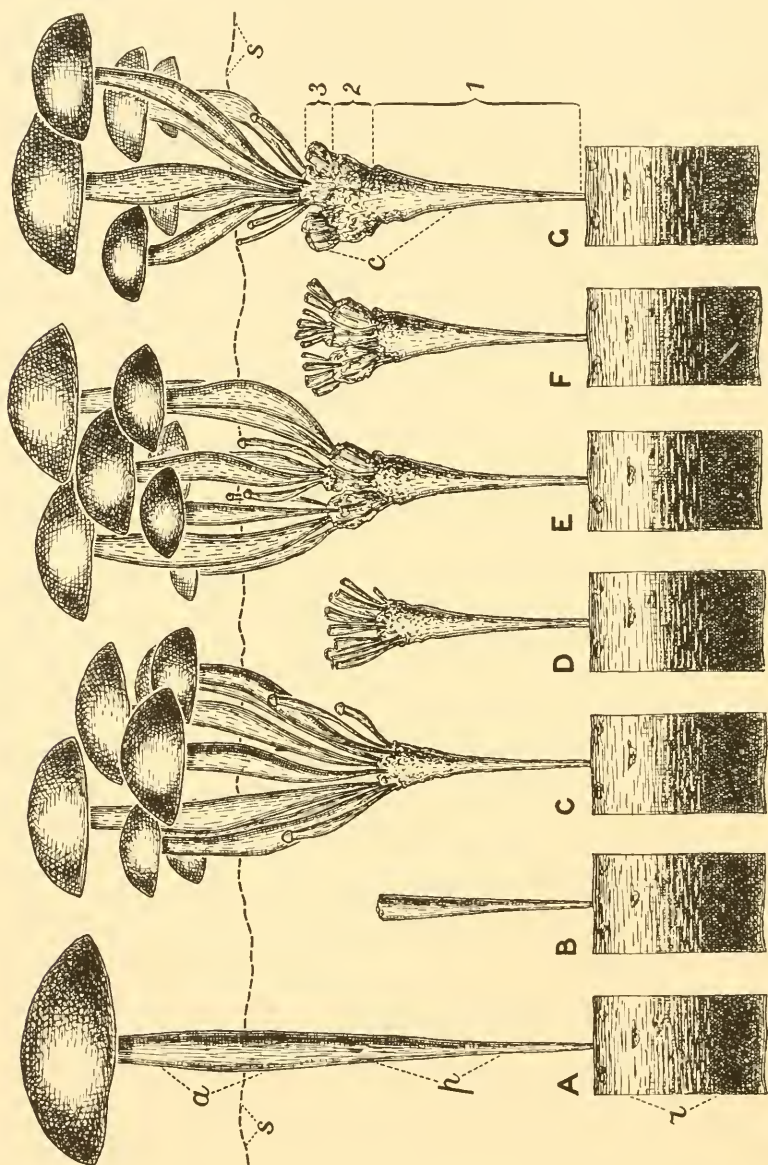
of pseudorhizae might have an appearance like that found for the fruit-body cluster shown in Fig. 195. It is possible that the compound pseudorhiza under favourable conditions might function for even one or more further years.

When in the first year of fruiting, instead of one fruit-body, several fruit-bodies are produced, several pseudorhizae persist through the winter ; and in the next summer several or all of these pseudorhizae produce new fruit-bodies ; and so on from year to year (Fig. 196). If, in such a case, the buried root is only about two inches below the surface of the ground, the pseudorhizae are all very short ; and there may be formed, in the course of several years, a black agglomeration of very numerous more or less laterally fused pseudorhizae, which may be more or less rounded peripherally and sclerotioid in general appearance. Such, doubtless, was the origin of the massive structure shown in Fig. 192 (p. 384). Its true nature was made out from vertical and transverse sections, and by comparison with a series of persistent pseudorhizae which led by gradations to the simplest and most unmistakable forms.

Lateral Grafting.—In an old compound pseudorhiza, one often finds that the persistent pseudorhizae of any one year have become irregular in outline, more or less tuberculate, and fused together laterally. This natural lateral grafting of pseudorhizae which were originally distinct appears to be due to a certain amount of renewed growth taking place in summers subsequent to the one in which the pseudorhizae were originally developed. It was doubtless such renewed growth and fusion of many scores of

Fig. 194.—*Collybia fusipes.* A diagrammatic representation of the development of a compound pseudorhiza during a series of years. A, a fruit-body in the summer of the first year ; *r*, a Beech root being rotted by the mycelium ; *s*, the general surface of the leaf-mould ; *p*, the simple pseudorhiza passing into *a*, the aerial stipe-shaft. B, the same fruit-body in the ensuing winter : the pseudorhiza alone is persistent. C, summer of the second year : the pseudorhiza has given rise to several new fruit-bodies, each of which has its own pseudorhiza. D, winter condition of C : the persistent pseudorhiza is now compound. E, summer of the third year : the compound pseudorhiza has given rise to several new fruit-bodies, each of which again has its own pseudorhiza. F, winter condition of E : the persistent pseudorhiza is now made up of the pseudorhizae of three successive generations of fruit-bodies. G, summer of the fourth year : new fruit-bodies are again being produced, but only from one part of the top of the pseudorhiza ; the numbers 1, 2, and 3 indicate the successive annual increments of growth of the compound pseudorhiza *c*. About two-thirds natural size.
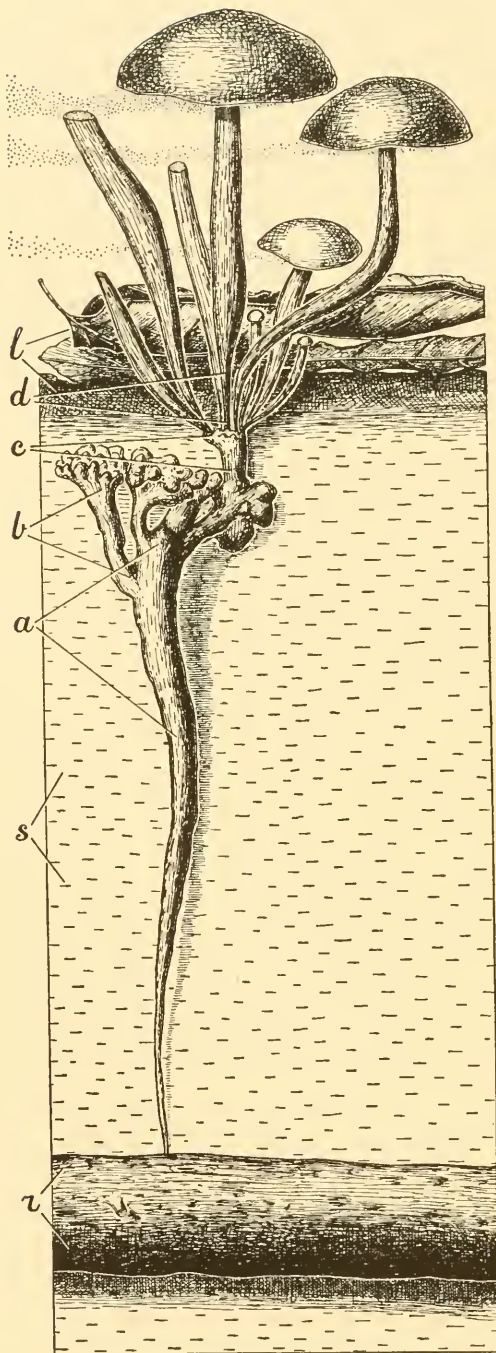
pseudorhizae produced in successive years that brought into being the pseudorhizal mass shown in Fig. 192 (p. 384).

Healing of Pseudorhizal Wounds.—It was discovered that, if a pseudorhiza which has been freshly dug up from the ground is broken across and kept in a moist place, the wound heals up within a few days. The hyphae at the wound-surface turn brown and form a dark smooth layer resembling that which one normally finds on the pseudorhiza's exterior. Such healing,

FIG. 195. *Collybia fusipes.* A much elongated compound perennial pseudorhiza arising from a deeply-buried root of a Beech in Kew Gardens. *l*, leaf-mould made of Beech leaves ; *s*, soil ; *r*, a deep-seated root ; *a*, the pseudorhiza of the first-year fruit-body ; *b*, a pseudorhiza of a second-year fruit-body ; *c*, a pseudorhiza of a third-year fruit-body; and *d*, a pseudorhiza of a fourth-year fruit-body. The root in which the mycelium was vegetating is represented here, for the sake of convenience, as only about 8 inches below the surface of the leaf-mould ; but its actual depth was about 12 inches. Clouds of spores are shown escaping from the pilei and being carried away by the breeze. One-half natural size.

doubtless, also takes place under natural conditions, whenever an

Fig. 196.—*Collybia fusipes.* (1) On the right, five fruit-bodies developing in 1916 from one of several pseudorhizae which have persisted since the summer of 1915. The 1915 fruit-bodies arose from the persistent (and now irregularly swollen) pseudorhiza of a fruit-body which developed in 1914. The pseudorhiza of the 1914 fruit-body is shown attached to a piece of wood which was part of a large decaying Beech root. (2) On the left, a living fungous mass consisting probably of a 1914 pseudorhiza and three 1915 pseudorhizae. It was found in 1916 beneath the ground attached to a Beech root. Had it been left undisturbed, it would have given rise, doubtless, to a new set of fruit-bodies. Photographed in August, 1916, at Kew by Miss E. M. Wakefield. Natural size.

aerial stipe-shaft dies down and rots away leaving a bare irregular exposed surface at the top of the subterranean pseudorhiza. The

wound-tissue, with which the pseudorhiza heals itself, serves to keep bacteria and other small organisms from making their way into the interhyphal air-spaces and to conserve moisture in dry weather.

The Mycelium and the Problem of Parasitism.—The continuity of a persistent pseudorhiza with the mycelium in the root of a Beech is well shown in Fig. 193 (p. 386) which is reproduced from a sketch made by Miss E. M. Wakefield. The root contained a paper-thin sheet of mycelium between the bark and the wood (*n*), and a thicker sheet of mycelium in the wood itself (*m*). With these sheets of mycelium the base of the pseudorhiza was in direct continuity. Moreover, Miss Wakefield found long tubes containing a dense liquid and therefore resembling latex vessels, not only in the pseudorhiza but also in both of the mycelial sheets. These observations, as well as others which there is no need to describe, clearly show that the mycelium of *Collybia fusipes* inhabits the roots above which the fruit-bodies are found.

In a few cases I observed that there was a sharp line of demarcation between the dead part of a buttress-root upon which the mycelium of *Collybia fusipes* was feeding and the living sap-filled part a few inches nearer the tree-trunk. What was observed suggested that the fungus is parasitic on Beeches and Oaks, and that its mycelium is able to kill and destroy progressively even their stoutest roots. On the other hand, it might perhaps be argued that the fungus is nothing more than a saprophyte, and that it destroys roots progressively toward the tree-trunk only after they have died from other causes. To determine with certainty whether or not *Collybia fusipes*, in addition to behaving as a saprophyte, may also behave as a parasite would require a detailed investigation such as I have not been able to make. It is possible that, if one inserted small pieces of Beech wood in which the mycelium was growing into a large sound root, the mycelium might grow into the wood of the sound root and kill the root as its hyphae advanced ; and, in a few successive summers, one might observe fruit-bodies coming up above the inoculated root and attached to it by pseudorhizae. If fruit-bodies were thus produced during two or three successive summers, one would doubtless find at the end of this time that the root had been killed and its wood destroyed for a distance of several

feet. The rate of progress of the mycelium along the root per annum might then be calculated. If such a series of positive observations should one day be made, there would be no alternative but to regard *Collybia fusipes* as a destructive root-parasite. On the other hand, if inoculation experiments were to yield only negative results, we might be obliged to consider the fungus merely as an innocent saprophytic scavenger, which rots Beech roots and Oak roots only after these have been killed by some other agency. Here, then, is an attractive problem for the phytopathologist.

The Functions of the Pseudorhiza and the Significance of its Persistency.—The pseudorhiza of *Collybia fusipes* has the same chief function as the pseudorhiza of *Collybia radicata, Coprinus macrorhizus*, etc., *i.e.* it serves as an organ for pushing upwards from the surface of the buried nutrient substratum (here a tree-root) through a non-nutrient medium (here soil and leaf-mould) to the surface of the ground the rudimentary stipe-shaft and pileus, thus enabling these parts of the fruit-body to expand subaerially. The pseudorhiza also (1) conducts food materials from the mycelium in the buried root to the stipe-shaft and pileus whilst these are developing subaerially, and (2) gives a certain amount of mechanical support to the aerial stipe-shaft and pileus, thus assisting these organs in taking up and maintaining their proper positions in space.

Just as in *Collybia radicata* and *Coprinus macrorhizus*, the pseudorhiza of *Collybia fusipes* increases in length by intercalary growth in a subterminal axial region which lies just below the rudimentary pileus and stipe-shaft. The upward direction of growth of the pseudorhiza is doubtless due to negative geotropism ; and probably the pseudorhiza, while able to grow in length freely in the dark soil, has its growth in length inhibited by light, so that the action of light regulates the length of the pseudorhiza and prevents this organ from emerging above the surface of the ground.

In *Collybia radicata* and *C. longipes* the pseudorhiza is strictly annual and dies shortly after the fruit-body of which it forms a part has shed its spores, but in *Collybia fusipes* it is perennial. In having a perennial pseudorhiza *Collybia fusipes*, so far as is at present known, is not only unique in the genus Collybia but also unique among the Agaricaceae in general.

In having a pseudorhiza which is persistent and perennial, *Collybia fusipes* gains a distinct advantage, for thereby it economises fruit-body material and so, in the end, increases its output of spores. As we have seen, the mycelium lives in a buried root and produces new stipe-shafts and pilei subaerially each year. If the new fruit-bodies produced in successive years by a single mycelium were each to develop a full-length pseudorhiza stretching upwards from the buried root to the surface of the soil, a great mass of fungus material would be expended in pseudorhizal production that is actually saved by the first-formed pseudorhiza and its branches being persistent and perennial (*cf.* Fig. 195, p. 390). There can be but little doubt that the fungus material saved by using the same pseudorhiza over and over again in successive summers is applied to the production of additional or of larger fruit-bodies and, therefore, to the production and liberation of millions of additional spores. Thus the persistence of the pseudorhiza of *Collybia fusipes* is a factor of considerable importance to the fungus in its struggle for existence.

The annual pseudorhiza of *Collybia radicata* and the perennial pseudorhiza of *Collybia fusipes* are comparable with the annual fruit-body of *Polyporus squamosus* and the perennial fruit-body of *Fomes applanatus* respectively. The perennial pseudorhiza and the perennial polyporous fruit-body must be considered as having been derived in the course of evolution from their annual counterparts in allied species, and therefore, as representing advances in specialisation and adaptation of structure to function. There can be no doubt that the persistence of the fruit-body in *Fomes applanatus* is a great economy and enables very many more spores to be produced than would be possible were it necessary to renew the fruit-body flesh in its entirety each year. Thus the persistence of the pseudorhizal portion of the fruit-body of *Collybia fusipes* and the persistence of the whole fruit-body in *Fomes applanatus* both serve to increase the number of spores produced and liberated, and therefore make for greater efficiency in the process of reproduction.

Sarcoscypha protracta.—This Discomycete (Fig. 197), as already set forth in a previous Chapter,[1] resembles *Collybia fusipes*

[1] This volume, pp. 239–240.

in that its mycelium vegetates in buried roots and its fruit-bodies develop a perennial pseudorhiza. Now that the mode of origin of the fruit-bodies of *C. fusipes* has been described in detail, a

Fig. 197.—*Sarcoscypha protracta*, a Discomycete with a perennial pseudorhiza. Each fruit-body or group of fruit-bodies shown was attached to a buried root in a Poplar wood. From each root a single fruit-body, like those shown at the extreme right and left, first came up. The darker portion of its stem—the *pseudorhiza*—was covered by earth and leaf-mould, while the lighter portion—the *aerial stipe*—terminating in a cup was developed in the air. The aerial part of each fruit-body died and rotted away, leaving behind most of the pseudorhiza. This pseudorhiza branched the next spring and produced several fruit-bodies. This condition is shown in specimens Nos. 3, 4, 5, and 6 from the left. No. 2 is doubtless in its third year : it exhibits a primary pseudorhiza produced the first year, three secondary pseudorhizae (the remains of three fruit-bodies) produced in the second year, and two fruit-bodies each with a tertiary pseudorhiza (attached to the end of a secondary pseudorhiza) produced in the third year. Obtained at Winnipeg, May 27, 1925. Natural size.

comparison between the fruit-bodies of *S. protracta* and *C. fusipes* can be made more readily.

A solitary fruit-body of *Sarcoscypha protracta* attached by a pseudorhiza to a buried Beech root is shown in Fig. 116 (p. 240) and is strictly comparable with the solitary fruit-body of *Collybia fusipes* attached to a buried Poplar root shown in Fig. 194, A (p. 388).

A persistent pseudorhiza which has branched in the second year is shown for *Sarcoscypha protracta* in Fig. 197, Nos. 3, 4, 5 and 6 from the left, and a similar persistent pseudorhiza is shown for *Collybia fusipes* in Fig. 194, C (p. 388).

A pseudorhiza in the third year, *i.e.* with primary, secondary, and tertiary parts, is shown for *Sarcoscypha protracta* in Fig. 197, No. 2 from the left, and a similar pseudorhiza is shown for *Collybia fusipes* in Fig. 194, E (p. 388).

The production of a perennial pseudorhiza in *Collybia fusipes* and *Sarcoscypha protracta*, both of which have a mycelium which vegetates in buried roots, affords another example of two very diverse plants having become adapted in the same way to meet the requirements of a similar set of external conditions.

Probably there are other Agaricaceae beside *Collybia fusipes*, and other Discomycetes beside *Sarcoscypha protracta*, which have a perennial pseudorhiza ; but, if so, they remain to be discovered by further field investigations.

CHAPTER III

Introduction.—*Omphalia flavida* is a Hymenomycete of peculiar interest in that : (1) it is a parasite which is able to attack the leaves of a great variety of plants ; (2) it reproduces itself not only by means of basidiospores but also by means of gemmae of unique structure (the so-called *stilbum*-heads) ; and (3) its mycelium is luminous.

Omphalia flavida and the American Coffee-leaf Disease.— *Omphalia flavida* first attracted attention owing to the fact that, under very moist climatic conditions, it causes a serious leaf-spot disease of the Coffee tree (Figs. 198, 199, and 200). The disease has been reported from Mexico, Central America, the Antilles, Trinidad, Venezuela, Brazil, and generally throughout the coffee-growing region of America,[1] and it has therefore been called the *American Coffee-leaf disease*.[2]

[1] G. L. Fawcett, " Fungus Diseases of Coffee in Porto Rico," Porto Rico Agri. Exp. Station, *Bull. No. 17*, 1915, p. 11.

[2] S. F. Ashby, " The Perfect Form of *Stilbum flavidum* Cke. in Pure Culture," *Bulletin of Miscellaneous Information*, Royal Botanic Gardens, Kew, 1925, p. 325.

The American Coffee-leaf disease, as it occurs in Porto Rico, has been thus described by Fawcett.[1] " The disease is characterised by the occurrence on the leaves of small spots usually circular in outline, but sometimes ovate along the veins. The newer ones are very dark, the older ones light colored. The spots are usually about 6 mm. in diameter, although many of the older ones become 12 to 13 mm. in diameter. Sometimes they fuse or give entrance to other tissue-destroying fungi which infect the intervening tissue, producing spots of considerable size. The worst affected leaves have from 30 to 40 or even more spots, so that a large proportion of the leaf tissue is destroyed. On the upper surface of many of the spots and also to some extent on the lower surface may be seen

Fig. 198.—A Coffee tree almost defoliated by *Omphalia flavida*. Only the youngest leaves remain on the twigs. Photographed by G. L. Fawcett at the Mayagüez Experiment Station, Porto Rico.

hair-like projections from 1 to 4 mm. long of a yellowish color, each bearing at the end a head so that they resemble minute pins. This is the reproductive or fruiting stage of the fungus [*cf.* Figs. 201 and 202, pp. 402 and 403]. Each spot produces a continuous crop of these hairs so long as weather conditions are favourable. The total

[1] G. L. Fawcett, *loc. cit.*, pp. 11–12.

number at any time is small and in an entire season but from 20 to

Fig. 199.—Leaf-spots caused by the mycelium of *Omphalia flavida* on Coffee leaves. Photographed by G. L. Fawcett at the Mayagüez Experiment Station, Porto Rico. Reduced to about one-half the natural size.

50 are produced in each spot, judging from the number of old filament bases. The largest number observed was 70 in a spot of

7 mm. diameter. As the leaf-spots become older, growth having stopped for any reason, such as the advent of the dry season, the diseased tissue falls away, leaving numerous circular openings in the leaf. In other leaf diseases the dead tissue remains.

" Sometimes the fungus attacks young stems, where it causes conspicuous scars and so weakens the points affected that they are easily broken by the wind. The berries also are attacked, a slight discoloration of the grain being frequently caused."

In Porto Rico the disease is restricted to the moister parts of the island, but there the best coffee is produced. The trees with leaf-spots yield fewer berries. " The injury to the trees," says Fawcett, " is not so much in the actual amount of the leaf tissue destroyed, although this may amount to one-fifth or even more of the entire amount in the worst cases, but in the defoliations which take place after a time. The diseased leaves drop

Fig. 200.—A Coffee leaf showing leaf-spots caused by the mycelium of *Omphalia flavida*. Each spot has arisen from a gemma, presumably blown on to the leaf by the wind or splashed on by the rain. The spots, apparently, have not yet produced any gemmifers. They are luminous in the dark. Photographed by G. L. Fawcett at the Mayagüez Experiment Station, Porto Rico. About the natural size.

sooner than those not affected and, owing to the weakened condition of the tree, are not soon replaced. After the first severe attack, the base of each tree may be seen surrounded by a pile of green leaves several inches deep. The disease never kills the trees. They live on with scanty foliage and are able to put forth new growth and bear a small amount of berries each year."

The tiny yellow pin-like fruiting structures described by Fawcett as projecting from the surface of the leaf-spots are the so-called *stilbum*-bodies. A *stilbum*-body never produces any spores ; but, when fully formed, its head, often called a *stilbum*-head, is readily detachable (*cf.* Figs. 205, A, p. 408, and 213, C, p. 424). " The fungus," says Fawcett, " is distributed by the heads at the ends of the filaments being caught by the wind or rain-drops and carried to near-by leaves, a process facilitated by the heads becoming loosened in the older filaments through the formation of cavities or ' lacunae ' near the point of attachment. The head is soon fastened to the leaf on which it happens to fall by the numerous threads which it sends out at the point of contact. Within less than a week a dark circular spot is formed and new filaments appear and new loosely attached heads are formed on these by means of which the spread of the disease is continued."

Recently Briton-Jones [1] has attempted to control the Coffee-leaf disease by heavy pruning followed by the removal of all the remaining leaves and by manuring and cultivation to induce the subsequent production of vigorous new growth.

Stilbum flavidum as a Stage in the Life-history of Omphalia flavida.—In 1880, some diseased Coffee leaves were sent to M. C. Cooke [2] from Venezuela by Dr. Ernst. On some of the coloured spots Cooke found the perithecia of *Sphaerella coffeicola* [3] and on others the tiny yellow Stilbum-like fruiting structures of another fungus which he called *Stilbum flavidum*. In some of the spots the two

[1] H. R. Briton-Jones, " Control of the American Leaf Disease (*Omphalia flavida*) on Arabian Coffee in Trinidad," *Memoirs of the Imperial College of Tropical Agriculture, Trinidad*, Mycological Series, No. 2, 1930, pp. 1–8.

[2] M. C. Cooke, " The Coffee Disease in South America," *Journ. Linn. Soc.*, Botany, Vol. XVIII, 1881, pp. 461–467, Pl. XVIII, Figs. 1, 5, 6.

[3] According to E. J. Butler (Note on Cooke's type specimens at Kew, 1915) *Sphaerella coffeicola* is a Didymella, for its perithecia contain paraphyses.

fungi grew together. The heads of the *stilbum*-bodies, as we have seen, serve to spread the American Coffee-leaf disease.
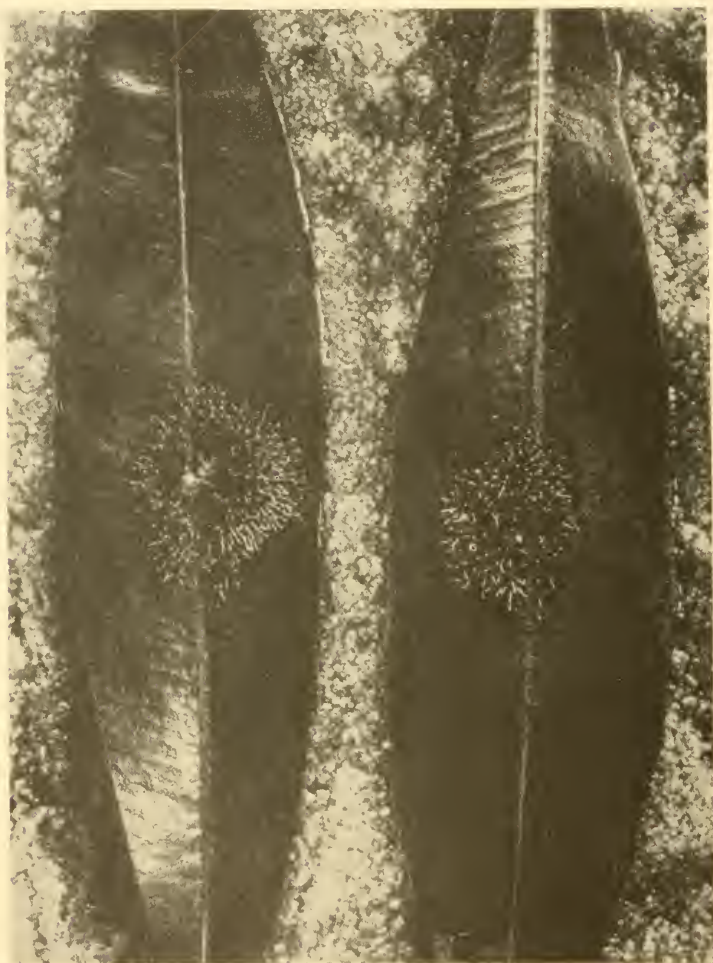
FIG. 201.—Two *Nerium Oleander* leaves infected with the mycelium of *Omphalia flavida* and bearing numerous gemmifers of the fungus. The leaves were gathered, dipped in 0·1 per cent. mercuric chloride for 2 minutes, rinsed in sterilised water, and placed in a large crystallising dish on moist sand. Each leaf was wounded by scratching the surface with a sterile needle and the wound was then inoculated with three or four gemmae. The gemmifers began to appear 10 days after inoculation. The photograph was taken 19 days after inoculation. The fungus strain was of Porto Rican origin. The divergence of the gemmifers from one another can be observed. Natural size.

Stilbum flavidum Cke., as we shall see, has nothing to do with the genus Stilbum but is merely a stage in the life-history of *Omphalia flavida* (Cke.) Maublanc et Rangel.

In 1914, Maublanc and Rangel[1] obtained some spotted leaves of the Loquat, *Eriobotrya japonica*, at Rio de Janeiro and placed them in an atmosphere saturated with water-vapour. On the surface of the spots there developed first of all a crop of *Stilbum flavidum* fruit-bodies and subsequently the much larger fruit-bodies of a small agaric to which they gave the name *Omphalia flavida*. Similar results were obtained with the leaves of undetermined species of Melastomaceae and Compositae. Beeause (1) the leaf-spots frequently produced the Stilbum and the Omphalia in succession, and because (2) the two fungi showed marked resemblances in colour and general structure, Maublanc and Rangel concluded that *Stilbum flavidum* Cke.
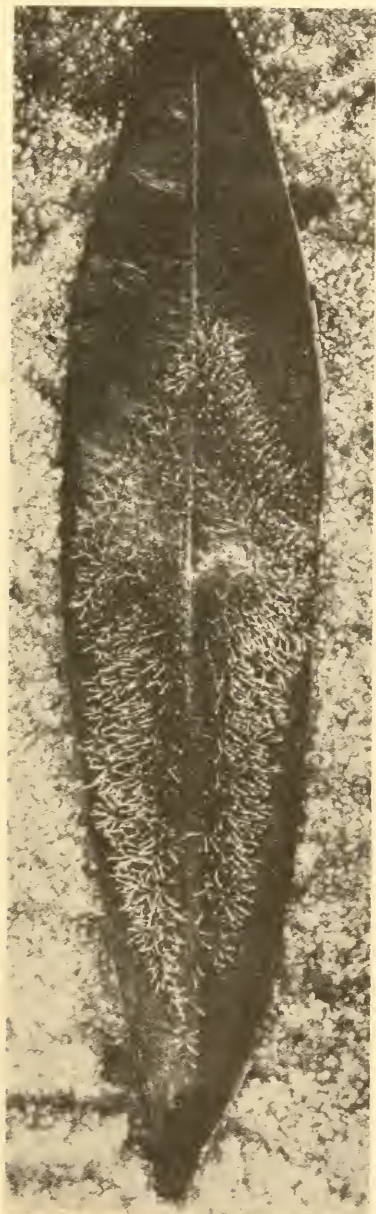
Fig. 202.—Numerous gemmifers on a leaf of *Nerium Oleander* artificially inoculated with *Omphalia flavida*. The same leaf as the one on the left in Fig. 201 photographed 10 days later and 29 days after inoculation with gemmae. The divergence of the gemmifers from one another can again be well seen. Natural size.

[1] A. Maublanc et E. Rangel, " Le *Stilbum flavidum* Cooke, forme avortée de l'*Omphalia flavida* n. sp.," *Bull. Soc. Myc. France*, T. 30, 1914, pp. 41–47.

is nothing more or less than an aborted condition (*état avorté*) of *Omphalia flavida*.

In 1925, Ashby,[1] making use of some diseased Coffee leaves obtained from a valley in the Northern Range of the island of Trinidad, proved by the pure-culture method that Cooke's Stilbum and the Omphalia of Maublanc and Rangel do actually arise from one and the same mycelium.

Ashby lifted ripe *stilbum*-heads from a leaf-spot, washed them in a weak solution of sulphuric acid for about a minute, and then placed them on corn-meal agar in a Petri dish. At room temperature (79° F.), after three days, a radiately spreading growth of fine hyphae had developed from most of the heads, only two out of ten mycelia having become contaminated by bacteria. Transfers of the mycelium were made to sterilised potato blocks, rice grains, and bread. On potato and rice there was fair growth and reproductive bodies began to develop but remained imperfect and sessile.

Fig. 203.—*Omphalia flavida*, growing on bread and water in a conical flask. In front, the white bread permeated by the mycelium; above, a forest of numerous yellow gemmifers; still higher, a group of thirteen yellow sporophores towering up above the gemmifers. Photographed by S. F. Ashby. Magnification, about 1·5.

On bread, however, development was completed. This substratum was used by Ashby because Westerdijk[2] had recommended it for the culture of Basidiomycetes. In a flask containing four parts of water to one part of fresh crumb of white bread, at room temperature, the mycelium spread steadily around the inoculum and, after a few days, became dotted with pale-yellow *Stilbum flavidum* initials which completed their development and formed a dense low forest

[1] S. F. Ashby, *loc. cit.*, pp. 325–328.
[2] J. Westerdijk, *Report Internat. Conf. Phytopath.*, Holland, 1923, pp. 165–169.

extending from the centre almost to the margin of the colony. After twelve days a few much larger bodies, having deep-yellow hemispherical heads, began to push up from the centre, and in fifteen days some of them had become perfect *Omphalia flavida* agarics of a light sulphur colour, with erect slender minutely hirsute stipes and membranous subhemispheric or broadly conical pilei (Fig. 203). As inocula for further subcultures on sterilised bread, Ashby used either (1) *stilbum*-heads or (2) fragments of the stipes or pilei of the agarics, with the result that the mycelium always gave rise to both *stilbum*-bodies and Omphalia fruit-bodies. Basidiospores, obtained from the gills of one of the agarics, were sown ; and the mycelium, after being transferred to a corn-meal agar slope, yielded many *stilbum*-bodies and a few perfect agarics. Thus Ashby conclusively proved that the mycelium of *Omphalia flavida* produces fruiting structures of two kinds : (1) ordinary hymenomycetous sporophores which liberate basidiospores, and (2) peculiar pin-shaped *stilbum*-bodies which are sporeless but each of which readily sets free its *stilbum*-head.

In the autumn of 1925, Mr. Ashby, at the Imperial Mycological Institute, Kew, kindly showed me his cultures of *Omphalia flavida* and gave me a subculture. I took this to Winnipeg and, in conjunction with T. C. Vanterpool, repeated some of Ashby's experiments. The mycelium was transferred to

Fig. 204.—*Omphalia flavida*, growing on an oat-meal agar slope in a test-tube. The mycelium has produced numerous gemmifers and a few perfect fruit-bodies. Culture several weeks old. Natural size.

sterilised bread (1 part bread, 4 parts water) where, within about three weeks, it gave rise first to a little forest of *stilbum*-bodies and afterwards to a number of the much larger perfect Omphalia agarics (*cf.* Fig. 204). Both *stilbum*-bodies and perfect agarics were also obtained by sowing *stilbum*-heads (1) on bread sterilised in conical flasks and (2) on living leaves of *Bryophyllum calycinum* (for details *vide infra*).[1] Thus Ashby's conclusions as to the behaviour of *Omphalia flavida* in artificial cultures have been confirmed.

The Structure of the so-called Stilbum-body.—The structure of the *stilbum*-body was first investigated and illustrated by Puttemans.[2] With the hope of throwing light on the mode of abscission of the head from the stalk, it has been carefully re-investigated by Miss Ruth Macrae and the author in the laboratory at Winnipeg. The description which here follows is based on observations made by us on living material grown on malt-agar and also on Bryophyllum and Oleander leaves.

A *stilbum*-body is, as we have seen, a minute pin-shaped structure consisting of a slender cylindrical stalk surmounted by a head which, when fully developed, can be readily detached (Fig. 205, A, B, C). Both stalk and head are yellow. The stalk, which tapers gradually from below upwards, is about $2 \cdot 0$ mm. in length, about $0 \cdot 12$ mm. in diameter at its base, and about $0 \cdot 05$ mm. in diameter just below the head, while the head has an average diameter of about $0 \cdot 36$ mm. varying up to $0 \cdot 4$ mm. A *stilbum*-body never produces any spores.[3]

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[1] A. H. R. Buller and T. C. Vanterpool, " The Bioluminescence of *Omphalia flavida*, a Leaf-spot Fungus," *Phytopathology*, Vol. XVI, 1926, p. 63.

[2] M. A. Puttemans, "Sur la maladie du Caféier produite par le *Stilbella flavida*," *Bull. Soc. Myc. France*, T. XX, 1904, pp. 158–164, Pl. XI.

[3] M. C. Cooke (*Grevillea*, Vol. IX, 1880–1881, p. 11) and Kohl (*vide infra*) thought that conidia are produced on the exterior of the head ; but the careful researches of Puttemans (*loc. cit.*, pp. 160–163), which are supported by the observations of Patouillard, Massee, Spegazzini, Delacroix, Ashby, and myself, lead to the conviction that Cooke and Kohl were mistaken. Cooke was probably misled by the Stilbum-like appearance of the fungus and, as Puttemans argues, by an optical illusion in respect to the appearance of the outer hyphae of the head. Kohl, whose description of the *stilbum*-head and its outer hyphae is imperfect and unsatisfactory, states that for months he sought in vain for conidia before finding any and that many attempts to infect Coffee leaves with the supposed conidia completely failed. He concluded that, under natural conditions, the fungus is propagated not by the conidia but by the detached heads.

The head is an oblate spheroid (a flattened globe) with an apophysis of smaller diameter below (Fig. 205, A, B, C). Its upper surface is slightly depressed in the centre. The apophysis encloses and clasps the stalk about 0·1 mm. from its extreme end. The head is tough and coriaceous, so that, when it dries, it is not appreciably deformed. With moderate magnification one can observe that the main oblate-spheroidal part of the head, but not the apophysis, is covered peripherally with a large number of aerial radiating hyphal filaments (Fig. 205, C, *l*). It is these filaments which serve to infect a new host-leaf when a head has fallen upon it and the weather is sufficiently moist.

The stalk is a solid cylinder (Fig. 205, B, C, D), and not hollow as described and illustrated by Puttemans.[1] When very young, it is quite straight and perpendicular to the substratum (Fig. 212, A, B, p. 419) ; but, as it grows in length, it usually becomes more or less bent or curved (Fig. 205, A and B). At maturity, *i.e.* when about 2 mm. long, it is thickest at its base, slightly attenuated from its base up to the apophysis and then, within the apophysis, distinctly contracted and always more or less sigmoidally bent (Fig. 205, B and C). The sigmoid terminal portion of the stalk is hidden from external view inside the head, but can readily be observed after the head has been removed from it.

The microscopic structure of a median-longitudinal section of the head and upper part of the stalk of a mature *stilbum*-body is shown in Fig. 205, C. The head consists of a central mass of relatively large pseudoparenchymatous cells (*m*), surrounded above and at its sides by a thin layer of smaller and more flattened cells from which radiate outwards a number of slender branched septate hyphae (*n*) which, at the sides of the head, terminate in large pear-shaped cells (*k*) and, at the top of the head, terminate in more slender clavate cells (*i*). A certain number of the swollen cells at the sides of the head give rise each to a single aerial hypha which is usually simple (*l*) but may be branched. The hyphae grow outwards from the head and give it the woolly appearance that can be seen externally with moderate magnification. The clavate cells on

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[1] Puttemans (*loc. cit.*, p. 159, Plate XI) in his Fig. 4 erroneously shows aerial hyphae just as thickly present on the top of the head as at the sides.
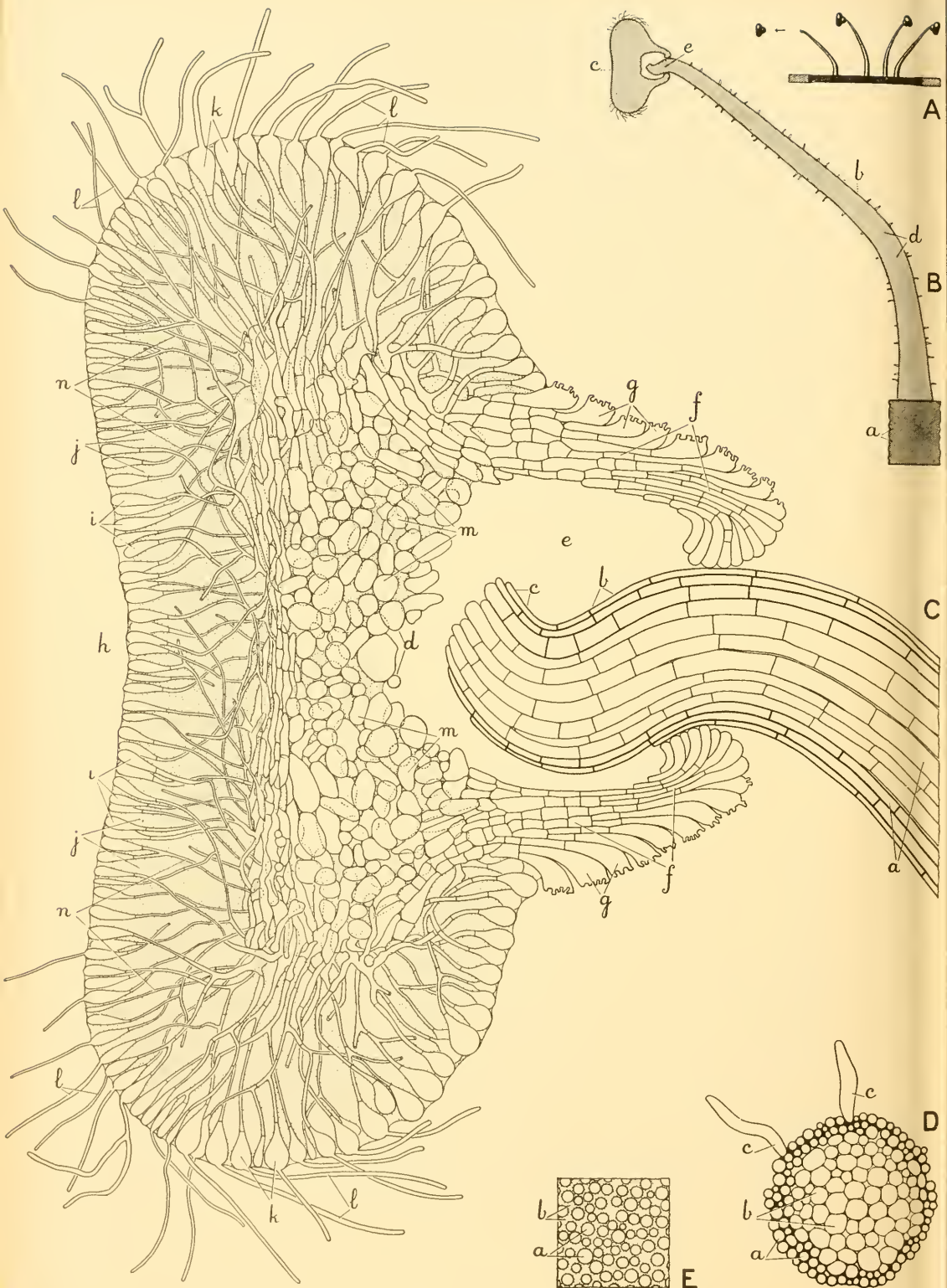
the somewhat depressed upper side of the head (*i*) are slightly separated from one another by the mucilage of their outer walls (*j*), and they do not bear aerial hyphae but remain naked.

The flesh of the whole head is *solid* ; for the space between the pseudoparenchymatous cells, the radiating hyphae, the clavate cells, etc., is filled with transparent mucilage which, doubtless, just as in the thallus of a Fucus, is derived from swollen outer cell-walls. When a head on its stalk is placed in water, no air-bubbles can be seen between any of the cells of the head. The only air-bubble to be observed is a large one which fills the cavity around the end of the stalk within the apophysis. The whole head, owing to its solidity and yellow colour, has a waxy appearance when seen in bright light

Fig. 205.—The structure and orientation of the gemmifers of *Omphalia flavida*. A, a group of four mature gemmifers on the upper side of a leaf-spot of a leaf of *Nerium Oleander*. During their development, these gemmifers diverged from one another and took up the positions here shown. Each gemmifer consists of : (1) a pedicel which tapers upwards and exhibits a divergence-curvature below its middle point ; and (2) a terminal gemma. The gemma of the gemmifer farthest to the left has just been blown away from its pedicel by the wind in the direction shown by the arrow. Magnification, 5.

B, a median-longitudinal section of a single gemmifer attached to an Oleander leaf, shown in its natural position : *a*, part of the leaf ; *b*, the pedicel, bearing hairs at its surface ; *c*, the gemma, produced peripherally into infection hyphae. The pedicel *b* exhibits two curvatures : (1) a divergence-curvature at *d*, and (2) a double or sigmoid geotropic curvature terminally at *e*. The sigmoid bending of the terminal part of the pedicel resulted in the end of the pedicel being torn away from the flesh of the gemma. The gemma is now attached to the pedicel merely by its basal collar or apophysis and, in this condition, it is ready for dislodgment and transportation by the wind. Magnification, 38.

C, the upper part of the gemmifer in B greatly enlarged, to show the histological structure. In this vertical median-longitudinal section the parts may be distinguished as follows : *a*, the pedicel, with a thicker-walled cortex and a thinner-walled looser medulla (*cf.* D) ; the terminal part of the pedicel is sigmoidally curved ; the downward curvature *b* was made in response to a positive geotropic stimulus, and the upward curvature *c* in response to a negative geotropic stimulus and, when the sigmoid curvature was being formed, the pedicel broke away from the flesh of the gemma to which it previously had been attached at *d* ; *e*, a large air-space between the terminal part of the pedicel and the apophysis (collar) of the gemma ; *f f*, the apophysis of the gemma which holds the gemma on to the pedicel until the wind blows it away ; *g g*, fimbriate cells which form the outer layer of cells of the apophysis ; *h*, the apex of the gemma, somewhat depressed ; *i i*, clavate cells separated by a gelatinous matrix *j j* derived from swollen cell-walls ; *k k*, more swollen clavate cells at the periphery of the gemma, which are produced terminally into aerial infection hyphae *l l* ; *m m*, pseudoparenchymatous cells embedded in a gelatinous matrix and forming the core of the flesh of the gemma ; *n n*, slender branched septate hyphae forming the cortex of the flesh of the gemma. Magnification, 405.

D, a transverse section of the pedicel a little below *a* in C ; *a*, the thicker-walled cortex ; *b*, the thinner-walled medulla; *c c*, two hairs. Magnification, 410.

E, a surface view of part of the apex of the gemma, as seen at *h* in C : *a*, clavate cells ; *b*, the gelatinous matrix formed of swollen cell-walls. Magnification, 405.

Drawn by A. H. R. Buller and Ruth Macrae.

and, owing to its mucilage, is slippery and difficult to hold in place for making hand-sections.

The apophysis or under part of the head is formed of hyphae which arise from the core of the oblate-spheroidal part of the head. These hyphae (Fig. 205, C, *f*) are extended outwards and downwards, so that at first they avoid the contracted upper part of the stalk, and then inwards and downwards so that finally many of them come to rest against the stalk, with the result that an annular air-space is formed between the upper part of the stalk and the apophysis by which it is surrounded (Figs. 212, D, p. 419, and 205, C, *e*).   The
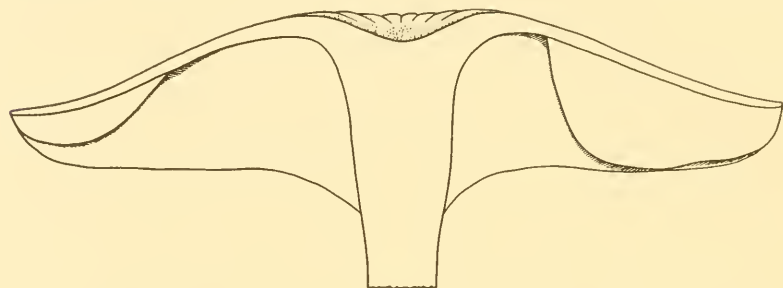
FIG. 206.—*Omphalia flavida*. Vertical section through pileus and upper part of the stipe of a large expanded fruit-body. The fruit-body was removed from a Bryophyllum leaf which had been inoculated with gemmae 38 days previously. Note the umbilicate pileus, the thin pileus-flesh, the decurrent gills, and the solid stipe. The pileus was 6·7 mm. in diameter. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, about 15.

outer hyphae of the apophysis end in outwardly curved, swollen, almost hyaline cells, each of which is extended into a number of short, slender, simple or bifurcated projections (Fig. 205, C, *g*).

From the above description it will be seen that the *stilbum*-body produces spores neither externally nor internally, *i.e.* it is completely non-sporogenous.

The Omphalia flavida Sporophore.—The external appearance of the sporophore of *Omphalia flavida*, which represents the perfect stage of the fungus and produces and liberates an abundance of basidiospores, is shown in Figs. 203, 204, and 213 (pp. 404, 405, and 424). It was first observed and described in 1914 by Maublanc and Rangel.[1] Their diagnosis, translated from the Latin, is as follows.

[1] A. Maublanc et E. Rangel, *loc. cit.*, p. 46.

" Very minute, yellowish ; pileus thin, membranaceous, hemi-spheric-campanulate, depressed or subumbilicate in the centre, then more or less flattened, glabrous, radially striate, 1·5–2·5 mm. in diameter, with an acute margin ; stipe setiform, straight, thin, of the same colour, very minutely velvety, about 1–1·5 cm. long,[1]
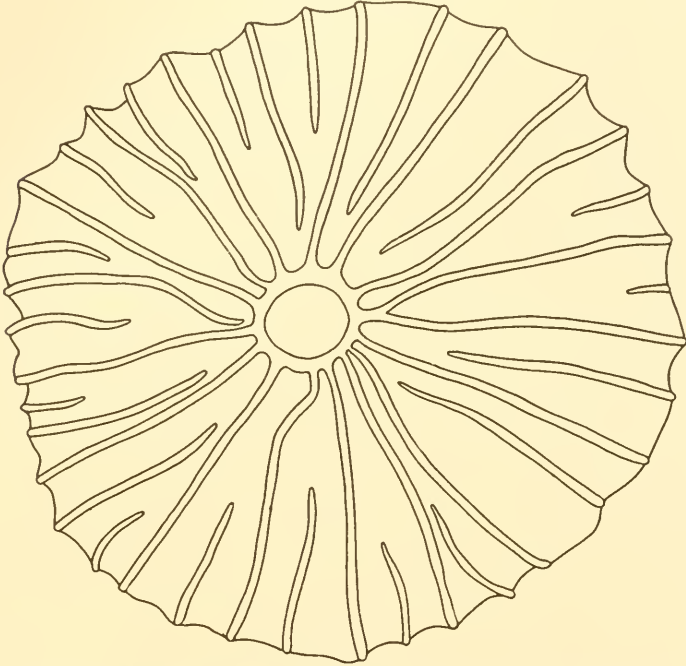
Fig. 207.—*Omphalia flavida*. Plan of under side of the pileus of an expanded fruit-body, to show the arrangement of the gills.   The fruit-body was removed from a Bryophyllum leaf which had been inoculated with gemmae 40 days previously.   Drawn by A. H. R. Buller and Ruth Macrae.   Magnification, about 15.

0·25 mm. thick, the base not swollen.   Gills few, rather distant, yellowish, somewhat waxy, triangular, attenuated at each end,

[1] Maublanc and Rangel, in their general description of the fungus (*loc. cit.*, p. 43), give the height of the fruit-body as about 1 cm. (haut d'environ un centimètre), but in their diagnosis they say the stipe is " circ. 1·5–3 mm. longo." This discrepancy seems to be due to a misprint in the diagnosis, as the authors' illustration shows the fruit-body a little more than 1 cm. high.   Ashby (*loc. cit.*, p. 327) states that the fruit-body is 1–1·5 cm. high, and my experience is the same as his.   I have therefore emended the diagnosis of Maublanc and Rangel by substituting 1–1·5 cm. for 1·5–3 mm.   Ashby gives the width of the pileus as 2–3·5 mm.

more or less decurrent. Basidia clavate, 14–17·4 × 5 μ ; spores minute, ellipsoid or ovoid, apiculate below, hyaline, eguttulate or with one guttule, 4–5 × 2·5–3 μ.

" On leaves of *Eriobotrya japonica*, Melastomaceae, Compositae, and Rubiaceae near Rio de Janeiro (Brazil) in company with *Stilbum flavidum* Cooke (its abortive form)."

The sporophore, as may be seen from Figs. 203, 204, and 213 (pp. 404, 405, and 424), is a much larger organ than the gemmifer. Its height was found to vary from 0·6 cm., as shown in one produced on a Bryophyllum leaf (Fig. 218, p. 429), to 1·5 cm. where growth took place on a medium of bread crumbs. The young stipe is only very slightly or not at all heliotropic and soon becomes negatively
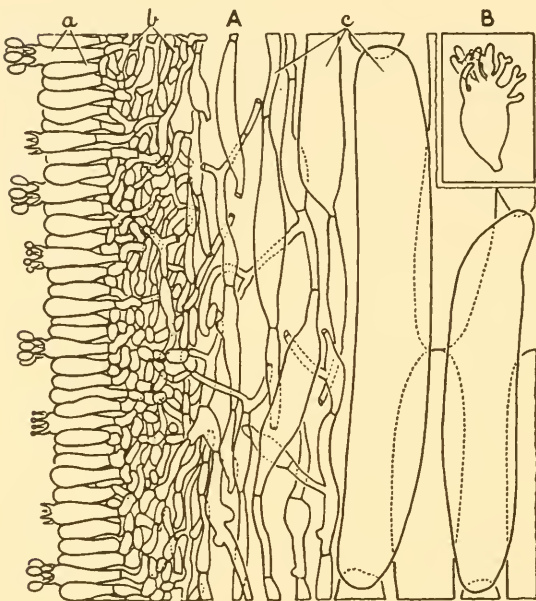
Fig. 208.—*Omphalia flavida*. A, vertical section through a lamella of a perfect fruit-body : *a*, the hymenium made up of basidia, each bearing four spores, and paraphyses ; *b*, the subhymenium ; and *c*, the trama. B, a cheilocystidium (cystidium on the free edge of a gill) ; it is fimbriate, like the clavate cells on the exterior of the pileus. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 540.

geotropic so that, in the end, the axis of the pileus becomes vertical (Fig. 213, p. 424). Two illustrations showing a median-vertical section through a pileus and the appearance of the under side of a pileus are reproduced respectively in Figs. 206 and 207.

In a gill (Fig. 208, A), the trama consists of large elongated cells, the subhymenium of smaller much more numerous cells, and the hymenium of basidia and paraphyses. The hymenium lacks cystidia (pleurocystidia) but there are numerous fringed cystidia

(cheilocystidia, Fig. 208, B) along the edge of each gill. Each basidium bears four spores, and the spores are minute, ovoid, apiculate below, as a rule eguttulate, 5–6·5 × 2·5–3 μ.

The upper end of the stipe expands obconically and to it are attached the decurrent gills (Fig. 206, also Fig. 213, p. 424). The
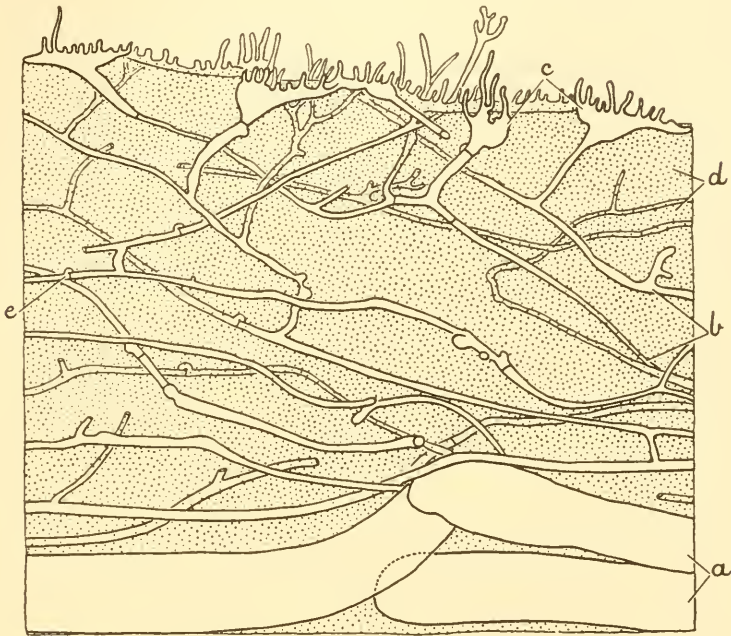
Fig. 209.—*Omphalia flavida*. Vertical section through the pileus-flesh above the gills of a large expanded fruit-body grown on a Bryophyllum leaf in a Petri dish : *a*, part of a layer of swollen cells ; *b*, loose, netted, thin hyphae, terminating on the exterior of the pileus in irregularly clavate, fimbriate cells *c* ; *d*, mucilage, derived from the outer hyphal walls, filling the spaces between the hyphae ; *e*, a clamp-connexion. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 467.

thin pileus-flesh above the gills consists below of two or three layers of swollen elongated cells (Fig. 209, *a*) and above of slender netted hyphae (*b*) terminating on the exterior of the pileus in irregularly clavate, fimbriate cells (*c*). Between these loose hyphae and between the clavate cells the spaces are filled, as in a *stilbum*-head, with mucilage (*d*) derived from the swollen outer cell-walls.

The pileus-flesh at the top of the stipe and below the umbilicate

pilear depression consists of netted gelatinous hyphae which terminate above in a layer of clavate cells (Fig. 210). These clavate cells are more club-shaped and less fimbriate than those which bound the flesh above the gills.

The spores are produced in considerable numbers when once the pileus has begun to liberate them, and fairly thick white spore-deposits were obtained on cover-glasses placed beneath the pilei of fruit-bodies growing normally on Oleander leaves.

Newly fallen spores were tested for germination. The spores on some cover-glasses were covered with sterilised tap-water, those

on other cover-glasses with malt-agar, and those on still other cover-glasses with malt-extract ($2 \cdot 5$ grams malt to 100 cc. water), and the cover-glasses, with their drops hanging downwards, were set on van-Tieghem cells containing a little water. The spores in the tap-water and in the malt-agar did not germinate,
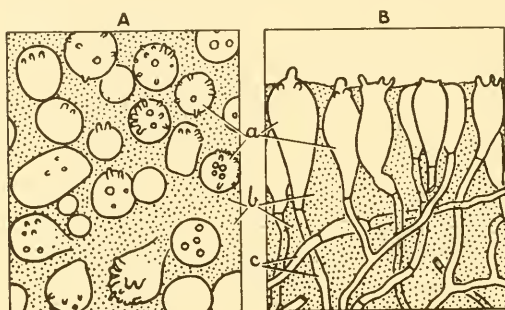
Fig. 210.—*Omphalia flavida*. A, surface view, and B, vertical section, of the pileus-flesh above the stipe and below the central umbilicate depression : *a*, clavate fimbriate cells ; *b*, mucilage ; *c*, netted hyphae. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 467.

but those in the malt-extract put out germ-tubes within 36 hours. Stages in the germination of some spores are shown in Fig. 211.

A pileus which was actively discharging spores was set in succession over two fresh wounds scratched in a Bryophyllum leaf, so that many spores must have fallen into each wound. The leaves were kept on very wet sand in a Petri dish covered with a bell-jar. The air in the Petri dish must have been saturated with water vapour. Nevertheless, the wounds healed up without becoming infected. Whether or not, therefore, germinating basidiospores can initiate the Coffee leaf-spot disease is still an open question. Since, under natural conditions, the perfect fruit-bodies appear to be but rarely developed, basidiospore infection, if it occurs at all, may be very infrequent.

The Origin of the Stilbum-bodies.—As first pointed out by Maublanc and Rangel,[1] the *stilbum*-bodies and the Omphalia sporophores show marked resemblances. Among these resemblances are the following. (1) The stalk and head of a *stilbum*-body correspond
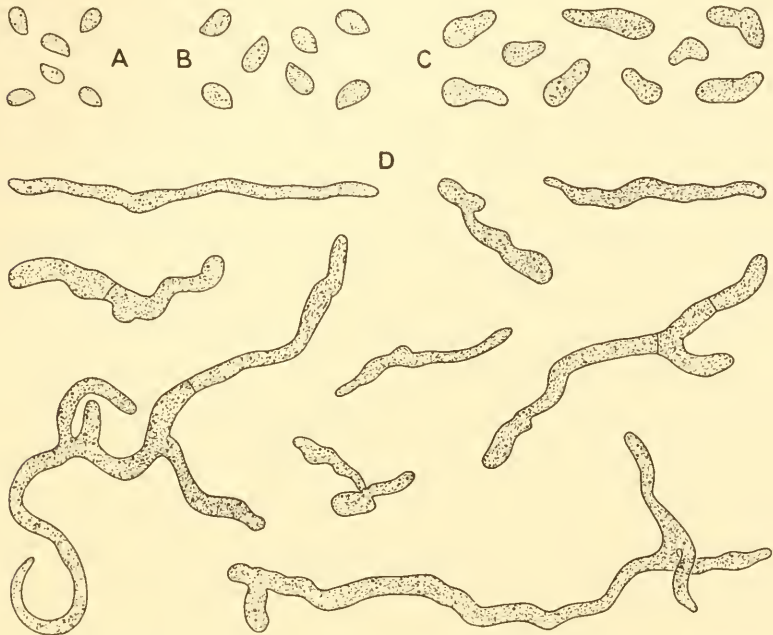
FIG. 211.—*Omphalia flavida.* Basidiospores and their germination in a malt solution (2·5 per cent.). A : spores freshly immersed, some in lateral view, others in face view. B : spores after being immersed 36 hours ; they are now much swollen. C : spores germinating after 42 hours immersion ; the germ-tubes have just been produced. D : other spores with longer germ-tubes, the seven upper ones after 42 hours immersion, the two lower ones after six days ; the spores cannot now be distinguished from the germ-tubes or mycelium ; the hyphae are filled with fine cytoplasm, and some of the sporelings have developed one or two septa. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 749.

respectively to the stipe and pileus of the agaric. (2) The stalk of a *stilbum*-body and the stipe of the agaric are both clothed with short simple hair-like outgrowths. (3) The palisade cells at the top of the pileus of the agaric correspond to the swollen cells which surround the oblate-spheroidal part of the head of a *stilbum*-body, but differ from them in being fimbriate and in not producing long

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[1] A. Maublanc et E. Rangel, *loc. cit.,* pp. 44–45.

filiform hyphae. (4) The large clavate palisade cells at the margin of the pileus of the agaric are fimbriate and thus correspond to the very similar cells which occur on the exterior of the apophysis of a *stilbum*-body, *i.e.* on a structure which may be regarded as forming the outer margin of a *stilbum*-head. (5) The spaces between the hyphae in a head of a *stilbum*-body and the spaces between the hyphae of the flesh of a pileus are both filled with mucilage. (6) The position of the annular air-chamber enclosed between the apophysis and the upper end of the stalk of a *stilbum*-body corresponds to the position of the gills in a young pileus. These resemblances and also the occurrence of what Maublanc and Rangel took to be intermediate forms [1] between the *stilbum*-bodies and the perfect sporophores led these authors to the conclusion that the *stilbum*-bodies are nothing but aborted Omphalia fruit-bodies.

The view that the *stilbum*-bodies have been derived from the rudiments of Omphalia fruit-bodies appears to me to be well based. Many Hymenomycetes produce far more fruit-body rudiments than ever come to perfection ; and it seems likely that, in *Omphalia flavida*, in the course of evolution, some of the rudiments have become progressively metamorphosed by means of structural and physiological changes so as to fit them for liberating their aborted pilei and thus disseminating the species in a novel but very effective manner.

The Stilbum-body as a Gemmifer.—The *stilbum*-body, whatever its origin in the course of evolution, is to-day an effective organ for bringing about the dissemination of *Omphalia flavida* from place to place, and it performs this function by liberating its tiny head. This is blown, splashed, or otherwise carried from diseased leaves to healthy ones, the latter readily becoming infected by means of the long filiform filaments—which we may call the *infection hyphae*— which radiate from the surface of the head and at once renew their growth when a head becomes attached by its gelatinous envelope to a new host-leaf.

The name *stilbum*-body is a misnomer ; for, as we have seen, a *stilbum*-body has nothing to do with a true Stilbum. A *stilbum-*

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[1] Intermediate forms between *stilbum*-bodies and perfect agarics have not been observed by either Ashby (*loc. cit.*, p. 328) or myself.

body, whatever its origin, cannot now be called a sporophore, for it produces no basidiospores or conidia ; and to call it an aborted sporophore is to neglect the fact that it is an active organ with a definite reproductive function and interesting structural characters of its own. A *stilbum*-body is in reality a gemmifer, and in what follows it will be thus designated.

The gemmifer of *Omphalia flavida* consists of two parts : (1) the stalk which we may now call the *pedicel*, and (2) the head which is in reality a *gemma*.

Under natural conditions, according to Maublanc and Rangel,[1] the gemmae (their *stilbum*-heads) are the actual means by which *Omphalia flavida* is disseminated and reproduced, for the perfect sporophores were seen by them only in artificial cultures in which the atmosphere was saturated with water vapour. While it is true that the leaf-spots on attacked leaves of Coffee, etc., give rise only to gemmifers, it is possible, as Maublanc and Rangel point out, that the perfect fruit-bodies are sometimes developed on fallen leaves in very wet situations on the ground, although, so far, apparently no one has ever seen them there. It therefore seems that, in *Omphalia flavida*, reproduction by gemmae has largely or wholly taken the place of reproduction by basidiospores. This suppression and replacement of basidiospores finds analogies elsewhere. Thus chlamydospores have completely or almost completely taken the place of basidiospores in Nyctalis[2] and in Ptychogaster.[3]

That the gemmifers of *Omphalia flavida* are well suited to act as reproductive organs is shown by the following considerations. (1) The gemmifers are produced in considerable numbers. Each spot on a Coffee leaf in an entire season may produce 20–50 gemmifers ; but as many as 70 have been observed on a leaf-spot 7 mm. in diameter.[4] (2) A gemma is easily detached from its pedicel by wind, rain, or other mechanical means. (3) The gemmae are very small, each being about one-third of a millimeter in diameter. Owing to their small size they are readily transported by mechanical

[1] A. Maublanc et E. Rangel, *loc. cit.*, p. 45.
[2] These *Researches*, Vol. III, 1924, pp. 447–448, 463.
[3] O. Brefeld, *Untersuchungen über Pilze*, Heft VIII, 1889, pp. 114–142.
[4] G. L. Fawcett, *loc. cit.*, p. 11.

means, *e.g.* wind, rain-splashes, etc., from one place to another. (4) The exterior layer of each gemma is mucilaginous, so that a gemma readily adheres to the upper surface of a leaf on which it may happen to fall.[1]   (5) The pear-shaped palisade cells all around the outer side of the oblate-spheroidal part of each gemma bear more or less radiately-directed filiform hyphae—the *infection hyphae*.   When a gemma falls on a leaf, these filiform hyphae grow over the surface of the leaf (Fig. 212, H), pierce the cuticle, pass through the epidermis, and soon invade the palisade cells and spongy mesophyll.[2]   In less than a week, the mycelium in the leaf-spot begins to form new gemmifers (Fig. 213, A and B, p. 424).

The Basal Curvature of the Pedicel and its Significance.— When a gemmifer growing on the upper side of a leaf or a malt-agar plate is very young, the pedicel projects outwards perpendicularly or almost perpendicularly from its substratum and the gemma is always situated symmetrically on the end of the pedicel (Fig. 212, A and B).   As the pedicel increases in length by intercalary growth just beneath the gemma, its lower or basal half usually becomes curved, so that the upper half of the pedicel and the gemma are turned away from the perpendicular at an angle which often varies from 30° to 45° (*cf.* Fig. 212, C, D, E.).

At first it was thought that the basal curvature of the pedicel just described might be due to the stimulus of gravity, but experiment soon disproved this supposition.   Gemmifers were grown on malt-agar : (1) in upright Petri dishes, (2) in inverted Petri dishes, (3) in vertically placed Petri dishes, and (4) in Petri dishes rotated in a vertical plane on a klinostat.   Under all these conditions the gemmifers at first grew out perpendicularly from their substratum, and then their pedicels became more or less curved in various directions (*cf.* Figs. 202, p. 403, and 213, p. 424).   In the inverted and vertical Petri dishes, the pedicels did not all curve downwards as they should have done had they responded to a

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[1] F. G. Kohl, " Untersuchungen über die von *Stilbella flavida* hervorgerufene Kaffeekrankheit mit Angaben der aus den Untersuchungen sich ergebenen Mass- regeln gegen diese Pilzepidemie," *Beihefte zum Tropenpflanzer*, Bd. IV, 1903, pp. 63–64.

[2] Kohl shows the infection hyphae growing from a gemma into a leaf in his Taf. II, Fig. 7.

positive geotropic stimulus, but some turned upwards, some downwards, and some sideways. Also, when rotated slowly on the
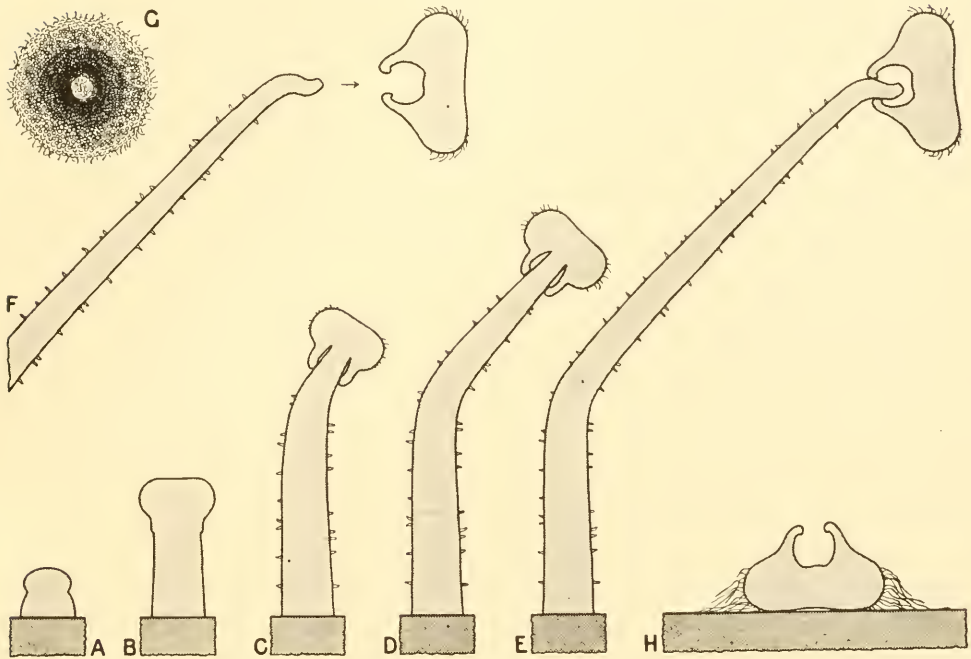
Fig. 212.—*Omphalia flavida.* Stages in the development of a gemmifer on a leaf of *Bryophyllum calycinum.* A, a very young stage in median-vertical section showing the primordia of the pedicel and the gemma. B, the pedicel, which is still straight, has elongated by intercalary growth at its upper end, and the gemma has become swollen. C: the pedicel has become bent (repulsion curvature) and is still elongating by intercalary growth under the gemma ; the gemma has developed a circular cavity between the pedicel and the apophysis ; hairs have grown out from the pedicel and infection hyphae from the periphery of the gemma. D, the circular cavity has enlarged and the pedicel is still growing in length. E : the pedicel is now full-grown ; its end has become sigmoidally bent (geotropic curvatures) and has broken away from the gemma which now has its axis horizontal ; the gemma is holding on to the end of the pedicel by its apophysis only. F, the gemma is being blown away from its pedicel by the wind and is travelling in the direction shown by the arrow. G, the underside of a ripe gemma after leaving the pedicel ; the central opening came into existence when the gemma slipped off the stipe. H, a gemma which has settled on a leaf and is infecting it by means of its infection hyphae which have grown in length. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 47.

klinostat during their development, the pedicels all became more or less curved, the direction of the curvature being usually away from the centre of the plate toward the edge of the radiately growing

mycelium. There can be no doubt, therefore, that the basal curvature of the pedicel is not due to a geotropic stimulus.

What, then, does cause the basal curvature of the pedicel ? A study of the disposition of gemmifers in a single group growing on agar in upright, inverted, or vertical fixed Petri dishes or in Petri dishes rotated in a vertical plane on a klinostat, or on a leaf-spot (Fig. 213), reveals the fact that the gemmifers *diverge from one another*. One cause of the general curvature of the stipe, therefore, is *the mutual repulsion of the gemmifers*. Where a gemmifer is growing alone and its pedicel is curved, it may be that the curvature is due to the mycelium in the substratum which in some way repels the gemmifer more on one side than on the other.

The bending of the stipe of a sporophore and the basal bending of the pedicel of a gemmifer are due to different causes and have different biological advantages. A stipe is negatively geotropic : as it grows in length, its upper end becomes more and more vertical, with the result that the median axis of the pileus becomes vertical and the pileus is given the most favourable orientation for the successful discharge of the spores (Fig. 213, p. 424). On the other hand, a pedicel (except at its apex, as we shall see in the next Section) is ageotropic and the different pedicels in a group of gemmifers bend away from one another owing to mutual repulsion, with the result that the gemmae, which are adhesive, avoid coming in contact with one another during their development and, when the time arrives for their dispersion, have a better chance of being carried off freely by the wind (Fig. 213).

The Sigmoid Curvature of the Pedicel and the Abscission of the Gemma.—A gemma, before being dispersed by the wind, undergoes abscission from the end of the pedicel whilst still attached to the pedicel subterminally by means of the clasping collar-like apophysis (*cf.* Fig. 212, D and E). The abscission process, which is effected in a unique manner, has been investigated in detail, and an account of it will now be given.

The extreme end of a pedicel (the last $0·2$ mm.), *i.e.* the part within and just below the clasping apophysis, is at first quite straight (Fig. 212, D) ; but, finally, as shown in Figs. 205, C (p. 408) and 212, E, it *becomes curved sigmoidally*. It is whilst the end of

the pedicel is becoming sigmoid that the top of the pedicel is torn away from the gemma, thus bringing about the abscission of the latter. After abscission of the gemma has been effected, the attachment of the gemma to the pedicel is maintained merely by the clasping apophysis (*cf.* Fig. 212, E).

Until the abscission of the gemma has taken place, the apophysis firmly clasps the pedicel, and it is this firm attachment which makes abscission of the gemma from the top of the pedicel mechanically possible. After abscission has taken place, the attachment of the apophysis to the pedicel weakens, and then the gemma can readily be blown off the pedicel by the wind (Fig. 212, F). Thus the apophysis functions in two ways : (1) it grasps the pedicel subterminally and thus permits of the abscission of the gemma from the top of the pedicel, and (2), after abscission, it holds the gemma on to the top of the pedicel until a blast of wind carries the gemma away and thus brings about its dispersion.

We have just seen that the sigmoidal growth-curvature of the end of the pedicel results in the abscission of the gemma from the top of the pedicel. We may now ask : what causes the end of the pedicel to assume its sigmoid form ? The answer to this question is to be found in the response of the pedicel to geotropic stimuli.

By observing gemmifers growing on malt-agar plates from surfaces looking upwards, downwards, and sideways, it was found : (1) that the part of the pedicel in the region of the collar of the apophysis always bends *downwards* through an angle, often of 45°, during the last stages of the development of the gemmifer ; (2) that, at the same time, the very top of the pedicel, hidden within the apophysis, always bends *upwards* (Fig. 212, E) ; and (3) that, whilst the pedicel is bending sigmoidally, the *upper* side of the apophysis elongates slightly more than the lower (Figs. 205, C, p. 408, and 212, E). The simplest explanation of all these growth phenomena is that they are due to geotropic stimuli : gravity stimulates the pedicel in the region of the collar of the apophysis to elongate more on its *upper* side than on its lower ; gravity stimulates the pedicel at its very top to elongate more on its *lower* side than on its upper ; and, finally, gravity stimulates the apophysis to elongate more on

its *upper* side than on its lower.   The opposite geotropic reactions of the subterminal and terminal parts of the pedicel produce two opposite curvatures which together give to the last 0·2 mm. of the pedicel its characteristic sigmoid form.   The sigmoid curvature, since it is due to the positive and negative geotropic stimuli, is necessarily always in a vertical plane (Figs. 205, C, p. 408, and 212, E, F).

That the stimulus of gravity is the true cause of the sigmoid curvature of the end of the pedicel and of the asymmetrical growth of the apophysis of the gemma is strongly supported by an experiment made with the help of a Pfeffer klinostat.   A malt-agar plate upon which the rudiments of gemmifers of *Omphalia flavida* were just beginning to appear was rotated on the klinostat in a vertical plane, and the rotation was continued for several days until the gemmifers were full-grown.   As a control, another malt-agar plate, prepared in the same way as the first, was kept in a fixed position (upside down on a table).   In the control, when the experiment came to an end, all the gemmae were turned slightly downwards on the ends of the pedicels and, when the gemmae were pulled off their pedicels, the ends of the pedicels were found to have the characteristic sigmoid curvature.   Moreover, the gemmae had become asymmetrical in form, the upper side of the apophysis, as usual, having become longer than the lower.   On the other hand, in the culture rotated on the klinostat, the ends of the pedicels were *quite straight* and none of them had developed even a trace of a sigmoid curvature, so that each gemma occupied a symmetrical position on the end of its pedicel. Furthermore, each of the gemmae which had been rotated on the klinostat, instead of displaying the usual asymmetry, was *symmetrical* in form, like a door-knob, the apophysis not having elongated more on one side of the gemma than on the other.

From the above observations and experiments we may conclude that the abscission of a gemma from its pedicel is effected by the end of the pedicel, toward the close of its development, taking on a sigmoid form, and that the sigmoidal curvature is due to the end of the pedicel giving a double response to the stimulus of gravity. It is evident that a gemmifer is beautifully organised as an organ for the production and liberation of its gemma.

The Detachment of a Gemma from its Pedicel.—Ripe gemmae could be readily blown away from their pedicels as follows. A hollow glass tube was drawn out to a slight nozzle at one end, the nozzle was then placed near some gemmifers growing on a Bryophyllum leaf, and then air was blown through the tube from the mouth. The gemmae upon which the blast of air impinged were very easily blown away from their pedicels to some distance. There can be no doubt that a slight wind would also have carried off these gemmae.

A test-tube containing a malt-agar slope which bore gemmifers was held horizontally with the gemmifers looking downwards and was then tapped. Several of the gemmae came off their pedicels and settled on the side of the tube below, others remained on their pedicels. This experiment affords further evidence that ripe gemmae can be detached from their pedicels without much difficulty by mechanical means.

The gemmae *never drop off their pedicels spontaneously* and, no doubt, under natural conditions in the open, they remain *in situ* until the wind is sufficiently strong to bear them away. This resistance to detachment except under conditions favourable for dispersion must be of no small value to a fungus which is a parasite on leaves. It finds its parallel in the fruits of the Manitoban Maple, *Acer Negundo*, which in Manitoba hang on the trees through the winter until some violent gale in March or April tears them from the branches and scatters them far and wide.

The mature gemma of a gemmifer situated on the top of a leaf-spot, owing to the basal and the sigmoid curvatures of the pedicel, comes to have its median axis disposed more or less parallel to the leaf surface (Fig. 213, C). This orientation of a gemma, as we have seen, is due to the reaction of the pedicel to certain stimuli. The basal curvature of the pedicel of a gemmifer in a group of gemmifers serves to remove the gemma from neighbouring gemmae, and the sigmoid curvature of the pedicel resulting in the turning downwards of the gemma seems to be significant merely as an incidental factor in the mechanism of abscission. On the other hand, it may be that the final orientation of the gemma contributes something to the ease of detachment of the gemma by the wind. The wind sweeping
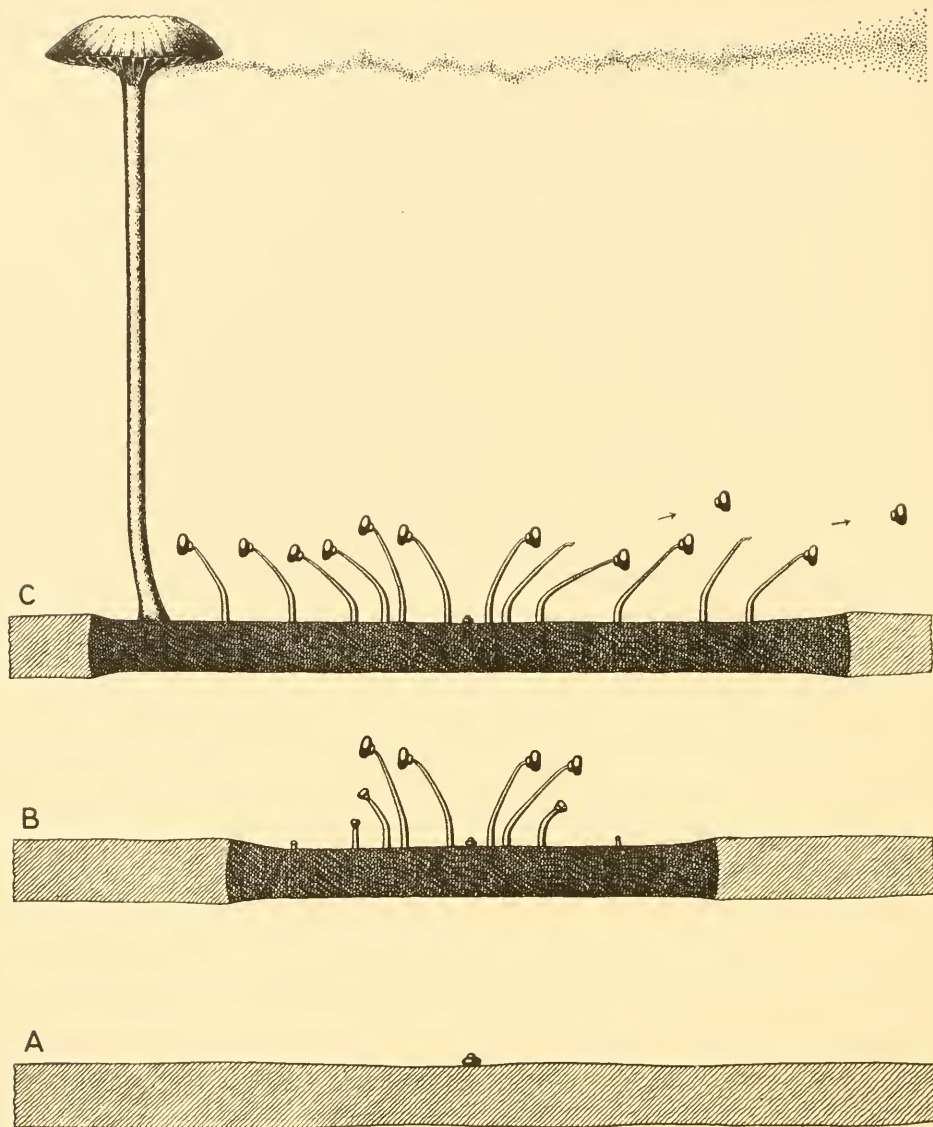
FIG. 213.—*Omphalia flavida* on a leaf of *Bryophyllum calycinum*. Semi-diagrammatic drawings (based on laboratory cultures on isolated leaves kept on wet sand), to show the development of a leaf-spot (which is luminous), the production of gemmifers and of a perfect fruit-body, and the dissemination of gemmae and basidiospores. A, a cross-section of part of a leaf upon which a gemma has just settled after being blown from its pedicel by the wind. B, the same, ten days later: from the gemma which fell on the leaf the infection hyphae grew into the leaf and the mycelium of the parasite then progressively killed the host-cells there, thus forming a necrotic lesion in the form of a blackish leaf-spot; from this spot gemmifers have developed and are still developing centrifugally; the younger gemmifers have straight pedicels; the older gemmifers have pedicels which are bent basally owing to mutual repulsion and bent above sigmoidally owing to a double reaction to the stimulus of gravity. The gemmae of the older gemmifers have nearly horizontal axes. C, the same as B, 3–4 weeks later: the leaf-spot has increased in size, but is no longer extending; the gemmifers are all mature and two of the gemmae have just been blown from their pedicels in the direction shown by the arrows; a perfect fruit-body has developed at the edge of the leaf-spot and is now discharging clouds of spores which are being carried away by the wind. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 6·6.

over the surface of a leaf must strike many gemmae on their broad under side instead of laterally and thus, perhaps, it may detach them from their pedicels more readily than if their axes had been perpendicular to the leaf surface.

The Attachment of a Gemma to a Leaf.—When a gemma falls on to a leaf, it usually settles on its slightly concave upper surface and readily becomes attached to the leaf, presumably by means of the mucilage in which the clavate palisade cells, which form the
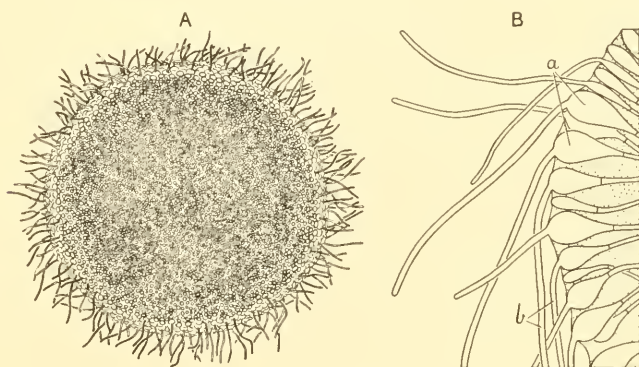
Fig. 214.—*Omphalia flavida* (from Trinidad). A, a gemma ripe and ready for dissemination, seen from above. It grew in moist air. Short infection hyphae can be seen at its periphery, but the central disc cells are free from hyphae. B, part of a longitudinal section of a ripe gemma (*cf.* Fig. 205), showing clavate peripheral cells *a* embedded in a gelatinous matrix, some of them extended into infection hyphae *b*. Drawn by A. H. R. Buller and Ruth Macrae. Magnification : A, 86 ; B, 466.

exterior layer of cells of the gemma, are embedded (Fig. 213, A). Gemmae which have fallen on to a leaf are not easily detached from the leaf ; for, when air was blown at them through the nozzle of the glass tube already used to blow them from their pedicels, they remained on the surface of the leaf and were not driven away from it. It thus appears that the ripe gemmae are well adapted for dispersion and for attachment : the wind can easily blow them from their pedicels and thus transport them from leaf to leaf ; but, when once they have settled on a leaf, they resist the action of the wind in removing them. Owing to the manner in which they attach themselves to a leaf, their chances of causing new leaf-spots are increased.

Mode of Germination of a Gemma.—Preparations for the germination of a gemma are made before the gemma is blown away
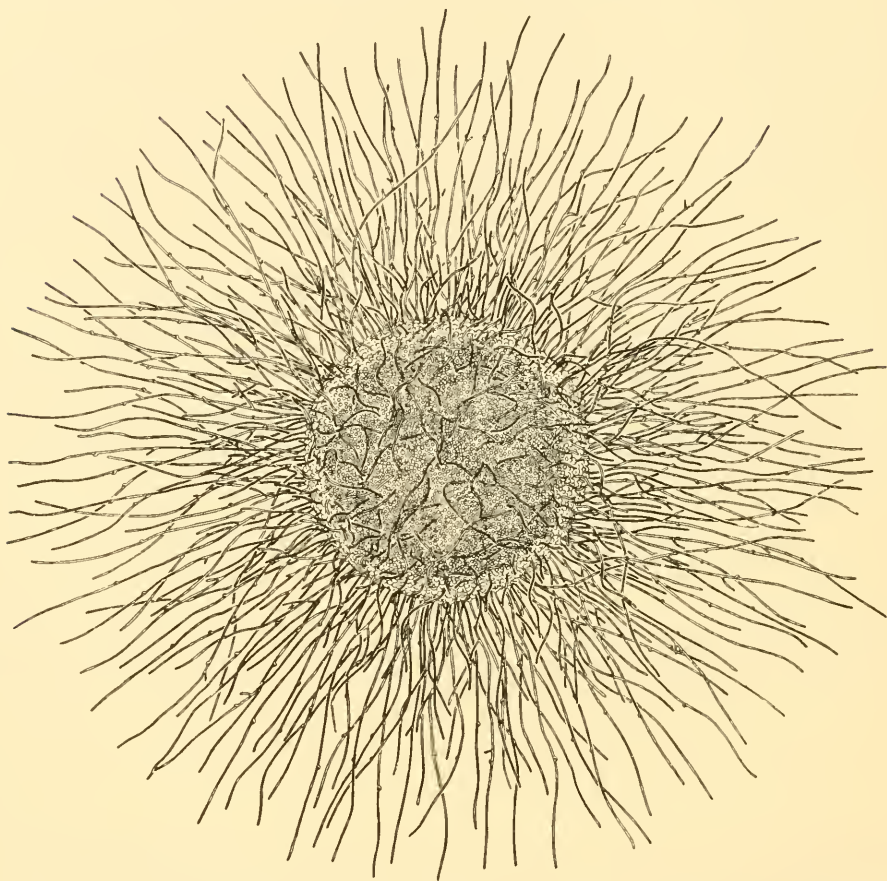
FIG. 215.—*Omphalia flavida* (from Trinidad). A gemma germinating. Drawn 24 hours after it was removed from its pedicel and placed in a hanging-drop of malt-agar. The upper surface of the gemma, which usually comes into contact with a leaf, is shown. The radiating hyphae, which are growing rapidly in length, are prolongations of the short infection hyphae which were produced by the pear-shaped peripheral cells and were already present on the gemma before its removal from the pedicel. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 88.

from its pedicel; for, when a gemma is mature and still attached to its pedicel, there grow outwards into the air from its pear-shaped or clavate cells all around the outer surface of·its oblate-spheroidal part a large number of what we have called *infection hyphae* (Figs.

205, C, p. 408, and 214, B). These hyphae come to project aerially from the gemma to a distance of 0·05–0·1 mm., and then they cease to elongate.

When a gemma (Fig. 214, A) is placed in tap-water or in malt-agar, the infection hyphae resume their growth and, in malt-agar, in the course of about 24 hours, they become 0·4–0·5 mm. long, thus surrounding the gemma with a radiating mycelium (Fig. 215). The individual hyphae of this mycelium are evenly cylindrical in form, 3–4 μ in diameter, septate, and provided with numerous clamp-connexions (Fig. 216). It is these hyphae which, under natural conditions, penetrate through the epidermis of a Coffee leaf, attack the internal leaf-tissues, and so produce a leaf-spot.

The Effect of Desiccation on the Vitality of a Gemma.—It is well known that the spores of many Hymenomycetes, *e.g.* species of Coprinus, will withstand desiccation for several weeks or months. It therefore seemed of interest to enquire whether or not the gemmae, which in Coffee plantations appear to be the normal reproductive bodies of *Omphalia flavida*, can also withstand desiccation.

Some ripe gemmae were taken from the pedicels of some gemmifers growing on an agar-plate and they were set on four cover-glasses A, B, C, and D. The gemmae on A were not allowed to dry up but, immediately after removal, were covered for two minutes with a minute drop of water, after which they were submerged in a drop of malt-agar. The cover-glass was then set on a van-Tieghem cell with water at the base and the drop of malt-agar hanging downwards. The gemmae on the cover-glasses B, C, and D
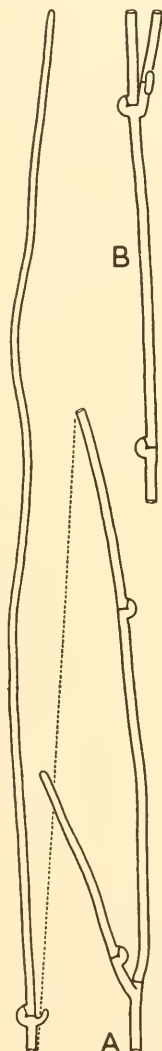
Fig. 216.—*Omphalia flavida* (from Trinidad). Hyphae from the radiating mycelium growing from a gemma placed in malt-agar for 76 hours. As indicated by the clamp-connexions, the mycelium is in the diploid nuclear condition. A, the terminal part of a hypha. B, an older part of a hypha. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 466.

were exposed to the air of the laboratory for half an hour, one hour, and 26 hours respectively, after which they were treated in the same manner as the gemmae on cover-glass A. The air in the laboratory was remarkably dry and the gemmae on the cover-glasses B, C, and D, when exposed to it, dried up within about a minute. Whilst undergoing desiccation, the gemmae became much shrunken. When treated with water, they rapidly expanded, and their expansion was doubtless due in part to the imbibition of water by the gelatinous matrix in which all the cells of each gemma are embedded.

FIG. 217.—*Omphalia flavida* on a leaf of *Bryophyllum calycinum*, two weeks after inoculation. The leaf was wounded by lightly scratching it in the centre and also near the left lower edge ; and then a few gemmae were sown on the wounds. The mycelium grew into the leaf-tissues and formed two leaf-spots. In the central leaf-spot can be seen a number of gemmifers each bearing terminally a tiny gemma. Natural size.

After the gemmae had been 24 hours in malt-agar, it was found : (1) that the gemmae on the cover-glass A, which had not been allowed to dry up, had all germinated perfectly, their infection hyphae having grown out radially from the gemma to a distance equal to the gemma's diameter ; (2) that the gemmae on the cover-glasses B and C, which had been dried for half an hour and one hour respectively, had germinated imperfectly, for only very few of the infection hyphae of each gemma had grown out radially into the culture medium ; and (3) that the gemmae on the cover-glass D, which had been dried for 26 hours, had not germinated at all.

From the above series of experiments it is obvious that exposure

to very dry air seriously injures the gemmae within $0 \cdot 5$–$1 \cdot 0$ hour and kills them within 26 hours.

Some gemmae were removed from their pedicels and set on a cover-glass in a Petri dish in which the air was saturated with water vapour for 24 hours and, at the end of this time, they were placed in a hanging drop of malt-agar. They germinated perfectly. From this we may conclude that gemmae, after being blown from their pedicels on to other leaves in a Coffee plantation when the weather

FIG. 218.—A leaf of *Bryophyllum calycinum* infected with the mycelium of *Omphalia flavida* which, in the course of about a month, produced gemmifers first and then, as here shown, a number of perfect fruit-bodies. The leaf was isolated, inoculated with gemmae, and kept moist on wet sand. The fungus has killed most of the leaf which, however, has succeeded in producing a few new shoots and roots at its edge. Natural size.

is moist, probably retain their vitality and ability to germinate for several days.

Inoculation Experiments with Living Leaves.—In 1901, Kohl [1] examined many hundreds of Coffee leaf-spots and observed that there was a gemma adhering to the epidermis above the middle of every one of them. With a little practice and under favourable light conditions, he succeeded in finding the gemmae even with the naked eye. Thus Kohl obtained strong evidence that, under natural conditions, *Omphalia flavida* is propagated by its gemmae. In 80–85 per cent. of the leaf-spots examined the gemmae were attached to the upper side of the leaf, while in the remaining 15–20 per cent. they were attached to the lower side of the leaf.

Kohl [2] also carried out some inoculation experiments. He

[1] F. G. Kohl, *loc. cit.*, p. 63. [2] *Ibid.*, pp. 64–65.

placed gemmae on living Coffee leaves and observed that a typical leaf-spot was formed beneath and around each gemma, thus proving beyond all doubt that the gemmae serve as sources of infection. Presumably, Kohl saw new gemmifers arise on his experimental leaf-spots, although he does not mention them.

In the autumn of 1925, T. C. Vanterpool and the writer, working in conjunction at Winnipeg, succeeded in infecting a number of living leaves with *Omphalia flavida*. One set of the experiments will now be described.

Two leaves of each of the following plants were gathered from the green-house : *Bryophyllum calycinum*, *Nerium Oleander*, and a species of *Ficus*. The leaves were then washed for about two minutes in 0·1 per cent. mercuric chloride, rinsed in distilled water, and set on very wet sand in a large crystallising dish. One leaf of each kind was then wounded by scratching it very slightly with a sterilised needle, and the other leaf of each kind was left intact. Then a very few gemmae were removed from a bread culture with the help of a sterilised needle and placed in particular spots on the upper surface of all the six leaves. In the case of the three wounded leaves the gemmae were placed on the

FIG. 219.—A leaf of a Fig (*Ficus* sp.) infected with the mycelium of *Omphalia flavida* which, in the course of five weeks, produced gemmifers first and then, as here shown, a number of perfect fruit-bodies. The leaf was isolated, inoculated with gemmae, and kept moist on wet sand. Natural size.

wounds. The crystallising dish was then covered and set in diffuse daylight on a table in the laboratory. The air within the dish very soon became saturated with water vapour.

Typical leaf-spots, which rapidly grew in size, were developed within a week on both the wounded and the unwounded Bryophyllum leaves and on the wounded, but not on the unwounded, Oleander and Ficus leaves. About ten days after inoculation, a crop of saffron-yellow gemmifers began to develop at the surface of the infected areas (Fig. 217, *cf.* Fig. 201, p. 402) ; and, after another ten days, at the edges of the same areas, a few typical

Fig. 220.—*Omphalia flavida* on an isolated leaf of *Nerium Oleander* resting on wet sand in a Petri dish. The leaf was dipped in 0·1 per cent. corrosive sublimate, washed in sterilised water, and placed on sterilised sand moistened with sterilised water. The surface of the leaf was then scratched with a needle and a piece of mycelium was laid over the wound. After about a week, a few gemmifers came up above the leaf. On the twenty-first day after inoculation, the sporophores here shown began to make their appearance. Photographed at Winnipeg twenty-seven days after inoculation. Natural size.

Omphalia flavida sporophores made their appearance (Figs. 218, 219, and 220). Thus the mycelium, by producing both kinds of fruiting structures, behaved in living leaves just as it had done in artificial bread cultures.[1]

In further experiments, Vanterpool and I succeeded in infecting the leaves of *Plumbago capensis* ; but, in this species, the leaf-spots developed gemmifers only, no sporophores ever making their appearance.

Omphalia flavida as a Non-specialised Parasite.—Many parasites, *e.g.* Uredineae, Ustilagineae, and most Peronosporeae and Chytridiaceae, are highly specialised, some of them being restricted

[1] *Cf.* Figs. 203 and 204, pp. 404 and 405.

to even a single host ; while other parasites, *e.g. Fomes pinicola, Polyporus squamosus, Peronospora parasitica,* and *Cystopus candidus,* are far less specialised and are found on many different hosts. *Omphalia flavida* is an extreme example of a non-specialised parasite ; for, in addition to Coffee, its chief host, it attacks plants belonging to the most diverse families of Flowering Plants and also various Ferns. It is probable that there are but few parasites which can attack Ferns as well as Flowering Plants.[1] The following list includes the host-families of *Omphalia flavida* and the observers by whom they have been recorded.

DICOTYLEDONES

Anacardiaceae	(*Mango*)	. . .	Fawcett.[2]
Apocynaceae	((*Nerium Oleander*)	.	Buller and Vanterpool.[3]
	((*Tabernaemontana*)	.	Puttemans.[4]
Begoniaceae	(*Begonia*)	. . .	Fawcett.
Compositae	(*unidentified spp.*)	.	Maublanc and Rangel.[5]
Crassulaceae	{(*Bryophyllum*)	. .	{McClelland.[6] {Buller and Vanterpool.
	{(*Bryophyllum pinnatum*)		Müller.[7]
Leguminosae	{(*Inga vera—Guava*)	.	Fawcett.
	{(*Andira inermis—Moca*).		McClelland.

[1] The species of the Rust genera Hyalospora, Milesia, and Uredinopsis have their aecidial stage on a Conifer (Abies) and their uredospore and teleutospore stages on Ferns (Blechnum, Scolopendrium, Polypodium, etc.).

[2] G. L. Fawcett, *loc. cit.*, p. 13.

[3] A. H. R. Buller and T. C. Vanterpool, *loc. cit.*

[4] A. Puttemans, " O *Stilbella flavida* parasita sobre Tabernaemontana," *Revista da Sociedade scientifica de São Paulo,* 1907, p. 95. Cited from Maublanc and Rangel.

[5] A. Maublanc et E. Rangel, *loc. cit.,* pp. 41, 43.

[6] T. B. McClelland, " The Coffee Leaf Spot (*Stilbella flavida*) in Porto Rico," Porto Rico Agric. Exp. Station, *Bull. No. 28,* 1921, p. 7. McClelland, in a badly affected, almost abandoned plantation in Porto Rico found the following plants acting as hosts for *Omphalia flavida* : achiotilla, bálsamo, bejuco de mona, bejuco de paloma, berugillo, bruja, cadillos, camasey blanco, camasey cimarron, china, helecho, guasabára, guayaro, guava, higüerillo, lechecillo, lengua de vaca, moca, nuez moscada cimarrona, palo de cucubano, palo de hueso, tostado, vinagrera, yerba hedionda. Owing to uncertainty as to the scientific equivalents of these local names, I have not added them to my list.

[7] A. S. Müller, at Mayagüez, Porto Rico, *in litt.,* 1928.

Melastomaceae	(*unidentified spp.*) .	. Maublanc and Rangel.
Moraceae	(*Ficus*) . .	. Buller and Vanterpool.
Plumbaginaceae	(*Plumbago*) . .	. Buller and Vanterpool.
Rosaceae	(*Eriobotrya japonica*—	
	Loquat) . .	. Maublanc and Rangel.
Rubiaceae	⎰(*Coffea*) . .	. Cooke and others.
	⎱(*Cinchona*) . .	. Kohl.[1]
Rutaceae	(*Orange*) . .	. Fawcett.

<div align="center">MONOCOTYLEDONES</div>

Araceae	(*Yautia*) . .	. Fawcett.
Commelinaceae	⎰(*Commelina spp.*) .	. Fawcett.
	⎱(*Zebrina pendula*) .	. Müller.[2]
Musaceae	(*Banana*) . .	. Fawcett.

<div align="center">PTERIDOPHYTA</div>

| Various Ferns | (*unidentified*) . | . Fawcett. |

Sterility of the Mycelium Induced by Prolonged Cultivation on Artificial Media.—S. F. Ashby isolated his pure culture of *Omphalia flavida* in Trinidad in 1925. Subcultures of this culture were used by Vanterpool and myself in the winter of 1925–1926, during which time the mycelium fruited well both on artificial media and on living leaves. Two years later, in the winter of 1927–1928, I procured another subculture of Ashby's pure culture from the Centralbureau voor Schimmel-cultures at Baarn, but found that the mycelium on bread and agar media, even after many weeks, produced neither gemmifers nor sporophores. This sterility appeared to be due to prolonged cultivation of the mycelium on artificial media.

The mycelium which, instead of producing gemmifers, developed an aerial fluffy hyphal mat was brought back into the fruiting condition by passing it through living leaves. Some Bryophyllum leaves, kept on wet sand in Petri dishes, were wounded by scratching with

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[1] F. G. Kohl, *loc. cit.*, pp. 66–68. Kohl, in Central America, examined 36 different kinds of plants and found ten of them affected by the leaf-spot disease. One was a *Quinabaum*. The others he was unable to identify.

[2] A. S. Müller, at Mayagüez, Porto Rico, *in litt.*, 1928.

a needle, and then the wounds were inoculated with mycelium attached to bits of agar. Infection took place ; but, in general, the leaf-spots failed to develop any gemmifers. At length, on one leaf-spot, two or three gemmifers made their appearance. The gemmae of these gemmifers were then removed with a needle and used as inoculum on a new wounded Bryophyllum leaf. The new leaf-spot produced gemmifers more freely than its predecessor, whereupon its gemmae were used as inoculum as before. After having been passed in this way through Bryophyllum leaves several times, the mycelium fruited freely, and thousands of gemmifers and some scores of sporophores were obtained from it on bread and malt-agar media as well as on living leaves of Bryophyllum and Oleander. Nevertheless, the mycelium did not seem to be in quite so good a condition as it had been three years before, because (1) it fruited rather slowly, and (2) it still showed a tendency to develop a fluffy mycelium at the surface of the culture medium, instead of gemmifers.

In the spring of 1928, through the kindness of Mr. J. A. B. Nolla, I obtained from Porto Rico a culture of *Omphalia flavida* which had recently been isolated by Professor Albert Müller of the Mayagüez Agricultural College. When portions of this Porto Rican culture were transferred to malt-agar plates, the mycelium, unlike that from Trinidad, did not develop an aerial fluffy layer of hyphae but rapidly produced gemmifers. Within five days after inoculation of the plates, which were exposed to light on a table, gemmifer production was in full progress. A comparison showed that the newly isolated Porto Rican culture fruited far more rapidly and vigorously than the Trinidad culture derived from an isolation made three years previously.

From the above observations we may conclude : (1) that the mycelium of *Omphalia flavida*, when newly obtained from gemmae developed in the open on Coffee leaves, fruits vigorously ; (2) that, when the mycelium is grown for several years on artificial media, it gradually loses its fruiting power ; and (3) that the fruiting power in an old culture can be restored in a large measure by passing the mycelium through living leaves.

The Effect of Light on the Formation of Gemmifers.—The mycelium of the Trinidad strain of *Omphalia flavida*, after it had

been brought back into the fruiting condition, was used to inoculate malt-agar in Petri dishes. The mycelium grew radially outwards from the centre of each dish. Certain of the cultures which were exposed to diffuse daylight on a table in the laboratory formed concentric zones in which broader fluffy sterile rings of mycelium
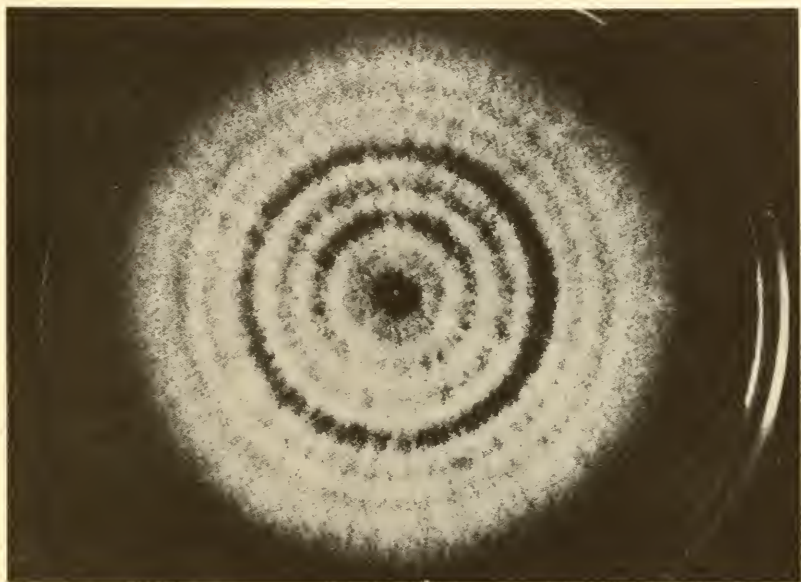
FIG. 221.—*Omphalia flavida.* Alternate zones of sterile fluffy mycelium (white) and of gemmifer-bearing mycelium (dark) in a malt-agar culture of *Omphalia flavida* originating from Trinidad. The culture was started by placing a single gemma in the middle of the agar. The sterile zones of mycelium were formed during the night and the fertile zones during the day. The last two dark zones are narrower than the previous ones because, on the days when they were formed, the plate was exposed to light only from 9.30 A.M. to 5.30 P.M. Natural size.

alternated regularly with narrower, non-fluffy rings of mycelium bearing gemmifers (Fig. 221). By marking the plates it was soon found that the sterile zones were formed during the night and the fertile zones during the day. These facts indicated that the production of gemmifers by the mycelium is initiated by a morphogenic stimulus of light. Some further observations supporting this deduction will now be recorded.

The Porto Rican culture, referred to in the last Section, was sent from Porto Rico in a dark wooden box and, after arrival in Winnipeg,

it was kept in the box in the dark. This culture grew well but did not produce any gemmifers in the dark in the course of several weeks.

Small pieces of agar bearing mycelium were taken from the Porto Rican culture and were used to inoculate the centres of three malt-agar Petri dishes on June 7, 1928. Two of these dishes were then exposed to daylight on a table, whilst the third was exposed to daylight for about one hour and was then enveloped in black paper and placed in a dark cupboard.

On June 13, *i.e.* six days after the beginning of the experiment, the condition of the plates was as follows. In the two plates which had been exposed to daylight numerous gemmifers had appeared not only on the mycelium on the pieces of Porto Rican agar used as inoculum but also on the new agar, and on the new agar three distinct concentric zones of gemmifers could be clearly observed. On the other hand, on the plate which had been exposed to light for about an hour and had then been placed in the dark, a certain number of gemmifers had developed on the old mycelium in the inoculum which had been exposed to the light for an hour, whilst no gemmifers whatever had appeared on the new mycelium which had grown out radially in the fresh agar in the dark. At the end of another week, the lighted cultures had developed many zones bearing gemmifers while the darkened culture had developed no such zones, the mycelium of the darkened culture, excepting that in the original inoculum which had been exposed to daylight for about an hour, having remained quite sterile.

From the above observations we may conclude : (1) that the formation of gemmifers is initiated by the stimulus of light ; and (2) that the mycelium is stimulated to produce gemmifers by an exposure to daylight of one hour or less.

Ashby [1] observed that both gemmifers and sporophores developed to maturity in bread cultures in flasks *kept in the dark.* The production of fruiting structures in this instance may well have been due to the stimulus of light given during the time when the flasks were being inoculated or were being examined from day to day. In

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[1] S. F. Ashby, " The Perfect Form of *Stilbum flavidum* Cke. in Pure Culture," *Bulletin of Miscellaneous Information,* Royal Botanic Gardens, Kew, 1925, p. 327.

the mould *Fusarium discolor sulphureum*, that shows typical zonation in Petri-dish cultures exposed to daylight, Bisby [1] observed that an exposure of the mycelium to bright daylight for *only one-fourth to one-half of a second* is sufficient to cause the formation of a ring of conidia which can be detected with the naked eye. As we have seen, exposure of the mycelium of *Omphalia flavida* for one hour or less stimulates it to produce gemmifers. Further experiment may show that an exposure of the mycelium to bright daylight for a very few minutes, or possibly less than one minute, is sufficient to initiate the production of gemmifers.

Luminescence of the Mycelium and its Value as a Diagnostic Character of the Coffee-leaf Disease.—For some years, in the hope of discovering luminosity in fungi in which it had not yet been observed, I examined systematically in the dark every fungus species grown in my laboratory and also many isolated agaricaceous fruit-bodies—but all in vain. One

Fig. 222.—The mycelium of *Omphalia flavida* growing on either moist bread-crumbs or corn-meal in a conical flask, photographed by its own light. Exposure of negative (a panchromatic plate), 75 hours. Aperture 4·5. Culture made by S. F. Ashby at the Imperial Mycological Institute at Kew, and negative by G. Atkinson, artist at the Royal Botanic Gardens, Kew. One-half the natural size.

evening in the autumn of 1925, however, my efforts were at last rewarded with success ; for, on taking a bread culture of *Omphalia flavida* contained in a conical flask into the dark room, I at once perceived that the mycelium emitted light (*cf.* Fig. 222). The glow was pale but quite distinct, and the culture was used to

[1] G. R. Bisby, "Zonation in Cultures of *Fusarium discolor sulphureum*," *Mycologia*, Vol. XVII, 1925, p. 92.

demonstrate the phenomenon of bioluminescence to students and friends.[1]

The mycelium appeared to emit light best when grown on bread (1 part bread, 4 parts water), but it is also luminous when grown on oatmeal agar or malt agar. The display of luminescence continues steadily both day and night so long as the mycelium is actively growing and only ceases when the mycelium has become old and exhausted. The colour of the light may be described as pale bluish-white.

As in *Panus stypticus luminescens*,[2] shadow photographs can be
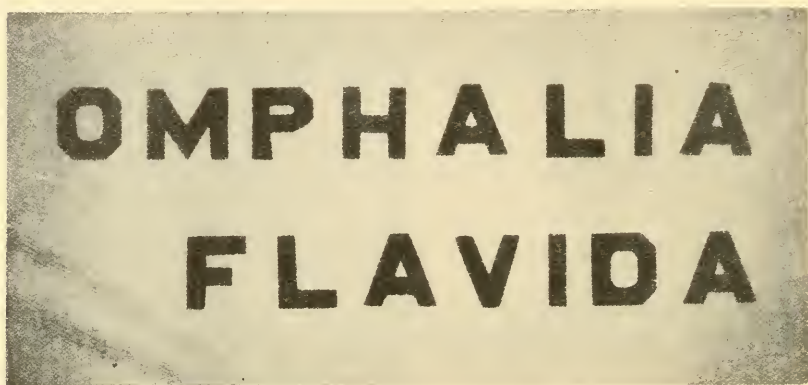
FIG. 223.—A shadow-photograph of the black-paper letters of the words OM-PHALIA FLAVIDA made with the light of the mycelium of *Omphalia flavida* growing on an agar medium in a Petri dish 7 cm. in diameter. The negative, covered by a glass plate bearing the black-paper letters, was exposed for 12 hours at a distance of 10 cm. from the Petri dish. Photograph made for the author by H. H. Thornbury. Reproduced the original size.

made with the light emitted by the mycelium of *Omphalia flavida*, and one such photograph, kindly made for me by Mr. H. H. Thornbury of the University of Minnesota, is reproduced in Fig. 223. The mycelium was grown on an agar medium in a Petri dish 7 cm. wide. The negative, covered by a sheet of glass to which the black-paper letters OMPHALIA FLAVIDA were attached, was exposed for 12 hours at a distance of 10 cm. from the Petri dish.

[1] In 1927, whilst visiting the Imperial Mycological Institute at Kew, by making use of some of Mr. Ashby's cultures I was able to demonstrate the bioluminescence of the mycelium of *Omphalia flavida* to Mr. Ashby himself.

[2] These *Researches*, Vol. III, 1924, pp. 388–390, Figs. 168–169.

Vanterpool and I [1] took the leaves of Bryophyllum, Nerium, Ficus, and Plumbago which we had artificially infected with *Omphalia flavida* into the dark room and there observed that the leaf-spots on all the four kinds of leaves emitted light.

The observations on luminosity so far recorded were all made with a strain of *Omphalia flavida* of Trinidad origin. In 1928, another strain, of Porto Rican origin, was examined and found to be equally, or perhaps even more, strongly luminous. The light emitted by the mycelium of Porto Rican origin, when the mycelium is growing on bread in a conical flask of 400 cc. capacity, was sufficiently strong to permit of the reading of large print with letters 6 mm. high.

Four leaves of *Nerium Oleander* which had been inoculated with the Porto Rican strain of *Omphalia flavida* showed infected areas which were irregularly elliptical and about two inches long (*cf.* Figs. 201 and 202, pp. 402 and 403). On examining these leaves in a dark-room I was able to detect the leaf-spots by their luminosity when the leaves were 12 feet distant from my eyes. A nearer view of the leaves revealed that the leaf-spots were beautifully defined by the light which they gave out. On examining the leaf-spots with a hand-lens, I clearly perceived that the luminosity was not confined to the mycelium in the leaf but extended also to the gemmae.[2] The young gemmae could be seen as bright spots. Some germinating gemmae which had rested for a few hours on a fresh Oleander leaf had the appearance of tiny stars. On the other hand, no luminosity could be detected in older cultures as coming from the little agarics which produce the basidiospores.

The discovery that the mycelium of *Omphalia flavida* growing in the leaf-spots of living leaves emits light has provided a means of diagnosing the American Coffee-leaf disease in the dark. Unfortunately, owing to my great distance from those parts of America

[1] A. H. R. Buller and T. C. Vanterpool, " The Bioluminescence of *Omphalia flavida*, a Leaf-spot Fungus," *Phytopathology*, Vol. XVI, 1926, p. 63.

[2] In the communication of Buller and Vanterpool (*loc. cit.*) it was stated that the gemmifers and sporophores " give out no light whatever." This statement, in so far as it applies to gemmifers, is erroneous ; for, as here recorded, it has been found that the gemmae are luminous.

where *Omphalia flavida* flourishes, I have not been able to go into Coffee plantations, pluck spotted leaves, examine them in the dark, and observe their luminescence ; but Professor Albert Müller of the Mayagüez College, Porto Rico, has recently done this,[1] thus proving beyond doubt that, so far as the emission of light is concerned, the spots on Coffee leaves in the open resemble the spots on leaves of Bryophyllum, Oleander, etc., in the laboratory at Winnipeg.

In the spring of 1928 and also on September 3 and December 4 of the same year, Professor Müller made field observations on the luminosity of infected leaves in a Coffee plantation in Porto Rico, and he described his observations to me in two letters, from one of which the following has been extracted. " My experience with *Omphalia flavida* has been very interesting. I saw luminosity for the first time when I entered my darkened laboratory and picked up a moist chamber containing leaves bearing the typical spots of the disease. This led to field expeditions for observing the phenomenon in the open. It was found necessary to select nights when the moon was absent and situations where the brightness of the tropical stars did not interfere. Another factor of the environment played a part in the form of fire-flies, large numbers of which flit about the Coffee trees or rest upon the leaves, giving off a very bright light. After the fire-flies had been dispersed, it was easy to observe luminosity in the numerous spots on the leaves. This luminosity was quite faint compared with the luminosity of a fire-fly placed on a leaf near the spot, yet quite bright compared with the luminosity of entire dead leaves of the Guava shade-trees found lying on the ground underneath, giving off a dull glow. An attempt was made to ascertain from what distances the luminosity of Coffee leaf-spots could be seen. From two to three feet the spots showed brightest. From six to ten feet one could state positively that luminosity was visible, but beyond ten feet imagination seemed to influence the decision of the observer."

It is possible that the luminosity of *Omphalia flavida* leaf-spots was actually observed by those engaged in coffee-growing half a century ago ; for, in 1880, Mr. C. Michelsen, the Commissioner of

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[1] *In litt.*, 1928.

Agriculture at Bogotá, wrote a letter to the Vice-consul of New Granada (Colombia), quoted by Dr. Ernst [1] in another letter to *Nature*, in which, after describing the Coffee leaf-spots and the yellow gemmifers which appear upon them, he adds : " This fungus is said to be phosphorescent at night ; and in places where it is very common a phosphoric smell is noted." That the Coffee leaf-spots should appear to be luminous in plantations after dark is exactly what might be expected in view of the laboratory observations of Vanterpool and myself and the Porto Rican observations of Professor Müller, but the idea that the fungus gives out a phosphoric smell is doubtless an accretion invented by the mythopoeic faculty. *Omphalia flavida*, it is true, is odoriferous, but its scent is faint and pleasant, reminding one of apricots [2] ; and, as stated in the discussion of bioluminescence in Volume III,[3] there is no reason to believe that the emission of light by fungi has any special relation with the element phosphorus.

There is another leaf-spot disease of Coffee caused by *Sclerotium coffeicola* which will be treated of in the next Section. Here it is only necessary to state that a comparison of artificial cultures made in the Winnipeg laboratory in the dark showed that, whereas the mycelium of *Omphalia flavida* is luminous, the mycelium of *Sclerotium coffeicola* is non-luminous. The luminosity criterion, therefore, should enable a phytopathologist to determine whether a particular Coffee leaf-spot is or is not caused by *Omphalia flavida*.

Omphalia flavida is not the only species of Omphalia which is luminous, for *O. Martensii* emits light also. *Omphalia Martensii* was described by Hennings [4] in 1893. This fungus grows on roots on the ground in Borneo ; and in March, 1863, near Bengkajang, it was observed to be luminescent by Professor O. Martens, a zoologist who had accompanied a Prussian expedition to eastern Asia.

[1] A. Ernst, " Coffee-Disease in New Granada," *Nature*, Vol. XXII, 1880, p. 292.
[2] The scent can be detected when the mycelium has just covered a malt-agar Petri dish and is producing gemmifers.
[3] These *Researches*, Vol. III, 1924, p. 364.
[4] P. Hennings, " Einige neue und interessante Pilze aus dem Königl. Botanischen Museum in Berlin," *Hedwigia*, Bd. XXXII, 1893, pp. 63–64, Pl. VII, Fig. 3. I am indebted to Mr. L. C. C. Krieger for calling my attention to this paper.

The fungus, on being examined by Hennings, proved to be an Omphalia, and it was named in honour of Professor Martens, its discoverer. The fruit-body has a stipe 2–2·5 cm. high and 0·5–1 mm. thick, while the pileus is 5–9 mm. in diameter. Evidently, the fruit-bodies of *O. Martensii* are more than double as large as those of *O. flavida*. The two fungi also differ in their luminescence, for the light is emitted by *O. Martensii* from the sporophore and by *O. flavida* from the mycelium and gemmifers.

The full description given by Hennings for *Omphalia Martensii*, translated from the Latin, is as follows : " Pileus membranaceous, campanulate, umbilicate in the centre, radiately striate, dirty yellowish, 5–9 mm. in diameter ; stipe thin, hollow, smooth and glabrous, yellowish, subfuscous at the base, 2–2·5 cm. long, 0·5–1 mm. thick ; gills subtriangular, broad, subdistant, pallid, decurrent ; spores not seen. Habitat : west Borneo, near Bengkajang, on roots."

In view of the fact that the mycelium of *Omphalia flavida* makes Coffee and other kinds of leaves luminous, one may well ask whether or not the mycelium of similar fungi is the cause of the luminosity which may be observed in dead leaves of Beech, Oak, Hornbeam, etc., forming part of the leaf-mould of the broad-leaved forests [1] very generally throughout the world.[2] This question has recently been answered in the affirmative by Bothe.

Bothe [3] has discovered that the mycelium of the following species of Mycena emits light : *M. polygramma* Bull. and *M. tintinnabulum*

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[1] Most observers have sought for luminous leaves only in broad-leaved forests, but Bothe (1931, in *Planta*, p. 753, *vide infra*) has discovered that they also occur in coniferous forests. In various places in the neighbourhood of Braunschweig and in the Harz under Pines, etc., he found needles of conifers, little twigs, and isolated " Tannen " cones, which gave out light just as strongly as decaying Beech and Oak leaves.

[2] In 1924, in Volume III of these *Researches* (pp. 421–427), to France, Germany, and Sumatra I added England, Canada, and the United States as countries in which luminous leaves occur. In 1926, S. R. Bose (*Nature*, 1926, p. 156) added India (Bengal). Here also it may be recorded that in 1929 I observed luminous Beech and Oak leaves in Scotland (Loch Awe) and in 1931 in Ireland (near Belfast).

[3] F. Bothe, " Ein neuer einheimischer Leuchtpilz," *Ber. d. D. bot. Gesell.*, Bd. XLVIII, 1930, pp. 394–399 ; also " Über das Leuchten verwesender Blätter und seine Erreger," *Planta*, Bd. XIV, 1931, pp. 752–765.

Fr., which grow on wood, and the following six species which grow on leaves :

M. dilatata Fr.   *M. sanguinolenta* A. et S.

M. epipterygia Scop.   *M. stylobates* Pers.

M. galopus Pers.   *M. zephira* Fr.

The mycelium of these leaf-fungi was obtained from spores and was then transferred to sterilised Oak and Beech leaves or Pine needles. The leaves were soon invaded by the fungi and in consequence they became luminescent. The mycelium of *Mycena galericulata* proved to be definitely non-luminous.[1] Certain, but not all, fruit-bodies of *M. epipterygia*, *M. galopus*, *M. pura*, *M. sanguinolenta*, and *M. zephira* collected in woods were found to have luminous gills. From Bothe's observations we may conclude that the luminescence of decaying leaves in forests is in part, if not entirely, due to the mycelium of various species of Mycena.

The Gemmifers of Sclerotium coffeicola.—*Sclerotium coffeicola* [2] Stahel is a parasitic fungus, probably of hymenomycetous origin, which is similar to *Omphalia flavida* in that it attacks Coffee plants and produces large numbers of gemmifers. A gemmifer of *Sclerotium coffeicola*, although differing in form, resembles in function a gemmifer of *Omphalia flavida*.

Our knowledge of *Sclerotium coffeicola* and of the disease which it causes is chiefly due to Stahel [3] who published his researches in 1921. I myself know the fungus only from spirit specimens on diseased Coffee berries and from a culture kindly sent to me by Mr. S. F. Ashby.

With a view to extending our knowledge of gemmifers in general,

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[1] F. Bothe, *loc. cit.*, 1931, p. 764. The mycelium of the following Mycenae, as so far cultivated, also appeared to Bothe to be non-luminous : *M. alkalina*, *M. crocata*, *M. haematopus*, *M. janthina*, *M. metata*, *M. vulgaris*, and four other undetermined wood-destroyers.

[2] Stahel wrote the specific name *coffeicolum*, but I have changed this to *coffeicola* in accordance with the Latin rule that the inhabitant of a place is denoted by a word ending in *cola*. Here the specific name is not an adjective but a noun in apposition. *Cf. Sclerotium lichenicola* Svendsen.

[3] G. Stahel,. " De Sclerotium-ziekte van de Liberiakoffie in Suriname," Departement van der Landbouw in Suriname, *Bull. No. 2*, 1921, pp. 1–34, Plates I–XI.

an account of the life-history of *Sclerotium coffeicola*, based on Stahel's investigations, will now be given.

Sclerotium coffeicola is the cause of the *Sclerotium disease* of the Coffee plant. The fungus, so far, is known to occur only in Dutch Guiana (Surinam),[1] British Guiana,[2] the island of Trinidad,[3] and Sierra Leone in west Africa.[4]

Martyn[5] has observed that in British Guiana *Sclerotium coffeicola* occurs as a leaf-parasite not only on *Coffea liberica* and one or two other Coffee varieties, but also on an ornamental shrub, *Gardenia*
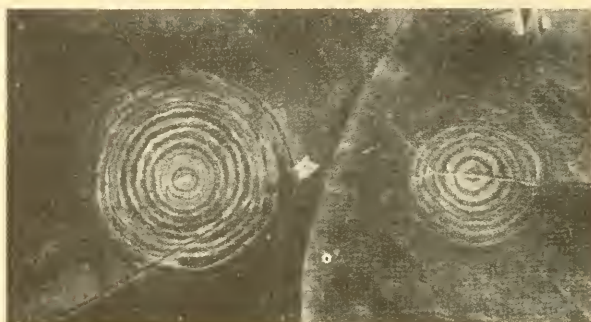
Fig. 224.—*Sclerotium coffeicola*. Leaf-spots caused by the fungus, seen from the upper side of pieces of two laminae of *Coffea excelsa*. Each spot is composed of a series of concentric rings. Gemmifers are absent, since they are produced only on the lower side of the spots. Photographed by G. Stahel. Natural size.

jasminoides, and on certain weeds growing in and about Coffee plantations, namely *Cecropia peltata*, *Commelina nudiflora*, *Vitis sicyoides*, an unidentified Melastomaceous plant, and a fern, *Blechnum serrulatum*. He also found the unmistakable bristles (gemmae) on the leaves of a shrub or young tree far removed from cultivation and, below the tree, on decaying leaves and fruits, the orange

[1] G. Stahel, " De Sclerotium-ziekte van de Liberiakoffie in Suriname," Departement van der Landbouw in Suriname, *Bull. No. 2*, 1921, pp. 1–34, Plates I–XI.

[2] E. B. Martyn, "The Sclerotium Disease of Coffee and its Occurrence in this Colony," *Agric. Journ. Brit. Guiana*, Vol. II, 1929, pp. 7–10 ; also " The Sclerotium Disease of Coffee," *ibid.*, Vol. III, 1930, pp. 28-34.

[3] In Trinidad *S. coffeicola* has been observed on *excelsa* Coffee leaves and fruits. Information communicated by S. F. Ashby.

[4] S. F. Ashby, personal communication.

[5] E. B. Martyn, *loc. cit.*, Vol. III, pp. 28-29.

sclerotia and the white feathery mycelium. The sclerotia and mycelium of *S. coffeicola* were observed by Martyn on decaying leaves not only in Coffee plantations but also in native forests. As a result of his observations Martyn is inclined to believe that *S. coffeicola* is to be regarded as a saprophyte on decaying leaf-tissues and, at the same time, as a potential parasite which, under suitable weather conditions, obtains a foot-hold on living plants. In being
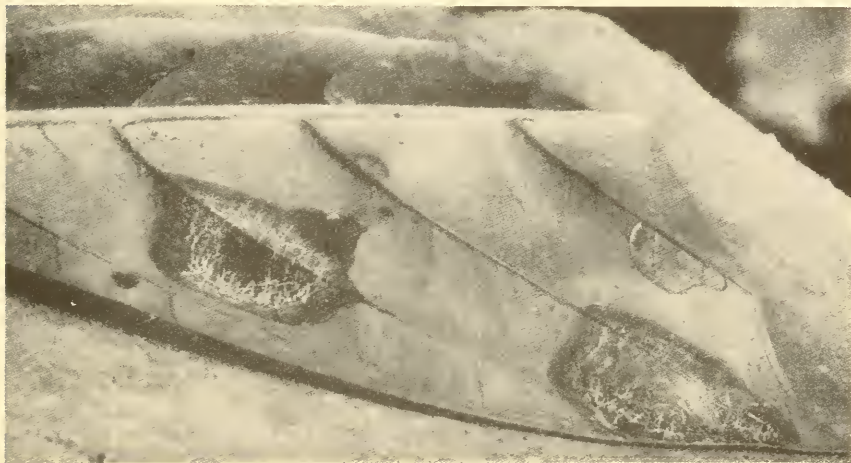
Fig. 225.—*Sclerotium coffeicola*. Leaf-spots caused by the fungus, seen from the under side of a leaf of *Coffea liberica*. The two large spots have produced numerous gemmifers which are arranged in concentric rings. The needle-like gemmae and the knob-like pedicels from which they break away can be easily recognised. The fungus progresses fastest along the larger veins of the leaf. Photographed by G. Stahel. Natural size.

able to attack the living leaves of various kinds of plants, including Ferns, *S. coffeicola* resembles *Omphalia flavida*.

The disease caused by *Sclerotium coffeicola* on the Coffee plant was first described by Kuyper [1] in 1913. He called it the *Coremium disease* because the little white needles (gemmae) which are produced on the leaf-spots and Coffee berries reminded him of coremia. However, these " coremia " do not produce any conidia and the presence of clamp-connexions in the mycelium affords evidence that the fungus is a Basidiomycete. Clearly, therefore, the name

[1] J. Kuyper, " Overzicht van de Koffieziekten in Suriname," Departement van der Landbouw in Suriname, *Bull. No. 31*, 1913, p. 58.

Coremium disease is a misnomer. Since the fungus produces characteristic sclerotia, it is better to follow Stahel and call the disease the *Sclerotium disease*.

Sclerotium coffeicola attacks both the leaves and the berries of the Coffee plant, and its ravages, in very moist weather, may even extend to the inflorescences and flower-buds. The berries are affected only when they are about three-quarters grown and are beginning to ripen. On the leaves, like *Omphalia flavida*, *Sclerotium coffeicola* causes the formation of brown leaf-spots (Figs. 224 and 225). These spots are characterised by the presence of concentric rings and by the production of gemmifers. The rings are due to the alternation of narrower lighter brown zones of dead tissue with broader darker brown zones. So long as the diseased leaves are hanging on the tree, the gemmifers are produced only on the under side of the leaf-spots. In the morning. after a dewy night, they can be seen on the lower surface of each spot standing up in large numbers and more or less in concentric circles (Fig. 225). The gemmifers are also produced in dense crowds on diseased berries and sometimes on the peduncles (Fig. 226).

On fallen leaves, in moist weather, the mycelium often grows out centrifugally from the leaf-spots over the surface of the leaves in the form of feathery branching strands which produce numerous gemmae (Fig. 227); and, in continuously moist weather, this may happen even on leaves still hanging on the trees.

Each gemmifer consists of a little white knob-like *pedicel*, attached to the substratum, and of an apical *gemma*. The gemma takes the form of a slender white cylindrical needle which ends in a blunt point. The gemmae on the leaf-spots are $1\cdot5-4\cdot0$ mm. long and $0\cdot05-0\cdot1$ mm. thick, *i.e.* about forty times as long as broad, while those on the fruits are shorter and thicker—about 1–2 mm. long and $0\cdot08-0\cdot2$ mm. thick.

There can be no doubt that the gemmifer of *Sclerotium coffeicola*, like that of *Omphalia flavida*, is entirely sporeless, so that, whatever its evolutionary origin, at the present day it does not serve as a sporophore. Since it is an active organ, obviously functioning by producing a multicellular body which is able to propagate the

species, we are justified, as in the analogous case of *Omphalia flavida*, in calling it a *gemmifer*.

When examined with the microscope, a gemma of *Sclerotium*
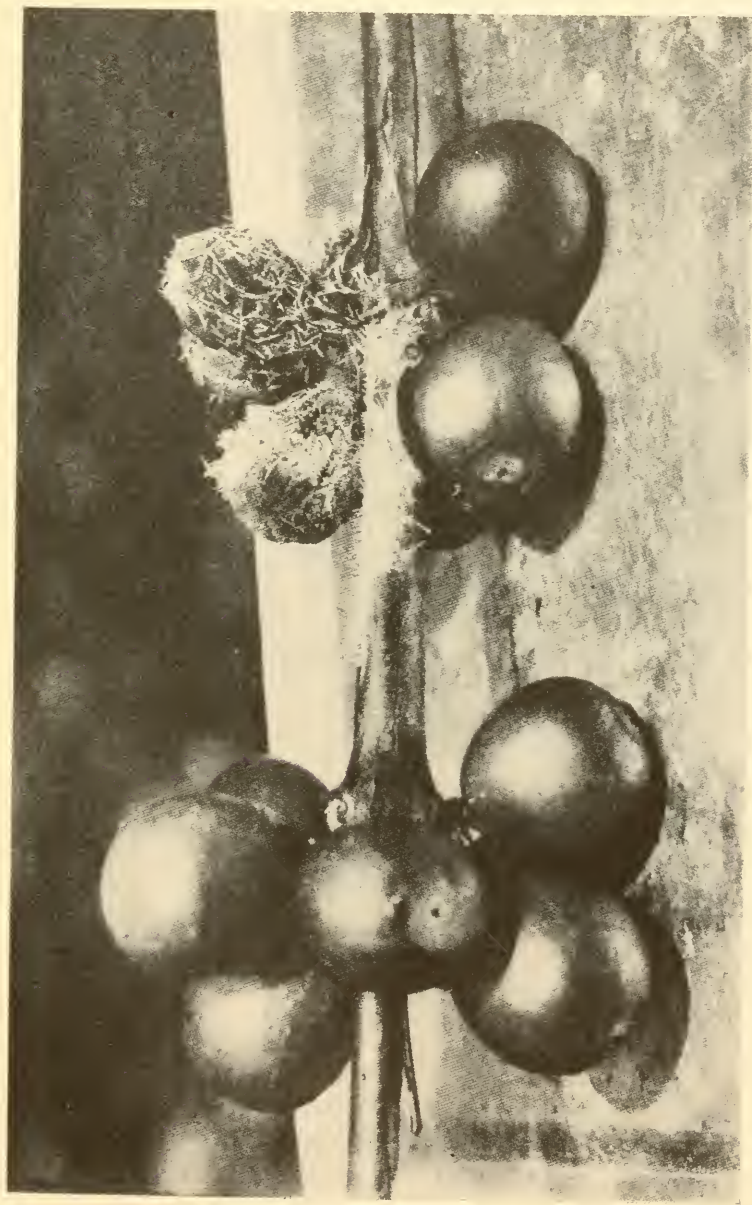
Fig. 226.—*Sclerotium coffeicola.* Branch of *Coffea liberica* bearing ripe fruits. To the right four dead berries covered with gemmifers. The mycelium has grown along the surface of the branch, has killed the bark below, and has produced a dense mass of gemmifers. Photographed by G. Stahel. Natural size.

coffeicola is seen to be composed of a bundle of hyphae, each 5–8 μ. thick, united with one another by numerous anastomoses. Furthermore, the outer surface of each hypha in a ripe gemma is verruculose owing to the presence of crystals of calcium oxalate. Owing to these structural features, a gemma is a very stiff rod, in consequence of which under slight pressure it readily breaks off just above the knob-like pedicel on which it stands. By the time a gemma is ready to be set free, its hyphae contain a rich store of glycogen.

Even a slight wind is sufficient to break off the gemmae from
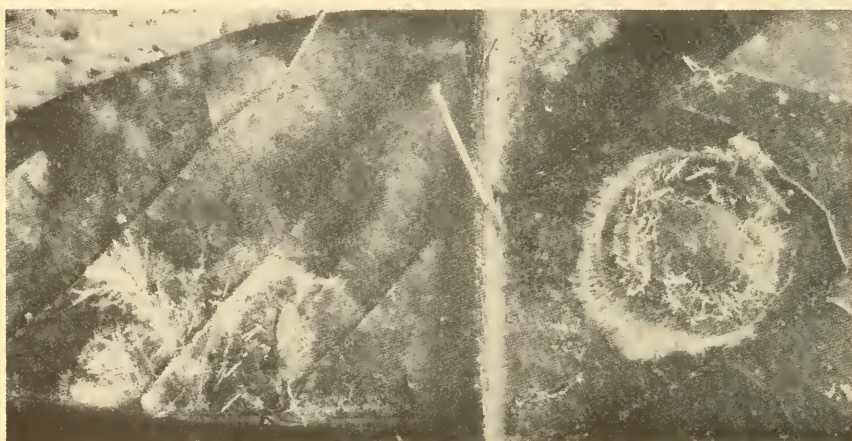
Fig. 227.—*Sclerotium coffeicola*. Infected leaves of *Coffea liberica* which had fallen upon the ground. The mycelium has grown out from the leaf-spots centrifugally over the surface of the leaf in the form of strands. The mycelial strands on the left-hand leaf have produced a number of gemmifers. Photographed by G. Stahel. Natural size.

their bases and carry them away. In plantations which are badly affected with the disease, a number of gemmae can be seen lying on every leaf, chiefly on the upper surface (Fig. 228), but not infrequently also on the lower surface. In damp weather it is possible to observe the gemmae infecting the leaves and forming new leaf-spots.

When a gemma which has been blown on to a leaf begins to develop, it first of all forms an *appressorium*, or sometimes two or more. An appressorium is a solid mass of hyphae which assumes a hemispherical shape and flattens itself out upon the epidermis. When full-grown, it has a diameter of 0·05–0·5 mm. The appres-

soria formed on the under side of leaves are usually smaller than

Fig. 228.—*Sclerotium coffeicola.* Upper side of pieces of leaves of *Coffea excelsa* with about thirty gemmae lying on the epidermis. Several of the gemmae have germinated and have penetrated into the leaf-tissue. The appressorium of some of the gemmae can just be seen. Photographed by G. Stahel. Natural size.

those on the upper side. Each appressorium becomes firmly attached to the cuticle by means of mucilage. In the middle of the

appressorium, on the upper side of a leaf, the hyphae converge to
a point. The hyphae at this central point then press against the
cuticle, force their way through it, and at once kill the epidermal
cells with which they come into contact. The invading hyphae, in
a compact mass, then force their way in between the cuticle and the
epidermis, kill more epidermal cells, press against and flatten the
dead epidermal cells, kill the subjacent palisade cells, and break
through the epidermis into the intercellular spaces of the palisade
layer. The palisade cells become compressed by the advancing
wedge of hyphae. The hyphae now kill the spongy parenchy-
matous cells below them. Then, still in a compact mass, they grow
downwards through the spongy parenchyma as far as the lower
epidermis. Already by this time a small brown leaf-spot has been
formed. Hyphae now grow out laterally through the intercellular
spaces of the mesophyll, and thus the leaf-spot, so long as the weather
is sufficiently moist, continues to enlarge centrifugally. The leaf-
cells a short distance in front of the advancing hyphae die before
the hyphae reach them, a fact which indicates that the mycelium
excretes a toxic substance. Since the hyphae of the gemmae and
of the mycelial fibrillae which spring from the sclerotia are coated
with calcium oxalate, Stahel has suggested that the toxic substance
is oxalic acid. If this supposition is correct, the leaf-spots ought
to give a pronounced acid reaction. Whatever the nature of the
toxic substance, its effect upon the leaf is such that the hyphae
which excrete it grow only in dead tissue.

The lower epidermis, unlike the upper, contains numerous
stomata, and here the invading hyphae which grow out from an
appressorium enter the leaf *via* the stomatic clefts. Stahel proved
by experiment that the gemmae on the under side of a leaf infect
the leaf more quickly and more easily than gemmae on the upper
side. Nevertheless, under natural conditions, as might be expected
from the fact that the gemmae settle more easily on the upper
side of leaves than on the under side, most infections take place
from the upper side. Stahel investigated 213 leaf-spots and found
that 175 (82 per cent.) had the infecting gemma on the upper side
of the leaf-spot and 38 (18 per cent.) on the lower side.

In his infection experiments, Stahel observed that the leaf-spots

began to produce gemmifers 10–12 days after the leaves had been inoculated with gemmae. A single leaf-spot may produce several scores of gemmae.

In dry weather the gemmae dry up, but they retain their vitality for some time even in the dried condition. Stahel placed a number of gemmae in a desiccator for varying periods of time and then tested them for germination. He found that they retained their vitality after two weeks in the desiccator, but not after three or four weeks except for a single gemma which germinated weakly.

Dry weather checks the progress of the Sclerotium disease ;

FIG. 22.ɔ.—*Sclerotium coffeicola.* Dead Coffee berries covered with sclerotia, lying on the ground. Photographed by G. Stahel. Natural size.

for, under these conditions, not only can new infections not take place, but the leaf-spots already in existence cease to grow in size and dry up. With the advent of dry weather, the leaf-tissues form a callus around each spot which effectually prevents the spots from increasing in size again when wet weather comes on. Stahel suggests that the callus sets a limit to the growth of the mycelium by preventing the toxic substance produced by the hyphae in the leaf-spot from diffusing centrifugally.

The sclerotia of *Sclerotium coffeicola* were discovered by Kuyper. They develop in considerable numbers on the outside of infected leaves and berries as these lie upon the ground under Coffee trees

(Figs. 229 and 230). Occasionally they arise within the tissues of a leaf between the two layers of epidermis. They are orange-yellow or brown in colour, 2–15 mm. broad, and mostly somewhat flattened. In continuously rainy weather sclerotia may be formed on the berries and more rarely on the under side of leaves, even before these organs fall to the ground.

The sclerotia, as observed by both Kuyper and Stahel, never give rise to any sporophores. In moist surroundings, *e.g.* under trees and in cultures, they send out feathery branching mycelial
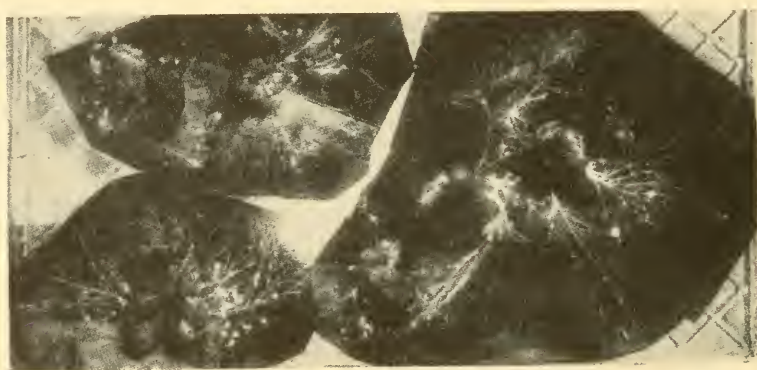
Fig. 230.—*Sclerotium coffeicola*. Germinating sclerotia on green leaves of *Coffea liberica*. The mycelial strands have produced numerous appressoria. Photographed by G. Stahel. Reduced to three-quarters of the natural size.

strands which grow over the surface of the dead leaves and fruits to which they are attached (Fig. 230). These mycelial strands, which Stahel refers to as rhizomorphs, under favourable weather conditions form gemmifers, the gemmae of which may be carried away by the wind and deposited on leaves and fruits, where they may form disease spots and thus reproduce the species. Some sclerotia, out in the open, were found by Stahel to have retained their vitality for one and one-third years. The sclerotia of *Sclerotium coffeicola* are thus well fitted for carrying the fungus over periods of drought and other adverse weather conditions.

In my laboratory at Winnipeg, a malt-agar plate was inoculated with the mycelium of *Sclerotium coffeicola*. The mycelium spread radially over the agar and, in the course of two or three weeks,

gave rise to three large sclerotia. The appearance of the plate one month after inoculation is shown in Fig. 231. This experiment and others make it evident that the sclerotia of *S. coffeicola* are readily formed in artificial cultures.

Since clamp-connexions are present in the mycelial strands which grow out from the sclerotia, it seems very probable that *Sclerotium*

Fig. 231.—*Sclerotium coffeicola* growing on 2·5 per cent. malt-agar in a Petri dish. A month after inoculation with mycelium. The mycelium has spread over the plate and has formed three sclerotia. The thickest mycelial cords lead to the sclerotia. Natural size.

coffeicola belongs to the Basidiomycetes and, in particular, to the Hymenomycetes. In the Hymenomycetes sclerotia are known in Typhula and other Clavariaceae, Hypochnus, Coprinus, Collybia, Lentinus, and Polyporus. Stahel is inclined to regard *Sclerotium coffeicola* as most like a Typhula. In this genus not only are sclerotia

known to be formed, but several species, *e.g. T. variabilis* on Sugar-beet, *T. varians* on Beet leaves, and *T. gyrans* on Cabbage, are parasitic. The gemmifers of *Sclerotium coffeicola* are rod-shaped in form and thus remind one of the fruit-bodies of Clavariaceae and particularly of the sterile branched structures of Anthina, a pseudo-genus placed among the Clavariaceae by de Bary.

It therefore seems not unlikely that the gemmifers of *Sclerotium coffeicola*, like those of *Omphalia flavida*, have been derived from sporophores or sporophore-rudiments which have become meta-morphosed and adapted to reproduce the species by means of gemmae instead of by basidiospores.

It is indeed a remarkable fact that there should be two unrelated gemmiferous species of Basidiomycetes, both of which form leaf-spots on the leaves and attack the berries of the Coffee plant, and in which the perfect sporophore has been either almost wholly or wholly suppressed. It may well be that, in the tropics, in the course of time, yet other gemmiferous Basidiomycetes will be discovered.

GENERAL SUMMARY

THE FOLLOWING IS A SUMMARY OF THE MORE IMPORTANT
RESULTS OBTAINED DURING THE INVESTIGATIONS

PART I

Chapter I.—The history of our knowledge of the discovery, development, general structure, sexuality, cytology, pigmentation, parasites, drop-excretion, reactions to light, and ballistics of Pilobolus has been reviewed.

The relation of larval Roundworms (Nematoda) to the fruit-bodies of Pilobolus has been discussed. These animals often swarm up the sides of the sporangiophore but do not penetrate into the subsporangial swelling. Sometimes they travel with a sporangium when this is shot away through the air.

Syncephalis nodosa van Tiegh., as a parasite on *Pilobolus longipes* and *P. Kleinii*, has been described and illustrated.

Chapter II.—The germination of the spores, the growth of the mycelium, and the formation of fruit-bodies of *Pilobolus longipes* have been described.

Spores of *P. longipes* were sown on fresh sterilised horse-dung balls in a closed crystallising dish, and many of the fruit-bodies which came up on the dung had colourless, instead of black, sporangial walls. This abnormality appears to have been caused by gaseous emanations given off by the horse dung.

Species of Pilobolus observed at Winnipeg were : *P. longipes, P. Kleinii, P. oedipus*, and *P. umbonatus*.

A series of successive stages in the development of a diurnal crop of fruit-bodies of *P. longipes*, as affected by external conditions, particularly light and darkness, has been represented diagrammatically.

The maximum vertical height to which sporangia of *P. longipes* and *P. Kleinii* were shot was 6 feet 0·5 inch.

The maximum horizontal range of the fruit-body gun was found to be : for *P. longipes*, 8 feet 7·5 inches ; and for *P. Kleinii*, 8 feet 0·5 inch.

The structure of the sporangiophore and of the sporangium of *P. Kleinii* and *P. longipes* has been described in detail as a preliminary to a

455

discussion of the factors which permit of the successful operation of the Pilobolus gun.

The sporangium-wall dehisces by splitting horizontally into two parts, a lower narrow band which remains attached to the base of the columella and an upper, much larger, cap-like portion which covers the spores. The escape of the spores from a sporangium which has just dehisced is prevented by a ring of jelly. This jelly, after the sporangium has been discharged, serves to stick the sporangium to the surface of a grass-leaf, etc.

The number of spores in a sporangium of *P. Kleinii* was estimated to be 30,000–90,000, varying with the sporangium's size.

The level of abscission in the sporangiophore when the sporangium is shot away is at the top of the subsporangial swelling just beneath the junction of the sporangium-wall with the wall of the columella.

The structure of a discharged sporangium has been described and illustrated in detail. As a discharged sporangium dries, the sporangium-wall becomes locally depressed above and circularly tucked-in beneath the spores below, while the spores contract in size and become polygonal.

A sporangium-wall, before the sporangium has been shot away, is always smooth and rounded. The more or less regular pattern of dimples, hexagonal areas, etc., which can often be seen on a dried discharged sporangium is formed as the sporangium dries up and contracts. The pattern on a dried discharged sporangium of *P. longipes* differs from that on a dried discharged sporangium of *P. Kleinii*; and, in general, the patterns are an aid in distinguishing these two species of Pilobolus from one another.

The drops excreted by the sporangium and subsporangial swelling of a Pilobolus fruit-body contain a colloidal mucilaginous substance, for they dry up with an irregular surface. The drops on the sporangium usually dry up before the sporangium is discharged; and, as they do so, they become darkly pigmented like the sporangium-wall. The drops on the upper part of the subsporangial wall tend to dry up sooner than those on the lower part, and their interference with the light rays which cause heliotropic response is thus diminished.

The wall of a Pilobolus fruit-body bears numerous very minute crystals of calcium oxalate. In *P. longipes* these crystals are arranged: most densely on the sporangium-wall, less densely on the wall of the subsporangial swelling, and far less densely on the wall of the stipe. The unwettability of the sporangium-wall may be due to the numerous close-set crystals imprisoning air and thus preventing water from coming into contact with the wall's surface.

The subsporangial swelling of the Pilobolus gun functions in two different ways: (1) as an ocellus which receives the heliotropic stimulus which causes the stipe to direct the free end of the gun toward the source of brightest light; and (2) as part of a squirting apparatus which,

by violently expelling cell-sap, shoots away the sporangium from the sporangiophore.

A subsporangial swelling is transparent and refracts light like the bulb of a Florence flask filled with water; and its diameter is always greater than that of the black sporangium which it supports. The lens action of a subsporangial swelling has been investigated directly and also by means of construction diagrams.

When the sunlight strikes upon one side of a subsporangial swelling, the light rays are refracted through it and converge so as to form a spot of light on the other side. When the incident rays of light strike the sporangium *head on* and are exactly parallel to the long axis of the swelling, the spot of light which is formed by the rays entering that part of the swelling which bulges out beyond and around the sporangium is symmetrically placed at the base of the swelling. Under these conditions there is physiological equilibrium and no heliotropic response takes place. When, however, the incident rays of light strike a swelling *obliquely*, the spot of light is placed on one side of the wall of the swelling in a manner which is asymmetrical for the swelling as a whole. Under these conditions, the protoplasm which is lighted by the spot of light sends a heliotropic stimulus down to the protoplasm at the top of the stipe just beneath the base of the swelling. The top of the stipe then reacts by growing in length most rapidly on the side nearest to the spot of light and thus bending as a whole. As a result of this reaction, the swelling is moved about its base through an angle and the spot of light passes downwards on the wall of the swelling until it comes to be symmetrically placed at the base of the swelling. As soon as the spot of light arrives at this terminal position, a physiological state of equilibrium becomes established in the sporangiophore and the heliotropic reaction ceases. At the end of the turning movement the gun is directed toward the source of the brightest light.

In *Pilobolus Kleinii* and other Piloboli the protoplasm in the lower part of the subsporangial swelling and at the top of the stipe contains a red pigment (carotin); and, at the top of the stipe just above the stipe's motor region, the protoplasm is heaped up so as to form a strongly bi-concave, very red, centrally perforated septum. As shown by direct observations and by theoretical diagrams, this protoplasmic septum is admirably shaped and situated for receiving the light rays converging on it when the gun is in a position of complete or almost complete physiological equilibrium. Its concave upper surface, its position in respect to the subsporangial lens, and its strong pigmentation suggest a comparison in function with the retina of the eyes (ocelli) of certain Mollusca.

The diameters of the sporangium, the subsporangial swelling, and the motor region of the stipe below the swelling in a well-grown fruit-body of *P. Kleinii* were observed to be 0·43 mm., 0·76 mm., and 0·16 mm. respectively. A simple calculation based on these data shows that the

sporangium, when head on to a beam of parallel light rays, casts a shadow which has an area $7\cdot2$ times that of a cross-section of the stipe. If there were no subsporangial swelling, the shadow of the sporangium would cut off the light from the top of the stipe before the ortho-heliotropic position had been completely attained. This would prevent the gun from being accurately directed toward the source of light. Evidently the difficulty of supplying the stipe with its required delicate heliotropic stimulus has been surmounted in the course of evolution by the intercalation between the sporangium and the stipe of the large light-collecting subsporangial swelling. That part of the swelling which bulges out beyond the black sporangium receives the light and concentrates it by refraction upon the base of the swelling ; and, when the spot of light so produced is asymmetrical in position, it provides the stimulus to which the motor region of the stipe can react.

A subsporangial swelling is capable of producing not only a spot of light but also a more or less well-defined image of the source of light or of an illuminated object. Such images have been observed and photographed.

The mechanism of heliotropic response in Pilobolus has been compared with that of (1) the leaves of certain Flowering Plants, (2) a free-swimming Volvox sphere, and (3) Man.

In the heliotropic reaction of Pilobolus, which is caused by the asymmetrical position of a spot of light in the subsporangial swelling with a consequent bending of the stipe, we have clear proof of the theory, first suggested by Haberlandt, that heliotropic reactions may take place in plants through ocellus action.

The sporangiophore of Pilobolus is the only ortho-heliotropic organ known which takes up its positively heliotropic position owing to the possession of a special light-perceiving cell structure.

Pilobolus may well be described as a fungus with an optical sense organ or simple eye (ocellus) ; and, in using its eye for laying its gun, it is unique in the plant world.

In bright sunlight directed perpendicularly to the long axis of the subsporangial swelling, a stipe of *P. longipes* was observed to turn the swelling and the sporangium through an angle of $90°$, and thus to complete its heliotropic reaction, in about one hour.

A solution of the problem why it is that a Pilobolus fruit-body turns so as to face directly one of two equal beams of light coming from sufficiently different directions, and does not take up a position of resultant reaction, has been given. It confirms Van der Wey's solution of the same problem, published by him in 1929, and is based on the assumption (1) that the subsporangial swelling acts as an ocellus and (2) that the pigment zone at the base of the swelling is the region of protoplasm where perception of light takes place. Two spots of light are formed by the two beams of light in the swelling : the spot of light nearer the base of

the swelling gives a greater stimulus to the motor region of the stipe than the spot farther away and, as a consequence, the stipe bends toward the source of light which has formed the lower spot of light and away from the source of light which has formed the higher spot of light. As this process continues, the lower spot of light moves toward the base of the swelling and the upper spot away from it. This results in the lower spot giving a greater and greater, and the upper spot a lesser and lesser, heliotropic stimulus to the stipe. Finally, the lower spot of light comes to rest directly on the perforate red protoplasmic septum at the top of the stipe. Thus heliotropic equilibrium becomes established and the sporangium faces one of the two beams of light (the one that produced the lower spot of light in the first instance) and one only.

A model for illustrating the Pilobolus fruit-body in its relations with light has been described.

The diurnal periodicity in the development of the fruit-body of Pilobolus enables the fruit-body to use the morning sun for directing its axis toward the source of strongest light and therefore toward an open space. Thereby the dispersion of the sporangia, which takes place during the noon hours, is accomplished under favourable conditions.

The second special function of the subsporangial swelling, that of acting as part of a squirting mechanism for the discharge of the sporangium, has been discussed. When a Pilobolus gun explodes, about one-half of the volume of the cell-sap in the stipe and subsporangial swelling is shot out of the opening in the top of the swelling. The cell-wall of the swelling is highly elastic, and its most contractile part is a band, called the *collar*, situated around what is to become the mouth of the swelling. The *abscission level*, *i.e.* the level at which the swelling is destined to split across, is indicated before the sporangium is discharged. As a Pilobolus fruit-body explodes, a jet of cell-sap is shot out of the mouth of the swelling. The first and largest drop of this jet carries off the sporangium and travels with it through the air. The other drops are smaller than the first one and have shorter trajectories.

Sometimes, when a Pilobolus gun explodes, the sporangiophore breaks across, not—as normally—just below the sporangium, but across the top of the stipe. When this happens, the projectile consists of a sporangium, a subsporangial swelling, and a drop of cell-sap.

By means of Barger's capillary-tube method it was found that the osmotic pressure of the cell-sap of *Pilobolus longipes* is approximately equal to $5\cdot5$ atmospheres.

The efficient working of the Pilobolus gun is due to a number of different factors, five of which have been specified.

A chemical analysis of the cell-sap of *Pilobolus longipes* suggests that the osmotic (turgor) pressure of the sap is largely due to phosphate and oxalate ions, but is also due in part to potassium, sodium, chloride, and sulphate ions, and in part due to some as yet unrecognised carbohydrate.

The problem why it is that a discharged sporangium normally lands on a grass-leaf, etc., so that its under gelatinous side is turned toward the surface of the substratum and its black upper convex side away from the surface of the substratum has been solved by taking into account the fact that (1) the lower gelatinous side of the sporangium can be wetted by water whereas (2) the upper convex side covered by the black sporangial wall is unwettable. That the black sporangial wall is unwettable has been demonstrated by various experiments. A Pilobolus projectile, whilst travelling through the air, consists of a sporangium and a large drop of cell-sap. The drop does not enclose the sporangium but is attached to the sporangium's wettable gelatinous under side. The projectile may rotate as it passes through the air. As the projectile lands on a grass-leaf, etc., the drop spreads out on the surface of the substratum. As it does so, it drags the gelatinous wettable under side of the sporangium toward the surface of the substratum and, at the same time, pushes away from itself and the substratum the black unwettable convex side of the sporangium.

Very occasionally one observes a sporangium which is attached to the surface of glass, etc., not by its gelatinous under side, but by its rounded black upper side. The taking up of this abnormal position by a sporangium may be due to the gelatinous ring having been torn away from the sporangium before the projectile struck the surface of the substratum or at the moment of impact.

The gelatinous material on the under side of a sporangium has remarkable adhesive properties, as may be shown by attempts to wash sporangia off sheets of glass, etc., to which they have become attached. Under natural conditions in pastures, the sporangia of Pilobolus are so effectively attached by their gelatinous sides to the herbage on which they have alighted that, in dry weather, they cannot be detached from their substratum by the strongest winds and, in wet weather, they cannot easily be detached by prolonged rain or even by violent thunderstorms.

Pilobolus is a highly specialised coprophilous fungus which is dependent for its existence : (1) on flowering plants which provide its sporangia with a temporary but prospectively favourable lodging-place ; and (2) on herbivorous animals which swallow the sporangia and herbage together, break open the sporangia and disperse the spores within their alimentary canals, and finally extrude the spores undamaged in their solid faeces.

The sporangial wall of Pilobolus, as opposed to that of Mucor, is (1) unwettable, (2) persistent (non-deliquescent), and (3) intensely black. The unwettability of the wall is a prime factor in causing the sporangium to alight on any object with its gelatinous side turned toward the object ; the persistency of the wall enables the wall, when the sporangium is attached for weeks or months to a flowering plant, to prevent the spores escaping from the sporangium even during rainy weather ; and the intensely black pigment in the wall enables the wall to absorb sunlight

and thus to act as a screen in cutting off injurious rays of light from the spores which lie beneath it.

Pilobolus gives to flowering plants and herbivorous animals nothing in return for their services in dispersing its spores. However, although Pilobolus does not pay for what it receives, it imposes on the organisms which assist it a burden which is so light as to be practically negligible.

Chapter III.—A new species of Pilobolus, *Pilobolus umbonatus*, has been described. It occurs on horse dung at Winnipeg and was observed by the late Dr. Roland Thaxter on sheep dung in the eastern part of the United States of America.

Pilobolus umbonatus, as compared with *P. longipes* and *P. Kleinii*, is a smaller and more delicate species. It is easily distinguished from all other species of Pilobolus by its decidedly umbonate sporangium and its minute ellipsoidal spores. With a hand-lens one can readily make out the acutely-pointed umbonate shape of the dried discharged sporangia when these are seen in lateral view.

A series of critical remarks on the Pilobolidae, made with a view to assisting future workers on this group, has been recorded.

Pilaira is regarded by the author as a good genus and not as " abnormal material of Pilobolus."

To the criteria so far employed by taxonomists for distinguishing species of Pilobolus should be added : (1) the exact shape of the subsporangial swelling, whether ellipsoid or pyriform, etc. ; (2) the ratio of the width of the sporangium to the width of the subsporangial swelling ; (3) the nature of the depressions or wrinkles on dried discharged sporangia when seen in strong reflected unilateral light ; and (4) the nature of the fringe of the sporangium-wall of dried discharged sporangia, in respect to form, colour, and disposition of crystals.

The spores of Piloboli, when seen in water, are *dichroic* : they are orange-yellow or yellowish in transmitted light and green in reflected light.

The form and the variability of the basal swelling of the fruit-bodies of Piloboli have been discussed.

The *width-ratio* of the sporangium, *i.e.* the ratio of the width of the sporangium to the width of the subsporangial swelling, has been measured in *Pilobolus longipes*, *P. Kleinii*, and *P. umbonatus*.

The pattern on the sporangium of *Pilobolus crystallinus*, as illustrated by Coemans, van Tieghem, and Zopf, has been treated of. Patterns develop on the discharged sporangia of *P. Kleinii*, *P. longipes*, and *P. umbonatus* as the sporangia dry up and flatten. Studies on the variability of patterns in *P. Kleinii* and *P. longipes* have been made. The pattern on a dried discharged sporangium, while fairly constant in its general aspect for each species of Pilobolus, is subject within each species to a large amount of variation in detail. The typical pattern for any particular species ought to be sought for in the larger sporangia rather than in the smaller ones.

Chapter IV.—This Chapter contains a systematic account and arrangement of the Pilobolidae, contributed by W. B. Grove, and it may be considered as a revision of the systematic part of his *Monograph of the Pilobolidae* published in 1884.

A history of our knowledge of the taxonomy of the Pilobolidae during nearly two hundred and fifty years is recorded. It shows how slowly and painfully a little accurate knowledge has been accumulated. From now on, with the modern technique of pure cultures and the art of photography at our disposal, progress should be more rapid. It is to be hoped that some younger mycologist may be stimulated by this presentation of the subject to make a life-study of the Pilobolidae and give us a comparative description of all the species that can be gathered together from different parts of the world.

The first observations on a Pilobolus, made by John Banister in Virginia, were recorded by Ray in 1688. The most recently found species, *Pilobolus umbonatus*, is described by Buller in this volume (1934).

An attempt has been made to arrange systematically all the known species of the Pilobolidae and to give to the group itself and to the two genera, Pilobolus and Pilaira, included within it, more precise definitions than have yet appeared.

Sixteen species of Pilobolus and five species of Pilaira are listed and described.

Of the species of Pilobolus the following are considered to have been adequately described and illustrated : *crystallinus, heterosporus, Kleinii, longipes, nanus, oedipus, roridus, sphaerosporus,* and *umbonatus* ; while the following are considered to have been insufficiently or negligently described : *argentinus, Borzianus, minutus, Morinii, pullus, roseus,* and *Schmidtii.*

A key to the species of Pilobolus is presented. It is based on characters of the sporangium, the trophocyst, and the spores.

The five known species of Pilaira are the following : *anomala, dimidiata, Moreaui, nigrescens,* and *Saccardiana.* A key to these species is also provided.

Appended is a bibliography which includes all those papers and books which refer more particularly to the species of Pilobolidae and their differentiation. Some additional works which treat of the physiology, ecology, and other aspects of the group are cited in the preceding Chapters written by Buller.

PART II

Chapter I.—Most Discomycetes exhibit the phenomenon of puffing, *i.e.* when they are subjected to certain changed conditions they pass from a quiescent to an active state and suddenly liberate a cloud of spores, visible to the naked eye. The fruit-bodies of Hymenomycetes, on the other

hand, never puff but liberate their spores in a steady stream which may continue for hours, days, or weeks.

Not only large Discomycetes but also small ones puff, so that puffing is not correlated with fruit-body size.

Puffing was first described by Micheli in 1729 and has been noticed by all mycologists since his time. In 1916, Falck divided the Discomycetes into : (1) the *radiosensitive* which emit spores when warmed by radiant heat given out by a lamp or the sun, and (2) the *tactiosensitive* which puff when touched or blown upon. In 1926, Ziegenspeck came to the conclusion that the opening of the asci at the moment of puffing is not due to a stimulus but is a purely mechanical phenomenon caused by a slight additional strain in the ascus walls at their apices.

Photographs, made by Dickson and Fisher, illustrating the spore-clouds of *Sclerotinia sclerotiorum* given off at the moment of puffing have been reproduced.

Conceivably, the asci of a Peziza might discharge their contents one by one in the order of ripening. Why then does a Peziza puff ? Falck has supposed that the dependence of Morchella, Gyromitra, Verpa, etc., on a sufficiently high temperature for the discharge of their spores causes spore-discharge to be delayed until the heat of the sun brings into existence air-currents which may assist in spore-dispersal ; and he also holds that the dependence of many Pezizaceae on the wind for the discharge of their spores causes spore-discharge to be delayed until the wind is sufficiently strong to be effective in carrying away the spores. In these ecological theories there may well be some truth. It was in the hope of throwing more light on the phenomenon of puffing that an investigation on *Sarcoscypha protracta* was undertaken.

Sarcoscypha protracta occurs in young Poplar bush (*Populus tremuloides*) in the suburbs of Winnipeg, and its fruit-bodies appear near the end of April and at the beginning of May. The cups are scarlet in colour and conical in form, and they look upwards toward the sky. They are attached to buried roots by a pseudorhiza ; and the pseudorhiza, like that of *Collybia fusipes*, is perennial.

When a fruit-body of *Sarcoscypha protracta* puffs, the spores are shot straight outwards from the conical hymenium in directions which are parallel to the cup's longitudinal axis. The distance to which the spore-cloud was shot before dispersing irregularly was found in three fruit-bodies to be 8 cm., 12 cm., and 17 cm. (= 7 inches) respectively.

Each ascus opens obliquely and the opening faces the mouth of the cup and consequently the sky. On this account, although the asci are straight and are obliquely inclined toward the long axis of the cup, the spores are shot not from one side of the cup to the other, but straight upwards through the mouth of the cup and parallel to the cup's long axis.

Owing to the contraction of the cell-wall, an ascus, when discharging

its contents, reduces its volume to about one-half. The half of the contents of the ascus which is discharged is the upper half which includes the eight spores and a mass of cell-sap.

The ascus, as a gun, is a squirt from which a jet of watery fluid containing eight spores is ejected by the pressure of a contracting elastic cell-wall. The wall contracts more per unit of distance in the circumferential direction than in the longitudinal direction.

Radial-longitudinal sections through a cup and surface views of the hymenium, (1) before puffing has taken place and (2) immediately after puffing has taken place, have been illustrated with a view to showing the exact positions of the asci, the opercula of the asci, the hinges of the opercula, the long axes of the spores, and the paraphyses, relatively to the position of the mouth of the cup as a whole.

Correlated with the conical form of the cavity of the apothecium of *Sarcoscypha protracta* and making for efficiency in the production and liberation of spores by the hymenium are : (1) the oblique upward-looking openings of the asci formed at the moment of spore-discharge ; (2) the obliquely upward inclination of the spores in each ascus ; and (3) the relatively distant separation of adjacent asci by paraphyses.

The paraphyses form at least one-half of the hymenial substance. They assist the discharge of the spores : (1) by holding the asci fixed in their positions whilst the asci are shooting away their spores obliquely through their apices ; and (2) by keeping adjacent asci sufficiently far apart, thereby preventing the spores of one ascus from hitting the protruding end of another ascus during spore-discharge.

It is probable that, in *Sarcoscypha protracta*, the mouth of an ascus comes to look upwards toward the mouth of the apothecium as a result of the end of the ascus making a heliotropic curvature.

By means of an experiment with a test-tube provided with a lateral opening, it has been proved that, when an apothecium puffs, it produces a blast of air. The blast of air is caused by tens of thousands of spores and sap-drops, which have been shot in the same general direction, striking the air and setting it in motion.

The significance of the blast of air in respect to the dispersal of the spores has been discussed. If the asci of a fruit-body of *Sarcoscypha protracta* were to explode one after another as they ripen, it is probable that the eight spores of each ascus would not be shot up into the air more than 3–4 cm., and no appreciable blast of air could come into existence to raise them still further. On the other hand, when a hundred thousand asci explode together, *i.e.* when puffing takes place, the bombardment of the air by the spores and sap-drops collectively is sufficient to create a blast of air which can carry the spores away with it for several inches after they have lost the velocity given to them by the ascus-guns. Under natural conditions in a Poplar wood, the stipe of the fruit-body is negatively geotropic and the apothecium looks upward to the sky. If the

asci were all to be discharged in succession as they ripen, none of the spores (we may suppose) would travel upwards more than an inch or two ; but, if the asci ripen one by one and then wait before exploding until some suitable stimulus sets them all off together, then the spores may be carried upwards by the blast of air, which they bring into existence, to a height of 5-7 inches. After being carried upwards into the air, the spores are borne away more or less horizontally by the wind and then dispersed. The nearer the ground, the less wind there is ; and, every extra inch the spores can be raised into the air by discharge from the apothecium, the stronger will be the wind which they will encounter and the greater the chance that they will be dispersed widely and find suitable conditions for propagating the species.

Our knowledge of the phenomenon of spore-discharge in the Discomycetes is still very incomplete. With a view to its extension individual apothecia of a series of typical species should be observed in the field. Such apothecia should be left *in situ* and not be touched by man ; and, for each apothecium, during the whole period of spore-discharge, a continuous record, not only of the times of spore-discharge, but also of the conditions of spore-discharge (temperature, moisture, wind-movements, etc.) should be made.

Chapter II.—In 1890, Zopf observed that the protuberant asci of *Ascobolus denudatus* and of a species of Saccobolus are heliotropic and bend toward the source of strongest light.

As a result of a series of his own observations the author has no doubt that the asci of *Ciliaria scutellata, Aleuria vesiculosa, Galactinia badia, Morchella crassipes*, and other similar Discomycetes, like those of the Ascoboleae, are heliotropic, and that the response of the asci to the directive stimulus of light serves to explain how it is that the ascospores discharged from the interior of hollow cups, such as those of *Aleuria vesiculosa, Galactinia badia*, etc., and from the interior of cavernous depressions at the surface of the caps of Morchellae are shot outwards into the external air without striking opposing chamber walls.

Richard Falck expressed the view that, when a Morchella or a Gyromitra fruit-body is caused to puff by irradiating it with heat from a lamp, air and water-vapour currents are produced in the hymenial chambers and passages and that these currents bear the spores along and thus enable the spores to escape from the fruit-body. The author is unable to accept this theory.

To explain the phenomenon of puffing in a Morchella or Gyromitra which has been irradiated by heat from a lamp we have only to suppose : (1) that the asci are sensitive to heat and, when ripe, always explode when their temperature is raised above a certain degree, so that puffing may be initiated by warming a fruit-body ; (2) that the asci are curved heliotropically toward the mouths of the hymenial cavities instead of being straight ; and (3) that the air-currents which arise at the moment

of puffing are due to the spores and sap-drops bombarding the air and setting it in motion mechanically and are not due to the fruit-body being hotter than the surrounding air and sending out blasts of warm air.

In *Ascobolus magnificus* about one-third of each ripe ascus protrudes freely into air above the hymenium. This terminal part of the ascus contains the eight spores and, when young, it executes a heliotropic curvature toward the source of light. The paraphyses of *A. magnificus* are anheliotropic.

On the basis of Corner's work and investigations by the author, the development and the structure of the apothecium of *Ascobolus stercorarius* have been described and illustrated. The asci of this species do not protrude so freely into the air as those of *A. magnificus*; but, as usual in the Ascoboleae, they are heliotropic.

Our present knowledge of the apothecium of *Ciliaria scutellata* (= *Lachnea scutellata*) has been summarised.

The apothecium of *Ciliaria scutellata* is discoid and its hymenium is flat or almost so. When an apothecium of *C. scutellata* develops in unilateral light, the asci all across the disc bend their free apical ends in the direction of the source of light; and, when puffing takes place, the spores are all discharged toward the source of light.

In respect to the heliotropism of their asci, the discoid fruit-bodies of *Melastiza miniata* and of *Cheilymenia vinacea* resemble those of *Ciliaria scutellata*. In all these three species, when an ascus which is curved at its end discharges its spores, it straightens considerably and the operculum, which was terminal, comes to occupy a more or less oblique position on that side of the ascus which is nearest to the source of light.

The fruit-bodies of *Aleuria vesiculosa* have been described and their varieties discussed. Previous observations on the discharge of the spores have been summarised.

In a hemispherical apothecium of *Aleuria vesiculosa* the asci are heliotropic, in consequence of which their free ends are all directed toward the apothecium's mouth. Hence, when puffing takes place, the spores are all shot out of the mouth of the apothecium and so escape freely into the air. The outer halves of the asci on the sides of an apothecium may be bent through an angle of 45°. The asci in the middle of the base of the apothecium may be quite straight. The amount of bending of an ascus varies with the position of the ascus in the hymenium and therefore with the angle at which the light strikes the end of the ascus as it is developing.

The paraphyses of *Aleuria vesiculosa* attain maturity before the asci and, like the asci, they are positively heliotropic. They bend toward the source of light and so point toward the mouth of the apothecium. This arrangement assists the asci in pushing their way up between the paraphyses as they develop.

Observations made by H. T. Güssow in a wood in Ontario, Canada,

and communicated to and recorded by the author, prove that apothecia of *Aleuria vesiculosa*, growing under purely natural conditions and untouched by man, may puff intermittently.

In respect to the heliotropism of their asci the cup-shaped apothecium of *Galactinia badia* resembles that of *Aleuria vesiculosa*. The asci on the sides of an apothecium of *G. badia* discharge their spores, not in directions which are perpendicular to the hymenium, but obliquely upwards at an angle of about 45°.

Each hymenium-lined alveolus of a fruit-body of a Morchella or Mitrophora is comparable with the single hymenium-lined cavity of one of the cupulate Pezizeae.

Investigations on fruit-bodies of *Morchella conica* and *M. crassipes* have shown that the asci in each alveolus are curved toward the mouth of the alveolus, and it is therefore concluded that the asci, like those of the Pezizeae, are heliotropic.

Since, in a fruit-body of a Morchella, the asci in each alveolus point toward the mouth of the alveolus and since the operculum of each ascus is situated symmetrically at the end of each ascus, it is clear that, when spore-discharge takes place, the spores must be shot through the mouths of the alveoli into the open air.

The folds or walls on a pileus in the Morchellaceae have been compared with the gills on a pileus in the Agaricaceae. The ascus, as a gun, has profoundly influenced the structure of discomycetous fruit-bodies; and the basidium as a gun has profoundly influenced the structure of hymenomycetous fruit-bodies.

In the Helvellaceae, more particularly in *Ptychoverpa bohemica*, evidence indicating that the asci are heliotropic has been obtained.

The asci of the Discomycetes and the basidia of the Hymenomycetes are structurally and physiologically two very different types of fungus guns. Whereas the asci are positively heliotropic, the basidia are anheliotropic. In the Discomycetes the positive heliotropism of the asci is advantageous for spore-dispersal, whereas in the Hymenomycetes a positive heliotropism of the basidia, if it occurred, would be disadvantageous for spore-dispersal.

Chapter III.—Various projectiles of fungi in order of size have been listed.

A Sphaerobolus gun gives out a distinct sound as it shoots away its gleba; and, when a gleba strikes a hard object near its place of discharge, the impact is audible. Of all fungus guns the Sphaerobolus gun is not only the largest and most powerful, but also the loudest.

When a Pilobolus gun is discharged, just as in Sphaerobolus, two sounds can be heard: (1) the sound of the exploding gun, and (2) the sound of the projectile striking some object.

A hissing sound emitted by the fruit-bodies of certain Discomycetes when puffing takes place has been noticed, among others, by Desmazières

(*Helvella ephippium*), de Bary (*Peziza acetabulum* and *Helvella crispa*), Stone (*Helvella elastica*), and Johnstone (*Otidea leporina*).

A simple method for rendering audible the puffing of Discomycetes has been described. One takes a fruit-body, which is ready to puff, out of the closed chamber where it has been confined for some hours, and one immediately places its mouth or upper hymenial side close against an ear. As soon as the fruit-body puffs (usually in 1–3 seconds), the sound of the puffing can be easily heard and one can feel one's ear being sprayed by spores and sap-drops.

By putting fruit-bodies to an ear in the manner just described, the author has heard the sound of puffing in the following species :

Aleuria repanda,	Pustularia catinus,
Aleuria vesiculosa,	Pyronema confluens,
Ascobolus stercorarius,	Rhizina inflata,
Caloscypha fulgens,	Sarcoscypha protracta,
Ciliaria scutellata,	Sarcosphaera coronaria,
Galactinia badia,	Urnula Craterium,
Peziza aurantia,	Urnula geaster.
Pseudoplectania nigrella,	

The sound produced by most large Discomycetes when puffing reminds one of hissing, but that produced by *Rhizina inflata* suggests effervescence.

The hissing sound produced by the puffing of *Pustularia catinus*, *Aleuria vesiculosa*, *Sarcoscypha protracta*, etc., is comparatively loud, but has a duration of only about two or three seconds. On the other hand, the effervescent sound produced by the puffing of *Rhizina inflata* is comparatively feeble, but has a duration of from one to several minutes. A puffing fruit-body of *R. inflata* can be passed from one person to another and thus be heard by several people in succession.

The discharge of conidia by Empusa and Entomophthora and of basidiospores by the Uredineae and Hymenomycetes, although inaudible to the unaided ear, might possibly be heard with the help of a microphone.

The sound emitted by fungus guns has no biological significance.

PART III

Chapter I.—In *Collybia radicata, Tricholoma macrorhizum, Pholiota radicosa, Coprinus macrorhizus*, and certain other Agaricaceae, the stipe of the fruit-body is prolonged downwards through the substratum for some distance by means of a so-called *rooting base*. For " rooting base " Fayod has substituted the excellent term *pseudorhiza* which has been adopted by the author.

A pseudorhiza forms a link between a mycelium vegetating in a buried root or other buried nutrient substance and the aerial part of the fruit-body which produces and liberates spores.

In most fungi with a pseudorhiza, *e.g. Collybia radicata*, the pseudorhiza is annual and unbranched; whereas in *C. fusipes*, which is exceptional, the pseudorhiza is perennial and branched.

Every fruit-body which develops as a pseudorhiza arises in the first instance as a tiny primordium at the surface of a buried root, etc. This primordium becomes differentiated into the primordium of the pileus and the primordium of the stipe. The primordium of the stipe then becomes differentiated into the primordium of the stipe-shaft and the primordium of the stipe-base or pseudorhiza. The pseudorhiza of the very young fruit-body by intercalary growth elongates just beneath the rudimentary stipe-shaft and pileus and, as it is negatively geotropic, it pushes the rudimentary stipe-shaft and pileus upwards through the soil, etc., until these organs reach the surface of the ground. Then the pseudorhiza ceases to grow in length, the stipe-shaft elongates aerially, the pileus expands, and the spores are liberated.

The length of a pseudorhiza varies with the depth of the soil overlying the buried root, etc., in which the mycelium of the fungus has vegetated.

As a rule, a pseudorhiza thickens as it grows upwards so that it is thickest just beneath the surface of the soil, *i.e.* in that position where it is subject to the greatest mechanical strain.

An account of the pseudorhiza of *Collybia radicata, Mycena galericulata*, and *Coprinus macrorhizus* has been given.

Collybia radicata lives upon the buried roots of Beeches, Oaks, Ironwoods, Horse Chestnuts and, doubtless, other trees. Its pseudorhiza may attain a length of 16 cm.

The mode of development of the pseudorhiza of *Coprinus macrorhizus* in all essentials resembles that of the pseudorhiza of *Collybia radicata*. The fruit-bodies of *C. macrorhizus* which are to develop a pseudorhiza arise as primordia upon dense straw-dung situated a little distance below the general surface of a manure pile, etc. The pseudorhiza by intercalary growth and response to the stimulus of gravity then pushes the rudimentary stipe-shaft and pileus through the overlying straw, etc., to the surface of the manure pile, and there the stipe-shaft and pileus complete their development aerially in the usual way.

In *Coprinus macrorhizus*, when a young pileus and enclosed aerial stipe-shaft are injured, the pseudorhiza may branch and give rise to several new but smaller fruit-bodies.

Chapter II.—The mycelium of *Collybia fusipes* vegetates in the wood of subterranean roots of Beeches and Oaks, and gives rise to fruit-bodies which are attached to these roots by means of a pseudorhiza.

The pseudorhiza of *Collybia fusipes* is perennial and, in the course of successive years, owing to branching and lateral fusion, may become

converted into a large obconic or irregular dark spongy structure. There is no good reason for regarding such a structure as a sclerotium.

Usually, in the first summer of fruiting of a *Collybia fusipes* mycelium, there comes up on a rotten root a solitary fruit-body which has a simple pseudorhiza like that of *C. radicata*. The pileus and aerial stipe-shaft of this fruit-body then die and decay, but the pseudorhiza buried in the ground persists and lives on through the winter. In the second summer the pseudorhiza, at its apex, gives rise to a cluster of new fruit-bodies, each of which has a pseudorhiza of its own. These new fruit-bodies eventually die down except for their pseudorhizae which persist. Thus in the second winter the persistent pseudorhiza is a branched structure. In the third summer, the pseudorhiza may, as before, give rise to new fruit-bodies, and so forth, so that, as several years go by, the pseudorhiza increases in size and becomes more and more branched.

The longest pseudorhiza of *Collybia fusipes* observed was about 12 inches in length. It was attached to a deeply buried Beech root and bore a cluster of new fruit-bodies on a tertiary branch. Presumably the pseudorhiza was three years old and the fruiting season was the fourth in succession.

In having a pseudorhiza which is persistent and perennial, *Collybia fusipes* economises fruit-body material and so, in the end, increases its output of spores. Thus the persistence of the pseudorhiza of *C. fusipes* is a factor of considerable importance to the fungus in its struggle for existence.

It is a remarkable fact that the fruit-body of *Sarcoscypha protracta*, a Discomycete whose mycelium vegetates in buried roots of Poplars, develops a pseudorhiza which, in its persistence from year to year and in its branching, exactly resembles the pseudorhiza of *Collybia fusipes*. We are here afforded another example of two very diverse plants having become adapted in the same way to meet the requirements of a similar set of external conditions.

Chapter III.—*Omphalia flavida*, the cause of the American Coffee-leaf disease, is a luminous and gemmiferous leaf-spot fungus.

In pure cultures on bread and other artificial media, as Ashby first observed, the mycelium produces in succession : (1) *gemmifers*, hitherto misnamed " *stilbum*-bodies," and (2) perfect agaricaceous *sporophores*.

Each *gemmifer* consists of a slender, solid, tapering *pedicel* about 2 mm. long and of a terminal, detachable, multicellular *gemma,* shaped like the knob of a door-handle and about 0·36 mm. in diameter.

The structure of a gemmifer has been redescribed in detail. The pedicel is solid, and not hollow as supposed by Puttemans.

Each gemma is an oblate spheroid (a flattened globe) with an *apophysis* of smaller diameter below. Its upper surface is slightly depressed in the centre. The apophysis encloses and clasps the top of the pedicel about 0·1 mm. from the pedicel's extreme end. Peripherally the oblate-

sphaeroidal part of the gemma is covered with aerial radiating filaments which the author has called *infection hyphae*. It is these hyphae which enter and infect a new host-leaf when a gemma has fallen upon it.

A sporophore of *Omphalia flavida* is a much larger structure than a gemmifer, for it is 0·6–1·5 cm. in height and its pileus is 1·5–2·5 mm. in diameter. It liberates an abundance of spores which readily germinate in malt-agar. Freshly-fallen spores were introduced into a wound in a Bryophyllum leaf, but no infection resulted. From the observations of others, it appears that the infection of Coffee leaves in Coffee plantations is due to gemmae and not to basidiospores.

A comparison of the external form and the internal structure of a gemmifer and of a sporophore of *Omphalia flavida* justifies the conclusion that a gemmifer is a highly specialised sporophore in which the pedicel is homologous with a stipe and the gemma homologous with a pileus. In a gemmifer the pileus has ceased to develop lamellae and basidia and has become detachable at maturity and thus converted into an organ of dissemination.

A pedicel of a gemmifer of *Omphalia flavida* is at first quite straight but, as it grows in length, it becomes bent at a little distance above its base through an angle of 30°–45°. This *basal curvature* is not due to gravity. When a number of gemmifers are growing together in a culture, the pedicels by means of basal curvatures come to diverge from one another. It thus appears that the pedicels of gemmifers mutually repel one another.

The abscission of a gemma of *Omphalia flavida* from the end of its pedicel in preparation for the detachment of the gemma from the pedicel by the wind takes place whilst the apophysis of the gemma firmly clasps the pedicel subterminally, and it is effected by the end of the stipe becoming sigmoidally curved and so withdrawing itself from the gemma. The *sigmoid curvature* of the end of the pedicel is always made in a vertical plane and, as experiments with a Pfeffer klinostat have shown, is due to a positive and a negative reaction to the stimulus of gravity.

A ripe gemma of *Omphalia flavida* which has undergone abscission from the extreme end of its pedicel and is holding on to the pedicel merely by means of its clasping apophysis is readily detached from its pedicel by the wind. When a gemma falls on to a leaf of a host-plant, it usually settles so that it rests on its slightly concave upper surface. Under moist conditions its peripheral infection hyphae then resume their growth and may penetrate into the leaf's interior tissues.

Detached gemmae of *Omphalia flavida* retain their vitality for over 24 hours under moist conditions but, when exposed to dry air, they soon die.

Wounded and unwounded leaves of *Bryophyllum calycinum* and unwounded isolated leaves of *Nerium Oleander* and of a species of *Ficus*, after having been inoculated with gemmae of *Omphalia flavida*, developed

leaf-spots which gave rise in succession to *O. flavida* gemmifers and sporophores. Similarly-treated isolated leaves of *Plumbago capensis* developed leaf-spots which produced *O. flavida* gemmifers only.

Omphalia flavida is able to form leaf-spots on the leaves of species belonging to the most diverse families of Flowering Plants and also on the leaves of various Ferns. A list of the plants which *O. flavida* is known to attack has been given.

The mycelium of *Omphalia flavida*, when newly obtained from gemmae developed in the open on Coffee leaves, fruits vigorously. When the mycelium is grown for several years on artificial media, it gradually loses its fruiting power, but this can be restored in large measure by passing the mycelium through living leaves.

The formation of gemmifers on a mycelium of *Omphalia flavida* is initiated by the stimulus of light and does not take place in complete darkness. When an agar-plate in which *O. flavida* is developing is exposed to daylight on a laboratory table, the mycelium forms alternate gemmiferous and non-gemmiferous zones corresponding with the diurnal hours of daylight and darkness.

The mycelium of *Omphalia flavida*, both in artificial cultures and in leaf-spots, is luminous. This discovery has provided a means for diagnosing the American Coffee-leaf disease in the dark. At night, in a Porto Rican Coffee plantation, Professor Albert Müller was able to see Coffee leaf-spots very clearly at a distance of 2–3 feet from the leaves and less clearly, although distinctly, at a distance of 6–10 feet.

Sclerotium coffeicola, which causes the Sclerotium disease of the Coffee plant in Dutch and British Guiana and in Trinidad, resembles *Omphalia flavida* in attacking many different kinds of plants, including Coffee and Ferns, and in producing gemmifers. An account of *S. coffeicola*, chiefly based on Stahel's observations, has been given.

A gemmifer of *Sclerotium coffeicola* consists of a little white knob-like *pedicel* and of a detachable slender white needle-like *gemma* 1·5–4·0 mm. long and 0·05–0·1 mm. thick. The gemmae of *S. coffeicola*, like those of *Omphalia flavida*, are blown about by the wind, settle on host-leaves, germinate there, and soon produce leaf-spots bearing new gemmifers.

On fallen leaves and on Coffee berries the mycelium of *Sclerotium coffeicola* produces sclerotia. On germinating, a sclerotium gives rise to a feathery mycelium which bears gemmifers. Perfect fruit-bodies of the fungus, so far as is known, are not produced.

The mycelium of *Sclerotium coffeicola* is provided with clamp-connexions, and this indicates that the fungus is a Basidiomycete. It is possible, as Stahel has suggested, that the gemmifers of *S. coffeicola*, which are rod-like in form and remind one of the fruit-bodies of certain Clavariaceae, may be modified fruit-bodies of a Typhula.

GENERAL INDEX